ASTMH
AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE
ADVANCING GLOBAL HEALTH SINCE 1903

VOLUME 101 NOVEMBER 2019 NUMBER 5 SUPPLEMENT

SIXTY-EIGHTH

ANNUAL MEETING

November 20–24, 2019 | **astmh.org** | **ajtmh.org** | **#TropMed19 #IamTropMed**

GAYLORD NATIONAL RESORT AND CONVENTION CENTER | NATIONAL HARBOR, MARYLAND, USA

ABSTRACT BOOK

Supplement to
The American Journal of Tropical Medicine and Hygiene

1

A PHASE IB STUDY TO INVESTIGATE THE ANTIMALARIAL ACTIVITY OF M5717, A FIRST-IN-CLASS INHIBITOR OF *PLASMODIUM* ELONGATION FACTOR 2, USING THE INDUCED BLOOD STAGE *PLASMODIUM FALCIPARUM* MALARIA MODEL

James McCarthy[1], Wilhelmina Bagchus[2], Arnand Odedra[1], Rebecca Webster[1], Claude Oeuvray[3], Aliona Tappert[4], Deon Bezuidenhout[5], Xiaoyan Yin[6], Akash Khandelwal[4], Oezkan Yalkinoglu[4]

[1]*QIMR Berghofer Medical Research Institute, Herston, Australia,* [2]*Merck Institute for Pharmacometrics, Lausanne, Switzerland,* [3]*The Global Health Institute of Merck, Eysin, Switzerland,* [4]*Merck KGaA, Darmstadt, Germany,* [5]*Merck (Pty), Modderfontein, South Africa,* [6]*emd Serono, Boston, MA, United States*

M5717 is a first-in-class antimalarial that targets *Plasmodium* elongation factor 2, thereby interrupting protein synthesis. A three cohort single-dose study was undertaken to define the antimalarial activity of M5717 in healthy volunteers using the induced blood-stage malaria (IBSM) model. Doses of 400 mg, 150 mg and 800 mg were successively selected by integrating preclinical data with the emerging human pharmacokinetic (PK) data from the ongoing phase Ia single ascending dose (SAD) study and, where relevant, the antiparasitic effect from the preceding IBSM cohort/s. Administration of 400 mg resulted in a lag of ~48 hrs prior to parasite clearance with a clearance half-life of 3.89 hrs (95% CI: 3.27-4.81). Recrudescence occurred in 2 out of 8 subjects, requiring artemether/lumefantrine rescue 12 days post dosing. Administration of 150 mg M5717 resulted in similar parasite clearance kinetics; the clearance half life was 3.52 hrs (95% CI: 3.12-4.03); recrudescence occurred in 2 out of 6 subjects 10 and 12 days after treatment. Administration of 800 mg M5717 resulted in complete clearance of parasitemia in all 8 subjects, without any recrudescence. PK parameters were similar to those observed in the phase Ia study. A PK/PD model is under development. There were no serious adverse events (SAEs) or severe adverse events (AEs). Clinical and laboratory deviations or AEs were mostly mild to moderate, malaria-related and were all transient in nature. In summary, this study has demonstrated that M5717 has a positive benefit/risk profile and supports its further clinical development of as a single dose treatment of malaria.

2

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE IB STUDY TO EVALUATE THE SAFETY, TOLERABILITY AND CHEMOPROTECTIVE ANTI-MALARIAL ACTIVITY OF P218 AGAINST CONTROLLED HUMAN MALARIA INFECTION BY DIRECT VENOUS INOCULATION (DVI) OF *PLASMODIUM FALCIPARUM* SPOROZOITE (PFSPZ-DVI) IN NON-IMMUNE HEALTHY ADULT VOLUNTEERS

Mohamed Farouk Chughlay

Medicines for Malaria Venture, Geneva, Switzerland

Globally, malaria causes substantial morbidity and mortality. With increasing drug resistance, there is an urgent need for new anti-malarial drugs for treatment and chemoprotection. P218 is a new candidate anti-malarial drug being developed for chemoprotection against *Plasmodium falciparum* malaria. Its mechanism of action involves selective inhibition of *Plasmodium* DHFR, an enzyme which catalyses the reduction of folates to tetrahydrofolates, essential for DNA biosynthesis in the malarial parasite. P218 demonstrated activity *in vitro* and *in vivo* activity on pyrimethamine *P. falciparum* resistant strains suggesting that this molecule may offer a favourable treatment advantage over pyrimethamine, a drug with similar mechanism of action. The present study which follows the First-In-Human study is investigating P218 (1000mg and 100mg) as a possible chemoprotective agent against *P. falciparum* in a standardised and model, using direct venous inoculation of aseptic, purified, cryopreserved, vialed *P. falciparum* sporozoites (*Pf*SPZ-DVI). Thirty-two healthy men and women aged 18 to 45 years are enrolled in 3 cohorts of 8, 12 and 12 subjects. Subjects are randomized in a 3:1 ratio, to receive two consecutive administrations of either P218 or placebo. Cohorts 1 (safety-1000mg) and 2 (1000mg/*Pf*SPZ) are completed and enrolment in Cohort 3 (100mg/*Pf*SPZ) is ongoing. Subjects are followed-up using a *Plasmodium* 18S rRNA targeted qRT-PCR treatment threshold to initiate rescue treatment after ≥250 estimated parasites/mL are detected. Pharmacokinetic data on P218, safety laboratory data, adverse events and parasite growth kinetics will be assessed. PK/PD modelling will be performed to identify the lowest chemoprotective P218 exposure. Data from the three completed cohorts will be presented. If the P218 activity against *P. falciparum* infection is clinically demonstrated in this study, a long acting injectable depot formulation will be considered, allowing for slow-blood-release of P218 over up to one month following single injection.

3

IDENTIFICATION OF IVERMECTIN METABOLITES

Phornpimon Tipthara[1], Kevin Kobylinski[2], Markus Winterberg[1], Joel Tarning[1]

[1]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

Preventing transmission is one of the targets in new drug discovery for malaria elimination. Ivermectin (IVM) mass drug administration (MDA) is used for controlling a number of neglected tropical diseases, but is now under consideration for malaria transmission suppression. A recent trial demonstrated mosquito-lethal effects of human venous blood from IVM-treated volunteers, with activity for several days beyond the expected half-life of IVM. This could be due to active IVM metabolites with mosquito-lethal effects. A commercial IVM tablet contains two racemic components. The major form is 25-secondary-butyl (IVM B_{1a}) (> 90%) and the minor form is 25-iso-propyl (IVM B_{1b}) (< 10%). In this study, a quadrupole time-of-flight (Q-ToF) mass spectrometer, coupled to ultra-performance liquid chromatography (UPLC), was used to identify IVM metabolites. Potentially important IVM metabolites were evaluated using *in vitro* techniques (human liver microsomes) and verified using *in vivo* clinical samples from healthy volunteers. Human liver microsomes were incubated with IVM racemic mixture (10 μM), pure IVM-B1a (10 μM), and pure IVM-B1b (10 μM). Metabolite fractions were collected one hour after incubation, and the reaction stopped with the additional of ice cold acetonitrile. Incubation at zero time, incubation without cofactor, and incubation without IVM were used as controls. For the *in vivo* study, whole blood was collected from volunteers after administration of IVM (400 μg/kg). IVM-B1a and IVM-B1b was separated successfully by the developed chromatographic method. Mass spectrometry analysis identified more than 10 potentially important metabolites in *in-vitro* samples. IVM metabolites were primarily oxidation, di-oxidation, and demethylation products. Of these, two were abundant in the blood of volunteers who had ingested IVM. To our knowledge, this is the first detailed description of IVM metabolites in human. Further evaluation of these potentially important metabolites and their mosquito lethal effects are ongoing.

4

PROPHYLACTIC EFFICACY OF POTENT AND BROADLY NEUTRALIZING, NON-CROSS-COMPETING FULLY HUMAN MONOCLONAL ANTIBODIES TARGETING PFRH5

Jonathan Viau, **Lisa Purcell**

Regeneron Pharmaceuticals, Tarrytown, NY, United States

Plasmodium falciparum reticulocyte-binding protein homolog 5 (PfRH5) is remarkably conserved, making it an ideal vaccine target. Passive immunization with monoclonal antibody (mAb) to a conserved epitope on the merozoite could provide protection throughout the malaria season. Human mAbs are ideally suited as therapeutics due to their tolerability and predictable pharmacokinetics. Recombinant PfRH5 protein and purified *Plasmodium falciparum* merozoites were used to immunize mice expressing fully human germline variable segments. We isolated a panel of human mAbs from these VelocImmune®mice that were broadly

neutralizing and potent. The mAbs were effective against both antimalarial drug-susceptible and -resistant laboratory and clinical strains *in vitro*. Both the IgG1 and IgG4 versions of these mAbs were also effective in the presence of human and *Aotus* normal serum. Growth inhibition was enhanced by the combination of mAb with chloroquine (CQ) in CQ-susceptible strains and the mAbs maintained effectiveness under CQ pressure with a CQ-resistant strain. The pharmacokinetic profiles of these mAbs in mice were consistent with that expected of human IgG specific for exogenous target. A six-week *in vitro* study with constant mAb pressure did not result in escape mutants for any one of the isolated mAbs, however, non-cross-competing RH5 mAbs from this panel could be combined to help circumvent the potential emergence of resistant strains. Nonhuman primates were used to assess the dose-dependent, prophylactic effectiveness of one of these mAbs against *P. falciparum* FVO infection. Passive immunization of PfRH5 mAb in the *Aotus* challenge model demonstrates the seasonal protection that could be afforded to humans in malarious regions.

5

DISSECTION OF HAPLOTYPE-SPECIFIC DRUG RESPONSE PHENOTYPES IN MULTICLONAL MALARIA ISOLATES

S**tandwell Nkhoma**, Amel Ahmed, Danielle L. Porier, Sharmeen Zaman, Timothy T. Stedman

ATCC, Manassas, VA, United States

Natural infections of *Plasmodium falciparum* often comprise multiple clonal lineages of the parasite. Drug susceptibility profiles of malaria isolates containing multiple parasite lineages (haplotypes) may yield variable susceptibility patterns depending on the abundance of each parasite haplotype present. We hypothesized that the observed variability in half-maximal drug inhibitory concentration (IC_{50}) of malaria isolates when measured on independent occasions is due to the presence of multiple parasite haplotypes with differing levels of antimalarial drug susceptibility. To test this hypothesis, we studied *in vitro* antimalarial susceptibility profiles of parasite haplotypes cloned from three *P. falciparum* Cambodian isolates (BEI Resources MRA-1236 IPC 3445, MRA-1240 IPC 5202, and MRA-1285 IPC 6403). Parasites were cloned by limiting dilution and typed at 23 highly polymorphic single nucleotide polymorphisms (SNPs) distributed across the *P. falciparum* genome to identify constituent haplotypes. The isolates harbored two to four co-infecting haplotypes. Isolate haplotypes were highly related, sharing up to 21 of the 23 SNPs. Individual haplotypes from two of the three isolates exhibited significant variability ($p<0.05$) in their *in vitro* susceptibility to chloroquine, mefloquine, lumefantrine and piperaquine as measured by standard growth inhibition assays. In most cases, the IC_{50} of the dominant co-infecting parasite haplotype reflected that of the uncloned parental isolate. This suggests that one co-infecting parasite haplotype often dominates the antimalarial susceptibility profile and may mask the effect of minor frequency haplotypes. We conclude that significant variability in the *in vitro* drug response phenotype of a multiclonal infection is often due to the presence of multiple parasite subpopulations with differing levels of susceptibility. We recommend cloning of isolates prior to use for *in vitro* drug susceptibility studies for screening candidate antimalarials. This practice should minimize variability in drug response across independent assays using standardized or clonal parasite lineages.

6

NANOMOLAR POTENCY INHIBITORS OF THE MALARIA PURINE UPTAKE TRANSPORTER KILL *PLASMODIUM FALCIPARUM* PARASITES

Yvett Darcie Sosa[1], Xiaoming Xu[2], Shi-Xian Deng[2], Donald Landry[2], Myles Akabas[1]

[1]*Albert Einstein College of Medicine, Bronx, NY, United States,* [2]*Columbia University, New York City, NY, United States*

Malaria is caused by infection with *Plasmodium* genus parasites. Due to spreading anti-malarial drug resistance, it is imperative to develop drugs with novel targets. One novel target is the purine uptake transporter, the *Plasmodium falciparum* Equilibrative Nucleoside Transporter (PfENT1). Previously, a yeast-based high throughput screen of the GSK compound library identified PfENT1 inhibitors. GSK transferred six hits with diverse chemical scaffolds to our lab as promising starting points for hit-to-lead medicinal chemistry. These compounds killed parasites with IC_{50} values under 15 μM. We focused medicinal chemistry efforts on one compound and synthesized 160 derivatives. 17 of these compounds had IC_{50} values under 50 nM in *P. falciparum* parasite cytotoxicity assays. All compounds killed a variety of drug sensitive and drug resistant *P. falciparum* strains with IC_{50} values between 0.2 to 158 nM. All compounds show efficacy against the *P. vivax* and *P. berghei* ENT1 homologues expressed in yeast. We tested the efficacy of compounds against human erythrocyte purine transporters, hENT1 and the human facilitated nucleobase transporter, hFNT. Four compounds did not inhibit hENT1. All other compounds inhibit hENT1 with IC_{50} values 4 to 12-fold greater than PfENT1. None of the compounds inhibited hFNT. Nine compounds did not display human hepatoma HepG2 cell cytotoxicity. Eight compounds had HepG2 cytotoxicity IC_{50} values between 10 to 30 μM. We demonstrate PfENT1 is a viable target for antimalarial drug development. These inhibitors have nanomolar potency against drug sensitive and resistant *P. falciparum* strains and represent attractive leads for further anti-malarial drug development.

7

ASPARAGINE ETHYLENEDIAMINES AS ANTI-MALARIAL *PLASMODIUM*-SELECTIVE PROTEASOME INHIBITORS

Wenhu Zhan[1], Joeseph Visone[1], Jacob Harris[1], Tierra Ouellette[1], Rong Wang[2], Hao Zang[1], Pradeep Singh[1], John Ginn[3], George Sukenick[2], Tzu-Tshin Wong[3], Judith Okoro[4], Ryan Scales[5], Patrick K. Tumwebaze[4], Philip J. Rosenthal[6], Bjorn Kafsack[1], Roland A. Cooper[5], Peter T. Meinke[3], Gang Lin[1], Laura Kirkman[1]

[1]*Weill Cornell Medical College, New York, NY, United States,* [2]*Memorial Sloan Kettering, New York, NY, United States,* [3]*Tri-Institutional Therapeutics Discovery Institute, New York, NY, United States,* [4]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [5]*Dominican University, San Rafael, CA, United States,* [6]*University of California San Francisco, San Francisco, CA, United States*

The *Plasmodium* proteasome (Pf20S) recently emerged as a target for antimalarials. Pf20S inhibitors are active at multiple stages of the parasite life cycle and synergize with artemisinins, suggesting that Pf20S inhibitors have potential to be prophylactic, therapeutic, transmission blocking as well as useful for combination therapy. We recently reported asparagine ethylenediamines (AsnEDAs) as human immunoproteasome inhibitors and modified AsnEDAs as selective Pf20S inhibitors. Here we report further structure-activity relationship study of AsnEDAs for selective inhibition of Pf20S over human proteasomes. Additionally, we show a new mutation in the Pf20S β5 subunit (β5A49S) that conferred resistance to AsnEDAs and collateral sensitivity to an inhibitor of the Pf20S β2 subunit, as in our previously identified mutation in the β6 subunit (β6A117D). This resistance could be overcome through the use of structure-guided inhibitor design. Thus, collateral sensitivity to inhibitors of one proteasome subunit can arise from point mutations in multiple other subunits, underscoring the potential value of treating malaria with combinations of inhibitors of different proteasome subunits to minimize the emergence of drug resistance.

8

THE COMPENSATORY RESERVE INDEX FOR PREDICTING SHOCK IN INTENSIVE CARE PATIENTS WITH SEVERE DENGUE

Trieu T. Huynh[1], Lam K. Phung[2], Tam T. Dong[2], Chau V. Nguyen[1], Quyen T. Nguyen[2], Ertan Deniz[3], Jane Mulligan[4], De Huynh[3], Brian Streng[3], Bridget A. Wills[2], Steven L. Moulton[5], Sophie Yacoub[2]

[1]*Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam,* [2]*Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,* [3]*Sierra*

Nevada Corporation, Sparks, NV, United States, [4]Flashback Technologies, Inc., Louisvill, CO, United States, [5]University of Colorado School of Medicine, Aurora, CO, United States

Severe dengue is defined by a plasma leak syndrome leading to intravascular volume depletion and shock. The majority of patients with dengue shock recover after a single fluid infusion, however, recurrent shock occurs in approximately 30% and carries a higher risk of poor outcomes. Early Identification of these patients would allow more intensive treatment. The compensatory reserve Index (CRI) is a new physiological parameter that tracks real-time changes in central volume. It is derived from feature analysis of the pulse arterial waveform. Arterial waveform data is processed by an algorithm, giving a CRI value between 1 and 0, where 1 represents normovolaemia and 0 represents decompensated shock (SBP < 80 mm Hg). We investigated the utility of CRI to predict re-shock compared to the current gold standards, including clinical and vital sign parameters in severe dengue. We performed a prospective observational study in the paediatric and adult Intensive Care Units (ICU) at the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. Patients were monitored with hourly clinical and vital sign parameters, and continuous recording of the arterial waveform using pulse oximetry. The waveform data was wirelessly transmitted to a laptop where it was synchronized with the patient's clinical data. 90 patients were enrolled, of which 55 had the minimum required dataset for analysis. The median age was 11 years (IQR 8-14 years). CRI had a moderate negative correlation with diastolic and systolic BP. Using logistic regression GEE models, CRI was found to predict shock (defined as PP<20mmHg) up to 5 hours before the event, ranging from the highest risk at 25 minutes prior to shock; OR 1.93, (95% CI 1.49-2.51), P<0.001 and AUC of 0.82, decreasing at 1 hour prior; OR 1.67, (95% CI 1.33-2.11), P<0.001, AUC of 0.77 and at 5 hours prior: OR 1.39, 95% (CI 1.11-2.76), P=0.004 and AUC of 0.68. A CRI cut-off of 0.4 provided the best sensitivity and specificity for predicting shock (0.8 and 0.72 respectively). In summary, CRI is a useful non-invasive method for monitoring intravascular volume status in severe dengue and can predict shock recurrence up to 5 hours before the event.

9

UPDATE FROM PAGODAS: PEDIATRIC ASSESSMENT GROUP OF DENGUE AND AEDES SALIVA TO INVESTIGATE VECTOR-BORNE DETERMINANTS OF *AEDES*-TRANSMITTED ARBOVIRAL INFECTIONS IN CAMBODIA

Rithea Leang[1], Daniel Parker[2], Dara Kong[1], Somnang Man[1], Sokunthea Sreng[1], Sreyngim Lay[1], Kimsour Nang[1], Shaden Kamhawi[3], Michael Fay[3], Emerito Amaro-Carambot[3], Stephen Whitehead[3], Stephen Whitehead[3], Seila Suon[1], Chea Huch[1], Rekol Huy[1], Thomas E. Wellems[3], Jesus G. Valenzuela[3], **Jessica E. Manning**[4]

[1]*National Center for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia,* [2]*University of California Irvine, Irvine, CA, United States,* [3]*National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [4]*National Institute of Allergy and Infectious Diseases, Phnom Penh, Cambodia*

Dengue virus is a serious public health concern in Cambodia. Defining the burden of dengue disease and the *Aedes aegypti* vector determinants that exacerbate disease transmission are critical for Cambodian public health authorities. In this longitudinal pediatric cohort based in community pagodas, we enrolled 771 healthy Cambodian children aged 2 to 9 years old in July and August 2018 to be followed twice per year (rainy and dry season) for serosurveillance for *Ae. aegypti* salivary gland homogenate antibody intensity determinations as well as dengue seroprevalence through a combination of ELISA and plaque reduction neutralization test (PRNT) assays. Traditional entomological surveys were also performed in 731 houses and 5053 water containers in the community. At baseline, dengue virus seroprevalence was: 19.5% (34/174; 95% CI 13.9-26.2) in 2 – 3 year olds, 22% (45/204; 95% CI 16.6- 28.4) in 4 – 5 year olds, 36.6% (67/183; 95% CI 29.6-44) in 6 – 7 year olds, and 65.8% (n=129/196; 95% CI 58.7-72.4) in 8 – 9 year olds. Full serotyping and PRNT titers will be available by presentation time. As the cohort data collection is still ongoing, initial geospatial analysis will be shown for baseline dengue seroprevalent participants (n=273), asyptomatic dengue cases and acute dengue cases as of October 2019 and their association (or lack thereof) with *Ae. aegypti* anti-saliva antibody intensity at wet and rainy season, mean larval density (from 5053 water containers at 731 houses), and Premises Condition Index (PCI) scoring. Our preliminary findings indicate that: 1) dengue seropositivity increases with age, with most children being seropositive by age 8; and 2) there is spatial overlap between house quality (from PCI), high *Ae. aegypti* salivary responses, and dengue seropositivity.

10

PHARMACOKINETICS OF TKM-130803 IN EBOLA VIRUS DISEASE IN SIERRA LEONEAN PATIENTS

J**anet T. Scott**[1], Raman Sharma[2], Luke W. Meredith[3], Jake Dunning[1], Catrin E. Moore[4], Foday Sahr[5], Steve Ward[2], Ian Goodfellow[3], Peter Horby[6]

[1]*MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*University of Cambridge, Cambridge, United Kingdom,* [4]*Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom,* [534] *Military Hospital, Freetown, Sierra Leone,* [6]*Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom*

TKM-130803 is a specific anti-ebola virus (EBOV) therapeutic comprising of two small interfering RNAs (siRNA) siLpol-2 and siVP35-2. The pharmacokinetics (PK) of these siRNAs was defined in Ebola virus disease (EVD) patients, with reference to efficacy (ET) and toxicology thresholds (TT). The relationship between PK and patient survival was explored. PK and pharmacodynamic (PD) data were available for seven participants with EVD in Sierra Leone who received 0.3 mg/kg of TKM-130803 by intravenous infusion over 2 hours daily for up to 7 days. Plasma concentration of siRNA was compared to survival at 14 days. PK data were fitted to two-compartment models then Monte Carlo simulated PK profiles were compared to ET (Cmax 0.04-0.57 ng/mL and mean concentration 1.43 ng/mL), and TT (3000 ng/mL). Viral loads (VL) were not significantly different at treatment onset or during treatment (p=0.1) in subjects who survived or died. siRNA was in quantitative excess of virus genomes throughout treatment, but the 95% percentile exceeded TT. Plasma concentration of both siRNAs were higher in subjects who died compared to subjects who survived (p<0.025 both siRNAs). The maximum AUC for which the 95% percentile remained under TT was a continuous infusion of 0.15mg/kg/day. TKM-130803 was circulating at concentrations considered sufficient for efficacy but given extremely high viral loads it seems likely that the patients died because they were physiologically beyond the point of no return. Subjects who died exhibited indications of impaired drug clearance, justifying caution in dosing strategies for such patients. This analysis has given a useful insight into the pharmacokinetics of the siRNA in the disease state and illustrates the value of designing PKPD studies into future clinical trials in epidemic situations.

11

HEARING LOSS ASSOCIATED WITH VIRAL HEMORRHAGIC FEVERS

Samuel C. Ficenec[1], Donald Grant[2], Robert Samuels[2], Susan D. Emmett[3], John S. Schieffelin[1]

[1]*Tulane School of Medicine, New Orleans, LA, United States,* [2]*Sierra Leone Ministry of Health and Sanitation, Freetown, Sierra Leone,* [3]*Duke University School of Medicine, Durham, NC, United States*

Hearing loss affects over 1.3 billion people worldwide. Despite well known social, economic, and neurologic consequences, this condition receives little attention. Ebola (EBV) and Lassa Fever (LF) have both been reported to cause hearing loss. However, the true burden of hearing loss secondary to these viruses is likely underestimated due to lack of standardized measurement and reporting. This is a cross-sectional study

of LF and EBV survivors and household controls. Upon recruitment into the study, survivors and controls were screened for hearing loss by determining Pure Tone Averages (PTAs) of air conduction thresholds using an AMBCO audiometer, according to WHO standards. Individuals found to have elevated PTAs were referred to confirmatory testing measuring both air and bone conduction thresholds using a SHOEBOX audiometer to differentiate between sensorineural and conductive hearing loss. As of March 2019, a total of 1329 individuals were recruited for this study, including 301 EBV survivors, 73 LF Survivors and 955 household controls. The mean age of the entire cohort was 25.3 years. Of the 301 participating Ebola survivors, 170 (56.5%) were found to have either bilateral or unilateral hearing loss vs 316 (44.4%, p <0.0001) of EBV controls . In addition, 52 (71.2%) of LF Survivors vs 120 (49.4%, p <0.001) of LF controls were to found have hearing loss on initial screening. Since the addition of confirmatory testing to the study protocol in August 2018, a total of 222 Ebola survivors and 537 contacts have been tested using this methodology. Confirmatory testing reveals 55 (24.8%) EVD Survivors vs 51 (9.5%, p <0.0001) of EVD controls have hearing loss. Confirmatory testing is still ongoing for LF Survivors and controls and will be completed by April 30, 2019.The results of this study demonstrate an association between hearing loss and survival of EVD or LF. Due to the chronic effects of this disability, it is imperative that greater emphasis be placed on characterizing EVD- and LF-related hearing loss not only to decrease the burden of disease and improve the lives of survivors.

12

MACHINE-LEARNING PROGNOSTIC MODELS FROM THE 2014-16 EBOLA OUTBREAK: DATA-HARMONIZATION CHALLENGES, VALIDATION STRATEGIES, AND MHEALTH APPLICATIONS

Andres Colubri[1], Mary-Anne Hartley[2], Mathew Siakor[3], Vanessa Wolfman[3], August Felix[1], Adam C. Levine[4], Pardis C. Sabeti[1]

[1]*Broad Institute, Cambridge, MA, United States,* [2]*University of Lausanne, Lausanne, Switzerland,* [3]*International Medical Corps, Los Angeles, CA, United States,* [4]*Brown University, Providence, RI, United States*

We created a family of prognostic models for Ebola virus disease from the largest dataset of EVD patients published to date. We incorporated these models into an app, "Ebola Care Guidelines", that provides access to recommended, evidence-based supportive care guidelines and highlights the signs/symptoms with the largest contribution to prognosis. We applied multivariate logistic regression on 470 patients admitted to five Ebola treatment units in Liberia and Sierra Leone during the 2014-16 outbreak. The models were then validated with two independent datasets from Sierra Leone. Viral load and age were the most important predictors of death. We generated a parsimonious model including viral load, malaria rapid test result, age, body temperature, bleeding, jaundice, asthenia, dyspnea, dysphagia, and referral time recorded at triage. We also constructed fallback models for when variables in the parsimonious model are unavailable. The performance of the parsimonious model approached the predictive power of observational wellness assessments by experienced health workers and was robust across the validation datasets, with Area Under the Curve (AUC) ranging from 0.75 to 0.83 and overall accuracy of 70% to 78%. Machine-learning models and mHealth tools have the potential for improving the standard of care in low-resource settings and emergency scenarios, but data incompleteness and lack of generalizable models are major obstacles. We showed how harmonization of multiple datasets yields prognostic models that can be validated across different cohorts. Similar performance between the parsimonious model and those incorporating expert wellness assessments suggests that clinically-guided machine learning approaches can recapitulate clinical expertise, and thus be useful when such expertise is unavailable. We also demonstrated with our guidelines app how integration of those models with mobile technologies enables deployable clinical management support tools that facilitate access to comprehensive bodies of medical knowledge.

13

CLINICAL PROFILE AND THERAPEUTIC RESPONSE OF MEROPENEM AND AZITHROMYCIN IN THE TREATMENT OF EXTENSIVELY DRUG RESISTANT (XDR) TYPHOID FEVER IN A LOW-MIDDLE INCOME COUNTRY

S**onia Qureshi**, Tahir Yousafzai, Abdullah Naveed, Khalil Ahmad, Sarwat Ansari, Heeramani Lohana, Farah Naz Qamar

Aga Khan University Hospital, Karachi, Pakistan

Enteric fever due to *Salmonella Typhi* is one of the leading causes of bacterial febrile disease in Pakistan and with the emergence of extensively drug resistant (XDR) *Salmonella Typhi*; it poses an even greater risk. Here we report the clinical manifestations and the treatment response of different antibiotics in treating patients with extensively drug resistant typhoid fever in Pakistan. We retrospectively reviewed the records of culture proven XDR typhoid patients who visited Aga Khan University Hospital, Karachi and Aga Khan Secondary Care Hospital, Hyderabad between April 2017 to June 2018. Only cases with complete records were included in this study. Clinicopathologic data was reviewed to assess antibiotic response used for treatment of XDR typhoid fever and frequency of different clinical symptoms was also documented. 60 patients were enrolled into this study. The two main antibiotics (azithromycin and meropenem) that were used were compared in terms of treatment response by dividing patients into 3 groups; meropenem only (n=10), azithromycin only (n=20) and combination of meropenem and azithromycin (n=30). For patients who were only administered azithromycin, their mean (±SD) time to defervescence was 7.5 (± 3.7) days with 2 treatment failures. Patients who were only administered meropenem had a mean (±SD) time to defervescence of 5.3 (± 3.5) days with 1 treatment failure. Patients who received combination of meropenem and azithromycin had a mean (±SD) time to defervescence of 7.0 (±4.0) days with 2 treatment failures. This observational study showed no significant difference in clinical outcomes when being treated with azithromycin, meropenem or both. Clinical trials are needed for further evidence.

14

HISTOPATHOLOGICAL GRADING OF ENVIRONMENTAL ENTERIC DYSFUNCTION (EED) IN THE SEEM STUDY

N**ajeeha Talat Iqbal**[1], Kamran Sadiq[1], Sana Syed[2], Zubair Ahmad[1], Romana Idress[1], Zehra Jamil[1], Kumail Ahmed[1], Junaid Iqbal[1], Shahida Qureshi[1], Aneeta Hotwani[1], Najeeb Rahman[1], Fayyaz Umrani[1], Sheraz Ahmed[1], Sean Moore[2], Asad Ali[1]

[1]*Aga Khan University, Karachi, Pakistan,* [2]*University of Virginia, Charlottesville, VA, United States*

Environmental Enteric Dysfunction (EED) is an acquired syndrome in children, a poorly defined entity of multi-factorial disease processes that encompasses environmental insults and repeated infections accompanied by structural and functional abnormalities of gut. Impoverished living conditions and inadequate nutrition markedly affect gut function that translates into linear growth faltering. Confirmation of EED requires a rigorous workup of malnutrition, including examination of GI mucosa. These studies are rare and results of few studies are primitive and equivocal. BMGF supported biopsy initiative to find treatable causes of malnutrition, and to characterize EED based on histological scoring. In this study, histological grading of EED is presented in 50 children with chronic malnutrition and failure to nutritional intervention. This longitudinal birth cohort was followed up to two years. Children were selected for upper GI endoscopy, if there was no recovery in growth (WHZ< -2.0) post educational counselling and nutritional rehabilitation. Child was assessed by physicians for further work up. Three punch biopsies were collected from D3/D2 portion of small intestine and were preserved in formalin for H&E. Biological samples were collected for exploratory biomarkers studies. Among several parameters of the grading system, acute and chronic inflammation, villous blunting, presence of IELs and detection of pathogen were analyzed by AKU histopathologists. Of 35 biopsies scored, the Mean

score was 7.0± 2.6 and range was 3 to12. Majority of cases (77%) were scored ≥ 5. *Giardia* and *H. pylori* mixed infection was observed in 18% of cases, H.pylori being most prevalent pathogen (48%), followed by Giardia (40%). Moreover, IELs were raised in 24% of cases. A negative correlation between Leptin (r-0.545; p=0.002) and IGF (r -0.293; p=0.08) were observed with increasing EED score.Further work require correlation of EED biomarkers with severity of disease, and whether EED pathology at site affects systemic or gut inflammatory biomarkers which can be captured through non-invasive EED biomarkers studies.

15

DROP-THE-LOSER ADAPTIVE INTERVENTIONS: AN INNOVATIVE DESIGN FOR FINDING THE OPTIMAL INTEGRATED MALARIA VECTOR CONTROL STRATEGIES

G**uofa Zhou**

University of California Irvine, Irvine, CA, United States

Vector control is the primary means of preventing mosquito-borne disease and has been implemented worldwide. Due to the large number of available and emerging interventions and the heterogeneous and dynamic nature of transmission, an innovative trial design must be developed to test interventions and find the optimal combination. One viable approach may be to build an adaptive design using a sequential multiple assignment randomized trial (SMART). We review the SMART design and highlight its advantages over alternative experimental designs in constructing and revising adaptive interventions. We used malaria vector control as an example to show how the new approach can be used to develop optimal integrated intervention strategies. We conducted the simulation study based on local vector ecology, malaria transmission characteristics, and environmental conditions. The simulated interventions included regular long-lasting insecticidal nets (LLINs), piperonyl butoxide-treated LLINs (PBO-LLINs), indoor residual spraying (IRS) with alternative insecticides, and long-lasting microbial larviciding (LLML). We used a drop-the-loser adaptive design with malaria infection prevalence (MIP) as the outcome measure. The simulation results indicate that, in an area with high pyrethroid resistance and moderate outdoor transmission, a) PBO-LLIN and alternative-insecticide IRS significantly reduced MIP compare to LLINs; b) when alternative-insecticide IRS or LLML were added to existing PBO-LLIN, both were effective in further reducing MIP; c) if only two interventions can be used, PBO-LLIN+LLML would be most effective in reducing MIP; and d) adding LLML on top of PBO-LLIN+IRS (with alternative insecticides) had a significant impact on MIP; however, if PBO-LLIN+LLML has already been implemented, adding IRS may not be recommended. Our simulation example provides a framework or new pathway for informing the optimal integrated intervention. The simulated results are in agreement with existing field trials, and the new strategy can be tested in field trials.

16

EFFECTIVENESS OF COMPLEMENTARY STRATEGIES ON MALARIA BURDEN AND TRANSMISSION: A FOUR-ARMED RANDOMIZED CONTROLLED TRIAL IN KORHOGO AREA, NORTHERN CÔTE D'IVOIRE

Barnabas Zogo[1], Bertin N'Cho Tchiekoi[2], Dieudonné Diloma Soma[3], Anthony Somé[3], Ludovic P. Ahoua Alou[2], Alphonsine A. Koffi[2], Florence Fournet[4], Amal Dahounto[3], Baba Coulibaly[2], Roch Kounbobr Dabiré[3], Lamine Baba-Moussa[5], Nicolas Moiroux[6], Cédric Pennetier[4]

[1]*Institut Pierre Richet/MIVEGEC (University Montpellier, CNRES, IRD)/ Université d'Abomey-Calavi, Bouaké, Côte D'Ivoire,* [2]*Institut Pierre Richet, Bouaké, Côte D'Ivoire,* [3]*Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso,* [4]*MIVEGEC (University Montpellier, CNRES, IRD), Bouaké, Côte D'Ivoire,* [5]*Université d'Abomey-Calavi, Abomey-Calavi, Benin,* [6]*MIVEGEC (University Montpellier, CNRES, IRD), Bobo-Dioulasso, Burkina Faso*

Communication for human behavioral changes, indoor residual spraying and larviciding belong to the vector control arsenal to fight against malaria. However, there are not conclusive evidences that their use in combination with the core vector control tool, long-lasting insecticide treated nets (LLINs) provide additional benefit. To help decision makers in policy making, we conducted a four-armed randomized controlled trial to assess whether the use of these tools in combination with LLINs provide additional protection against malaria in an area of high pyrethroid-resistance. The trial was conducted in 28 villages in Korhogo area, Northern Côte d'Ivoire from September 2017 to July 2018, after one year of baseline survey. We selected the villages based on the population size and a minimum distance between villages of 2 km. Eight villages were randomly allocated to larviciding with *Bacillus thuringiensis israeliensis*, six villages to IRS with pyrimiphos-methyl and six other villages to intensive communication for human behavioral changes. All these villages as well as the remaining 8 villages (control group) were covered with LLINs before the implementation of complementary strategies. We carried out four (4) entomological cross sectional surveys and five (5) epidemiological cross sectional surveys for the measurement of entomological and epidemiological outcomes after the implementation of strategies. The analysis of malaria incidence, prevalence and transmission in Korhogo will be presented.

17

THE DISTRIBUTION AND INSECTICIDE RESISTANCE STATUS OF *ANOPHELES STEPHENSI* IN EASTERN ETHIOPIA

M**eshesha Balkew**

Abt Associates Inc., Addis Ababa, Ethiopia

Anopheles stephensi was detected in the Horn of Africa for the first time in 2012 in Djibouti and in the Somali region of Ethiopia in 2016. To understand the extent of its geographic distribution and insecticide resistance status, the PMI VectorLink project in collaboration with Jigjiga and Diredawa Universities conducted a cross-sectional survey between August and November 2018, in ten urban localities in Eastern Ethiopia: Dire Dawa, Erer, Jigjiga, KebriDehar, Degehabur, Godey, Bati, Gewane, Semera and Awash Sebat Kilo. Adult mosquitoes were sampled using Human Landing Catches (HLCs), Centers for Disease Control and Prevention Light Traps (CDC LTs) and Pyrethrum Spray Catches (PSCs). Larvae and pupae were sampled from potential mosquito breeding sites using standard dippers. Morphology-based species identification was evaluated with sequence analysis at Baylor University. Susceptibility tests against six insecticides: pirimiphos-methyl, propoxur, bendiocarb, deltamethrin, permethrin and alphacypermethrin and synergist tests using piperonyl butoxide were conducted in two of the ten sites, Diredawa and Kebridehar using WHO tube test. The study revealed the presence of *An. stephensi* in all the ten study sites. The survey produced a total of 90 adult-caught and 2,149 larval-collected *An. stephensi* Cisterns, tanks, barrels and tires were found to be important larval habitats in the study areas. The two populations of *An. stephensi* were resistant to all insecticides tested (19-80% mortalities) with the exception of pirimiphos-methyl (100% mortality). Pre-exposure to PBO fully or partially restored *An. stephensi* susceptibility to deltamethrin and permethrin indicating involvement of oxidases as a resistance mechanism. The distribution of *An. stephensi* appeared to be wider than expected, based on the initial finding in one location, and warrants a nationwide survey to understand its spatial distribution in Ethiopia. Further study is also needed to determine physiological resistance, behavioral patterns and its role in malaria transmission in Ethiopia with the goal of design of appropriate vector control interventions.

18

THE PROTECTIVE GAP OF INDOOR RESIDUAL SPRAYING: WALL MODIFICATIONS AFTER SPRAYING AFFECTS ACTUAL COVERAGE AND HAMPERS MALARIA ELIMINATION EFFORTS

M**ercy Opiyo**[1], Charfudin Sacoor[2], Mara Maquina[2], Celso Alafo[2], Pedro Aide[2], Ariel Nhacolo[2], Lucia Fernandez-Montonya[3], Helena Marti[1], Francisco Saute[2], Krijn Paaijmans[4]

[1]*Barcelona Institute for Global Health, Barcelona, Spain,* [2]*Manhica Health Research Centre, Maputo, Mozambique,* [3]*World Health Organization, Geneva, Switzerland,* [4]*Arizona State University, Tempe, AZ, United States*

Though bednets and indoor residual spraying (IRS) have made a significant contribution to the reduction of malaria transmission in Africa, transmission persists, indicating that these tools alone are not sufficient for this disease. This is under the assumption that tools are implemented to full effect. Whilst the residual effect of IRS insecticides and changes in mosquito traits are being monitored, human behaviors that may affect IRS efficacy such as replastering, painting or washing of treated wall surfaces are never. Yet estimates on the number of people protected, as well as large-scale efficacy studies on the additional effect of IRS on top of nets continue. The purpose of the present study is to quantify the frequency of those human behaviors over time. Together with data on the residual effect of insecticides and information on mosquito bionomics, the protective gap of IRS can be properly quantified, which will allow us to design appropriate mitigation strategies. The study is conducted in two districts in southern Mozambique: Matutuine sprayed with Actellic® (300CS, active ingredient: pirimiphos-methyl, Syngenta) and Boane with SumiShield® (50WG, active ingredient: clothianidin, Sumitomo Chemical). Three hundred households were selected per district, and interviewed monthly using structured questionnaires to collect information related to human behavior. These included factors such as changes made to the walls since spraying, type of wall modification, number of rooms modified, specific rooms modified, adults and children sleeping in such modified spaces, reasons for wall modifications, number of rooms added since spraying, as well our their appreciation of long-lasting insecticide-treated nets, indoor residual spraying and other vector control tools. Preliminary data shows that wall modifications following a successful IRS implementation are common, with people mostly modifying bedrooms and living rooms. Final data are being collected, and will be presented during the meeting in November.

19

THE COST OF MEASURING IMPACT: RANDOMIZED CONTROL TRIAL (RCT) METHODOLOGIES FOR VECTOR CONTROL

Molly Robertson[1], Joe Wagman[1], Rose Zulliger[2], Abuchahama Saifodine[3], Baltazar Candrinho[4], Jason Richardson[5], Laurence Slutsker[6], Carlos Chaccour[7], Francisco Saute[1]

[1]*PATH, Washington, DC, United States,* [2]*President's Malaria Initiative, Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention, Maputo, Mozambique,* [3]*President's Malaria Initiative, US Agency for International Development, Maputo, Mozambique,* [4]*Programa Nacional do Controlo da Malaria, Maputo, Mozambique,* [5]*Innovative Vector Control Consortium, Liverpool, United Kingdom,* [6]*PATH, Seattle, WA, United States,* [7]*ISGlobal, Barcelona Centre for International Health, Research Hospital Clínic - Universitat de Barcelona, Barcelona, Spain*

To estimate the cost-effectiveness of combining third generation indoor residual spraying with long-lasting insecticidal bed net universal coverage campaigns, a cluster randomized control trial (RCT) was conducted over two years in Mopeia district, Mozambique from Nov 2016 - Oct 2018. The trial measured malaria burden and the effect of seasonal IRS campaigns conducted by PMI/Abt Associates using five methodologies: entomological monitoring, a monthly infection incidence cohort of ~1,500 children under 5 years old at enrollment, yearly, all-age cross-sectional surveys with parasite prevalence measured through rapid diagnostic tests, enhanced routine passive case surveillance linked to each study cluster, and costing. All of the methodologies help shape an understanding of malaria burden and the cost-effectiveness of the intervention in different ways. Some results closely aligned, for example, there were similar trends in decreased monthly vector densities as well as decreased cohort infection incidence and passive case incidence rates in communities that received IRS. But there was some discordance between the incidence and prevalence trends: during the first five months of the study incidence rates of new malaria infections in the cohort and confirmed malaria cases from district health facilities both declined significantly in IRS communities but there was no corresponding difference in malaria infection prevalence measured between IRS and non-IRS communities during the 2017 cross sectional survey. While the use of complementary impact measures provides nuanced and in-depth understanding of the effectiveness of the IRS intervention, each measure also has important analytical and cost implications. This talk will describe the details and contribution of each approach to overarching project objectives and discuss the decision, in the continuation of the study to a 3rd year, to alter the methodology. Given the cost of each method, and of RCTs in general, this discussion is important for others considering which strategies to employ when conducting studies to estimate the public health impact of vector control interventions.

20

LESSONS LEARNED, CHALLENGES AND IMPLICATIONS FOR DECISION-MAKING AFTER A DECADE OF EXPERIENCE MONITORING THE IMPACT OF INDOOR RESIDUAL SPRAYING IN BENIN, WEST AFRICA

M**artin Akogbeto**

Cotonou Research Entomology Center, Cotonou, Benin

Benin has performed Indoor Residual Spraying (IRS) in 19 communes since 2008: 4 communes in southern Benin (2008-10) and 9 communes in Atacora (2011-16) and 8 communes in Atacora, Alibori, and Donga (2017-18) in northern Benin. However, Benin still struggles with questions about IRS' costs, benefits, and epidemiological impact. We discuss lessons learned and challenges from 10 years of IRS in Benin. CREC has assessed entomological parameters in IRS districts since 2008. Compared to controls, significant decreases in *Anopheles gambiae* human biting rate*, An. gambiae* carrying *Plasmodium falciparum* in salivary glands, blood feeding, and entomological inoculation rate (EIR) occurred in all IRS districts. This encouraging 80-90% reduction in EIR should be observed with caution because: (i) it may be insufficient to decrease epidemiological indicators given the residual EIR in IRS districts was still higher than in some regions of stable malaria; (ii) it is based on comparisons with control districts, but it is difficult to select control areas with the same environmental characteristics as intervention areas; and (iii) half of all mosquitoes that entered IRS-treated houses still succeeded in taking human blood meals. Further, human behaviors limit IRS efficacy. Recent data show that >90% of Benin citizens are not fully protected by IRS from 7-10 PM because they remain outside and that most people are not fully protected from mosquito bites after 10 PM because they either sleep outside without IRS protection or inside without an ITN. People also have large amounts of items hanging on walls where mosquitoes rest, avoiding IRS-treated walls. Finally, three other issues are important to consider: (i) Vector resistance management strategies are sometimes poorly understood. Management aims to prevent the emergence of resistance; this is different from replacing an insecticide by another after resistance emerges. (ii) Before IRS begins, communities should be informed that IRS will not be a permanent measure. Clear communication and expectations are important. (iii) African countries should prepare to finance IRS and other vector control interventions themselves.

21

COST-EFFECTIVENESS OF COLLABORATING WITH THE TOGOLESE ARMED FORCES FOR LONG-LASTING INSECTICIDE-TREATED MOSQUITO NET (LLIN) MASS DISTRIBUTION CAMPAIGN

T**chaa A. Bakai**[1], Tchassama Tchadjobo[1], Josée Gnamien-Koudou[1], Jean-Emmauel Julo-Réminiac[2], Stéphane d'Almeida[3], Komi Kusiaku[3], Komla D. Kadzahlo[1], Agnidouféyi Aawi[1], Aféignitou Boukpessi[1], Batoma Tombegou-Pana[1], Esso-Kilina Tako[1], Kossi Yakpa[1], Ahoefan Djossou[1], Kansame Labarboré[1], Ley-Bawé Tchamoussa[1], Bana Botcholi[1], Batawa Akakpo[1], Kokoe D. d'Almeida[1], Afolabi Eliassou[1], Tinah Atcha-Oubou[1]

[1]*National Malaria Control Program, Lome, Togo,* [2]*HRH*[2030]*-Capacity Building for Malaria, Chemonics International, Arlington, VA, United States,* [3]*Global Fund Project Management Unit (PMU), Lome, Togo*

Malaria is the primary cause of premature death in Togo (Global Burden of Disease 2017). Aiming to maintain progress in universal coverage, Togo's National Malaria Control Program (NMCP) initiated their 2017 national long-lasting insecticide-treated mosquito net (LLIN) mass distribution campaign. The NMCP sought to distribute 4,770,250 LLINs acquired by the National Coordination Committee (NCC) with contributions from the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) (64%), the Against Malaria Foundation (26%), and the national government (10%). Due to prolonged procurement processes, unavailable or inadequate central warehouse space and distribution vehicles, and socio-political instability and insecurity, the NMCP estimated that 85% of LLINs would need to be distributed directly to the districts. By engaging high-level State authorities including the President, the NMCP facilitated a collaboration with the Togolese Armed Forces (FAT). The NCC and Ministry of Defense and Veterans Affairs signed of a memorandum of understanding with the Prime Minister (Principal Recipient of GFATM), the World Food Program (WFP) and the FAT. This allowed for the requisition of WFP-managed, State-owned warehouses for interim LLIN storage at the central level. Using army trucks staffed with one military driver and three additional soldiers, the FAT successfully transported 3,668,200 LLINs from the central warehouses to 37 district-level warehouses. The collaboration with the FAT for the LLIN distribution campaign achieved substantial savings of 186,166,637 FCFA in storage and transportation costs (more than $300,000 USD). The Alliance for Malaria Prevention lauded the Togo NMCP for this initiative with a special innovation award at its 2019 annual meeting in Geneva. Resource-constrained disease programs could benefit from considering Togo's approach to collaborate with non-health actors to strengthen the State's involvement in the fight against malaria.

22

DISSECTING THE MECHANISMS OF MALARIA INDUCED ANEMIA IN RODENT MALARIA MODELS

Keyla C. Tumas[1], Jian Wu[1], Sittiporn Pattaradilokrat[1], Lu Xia[1], Yu-Chih Peng[1], Timothy G. Myers[2], Xin-zhuan Su[1]

[1]*National Institutes of Health, Rockville, MD, United States,* [2]*National Institutes of Health, Bethesda, MD, United States*

Plasmodium parasites cause human malaria infections with symptoms ranging from no complications to deadly complications including severe anemia. Malaria induced severe anemia is likely multifactorial, arising from clearance of infected and uninfected red blood cells and an inhibition in erythropoiesis, the production of new red blood cells. However, the exact molecular mechanisms of malaria induced anemia are still largely unknown. Using *Plasmodium yoelii* N67C and 17XNL strains in C57BL/6 mice as models, we are dissecting the molecular mechanisms of malaria induced anemia and stimulation of hematopoiesis. From microarray analysis, mice infected with N67C strain have decreased expression of erythropoietic associated genes at day 4 post infection. In contrast, those infected with 17XNL strain have an initial inhibition of erythropoietic associated genes but later in the infection had an increase in expression. Using flow cytometry analysis of cells from bone marrow and spleen, where production of new red blood cells occurs during malaria infections, we found differences in progenitor cell populations of erythropoiesis. In particular, the N67C strain had decreased cell frequencies for proerythroblast and other early cell stages of erythropoiesis on day 4 post infection. In contrast, mice infected with 17XNL strain had increased cells of erythropoiesis in the spleen and bone marrow, including reticulocytes, 10 days post infection. The uninfected red blood cells from 17XNL infected mice were removed at a faster rate than those of N67C by phagocytic cells, which may explain production of more reticulocytes in the 17XNL infected mice. Future studies will aim to elucidate the molecular mechanisms contributing to the changes in erythropoiesis and red blood cell clearance, ultimately to advance our understanding of malaria induced anemia.

23

EXPERIMENTAL MALARIA IN PREGNANCY IS ASSOCIATED WITH NEUROPSYCHIATRIC DISORDERS IN OFFSPRING IN A DISEASE SEVERITY-DEPENDENT MANNER

A**ndrea Weckman**[1], Vanessa Tran[2], Chloe R. McDonald[2], Kevin C. Kain[3]

[1]*University of Toronto, Toronto, ON, Canada,* [2]*Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Toronto, ON, Canada,* [3]*Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Tropical Disease Unit, Department of Medicine, University of Toronto, Toronto, ON, Canada*

Each year ~125 million pregnant women are at risk for malaria infection. Malaria in pregnancy (MiP) has a profound impact on mother-child health, including delivery of low birth weight (LBW) infants. Even in the absence of LBW, epidemiological studies show a link between maternal infection and increased susceptibility of offspring to neuropsychiatric disorders later in life. There is preclinical evidence for a correlation between severity of maternal immune activation and severity of psychiatric outcome. The two-hit hypothesis of neuropsychiatry posits that a first "hit" (i.e. maternal infection) primes offspring for increased susceptibility to psychiatric disease in response to a second "hit" (i.e. stress) later in life. The impact of malaria exposure *in utero* on long-term vulnerability to psychiatric disease has not been reported. We hypothesize that exposure to MiP will prime offspring to an increased risk of psychiatric disorders in a disease severity-dependent manner. We used the established experimental mouse model of MiP (EMIP) with *Plasmodium berghei* ANKA (PbA). Uninfected adult offspring of dams infected with PbA underwent a battery of standardized behavioural tests. Exposure to EMIP *in utero* induced anxiety-like behaviour and hypersensitivity to amphetamine, and deficits in prepulse inhibition compared to unexposed offspring ($p<0.05$). In dams with less severe EMIP, which was characterized by lower parasitemia and lower maternal IL-6 ($p=0.01$), only offspring exposed to both EMIP plus a second hit of stress in puberty exhibited increased anxiety-like behaviour and hypersensitivity to amphetamine compared to EMIP alone ($p<0.05$). While exposure to more severe EMIP induced psychiatric disease in offspring alone, less severe EMIP acted synergistically with stress in puberty to induce a deficit. Our data implicate MiP as a modifiable risk factor for psychiatric disorders in offspring, even in instances of less severe disease. This concept represents a paradigm shift in our understanding of mental illness in malaria-endemic settings and may shift global health priorities from costly rehabilitation to prevention.

24

COMPARATIVE TRANSCRIPTOMICS IDENTIFIES PHENOTYPIC SIMILARITIES BETWEEN MOUSE MODELS AND HUMAN SEVERE MALARIA

A**thina Georgiadou**[1], Pablo Soro Barrio[1], Claire Dunican[1], Hyun Jae Lee[2], Michael Levin[1], Myrsini Kaforou[1], Aubrey Cunnington[1]

[1]*Imperial College London, Section of Paediatrics, London, United Kingdom,* [2]*Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia*

Mouse models are widely used in severe malaria research. However, the relevance of these models to human severe malaria (SM) pathophysiology is frequently disputed. New approaches are needed to objectively quantify and improve the translational relevance of mouse models. Comparative transcriptomics provides an unbiased assessment of similarities of biological processes between species.We performed RNA-sequencing on whole blood of C57BL/6 mice infected with each of 5 commonly-used rodent malaria parasites (*Plasmodium berghei* ANKA, *P. berghei* NK65, *P. yoelii* 17XL, *P. yoelii* 17XNL, and *P. chabaudi* AS). We identified differentially expressed genes (DEGs) in the whole blood transcriptomes between uninfected or early stage infections vs. late stage infections. In corresponding analyses in multiple datasets from African children we identified DEGs between healthy controls or uncomplicated malaria (UM) vs. different SM phenotypes including hyperlactatemia and / or cerebral malaria (CM). Unsupervised clustering analyses on orthologous DEGs in humans and mice revealed that *P. yoelii* 17XL was the most similar to human hyperlactatemia at a transcriptomic level, whilst *P. berghei* ANKA (experimental CM) infection was the most similar to human CM. However in both cases similarities between human and mouse were not absolute, with both concordant and discordant DEGs noted. No mouse model of hyperlactatemia has previously been reported, therefore we investigated blood lactate concentrations in each model and found that *P. yoelii* 17XL infection caused extreme hyperlactatemia (18-21mmol/L) with similar concentrations to the maximum values observed in human SM, whilst other models induced only modest or no increase in blood lactate. These findings demonstrate the potential of unbiased comparative transcriptomics to identify the most relevant mouse models for translational research. Concordant and discordant differential gene expression may be used to efficiently identify mechanisms and therapeutic targets for translational investigations in mouse models, and to exclude those which are unlikely to have translational relevance.

25

EXPRESSION PROFILING PATIENT SAMPLES IDENTIFIES GAMETOCYTE-COMMITTED RING BIOMARKERS

S**urendra K. Prajapati**[1], Ruth Ayanful-Torgby[2], Michelle C. Barbeau[1], Festus K. Acquah[2], Elizabeth Cudjoe[2], Courage Kakaney[2], Jones A. Amponsah[2], Evans Obboh[3], Benjamin K. Abuaku[2], Linda E. Amoah[2], Kim C. Williamson[1]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana,* [3]*University of Cape Coast, Cape Coast, Ghana*

The sequestration of immature stage *Plasmodium falciparum* gametocytes (stages I-IV) has made it challenging to track gametocyte development in peripheral blood samples and complicated the analysis of factors that contribute to *in vivo* gametocytogenesis. This challenge could be overcome by developing a gametocyte committed (gc)-ring biomarker. To identify potential markers, we used our *ex vivo* gametocyte culture assay to evaluate gc-rings in Ghanaian malaria patients (n=367) from 2015-2018 and observed a wide range of gametocyte conversion rates (GCR: sexual- to asexual-ring ratio) from 0 to 78%. Microarray analysis of the day 0 (D0) ring stage parasite transcriptome from high GCR (~3-66%; n=9) and low GCR (0%; n=8) samples revealed increased transcripts from only 8 genes (FDR=<0.045) in the high GCR samples. These 8 genes included a transcription factor (*ap2-g),* three enzymes *(hda1, lshd* and *arom),* and four proteins predicted to be exported to the RBC (3 *surfins* and one uncharacterized exported protein). *In vitro,* the ratio of the relative abundance of transcripts in ring stages and gametocytes was high (>11) only for *ap2-g* and two *surfins*, suggesting potential as specific biomarkers. Further *in vitro* testing using gametocyte-deficient and -producer strains demonstrated a correlation between the D0 RNA levels and D8 gametocyte production as well as D8 *pfs25* or *pfs230* RNA levels (R^2=0.56-0.77). The expression levels of all 3 genes are tightly correlated (R^2=0.99) and both *surfin*s were found to be AP2-G-dependent using an AP2-G inducible line. Importantly, in D0 RNA harvested from 224 malaria patient blood samples, the relative abundance of transcripts for all three genes correlated with the patient's D8 GCR (R^2=0.41-0.68) as well as *pfs230* transcript levels in D8 *ex vivo* culture (R^2=0.63-0.711). Further, transcripts for these genes were detectable in asymptomatic carriers. Together this work suggests these three genes are reliable early gametocyte biomarkers to study *in vivo* gametocyte production and, potentially, to use as a tool to predict malaria transmission 10-12 days before the circulation of mature gametocytes in the peripheral blood.

26

HEPATOCYTE BINDING PEPTIDE HP1 TARGETS *PLASMODIUM* SPOROZOITE-HEPATOCYTE INTERACTION

Sung-Jae Cha, Marcelo Jacobs-Lorena

Johns Hopkins University, Baltimore, MD, United States

Previously, using a phage peptide display library, our group has identified *Plasmodium* parasite ligands and corresponding host cell receptors important for ookinete-mosquito midgut interaction, sporozoite-mosquito salivary gland interaction and sporozoite-Kupffer cell interaction in the mammalian liver. Here we report on a phage display library screen for peptides that bind to hepatocytes, the sporozoite target for infection of the liver. We hypothesize that such peptides may be mimotopes (structural mimics) of *Plasmodium* sporozoite ligands for hepatocyte interaction. A prime candidate peptide from this screen - HP1 - binds to a ~25 kDa hepatocyte membrane protein (a candidate receptor) and an anti-HP1 antibody recognizes a ~45 kDa sporozoite surface protein (a candidate ligand). Immunization with HP1 protected 50 % mice from *P. berghei* challenge. Significantly, anti-HP1 antibody inhibited *P. falciparum*-human hepatoma cell (HepG2) interaction by 87 % compared to control antisera. This sporozoite ligand is a potential vaccine antigen targeting malaria liver invasion.

27

RISK OF READMISSION IN UGANDAN CHILDREN WITH SEVERE MALARIAL ANEMIA

Samina Bhumbra[1], Gregory S. Park[2], Robert O. Opoka[3], Dibyadyuti Datta[1], Chandy C. John[1]

[1]*Ryan White Center for Pediatric Infectious Disease and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Office of the Vice President for Research, University of Minnesota, Minneapolis, MN, United States,* [3]*Department of Paediatrics and Child Health, Makerere University, Kampala, Uganda*

Post discharge morbidity from severe malarial anemia (SMA) remains significant for survivors. As many as 16% of children diagnosed with SMA who survive are readmitted or die by 6 months. Identification of biomarkers predictive of recurrent severe malaria in children with SMA could help to define pathophysiologic factors that put children at risk for recurrent severe malaria. We assessed plasma concentrations of 25 pro- and anti-inflammatory cytokines and chemokines, markers of endothelial activation, and hematopoietic/vascular growth factors by cytometric bead assay in a cohort of Ugandan children aged 18 months to 12 years with severe malarial anemia (n=119). We then compared plasma concentrations of these factors in children with recurrent severe malaria (RSM, n=12) vs. no recurrent severe malaria (n=107) in the 6 months following the SMA episode. Children with RSM had lower baseline levels of PDGF bb (median [IQR], 429.15 pg/mL [269.93-649.47]), VEGF (33.43 pg/mL [19.12-49.93]), and EPO (2717.57 mU/mL [968.68-3410.17]) compared to children who

did not have RSM (PDGF bb 1193.30 pg/mL [508.68-2114.47], VEGF 67.5 pg/mL [34.2-102.94], EPO 4974.0 mU/mL [2979.7-10353.1]; all P<0.05). Children with RSM also had an increased Ang-2:Ang-1 ratio, an indicator of endothelial dysfunction associated with higher mortality in severe malaria (0.60 [0.47-1.13]), than children without RSM (0.31 [0.13-1.05], P=0.0477). Suppression of factors associated with angiogenesis (PDGF-bb, VEGF) and erythropoietic drive (EPO), and an increase in the Ang-2/Ang-1 ratio, leading to endothelial dysfunction, may put children with SMA at higher risk for recurrence of severe malaria.

28

MALIAN CHILDREN WITH SEVERE MALARIA SUBTYPES HAVE DISTINCT ANTIBODY GAPS TO VIRULENT PFEMP1S

M**ark A. Travassos**[1], Paul Han[1], Drissa Coulibaly[2], Albert E. Zhou[1], Antoine Dara[1], Biraj Shrestha[1], Rie Nakajima[3], Aarti Jain[3], Omid Taghavian[3], Algis Jasinskas[3], Matthew B. Laurens[1], Amadou Niangaly[2], Amed Ouattara[1], Andrea A. Berry[1], Matthew Adams[1], Shannon Takala-Harrison[1], Bourema Kouriba[2], Abdoulaye K. Kone[2], J. Alexandra Rowe[4], Ogobara K. Doumbo[2], Kirsten E. Lyke[1], Philip L. Felgner[3], Christopher V. Plowe[5], Mahamadou A. Thera[2]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Sciences, Techniques and Technologies, Bamako, Bamako, Mali,* [3]*University of California Irvine, Irvine, CA, United States,* [4]*University of Edinburgh, Edinburgh, United Kingdom,* [5]*Duke University, Durham, NC, United States*

Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) antigens play an important role in parasite sequestration and host immune system evasion. Acquired antimalarial immunity is at least partially due to antibodies directed against highly variable antigens like PfEMP1 that are present on the erythrocyte surface. PfEMP1 antigenic domains that drive immune-mediated protection in severe malaria have not been identified. We hypothesized that Malian children with severe malaria subtypes have lower seroreactivity to subsets of non-CD36-binding PfEMP1s containing domain cassettes (DCs) associated with severe malaria pathogenesis compared to matched uncomplicated malaria controls. We developed a custom protein microarray including 158 PfEMP1 fragments from the 3D7 reference genome and 78 fragments from PfEMP1s associated with severe malaria, including DCs 1, 5, 8, 13, and 15 from the IT4, HB3, and DD2 reference strains and DC8- and DC13-containing Malian PfEMP1s sequenced from field isolates. We measured serological responses for 34 cerebral malaria cases, 18 severe malarial anemia cases, and 9 cases of both cerebral malaria and severe malarial anemia, as well as age-matched controls who had uncomplicated malaria. Compared to controls, children with cerebral malaria had lower seroreactivity to PfEMP1s featuring DC1, DC5, DC8, DC13, and CIDRα1.7, but not to any 3D7 PfEMP1s known to bind CD36. In contrast, children with severe malarial anemia had lower seroreactivity to PfEMP1s featuring DC1, DC4, DC5, DC6, DC8, DC13, and CIDRα1.7, as well as several 3D7 CD36-binding PfEMP1s. Interestingly, children with both cerebral malaria and severe malarial anemia did not differ in PfEMP1 seroreactivity with controls, although low numbers preclude definitive conclusions. Acute and convalescent serologic comparisons for severe malaria are ongoing. These results suggest that immune-mediated protection against cerebral malaria may overlap with immune-mediated protection against severe malarial anemia, but the two are not identical, a finding that should be accounted for in severe malaria vaccine and treatment development.

29

POLYMORPHONUCLEAR, BUT NOT MONOCYTIC, MYELOID-DERIVED SUPPRESSOR CELLS CONTRIBUTE TO IMMUNOMODULATION IN CHRONIC LOIASIS

Rafiou Adamou[1], Gerrit Marwin Burger[1], Ruth Kreuzmair[1], Carlos Calle Lamsfus[2], Luzia Veletzky[3], Wolfram Metzger[2], Benjamin Mordmüller[2], Michael Ramharter[3], Ghyslain Mombo-Ngoma[1], Ayola Akim Adegnika[1], Rella Manego Zoleko[1], Matthew B. McCall[1]

[1]*Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon,* [2]*Institut für Tropenmedizin, Tübingen, Germany,* [3]*Bernhard Nocht Institut für Tropenmedizin, Hamburg, Germany*

Loa loa, known also as eye worm, is a filarial disease in which adult nematodes can migrate through subcutaneous tissues for many years, producing microfilaria that circulate in blood at concentrations of up to 100,000/mL, yet generally without triggering profound inflammation. The regulatory mechanisms involved in this paragon of immunological tolerance have nevertheless received almost no attention. Monocyte-like (M-) and polymorphonuclear (PMN-) myeloid-derived suppressor cells (MDSCs) are capable of displaying immunosuppressive features and play important roles in regulating the immune response against cancer and some infectious diseases. Here we compared the magnitude and immunosuppressive phenotype of M-MDSC and PMN-MDSC populations by flow cytometry in the peripheral blood of *L. loa* microfilaremic (MF+) and non-microfilaremic (MF-) subjects in an ongoing cross-sectional study in Moyen-Ogooué province in Gabon. The functional immunosuppressive activity of PMN-MDSC was assessed using *in vitro* T cell proliferation assays. We also assessed the dynamics of MDSCs in MF+ subjects undergoing treatment with albendazole. Our primary results show that (i) PMN-MDSC but not M-MDSC numbers are higher in MF+ than in MF- subjects; (ii) PD-L1 expression on PMN-MDSC, but not M-MDSC, is higher in MF+ than in MF- subjects; (iii) PMN-MDSC from these subjects show patent suppressive effects on T cell proliferation *in vitro*; (iv) PMN-MDSC but not M-MDSC numbers show a transient increase during albendazole treatment, followed thereafter by a decrease to below baseline. PMN-MDSC thus appear to be key players in the critical balance between anti-parasitic effector responses and immunopathology in chronic loiasis. The molecular pathways by which they are induced by this parasite and their relative cost/benefit to the host, including e.g. bystander effects on immune responses against other prevalent pathogens, remains to be determined.

30

MICROFILARIAE TRIGGER MURINE AND HUMAN EOSINOPHIL EXTRACELLULAR TRAPS IN A DECTIN-1-DEPENDENT MANNER

A**lexandra Ehrens**[1], Benjamin Lenz[1], Anna Lena Neumann[1], Samuela Giarrizzo[1], Stefan J. Frohberger[1], Wiebke Stamminger[1], Benedikt C. Bürfent[1], Frederic Fercoq[2], Coralie Martin[2], Daniel Kulke[3], Achim Hoerauf[1], Marc P. Hübner[1]

[1]*University Hospital Bonn, Bonn, Germany,* [2]*Muséum National d'Histoire Naturelle, Paris, France,* [3]*Bayer Animal Health GmbH, Monheim, Germany*

During primary filarial infection, eosinophils mediate protection against adult worms and microfilariae, but not against infective third-stage larvae. A recently described defense mechanism by eosinophils is the production of extracellular DNA traps (EETosis), a form of cell death where intracellular DNA is explosively released, entrapping pathogens and supporting their killing. The results of the present study demonstrate that microfilariae, but not third-stage larvae of the filarial nematode *Litomosoides sigmodontis*, trigger DNA release by eosinophils as analyzed by scanning electron microscopy, confocal microscopy and quantitative fluorescence DNA assay. *In vitro*, these eosinophil DNA traps, consisting of nuclear and mitochondrial DNA, inhibit microfilariae motility in a DNA- and direct cell contact-dependent manner. Eosinophils isolated from the gut of naïve and gut and pleura of filariae-infected-mice demonstrate an enhanced microfilariae motility reduction compared to bone-marrow-

derived eosinophils, whereas gut- and pleura-derived eosinophils primed during *L. sigmodontis* infection and gut-derived eosinophils from naïve animals showed comparable DNA-trap-dependent microfilarial motility reduction. *In vitro* assays further reveal that microfilariae-induced EETosis is independent of the presence of antibodies. Dectin-1 recognition was identified as the underlying signaling pathway involved in triggering the microfilariae-induced EETosis. DNA-dependent inhibition of microfilariae motility and microfilariae-induced DNA release by eosinophils appears to be a conserved mechanism since murine as well as human eosinophils respond to microfilariae derived from the rodent filarial nematode *L. sigmodontis* and the canine heartworm *Dirofilaria immitis*. *In vivo* studies reveal an increase in local DNA concentration upon *L. sigmodontis* infection in mice. Intravenous microfilariae injection raised systemic DNA concentrations, which is partly mediated by eosinophils, indicating a potential role of EETosis as an *in vivo* effector mechanism as well.

31

A PARASITE-ENCODED HUMAN IL-10 RECEPTOR ANTAGONIST REVEALS A NOVEL STRATEGY USED TO MODULATE THE HOST RESPONSE IN FILARIAL INFECTIONS

A**lessandra Ricciardi**, Thomas B. Nutman

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

IL-10 has been shown to be the primary immunoregulatory cytokine driving the modulation of the host response in filarial infections. To determine whether parasite-derived molecules could modulate the host IL-10/IL-10R pathway, we performed solid-phase immobilization of human IL-10Rα followed by binding assays with *Brugia malayi* (Bm) adult antigen extracts. Bm proteins that bound to human IL-10Rα were eluted and analyzed by liquid chromatography-tandem mass spectrometry. Our analysis identified 5 Bm molecules that bound human IL-10Rα. The top hit, Bm5539, a 114kDa GTPase activator, contains PH, Rho-GAP, and SH3 domains as well as coiled coil motifs. Using a structural alignment program, we identified a 164 amino acid sequence from Bm5539 that shared high structural homology with the human IL-10 functional dimer. This truncated sequence was also structurally similar to EBV- and CMV-encoded viral IL-10. Furthermore, predicted functions using COFACTOR and COACH included cytokine receptor binding as well as growth factor receptor binding. Sequence comparisons revealed that other filarial parasites (*Wuchereria, Loa, Onchocerca*) possess Bm5539 orthologues with 80-90% homology. Moreover, the truncated IL-10-like sequence is conserved among these other filarial species. Using a baculovirus-expressed truncated form of Bm5539, we examined its ability to signal through the human IL-10R, using phosphorylation of STAT3 (pSTAT3) in human monocytes by flow cytometry as the readout. Despite its ability to bind to human IL-10R, Bm5539 did not induce pSTAT3. In contrast, when we incubated monocytes in the presence of both rBm5539 and human IL-10, we observed an 88% decrease in pSTAT3. Furthermore, this effect was Bm5539 dose-dependent. Thus, *in vitro*, recombinant truncated Bm5539 abrogated IL-10 signaling through its receptor, which demonstrates that Bm5539 acts as an IL-10R antagonist most likely through competitive binding to the human receptor. This class of parasite-encoded cytokine receptor agonists and antagonists provides an additional lens through which parasite-induced modulation of the host immune response can be examined.

32

INTERLEUKIN-4 SIGNALING PLAYS A MAJOR ROLE IN UROGENITAL SCHISTOSOMIASIS-ASSOCIATED BLADDER CARCINOGENESIS

Evaristus C. Mbanefo[1], Chi-Ling Fu[2], Christina P. Ho[3], Loc Le[1], Kenji Ishida[1], Michael H. Hsieh[1]

[1]*Biomedical Research Institute, Rockville, MD, United States,* [2]*Pharmacyclics, Sunnyvale, CA, United States,* [3]*Children's National Medical Center, Washington, DC, United States*

Urogenital schistosomiasis (UGS) is a class 1 carcinogen, but the exact mechanism of schistosomiasis-induced bladder carcinogenesis is largely unknown. Although the mechanistic role of IL-4 signaling and the IL-4 inducing principle from schistosomes eggs (IPSE) in driving the type-2 granulomatous chronic inflammatory and fibrotic pathogenesis due to urogenital schistosomiasis is well recognized, we are yet to demonstrate the important causative role or otherwise of IL-4R signaling in driving bladder carcinogenesis. Using our previously described intramural bladder wall injection-based mouse model of urogenital schistosomiasis, we examined the mechanistic role of IL-4 signaling in the induction of bladder pathogenesis and carcinogenesis during urogenital schistosomiasis. Readouts include histopathological comparison, assessment of urothelial proliferation and urothelial chromosomal structural abnormalities between intramural bladder wall egg-injected IL-4 receptor alpha knockout (IL-4RαKO) mice versus wildtype BALB/c. To further assess the role of IL-4 in these oncogenic urothelial changes, we assessed the role of exogenous IL-4 on urothelial cell (HCV-29) proliferation, including assessment of phosphorylation pattern of downstream regulators in the IL-4 signaling pathway. Our results show that IL-4 receptor signaling is required for the recapitulation of the pathogenic features of urogenital schistosomiasis. There was significant decrease in the size and intensity of granulomatous response to the bladder-wall injected parasite eggs in the IL-4RαKO group (p= 0.0347). Next, we showed parasite egg-induced urothelial proliferation, including evidence of urothelial hyperdiploidy in wildtype mice following bladder wall egg injection. Again, these urothelial changes were found to be dependent of IL-4 receptor signaling. We further observed features consistent with oncogenesis following urothelial exposure to IL-4 and showed that IL-4 induces urothelial cell proliferation changes and potentially bladder carcinogenesis mainly via PI3K/AKT signaling cascade.

33

TARGETING THE HEDGEHOG PATHWAY IS A NOVEL THERAPEUTIC STRATEGY TO TREAT SCHISTOSOMIASIS FIBROSIS AND PORTAL HYPERTENSION

Thiago de Almeida Pereira[1], Paula Vidigal[2], Izabela Voieta[2], Vivian Resende[2], Rafal Witek[3], Anil Jegga[4], Joseph Arron[5], Satish Madala[4], José Roberto Lambertucci[2], Anna Mae Diehl[6], Thomas Wynn[7], Philip Beachy[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*Federal University of Minas Gerais, Belo Horizonte, Brazil,* [3]*Thermo Fisher Scientific, Frederick, MD, United States,* [4]*Cincinatti Children's Hospital Medical Center, Cincinatti, OH, United States,* [5]*Genentech Inc., South San Francisco, CA, United States,* [6]*Duke University, Durham, NC, United States,* [7]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States*

IL13 and Hedgehog (Hh) signaling pathways have both been implicated in the pathogenesis of fibrosis. Our aims were to determine if there is cross-talk between IL13 and Hh pathways and if Hh pathway inhibitors could be used as anti-fibrotic therapy in schistosomiasis mansoni. Hh/IL13 signaling were investigated by qRT-PCR, immunohistochemistry and ELISA in uninfected healthy transplant donors (n=22), infected hepatointestinal schistosomiasis patients (liver granulomas, low fibrosis, n=17), infected hepatosplenic patients (advanced fibrosis and portal hypertension n=72); in *Schistosoma mansoni* infected mice (wild-type, IL13Rα1-/- and TKO (IL-10-/- IL12p40-/-IL13Rα2-/-) treated with anti-IL13 antibody, Hh

pathway inhibitors (Vismodegib or AsO3 or HPI1-4) or vehicle; in mice overexpressing IL13 (plasmid) and in human liver cells stimulated with recombinant IL13 (rIL13) and treated with STAT6 siRNA or Vismodegib. Hh signaling is upregulated in human schistosomiasis and correlates with IL13, fibrosis stage and severity of portal hypertension. Overexpression of IL13 (plasmid, infected TKO mice, rIL13) induced Hh ligand production/pathway activation; lack of IL13 signaling (IL13Rα1-/- infected mice, anti-IL13 antibody, STAT6 siRNA) implicated in reduced Hh pathway, indicating that Hh signaling is dependent on IL13. STAT6 Chromatin Immunoprecipitation assay further demonstrated that STAT6 directly bind to the promoter region and regulate the transcription of Hh ligands (Ihh, Dhh) and transcription factors (Gli1, Gli2, Gli3). Smoothened antagonist Vismodegib effectively blocked fibrosis during acute schistosomiasis but failed to inhibit Hh signaling/fibrogenesis when treatment was initiated in chronic phase due to Smoothened-independent IL13-mediated Gli2 activation. Gli2 inhibition with AsO3 or HPI1-4 in the chronic phase impaired Hh signaling, fibrogenesis and inflammation. Activation of the Hh pathway in schistosomiasis is highly dependent on IL13-mediated signaling. Targeting Hh pathway with Gli2 antagonists may be a novel therapeutic strategy to treat schistosomiasis fibrosis and portal hypertension.

34

FROM SATELLITES TO SNAILS IN NORTHERN SENEGAL: HONING IN AN HIGHLY PRODUCTIVE SNAIL HABITATS USING REMOTE SENSING TECHNOLOGIES FOR TARGETED AND INTEGRATED VECTOR CONTROL OF SCHISTOSOMIASIS

Caitlin M. Wolfe[1], Christopher J. Haggerty[1], Andy Chamberlin[2], Isabel J. Jones[2], Raphael Ndione[3], Sidy Bakhoum[3], Nicolas Jouanard[3], Gilles Riveau[3], Chelsea Wood[4], Sanna Sokolow[2], Giulio De Leo[2], Jason R. Rohr[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Stanford University, Palo Alto, CA, United States,* [3]*Espoir Pour la Sante, Saint-Louis, Senegal,* [4]*University of Washington, Seattle, WA, United States*

Schistosomiasis often evades common control efforts, in part due to poor understanding of the spatial distribution of inherent transmission risk. While praziquantel effectively clears infection, it does not provide prophylactic protection and humans are re-infected when they return to the same waterbodies. According to WHO, administration of praziquantel covers less than 10% of the at-risk population in Senegal, where the logistics of mass drug administration preclude effective implementation. To address this, we present preliminary data revealing an innovative approach using remote sensing technology and artificial intelligence to schistosomiasis vector hotspots to facilitate geographically-targeted schistosomiasis control. Previous work across 16 villages in northern Senegal, conducted seasonally from summer 2016 to present, found that the removal of submerged vegetation at water access sites resulted in a 103- and 16-fold reduction in *Bulinus* and *Biomphalaria* snails, respectively, relative to controls. This suggests that mapping locations of *Ceratophyllum demersum* via remote sensing can identify hotspots of transmission risk. Through a grant from the Digital Globe Foundation (WorldView-2 imagery) combined with fine scale drone imagery of open water, *C. demersum,* other submerged vegetation, and emergent vegetation at the study sites, we developed the ability to discriminate submerged vegetation from open water and emergent vegetation, allowing for aerial identification of schistosomiasis habitats using image classification and training tools within ArcMap 10.6. These abilities were enhanced when spectral signatures (mean RGB values and NDVI scores) for sampled sites with *C. demersum* and many vector snails and those with *C. demersum* but few (less than 10) to no vector snails were compared, with preliminary analyses indicating significant differences between the mean RGB values and NDVI scores. Ongoing analyses are identifying specific signatures to interpolate over new locations in order to identify additional productive snail habitats. The resulting model is set for ground validation in the summer 2019 field season.

35

THE ROLE OF IRRIGATED AGRICULTURE IN SCHISTOSOMIASIS RISK IN A DAMMED LANDSCAPE IN WEST AFRICA

Andrea Lund[1], David Rehkopf[1], Susanne Sokolow[2], Nicolas Jouanard[3], M. Moustapha Sam[3], Assane Fall[3], Gilles Riveau[3], Jason Andrews[1], Giulio De Leo[2], David Lopez-Carr[4]

[1]*Stanford University, Stanford, CA, United States,* [2]*Hopkins Marine Station, Stanford University, Pacific Grove, CA, United States,* [3]*Centre de Recherche Biomedicale - Espoir Pour La Sante, Saint-Louis, Senegal,* [4]*University of California Santa Barbara, Santa Barbara, CA, United States*

Since dam development in the late 1980s, the Senegal River remains hyperendemic for schistosomiasis. The dams were conceived in response to a decade-long drought and designed to improve food security in an arid region vulnerable to climate change. The expansion of irrigated agriculture, while economically beneficial, is offset by a high burden of debilitating disease. This study investigates whether the choice to pursue irrigated agriculture at the household level affects risk for schistosomiasis. Presence and intensity of re-infection following treatment were discerned from urine samples from 1414 school-aged children (SAC) in 16 study villages in February 2017. Household surveys were conducted the preceding August in the 655 households where the 1414 SAC lived. Use and characteristics of agricultural land were self-reported for each household, along with demographic and socio-economic indicators. Logistic and Poisson regression were used to determine the relationship between agricultural livelihoods and infection presence and intensity, respectively, controlling for age, sex, geographic location, domestic water contact, wealth and the presence of other high-risk occupations. Area of irrigated land was marginally significantly associated with the presence of urinary schistosome infection (odds ratio = 1.04, p = 0.057), after controlling for covariates listed above. Area of irrigated land was positively and significantly associated with the intensity of urinary schistosome infections (rate ratio = 1.05, p < 0.001). The risk of urinary schistosome infection is elevated in children living in households actively pursuing irrigated agriculture. In particular, infection intensity appears to increase with increased agricultural activity, after controlling for a number of covariates. These results suggest that the schistosomiasis risk may be as much a result of the economic choices made at the household level as a result of landscape-scale environmental change, supporting the notion that people in this setting face a trade-off between health and livelihoods.

36

DEVELOPMENT AND APPLICATION OF A COMPLETE TRI- AND TETRAMER REPEAT MICROSATELLITE CATALOG TO BRAZILIAN AND KENYAN *SCHISTOSOMA MANSONI* POPULATIONS

J**effrey D. Kovach**[1], Lúcio M. Barbosa[2], Luciano K. Silva[3], Ana Rafaela Kruemmel[4], Mitermayer G. Reis[3], Ronald E. Blanton[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Bahiana School of Medicine and Public Health, Salvador, Brazil,* [3]*Oswaldo Cruz Foundation, Gonçalo Moniz Institute, Salvador, Brazil,* [4]*Georgia State University, Atlanta, GA, United States*

Analysis of population structure is necessary for understanding how schistosomes respond to control measures. Microsatellites remain a central tool for population genetics due to their high information content, low cost, high throughput and good resolution for most questions of diversity and differentiation. Issues that remain are which markers are best and how many are needed. A majority of the published >290 microsatellites are dinucleotide repeats, which are problematic for our approach of genotyping pooled eggs from feces. A literature review identified 52 tri- and tetramer repeat microsatellites, but many were never validated. Based on previous work and new assays using clones and lab strains, this list was refined to 28 markers that produced easily read peaks, were polymorphic for lab strains and were single locus. Markers were localized to all autosomal chromosomes, nevertheless, not all of these markers were successful for all field populations. Infrapopulations from

two rural Brazilian communities (n=354) were identified that had >85% amplification success using 25 markers. To determine the ideal marker number, random sets of 5, 10, 15, and 20 markers were used to calculate differentiation between infrapopulations (Di), component populations (Dc) and between each infrapopulation and component population (Dic). As marker number increased, variance markedly decreased between 10-15 markers for all measures. We developed a Dic Assignment Ratio Test that successfully assigned infrapopulations to geographic locations only 6 km apart (98% accuracy). Assignment score variance decreased with 15-20 markers. PCA of Dic values that included a Kenyan population could assign origin 100% correctly, including between the 2 neighboring Brazilian populations. This further validated the ability of the markers and the approach to identify epidemiologically and biologically relevant features. It was difficult to infer a marker's potential usefulness prior to genotyping based on characteristics of the lab strains or any distantly related population. However, using between 10-20 randomly selected markers give results near true values.

37

IMPACT OF BIANNUAL COMMUNITY-WIDE AND SCHOOL-BASED TREATMENT ON UROGENITAL SCHISTOSOMIASIS IN NIGER

Anna E. Phillips[1], Neerav Dhanani[2], Amadou Garba[3], Amina A. Hamidou[4]

[1]*Imperial College, London, United Kingdom,* [2]*Schistosomiasis Control Initiative, London, United Kingdom,* [3]*World Health Organisation, Geneva, Switzerland,* [4]*Riseal Niger, Niamey, Niger*

This was a five-year cluster-randomized trial that compared the impact of treatment strategies in areas with high and moderate *Schistosoma haematobium* prevalence. Each village was randomly allocated to one of six possible combinations of annual or biannual community-wide treatment (CWT) or school-based treatment (SBT). Data was collected annually among children aged 5-to-8 years in first-year of school, school-aged children 9-to-12-years and 50 adults (aged 20-to-55). In total, data was collected from 167,500 individuals across 225 villages in nine districts within the Niger River valley. Overall, treatment resulted in a decrease in prevalence of infection from baseline (15.7%) to Year 5 (8.85%) across all arms. The proportion of heavily infected was low but reduced from 1.46% to 0.76% over five years. The only significant difference between study arms was seen between annual and biannual SBT in areas with a high starting prevalence. Interestingly, although adults were not targeted for treatment in SBT, a statistically significant decrease in prevalence among adults was seen in moderate prevalence areas receiving biannual SBT (10.7% to 4.8%). Although treatment was successful in reducing the burden of active infection, there was no statistically significant difference between arms with once- and twice-yearly CWT or SBT in areas with low *S. haematobium* endemicity, despite high treatment coverage. There was, however, a significant impact on infection reduction from biannual versus annual treatment in areas of moderate prevalence. These findings are an important consideration for control programs that are considering elimination, as scaling up the frequency of treatment is a commonly proposed strategy in areas of low prevalence. These findings support the idea that preventive chemotherapy alone will not eliminate schistosomiasis. Interestingly, the finding that prevalence decreased among adults in SBT arms suggests that transmission in the community can be reduced, even where only school children are being treated, which could have logistical and cost-saving implications for the national control programmes.

38

SCHISTOSOMIASIS AT DELIVERY IS ASSOCIATED WITH A HIGHER RISK OF SMALL-FOR-GESTATIONAL AGE AT BIRTH AND INFANT'S WEIGHT DURING THE FIRST YEAR OF LIFE IN BENIN

Gino C. Agbota[1], Frank T. Wieringa[2], Maiza Compos-Ponce[3], Nadine Fievet[4], Manfred Accrombessi[1], Emmanuel Yovo[1], Clémentine Roucher[5], Achille Massougbodji[1], Michel Cot[4], Valérie Briand[4], Katja Polman[5]

[1]*CERPAGE/UMR*[216]*/IRD, Cotonou, Benin,* [2]*Nutripass, UMR*[204]*, IRD, Montpellier, France,* [3]*Vrije University, Amsterdam, Netherlands,* [4]*UMR*[216]*/IRD, Paris, France,* [5]*Institute of Tropical Medicine, Antwerp, Belgium*

Schistosomiasis represents one of the most prevalent and disabling parasitic infection in sub-Saharan Africa (SSA). While schistosomiasis has been related to anemia, stunting, neurocognitive disorders in infancy, little is known about the effect of schistosomiasis during pregnancy on birth and postnatal outcomes. In animal models, maternal schistosomiasis has been associated with an increasing risk of anemia, preterm birth and low birthweight. This study aims to assess the effect of maternal schistosomiasis on the risk of small-for-gestational age (SGA) and child's weight growth from birth to year one. From 2014 to 2018, in Benin, women were followed from the pre-conception period until delivery (RECIPAL study). A sub-sample of their children was followed from birth to year one (SEPSIS study) with weight measurements at birth and each quarter. Gestational age was accurately determined by ultrasound at the 1st trimester. Maternal schistosomiasis was defined as a urinary detection of *S. haematobium* eggs at delivery. In the child, SGA was defined according to the INTERGROWTH-21st standards and the postnatal growth was assessed using weight variation from birth to year one. A logistic and mixed linear regression models were used to assess the effect of maternal schistosomiasis on child growth at birth and during the first year of life, respectively. A total of 127 mother-child pairs were included. The prevalence of schistosomiasis at delivery was 11.8%. At birth, 20.5% of newborns were SGA. From birth to year one, the mean (standard deviation) child's weight increased from 3056 (397) to 8384 (1158) g. After adjustment for potential maternal and infant confounding factors, maternal schistosomiasis was significantly associated with a higher risk of SGA (adjusted odds ratio=4.8, [1.4; 16.5], p=0.014). From birth to year one, maternal schistosomiasis was significantly associated with a lower child's weight (adjusted β= -548 g [-973; -123], p=0.011). In conclusion, these results highlight the importance of maternal schistosomiasis in SSA and reinforce the need for the preventive strategies before and during pregnancy.

39

A THEATRE-BASED APPROACH FOR ASSESSING AND INFLUENCING HIGH-RISK WATER CONTACT BEHAVIORS OF SCHISTOSOMIASIS-ENDEMIC COMMUNITIES IN ETHIOPIA AND TANZANIA

May N. Sule[1], Safari M. Kinung'hi[2], Teshome Imana[3], Emma Bewley[1], Justina Mosha[2], Teckla Angelo[2], Kamran Rafiq[4], Alex Dower[4], Feleke Zewge[3], Michael R. Templeton[1]

[1]*Imperial College London, London, United Kingdom,* [2]*National Institute for Medical Research, Mwanza Centre, Mwanza, United Republic of Tanzania,* [3]*Addis Ababa University, Addis Ababa, Ethiopia,* [4]*Acting for Health, London, United Kingdom*

This study aimed to assess the effectiveness of a theatre-based behaviour change technique to reduce high-risk water contact behaviour in schistosomiasis-endemic communities. The study was carried out in three communities: Kemise in Ethiopia; Kigongo and Mwakalima in Tanzania. Initial baseline data on knowledge, perceptions, and behaviours of the communities used mixed quantitative and qualitative research methods including questionnaires, in-depth interviews and focus group discussions. For each theatre workshop, the cohort included 18-20 community representatives, occupationally exposed people (fishermen, paddy

farmers and horse cart men), and regional government representatives participating for 4 or 5 days. The Acting for Health methodology involves drawing out the disease 'complex' i.e. everything associated with schistosomiasis; this includes the life cycle, symptoms, treatment, beliefs, water contact, use of alternative safe water supplies and improved sanitation. Findings from the complexes progressed into solutions for the prevention and control of schistosomiasis, which were then developed into sketches and scenes with relevant messaging. Characters were created from among the cohort, who developed the narrative. The play was rehearsed and performed for the local community. Questionnaires were conducted with the cohort before and after the workshops, to measure changes in perceptions and understanding. The cohort made plans for peer education and communal dissemination of the workshop messages. There is ongoing cohort follow-up through monthly community visits and social media platforms. Quantitative and qualitative surveys will be repeated in the communities after three months (in June 2019) and results will be compared against the initial baseline data to assess changes in perception and behaviours towards water contact and alternative safe water supply. The conclusions from this study will provide information to identify effective and sustainable behaviour change communication strategies to accompany water and sanitation interventions that will reduce transmission of schistosomiasis.

40

ADAPTIVE STRATEGIES FOR SCHISTOSOMIASIS CONTROL AND ELIMINATION IN HETEROGENEOUS ENVIRONMENTS: A MODEL-BASED ANALYSIS OF PUBLIC HEALTH GUIDELINES

David Gurarie[1], Charles H. King[1], Nathan C. Lo[2], Qimin Huang[1], Emily Li[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Stanford University, Stanford, CA, United States*

Schistosoma infection is endemic in many parts of the world, and WHO has made its control and elimination a high priority. Recently, a large-scale trial and multiple country control-surveillance programs have been conducted, including SCORE (Schistosomiasis Consortium for Operational Research and Evaluation, U. Georgia) and SCI (Schistosomiasis Control Initiative, Imperial College, London). The 5-year SCORE trial explored several control strategies with different target age groups and drug regimens. However, these studies have yielded mixed results in seemingly similar settings; whereas some communities achieved substantial gains, others deemed "hotspots" were highly resilient to the effects of mass drug treatment (MDA). We developed a simulation-based study with dynamic transmission models to investigate how complex environmental and life cycle aspects (e.g. host demographics, in-host biology, transmission environment, and host-vector interactions) can drive the differential response to MDA seen in SCORE and country programs, and compared new adaptive decision making strategies (updating strategy based on initial treatment response) to current WHO guidelines and targets. The models were fit to datasets from SCORE villages and related studies. Our model-based analysis revealed many key predictors of response to MDA, including those of intermediate snail host biology and infection dynamics. The new set of adaptive strategies achieved more rapid control of schistosomiasis with fewer treatment rounds compared to WHO guidelines. Finally, an adaptive strategy that included an integrated approach (MDA + snail control with molluscicide) was found to be optimal in control.This study found that across a broad range of host communities and environmental settings that adaptive decision making with integrated strategies (MDA + molluscicide) could achieve control of schistosomiasis in shorter time periods compared to current WHO guidelines and targets. Future policy decisions should consider adopting adaptive decision making to address "hot spot" settings.

41

CEREBRAL SPINAL FLUID IN SUBARACHNOID NEUROCYSTICERCOSIS IS CHARACTERIZED BY PROINFLAMMATORY CYTOKINES AND CHEMOKINES THAT FAIL TO FULLY NORMALIZE FOLLOWING CURE

Elise M. O'Connell, Sarah Harrison, Theodore E. Nash, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

When treatment for racemose subarachnoid neurocysticercosis (SANCC) commences or other factors cause breakdown of the parasite integrity, an exuberant inflammatory response ensues that drives the majority of pathology, including hydrocephalus, vasculitis, and cranial nerve damage. The standard of care for SANCC includes global immune suppression with high doses of corticosteroids and concomitant anthelmintics, both often required over months to years after initial diagnosis. As an improved understanding of both the mediators required for parasite control and those primarily responsible for the host damage is necessary, we sought to measure the major pro- and anti-inflammatory cytokines and chemokines in SANCC. To this end, 16 patients with active SANCC and 16 patients with cured SANCC had CSF and serum subjected to multianalyte profiling on a Luminex™ platform. Likewise, 9 normal patients' CSF was tested in 3 pools of 3. When levels of proinflammatory cytokines/chemokines from those with SANCC were compared to CSF from healthy individuals the Th1- (IFNy, IL-12p40, IL12-p70) and Th2- (IL-10, IL-13) associated cytokines, along with GM-CSF, IL-1ra, IFN-α2b, VEGF, and the chemokines eotaxin-1, IL-8, IP-10, MIP-1α and MIP-1β were significantly elevated. Moreover, all but 4 of these same analytes (eotaxin-1, VEGF, MIP-1α and MIP-1β) were found at significantly higher levels in the CSF than in the serum from the same patients. In assessing paired CSF samples from patients before and following cure, while all analyte levels fell significantly following cure (average of 133 days post anthelmintics, range 0-452 days), IL-12p40 and IL-10 still remained statistically significantly elevated compared with normal CSF. These data suggest that neuroinflammatory processes underlie SANCC. Moreover, we have identified multiple pathways that could be targeted by specific cytokine/chemokine inhibitors reduce the pro-inflammatory milieu seen in the CNS of those with active SANCC. These findings also raise questions about the influence of persistent neuroinflammation in the pathology associated with SANCC.

42

COMBINED USE OF ANTIBODY AND ANTIGEN DETECTION TO IMPROVE THE ACCURACY IN THE DIAGNOSIS OF VIABLE INFECTION IN PATIENTS WITH PARENCHYMAL CEREBRAL CYSTICERCOSIS

G**ianfranco Arroyo**[1], Javier A. Bustos[1], Andres G. Lescano[1], Pierre Dorny[2], Erika Perez[3], Yesenia Castillo[1], Isidro Gonzales[3], Herbert Saavedra[3], E. Javier Pretell[4], Saul Santivañez[5], Robert H. Gilman[6], Armando E. Gonzalez[7], Hector H Garcia[1]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Institute of Tropical Medicine, Antwerp, Belgium,* [3]*Cysticercosis Unit, Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [4]*Department of Neurology, Hospital Nacional Alberto Sabogal, Callao, Peru,* [5]*Instituto Peruano de Parasitología Clínica y Experimental, Lima, Peru,* [6]*Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States,* [7]*School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru*

Anthelmintic therapy in parenchymal neurocysticercosis (NCC) depends on a proper diagnosis of viable cysts by neuroimages, and is confirmed by serology. The EITB assay detects serum antibodies and is highly sensitive, but poorly specific to differentiate old from viable infections, whereas antigen-detection discriminates viable cyst infections, although its performance is lower compared to EITB. Nonetheless, their combined use may improve diagnostic accuracy for viable parenchymal NCC. Neuroimages (magnetic resonance [MR] and computed tomography [CT]), and sera of 222 patients with parenchymal NCC were evaluated. Cases

were classified with viable parenchymal NCC according to neuroimages. Sera were processed by EITB and ELISA for antibody and Ag-detection. We assessed the performance of different EITB-positive cutoffs (≥1, ≥3, and ≥4 bands), alone or combined with Ag-detection in terms of their accuracy to diagnose viable parenchymal NCC (ROC-curve areas, sensitivity, and specificity) using logistic regression models. A total of 135 cases (60.81%) had viable parenchymal NCC. Adding ELISA Ag-detection to a EITB diagnosis with ≥1 reactive bands improve its accuracy to discriminate viable cyst infections (ROC-areas 0.84 versus 0.72, $p<0.001$), and a similar effect was obtained for EITB diagnosis with ≥3 reactive bands (ROC-areas 0.87 versus 0.77, $p<0.001$). Also, specificity levels significantly increased when Ag-detection was added to EITB cutoffs ≥1 bands (82.75 versus 52.87, $p<0.001$), and ≥3 bands (85.06 versus 64.37%, $p=0.001$), whereas sensitivity levels were not significantly affected. Using an EITB cutoff ≥4 bands, the addition of Ag-detection did not improve assay performance. In patients reacting to 1 to 3 bands on EITB, the addition of ELISA Ag-detection improves the accuracy to diagnose viable parenchymal NCC by increasing specificity levels in 30% or more. Combined use of antibody and antigen detection may serve to correctly diagnose NCC in patients that require anthelmintic therapy, and in rural settings to identify cases that require further neuroimaging assessment.

43

RISK FACTORS FOR BREAKTHROUGH SEIZURES IN PATIENTS WITH EPILEPSY DUE TO CALCIFIED NEUROCYSTICERCOSIS

Javier A. Bustos[1], Gianfranco Arroyo[1], Isidro Gonzales[2], Herbert Saavedra[2], Robert H. Gilman[3], Armando E. Gonzalez[1], Hector H. Garcia[1], for the Cysticercosis Working Group in Peru[1]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Neurocysticercosis is one of the main causes of epilepsy in endemic areas. Viable brain cysts follow a degenerative progression and finally resolve completely or leave a calcified scar. Some patients develop epilepsy and receive antiepileptic drugs (AED), however a proportion of these patients would still present breakthrough seizures. We performed a prospective cohort study to assess the incidence and associated risk factors of breakthrough seizures in adult patients with epilepsy and calcified NCC under AED treatment. At baseline, patients had a clinical examination, EEG test, and EITB Western Blot. Participants were under AED as indicated by their attending neurologist. Then Patients had a clinical evaluation every three months and the study team contacted them by phone every two weeks. All reported seizure events were classified according to the ILAE guideline. 210 patients that fulfilled inclusion criteria composed the final cohort and 101 of them presented new seizure during the follow-up. Maximum follow-up time was 69 months with a median of 11 months. Mean age was 36.34 (SD 13.04) years and 48.57% were males (102/210). The time-to-event analysis shows that 35.5% (95% CI 27.2%-46.5%) remains free of seizure and most of the events occurred within the first 2 years of follow-up (93%, 94/101). In the bivariate Cox analysis we found as protective factors: being male (HR: 0.67 p=0.05), history of antiparasitic treatment (HR 0.68 p=0.06), and to remain free of seizures during the last two years (HR: 0.38 p=0.001) and as risk factors: history of 10 or more seizures (HR: 1.65, p=0.01) and to present an abnormal EEG (HR: 2.52 p=0.03 On multivariate analysis, we found the same tendencies, but only a history of ten or more seizures (HR: 1.68 p=0.02) and abnormal EEG (HR 2.22 p= 0.06) remain as significant risk factors and be seizure free in previous 2 years is protective factor (HR: 0.49 p=0.02). Seizures control in calcified NCC is poor. Only 35.5% of patients remain remains free of seizure in a 5-years follow-up time, and the main risk factors are having a history of ten or more seizures, having an event within the previous 2 years and an abnormal EGG at enrolment.

44

MOVING TOWARDS IMPLEMENTATION: A COMMUNITY-BASED PARTICIPATORY RESEARCH PILOT TO PROMOTE CYSTICERCOSIS PREVENTION AND RING SURVEILLANCE IN NORTHERN PERU

M**ichelle Beam**[1], Angela G. Spencer[1], Ruth Atto[2], Roberto Camizan[2], Lauralee Fernandez[1], Ian Pray[1], Brian Garvey[1], Percy Vilchez[2], Claudio Muro Ecca[2], Ricardo Gamboa[2], Luz Maria Moyano[2], Josefina Coloma[3], Hector H. Garcia[4], Seth E. O'Neal, for the Cysticercosis Working Group[1]

[1]*Oregon Health & Science University, Portland, OR, United States,* [2]*Center for Global Health Tumbes, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*University of California Berkeley, Berkeley, CA, United States,* [4]*Universidad Peruana Cayetano Heredia, Lima, Peru*

Taenia solium is a common cause of preventable epilepsy. Ring treatment (RT), a control strategy that targets treatment for taeniasis to households in close proximity to heavily infected pigs, has been shown to be effective when surveillance is carried out by research teams. We carried out a two-year mixed-methods pilot study in 4 intervention villages (pop. 819) in Peru to promote community participation in RT, using participatory research methods previously effective for dengue prevention in Nicaragua. The intervention included a workshop in which villagers observed cyst evagination with microscopes, as well as 3 focus groups and 16 working groups in which community-identified leaders worked with study staff to design and adapt RT for their village. In 3 control villages (pop. 992), the same surveillance, reporting, and response structures were implemented without participatory development of local champions or workgroups. Key measurements included augmented knowledge, attitudes and practices (KAP) survey administered in all study households every 4 months, audio-recorded and transcribed focus groups analyzed with *in vivo* thematic coding, and seroincidence in pigs measured using EITB LLGP. The community-engaged RT intervention was effective, achieving 40% decrease in seroincidence among pigs over 16 months vs. 4% increase in the controls. Household survey results demonstrated *T. solium* lifecycle knowledge of human-to-pig transmission and pig-to-human transmission improved among adults who attended the evidence workshop compared to non-attendees, OR=2.67 (CI=1.23-5.78) and OR=3.50 (CI=1.76-6.9), respectively. Preliminary analysis of the KAP survey also demonstrated community-level behavior change. In this pilot study, we showed that surveillance and reporting can be successfully managed by community members in partnership with local health posts, when supported by research teams. Final results, salient lessons learned, and plans to refine, scale up, and test community-based RT strategy regionally through implementation research will be presented.

45

PROLONGED CYSTICIDAL TREATMENT AND CONTROL OF INFLAMMATION LEADS TO SUSTAINED INACTIVE DISEASE IN SUBARACHNOID RACEMOSE NEUROCYSTICERCOSIS

Theodore E. Nash[1], Elise M. O'Connell[1], Dima A. Hammoud[1], Lauren Wetzler[1], JeanAnne M. Ware[1], Siddhartha Mahanty[2]

[1]*National Institutes of Health, Bethesda, MD, United States,* [2]*The Peter Doherty Institute for Infection and Immunity, University of Melbourne and The Royal Melbourne Hospital, Melbourne, Australia*

Subarachnoid racemose NCC (SUBNCC), the most morbid form of NCC, is caused by an aberrant proliferative form of *Taenia solium* that grows and invades the subarachnoid spaces of the brain and spinal cord. The best way to treat this form of NCC is unclear. Here we summarize the clinical course and outcomes of 34 SUBNCC patients evaluated and treated at the National Institutes of Health employing prolonged cysticidal and anti-inflammatory regimens and long term follow up. The median ages at the time of first symptom, diagnosis and enrollment were 29.7, 35.6 and 37.9 yrs, respectively. Twenty participants (58.8 %) were male and 28 (82.4%) Hispanic. The median time from immigration to development of symptoms (minimal incubation period) was 10 yrs, with an estimated median

incubation period of 22.2 yrs. Other NCC involvement was detected in half, including 20.6% with ventricular cysts, 8.8% with parenchymal cysts and 44.1% with parenchymal calcifications. Hydrocephalus, shunt insertions, infarcts and symptomatic spinal disease occurred in 55.9%, 41.2%, 17.6% and 14.7 %, respectively. Thirty required prolonged treatment with albendazole (88.2%, median duration of 0.55 yr) and/or praziquantel (61.8%; median of 0.96 yr), high dose corticosteroids (88.2%, median of 1.09 yr.), methotrexate (50%, median of 1.37 yr.), and etanercept (34.2%, median of 0.81 yr.), which led to inactive disease in 29 of the 30 treated (96.7%) a median of 4.2 yrs post treatment (range of follow up: 15 for ≥4 yrs, 20 ≥ 2 yrs, 26 > 1 yrs, 3< 1 yrs). Three patients had successfully retreated recurrent disease and 1 has continuing infection. Normalization of CSF parameters and cestode antigen levels guided treatment decisions. None of the 15 patients who reached undetectable cestode antigen values had recurrent disease. There were no deaths and, in general, moderate morbidity. Corticosteroid related side effects were common, avascular necrosis of joints (8 of 33, 24.2 %) being the most long lasting. Prolonged cysticidal treatment combined with effective control of inflammation led to good clinical outcomes and sustained inactive disease, which is likely curative.

46

PREDICTORS FOR THE DEVELOPMENT OF RESIDUAL CALCIFICATIONS AFTER ANTIPARASITIC TREATMENT OF PARENCHYMAL BRAIN CYSTICERCOSIS

J**avier A. Bustos**[1], Gianfranco Arroyo[1], Percy Soto-Becerra[1], Robert H. Gilman[2], Isidro Gonzales[3], Herbert Saavedra[3], Armando E. Gonzalez[1], Hector H. Garcia[1], for the Cysticercosis Working Group in Peru .[4]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [4]*Universidad Peruana Cayetano heredia, Lima, Peru*

Neurocysticercosis (NCC) is a main cause of secondary epilepsy in endemic areas. Along the natural course of evolution or as a effect of antiparasitic treatment the host´s inflammatory response leads to cyst degeneration, which in some cases ends as a calcified scar. This study assessed the proportion of residual calcification at one year in cerebral cysts that resolved after antiparasitic treatment. We evaluated data of 220 adult patients with 1 to 20 viable parenchymal NCC from three previous clinical trials were patients received standard Albendazol (ABZ-S 15mg/kg/d), increased ABZ (ABZ-I 22.5mg/kg/d) or ABZ-S plus Praziquantel (PZQ 50mg/kg/d), and corticosteroids. Patients had MRI exams at baseline and at day 180 to assess cyst resolution and a CT scan at day 360 to assess calcification. We selected cases with completed follow up (MRI at day 180 and CT at day 360). We used Poisson regression with random intercepts adjusted by covariates at the patient and cyst levels to estimate risk ratios of calcification. A total of 495 cysts in 147 patients were assessed, with an overall percentage of calcification of 38.2% (189/495 95%CI 33.9%-42.6%). Main positive predictors of cyst calcification were more than 24 months with seizures (RR: 1.24 p=0.004) and presence of perilesional edema at baseline (RR: 1.40 p=0.013). Protective factors included a higher dose of dexamethasone (RR: 0.73 p=0.032 for >6.5 mg/day), using combined antiparasitic treatment (ABZ + PZQ) (RR: 0.91 p<0.58 compared to ABZ-S and RR: 0.77 p=0.001 compared to ABZ-I), re-treatment in patients with partial cure who need an additional course of antiparasitic treatment after 6-month MRI evaluation (RR: 0.64 p<0.001 compared to patients with complete cure who do not need retreatment and RR: 0.68 p=0.024 compared to patients with incomplete cyst cure and not re-treated). This study defined factors associated with frequency of residual calcifications. These factors (such as to enhance corticosteroids dose, use of combined treatment (ABZ+PZQ), or additional courses of antiparasitic re-treatment) could be targeted in future studies intended to decrease the proportion of calcification.

47

SEASON PATTERNS IN RISK FACTORS FOR *TAENIA SOLIUM* TRANMISSION: A GPS TRACKING STUDY OF PIGS AND OPEN HUMAN DEFECATION IN NORTHERN PERU

Ian W. Pray[1], Claudio Muro[2], Percy Vilchez[2], Ricardo Gamboa[2], Hector H. Garcia[3], Seth E. O'Neal[1]

[1]*Oregon Health and Science University, Portland, OR, United States,* [2]*Center for Global Health Tumbes, Tumbes, Peru,* [3]*Universidad Peruana Cayetano Heredia, Lima, Peru*

Taenia solium (cysticercosis) is a parasitic cestode that is endemic in rural populations where open defecation is common and pigs are allowed to graze on human feces. The purpose of this study was to examine the roaming patterns of free-range pigs, and identify areas where *T. solium* transmission could occur via contact with human feces. We did this by using GPS trackers to log the movement of 108 pigs in three villages of northern Peru. Pigs were tracked for 6 days each, and tracking was repeated in the rainy and dry seasons. Maps of pig ranges were analyzed for size, distance from home, land-type, and contact with human defecation sites, which were assessed in a community-wide defecation survey. Consistent with prior GPS studies and spatial analyses, we found that the majority of pigs remained close to home during the tracking period and had contact with human feces in their home areas - pigs spent a median of 79% (IQR: 61-90%) of their active roaming time within 50 meters of their homes, and 60% of contacts with open defecation within 100 meters of home. Extended away-from-home roaming was predominately observed during the rainy season; and, overall, home range areas were 61% larger during the rainy season compared to the dry season (95% CI: 41-73%). Both home range size and contact with open defecation sites showed substantial variation between villages, and contact with open defecation sites was more frequent among pigs with larger home ranges, and pigs living in higher density areas of their village. Our study builds upon prior work showing that pigs predominately roam and have contact with human feces within 50-100 meters of the home, and that *T. solium* transmission is most likely to occur in these concentrated areas of contact. This finding, therefore, supports control strategies that target treatment resources to these areas of increased transmission. Our finding of a seasonal trend in roaming ranges may be useful for control programs relying on pig interventions, and in the field of transmission modeling, which require precise estimates of pig behavior and risk.

48

PARENTERAL ARTEMISININS ARE ASSOCIATED WITH REDUCED MORTALITY AND IMPROVED LONG-TERM BEHAVIORAL OUTCOMES BUT INCREASED HOSPITAL READMISSION IN UGANDAN CHILDREN WITH SEVERE MALARIA

A**ndrea L. Conroy**[1], Robert O. Opoka[2], Paul Bangirana[2], Richard Idro[2], Chandy C. John[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Makerere University, Kampala, Uganda*

In 2011 the World Health Organization recommended injectable artesunate as the first-line therapy for severe malaria (SM) due to its reduction of mortality compared to quinine. From 2008-2013, we enrolled 718 children in a prospective cohort study to assess long-term neurocognitive and behavioral outcomes following cerebral malaria or severe malarial anemia. Behavior was assessed one week after discharge and at 6, 12 and 24 months after discharge by the Child Behavior Checklist (CBCL) and Behavior-Related Inventory of Executive Function (BRIEF). Higher scores indicated poorer behavioral outcomes. Artemisinin use increased gradually from 0% to 91.9% from 2008 to 2013, with 156 children with severe malaria (31.1%) receiving parenteral artemisinins. Children receiving quinine vs. artemisinins were similar in demographics. All analyses including adjustment for potential confounding factors. Children receiving artemisinins compared to quinine had reduced in-hospital mortality (3.9% vs. 8.4% adjusted odds ratio (aOR) [95%

confidence interval (CI)] 0.25 [0.09, 0.72]) and reduced neurologic deficits at discharge (23.7% vs. 41.7%, aOR 0.25 [0.12, 0.54]) but no differences in neurologic deficits in follow-up. However, children receiving artemisinins compared to quinine were more likely to be readmitted to hospital multiple times (incidence rate ratio 2.01 [95% CI, 1.28-3.17]). Artemisinin compared to quinine treatment was associated with improved total behavior scores on the CBCL (beta, 95% CI: -0.28, -0.47, -0.09, p=0.004), and improved global executive function (beta, 95% confidence interval (CI): -0.36, -0.62, -0.10, p=0.007), and metacognition scores (beta, 95% CI: -0.34, -0.61, -0.08, p=0.012) on the BRIEF in children ≤5 years of age, over 24-month follow-up. No differences were observed in children over five years of age. Artemisinin compared to quinine treatment of severe malaria is associated with reduced mortality and improved long-term behavioral outcomes but increased risk of hospitalization readmission. Additional studies are needed to understand how artemisinins may increase risk of recurrent hospitalizations.

49

ACUTE KIDNEY INJURY DURING AN EPISODE OF SEVERE MALARIA IS ASSOCIATED WITH RECURRENT SEVERE MALARIA IN UGANDAN CHILDREN

Ruth Namazzi[1], Robert Opoka[1], Richard Idro[1], Paul Bangirana[1], Dibyadyuti Datta[2], Andrea Conroy[3], Chandy John[3]

[1]*Makerere University College of Health Sciences, Kampala, Uganda,* [2]*Indiana University School of Medicine., Indianapolis, IN, United States,* [3]*Indiana University School of Medicine, Indianapolis, IN, United States*

Severe malaria is associated with high post discharge morbidity and mortality. Few studies have examined the burden and risk factors for repeated malaria episodes in children discharged after an episode of severe malaria. 600 children ages 6-48 months with severe malaria (SM), and 120 age-matched healthy community children (CC) were enrolled and followed for 12 months post discharge at two hospitals in Uganda. These children were assessed for malaria whenever they presented to the hospitals with a complaint of fever or had an axillary temperature of ≥37.5˚C. Among all the enrolled children, there were a total of 2144 sick visits over the 12 months follow-up period of which 950/2144 (44.3%) were positive for malaria. Of these, 625/950 (65.8%) were severe episodes requiring hospitalization. The incidence of malaria (episodes per 100 person-years) was 0.46 for children with SM, compared to 0.29 for CC, yielding an incidence rate ratio (IRR) of 1.57 (95% confidence interval (CI) 1.29, 1.93, p<0.0001) for malaria episodes in children with SM compared to CC. The incidence of severe malaria (malaria requiring hospitalization, episodes per 100 person-years) was 0.32 for children with SM compared to 0.13 for CC (IRR, 2.47, 95% CI 1.85, 3.37). In a model that included site, age, sex, nutritional status (weight-for-age and height-for-age z scores), severe anemia, hyperparasitemia, coma, acute kidney injury (AKI), and hyperlactatemia, site and AKI at enrollment was associated with an increased risk recurrent SM in children with prior SM (IRR, 1.64; 95% CI, 1.23-2.19, p=0.0008). When analyses were stratified by site, AKI remained an independent risk factor for recurrent SM in both the low transmission site (IRR, 1.76; 95% CI, 1.09-2.86, p=0.022) and high transmission site (IRR, 1.45; 95% CI, 1.02-2.07, p=0.040). Children with SM in malaria endemic areas are at high risk for recurrent SM, and AKI at the time of first SM episode is associated with increased risk of recurrent SM. Future studies should assess how AKI and other factors lead to an increased risk of recurrent SM, to allow for prevention of future SM episodes in these children.

50

ATOVAQUONE-PROGUANIL EXPOSURE IN PREGNANCY AND RISK FOR ADVERSE FETAL AND INFANT OUTCOMES

Julie R. Gutman[1], Clinton Hall[2], Zeina G. Khodr[2], Anna T. Bukowinski[2], Gia R. Gumbs[2], Ava Marie S. Conlin[3], Natalie Y. Wells[4], Kathrine R. Tan[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Naval Health Research Center, Deployment Health Research Department and Leidos Inc., San Diego, CA, United States,* [3]*Naval Health Research Center, Deployment Health Research Department and Innovative Employee Solutions, San Diego, CA, United States,* [4]*Naval Health Research Center, Deployment Health Research Department, San Diego, CA, United States*

Malaria infection in pregnancy can lead to maternal and fetal complications. Only chloroquine (CQ) and mefloquine (MQ) are recommended for chemoprophylaxis in pregnancy, but parasite resistance and contraindications may leave some women with no recommended options. Limited data suggest atovaquone-proguanil (AP), a highly effective antimalarial, might be suitable for malaria prevention in pregnancy, but more evidence is needed. Data for pregnancies and live births among active duty women, 2003-2014, from the Department of Defense Birth and Infant Health Research program were linked with pharmacy data to determine antimalarial exposure, defined as a drug dispensation date in pregnancy. Multivariable Cox and logistic regression models were used to assess the relationship between antimalarial exposure and fetal and infant outcomes, respectively. Among 199,017 pregnancies, 51 were exposed to AP, 159 to MQ, and 133 to CQ. Overall, 15.1% of unexposed pregnancies and 27.5%, 13.8%, and 4.5% of pregnancies exposed to AP, MQ, and CQ, respectively, ended in miscarriage (adjusted hazard ratios [aHR]=1.72, 95% confidence interval [CI]=1.02-2.90; aHR=1.03, 95% CI=0.68-1.57; and aHR=0.38, 95% CI=0.17-0.85, respectively); similar results were seen for the outcome "fetal loss," which included both miscarriage and stillbirth. Among 160,944 live births, 36 were exposed to AP, 130 to MQ, and 122 to CQ. Compared with unexposed infants, there was a statistically insignificant increased risk for a composite poor live birth outcome (preterm birth, low birthweight, or small for gestational age) among AP exposed infants (adjusted odds ratio=2.02, 95% CI=0.88-4.60), but not MQ or CQ exposed infants. Birth defects were seen in 3.0% of unexposed and 5.6%, 0.8%, and 0.8% of infants exposed to AP, MQ, and CQ, respectively. The small number of AP exposed pregnancies highlights the difficulty in assessing safety. While definitive conclusions are not possible, these data suggest further research of AP exposure in pregnancy and fetal loss is warranted.

51

AETIOLOGIES OF ACUTE FEBRILE ILLNESS AMONG CHILDREN IN A CONTEXT OF DECLINING MALARIA TRANSMISSION

T**echalew Shimelis Woldkiros**[1], Birkneh Tilahun Tadesse[2], Fitsum Belay[2], Gill Schierhout[3], Susana Vaz Nery[1], John Kaldor[1]

[1]*University of New South Wales, Sydney, Australia,* [2]*Hawassa University, Hawassa, Ethiopia,* [3]*The George Institute, Sydney, Australia*

The introduction of rapid malaria rapid diagnostic tests greatly improved malaria case management and reduced unnecessary anti-malaria drug prescription. However, the management of non-febrile illnesses, which now represent a higher proportion of cases, remains problematic in resource-constrained countries where laboratory facilities are limited, with a particular issue being over-prescription of antibiotics. In Ethiopia, the relative importance of various infectious causes of non-malaria febrile illnesses is unknown. This study aimed to assess aetiologies of fever (temperature of ≥37.5 ^{0}C or a history of fever in the past 48 hours) in children aged ≥2 months and <13 years presenting to Hawassa University Hospital, southern Ethiopia from May 2018 to February 2019. Clinical and demographic data were gathered from 433 participants using a clinical case report form. Various samples (blood, urine, stool, throat swab, CSF) were analysed using culture, microscopy and rapid diagnostic tests (RDTs) to identify pathogens. In addition to fever, the most common symptoms

were cough (53.1%, 230/433) and diarrhoea (18.3%, 79/433). Malaria and HIV infection were diagnosed in 3.2% (14/431) and 0.7% (3/431) of children, respectively. Blood stream bacterial infections were detected in 5.5% (23/421) of children, with *S. aureus* (3.8%, 16/421) the leading isolate. Urinary bacterial pathogens were isolated in 18.2% (73/402) of children, with *E. coli* (9.2%, 37/402) and *Klebsiella* species (4%, 16/402) predominant. Among 56 children with gastroenteritis from whom stool specimens were obtained, there were findings of rotavirus/adenovirus (17 cases, 30.4%) by RDT, intestinal parasites (9 cases, 16.1%) by microscopy and *Salmonella/Shigella* (2 cases, 3.6%) by culture. Group A *Streptococci* (16.1%, 28/174) and *S. pneumonia* (16.9%, 30/178) antigens were detected via RDTs in children with acute respiratory illness. This study represents the first systematic investigation of fever causes in children in Ethiopia, and supports the need for improved access to routine laboratory services to ensure appropriate management of specific pathogens.

52

ALGORITHM IN THE DIAGNOSIS OF FEBRILE ILLNESS USING PATHOGEN-SPECIFIC RAPID DIAGNOSTIC TESTS

Sunil Pokharel[1], Lisa J. White[2], Ricardo Aguas[2], Olivier Celhay[2], Karell G. Pelle[3], Sabine Dittrich[3]

[1]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [2]*Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [3]*Foundation for New Innovative Diagnostics (FIND), Geneva, Switzerland*

Acute Undifferentiated Febrile Illnesses (AUFI) caused by malaria, dengue, scrub typhus, typhoid and other aetiologies are common presentations in developing countries. In the lack of clinical expertise and reliable laboratory tests, patients are empirically treated with antimicrobials. Rapid diagnostic tests (RDTs) are increasingly available and used but lack optimal diagnostic accuracy. There are no guiding protocols and algorithms for the application of these tests. Using prevalence data of five common febrile illnesses from India and Cambodia and performance characteristics (sensitivity and specificity) of relevant pathogen-specific RDTs, we used a mathematical model to predict the probability of correct identification of each disease when diagnostic testing occurs either simultaneously or sequentially in various algorithms. We developed a web-based application of the model to visualise and compare output diagnostic algorithms when different disease prevalence and test performance characteristics are introduced in the model (https://moru.shinyapps.io/diagnostic-algorithm-app/). Diagnostic algorithms with appropriate sequential testing predicted better diagnostic outcomes in both settings when compared to simultaneous testing. The best performing sequential diagnostic algorithms were different in India and Cambodia due to varying disease prevalence. Simultaneous testing is not appropriate for diagnosis of AUFIs with presently available tests, which discourages the unsupervised use of multiplex diagnostic tests. The implementation of adaptive algorithms can predict better diagnosis and add value to the available RDTs. The web application of the model can serve as a tool to identify the optimal diagnostic algorithm in different epidemiological settings, whilst taking into account local epidemiological variables and accuracy of available tests.

53

AN EPIDEMIOLOGICAL STUDY OF *TOXOCARA* SPECIES IN HOUSTON PARKS USING A NOVEL PCR-BASED METHOD

David McCormick[1], Timothy Erickson[1], Donna L. Tyungu[2], Rojelio Mejia[1]

[1]*Baylor College of Medicine, Houston, TX, United States,* [2]*The University of Oklahoma Health Sciences Center, Oklahoma, OK, United States*

Toxocariasis is a parasitic infection caused by *Toxocara* species that can lead to visceral and ocular migrans and cognitive impairment. These parasites are found in the feces of dogs (*T. canis*) and cats (*T. cati*) and excreted into the soil, where they can survive for many years. Toxocariasis is an underdiagnosed neglected parasitic infection in the United States. Persons living in low socioeconomic communities are at increased risk. We examined soil samples from 15 urban and suburban parks in Houston, Texas using molecular methods. Approximately 30-70 grams of soil collected from the upper centimeter of dirt in two separate locations for each park. Soils were concentrated and DNA extracted using a novel method. Including mechanical disruption in a bead-beating device, and then processed using the MP FastDNA spin kit for soil preparation. Multi-parallel real-time quantitative PCR (qPCR) was performed using primers specific to *T. canis* and *T. cati* qPCR performed in duplicate for all samples, and a positive control with known amounts of *T. canis* and *T. cati* eggs used for each experimental run. *Toxocara canis* DNA was detected in 20% of parks located in the zip code with the lowest median income ($22,424/year). No *Toxocara* spp DNA was identified from parks located in zip codes with median income >$50,000/year. No samples contained DNA from *T. cati.* This study suggests that *T. canis* is an environmental contaminant in public spaces in Houston and associated with the socioeconomic status of the surrounding population. Use of a novel molecular method will allow for rapid screening of soil samples to further characterize this public health problem. Further studies will include a total of 45 parks and school playgrounds with quantification of the contamination burden. Environmental data will be cross-referenced to patient chart records with toxocariasis at Texas Children's Hospital and Harris Health systems. Comparisons of the socioeconomic status of those infected to the presence of *Toxocara* contamination will further show how parasites unequally affect those living in poverty.

54

EFFECTS OF IVERMECTIN ON INTRAOCULAR MICROFILARIAE IN PERSONS WITH ONCHOCERCIASIS IN EASTERN GHANA

Hong Augustine[1], Nicholas O. Opoku[2], Charles W. Goss[1], Christopher L. King[3], Gary J. Weil[1], Michael E. Gyasi[4]

[1]*Washington University School of Medicine, St. Louis, MO, United States,* [2]*University of Health and Allied Sciences, Hohoe, Ghana,* [3]*Case Western Reserve University, Cleveland, OH, United States,* [4]*St. Thomas Eye Hospital, Accra, Ghana*

Ivermectin (IVM) is widely used for treatment for onchocerciasis. We performed a prospective cohort study in Ghana to assess the kinetics of microfilarial (Mf) clearance from the eye after IVM 150 ug/kg in 261 patients with palpable nodules and Mf-positive skin snips. This study is the first to use optical coherence tomography (OCT) in the setting of onchocerciasis. OCT is analogous to an optical ultrasound that composes microscopic, high-resolution cross-sectional images of the retina. Skin Mf counts (geo mean Mf /mg and range) were 12.6, 2-86 at baseline and 0, 0-11 at 3 months. 28.5% of participants had at least 1 Mf in their anterior chambers (MfAC, total for both eyes, range 1-150) at baseline, and 3.2% of patients had at least 1 MfAC three months after treatment. 97.8% of participants with 1-9 MfAC and 95% of those with at least 10 MfAC at baseline had complete clearance at 3 months. No Mf were observed in the posterior segment. MfAC counts were correlated with baseline skin Mf counts ($p<0.001$). 52.1% of persons with high skin Mf counts (at least 30 Mf/mg) had MfAC compared to 26.5% of subjects with skin Mf counts between 1 and 10 Mf/mg. 84% of participants experienced at least one adverse event (AE) after IVM, with itching skin (46.4%), headache (28.6%) and joint or muscle pain (26%) being most common. Ocular itching (9.4%), eye pain (5.7%) and watery eyes (1%) were the most common ocular AEs. No severe or serious AEs were observed. OCT was useful for evaluating and monitoring glaucoma, chorioretinal atrophy, retinal edema and retinal anatomy. Subjects with severe eye disease or media opacities were excluded prior to enrollment. Among enrolled participants, baseline eye diseases (not including uncorrected refractive error) included glaucoma (7.3%), macular degeneration (2.3%), macular scarring (3.1%), severe cataract (1.9%), unexplained vision loss (1.5%), central corneal scarring (1.1%), and other (2.7%). Minimal changes occurred in ocular anatomy following IVM. In conclusion, our study demonstrated that IVM is safe and effective for reducing MfAC counts at 3 months; 6 month data will be presented at the meeting.

55

CHIKUNGUNYA VIRUS DISSEMINATION FROM THE MIDGUT OF *AEDES AEGYPTI* - INSIGHTS INTO THE MECHANISM

Alexander W.E. Franz, Yingjun Cui, Asher M. Kantor, DeAna G. Grant, Tommi A. White

University of Missouri, Columbia, MO, United States

The mosquito, *Aedes aegypti*, is a vector for emerging arboviruses such as chikungunya (CHIKV). The midgut epithelium is the initial tissue of the mosquito to be infected with an arbovirus following its oral acquisition from a vertebrate host. Following its replication within the midgut epithelium, the virus disseminates to secondary tissues including the salivary glands. The tissue barrier separating the midgut epithelium from the hemocoel, the midgut escape barrier, is an important determinant of mosquito vector competence for arboviruses. The midgut epithelium is surrounded by a multi-layered basal lamina (BL) composed of collagen IV and laminin. In ultrastructural (TEM and FIBSEM) time course studies, we showed that CHIK virions accumulated at the BL around 24 h pbm and were found between BL strands until 32 h pbm. However, at 48 h pbm, virions no longer strongly amassed towards the BL. This indicates that there is a time window post-bloodmeal acquisition during which CHIKV is disseminating from the midgut. An accompanying SEM time course study indicated that following bloodmeal acquisition, the midgut tissue and the BL were overstretched and partly damaged, which could be critical factors allowing virions to escape the midgut through the BL. We now show that fluorescent dye conjugated gold nanoparticles with a 5 nm diameter efficiently diffused through the BL of midguts dissected from sugarfed and BSA-fed mosquitoes. In contrast, 50 nm (approximate virion size) gold nanoparticles diffused only through the BL of those midguts that were obtained from BSA-fed mosquitoes. Importantly, one week after receiving the BSA meal, the midgut BL was still permissive for both 5 and 50 nm gold nanoparticles, whereas midguts of sugarfed mosquitoes, independent of their age, remained permissive for 5 nm gold nanoparticles only. These findings suggest that although the overall midgut structure relaxes during gradual meal digestion, the pore size exclusion limit of the BL remains (at least 10x) increased for a prolonged period following complete meal digestion.

56

SAFETY OF THE MEASLES-VECTORED CHIKUNGUNYA VACCINE (MV-CHIK) IN HEALTHY VOLUNTEERS PREVIOUSLY EXPOSED TO CHIKUNGUNYA VIRUS

Katrin Ramsauer[1], Clemente Diaz[2], Irma Febo[2], James Powell[2], Aileen Rivera Maldonado[2], Raimund Vielnascher[1], Paul B. Keiser[3]

[1]*Themis Bioscience GmbH, Vienna, Austria,* [2]*University of Puerto Rico, San Juan, Puerto Rico,* [3]*Walter Reed Army Institute of Research, Silver Spring, MD, United States*

The pathophysiology of chronic chikungunya is not well understood but several mechanisms have been proposed including immunopathology triggered by chikungunya antigens. The vaccine candidate MV-CHIK consists of a measles vaccine Virus expressing the polyprotein gene of chikungunya, resulting in production of chikungunya virus-like particles. We have recently shown safety and immunogenicity of the vaccine in phase 1 and phase 2 clinical trials. In anticipation of field trials and vaccination campaigns in areas of on-going or previous chikungunya transmission, we sought to evaluate the safety of MV-CHIK among subjects primed by natural infection. The study was conducted in Puerto Rico, which has a relatively high seroprevalence of chikunugunya following an epidemic in 2014 but negligible chikungunya transmission during the study period. A total of 34 healthy volunteers ages 21 to 50 were enrolled. After openly cohorting by baseline chikungunya IgG (16 seropositive and 18 seronegative) volunteers were randomized to receive either MV-CHIK or MMR in a 4:1 ratio. Adverse events (AEs) were solicited for 28 days after each of two intramuscular doses and unsolicited AEs were collected for a full year. The percentage of individuals reporting solicited AEs by unblinded cohort was not significantly higher in subjects previously exposed to chikungunya: fatigue (31% among pre-exposed vs 33% of unexposed), malaise (13 vs 44%), headache (31 vs 72%), diarrhea (19 vs 33%), nausea/vomiting (0 vs 28%), joint pain (31 vs 22%) and injection site pain (19 vs 61%). There were no related clinically significant laboratory abnormalities and no grade 3 or serious adverse events. C-reactive protein levels after the first vaccination did not increase significantly in either cohort suggesting that vaccine-induced inflammation was negligible. This study supports the safety of MV-CHIK in healthy volunteers primed by prior chikungunya infection. Additional studies to assess the safety of MV-CHIK in previously exposed populations are planned.

57

AEDES AEGYPTI SIALOKININ I MODULATES SIGLEC-1 EXPRESSION ON HUMAN MONOCYTES AND MACROPHAGES DURING CHIKUNGUNYA VIRUS INFECTION

Siew-Wai Fong[1], Jeslin J.L Tan[2], Vaishnavi Sridhar[1], Tze-Kwang Chua[2], Siti Naqiah Amrun[2], Guillaume Carissimo[2], Fok-Moon Lum[2], Kini R Manjunatha[1], Lisa F.P Ng[2]

[1]*National University of Singapore, Singapore, Singapore,* [2]*Singapore Immunology Network, Agency for Science, Technology and Research, Singapore (A*STAR), Singapore, Singapore*

Chikungunya virus (CHIKV) infection is initiated by the bite of infected female *Aedes* mosquitoes when viruses along with saliva are released below the skin of human host. Several reports have indicated that mosquito bites can enhance arbovirus infection and enhancement is attributed to proteins in the mosquito saliva. The saliva of female *Aedes aegypti* mosquito contains a 1400-Da Sialokinin peptide with vasodilatory properties typical of a tachykinin. Here, the effect of Sialokinin I was studied in primary human monocytes and monocyte-derived macrophages (MDMs). The expression of Siglec-1 (or CD 169) on human monocytes/macrophages is critical for activation of immune responses to viral infections in the human host. Increased expression of Siglec-1 on peripheral CD14+ monocytes was observed in CHIKV-infected patients. Interestingly, Sialokinin I significantly down-regulated Siglec-1 expression in both primary human monocytes and MDMs. The modulation effect of Siglec-1 expression by Sialokinin I was abrogated by antagonism of human neurokinin receptors of which the nonpeptide neurokinin receptor antagonists (CP-96345 and GR-159897) potently restricted downregulation of Siglec-1 expression by Sialokinin I on both monocytes and MDMs. Collectively, our findings reveal a novel role for Sialokinin I, which regulates Siglec-1 expression on monocytes/macrophages, affecting human host immune response to CHIKV infection. Modulation of Sialokinin I activity and neurokinin receptors may offer a new approach to the design of anti-CHIKV therapy.

58

RISK FACTORS FOR INFECTION WITH CHIKUNGUNYA AND ZIKA VIRUSES IN A COMMUNITY-BASED COHORT STUDY IN SOUTHERN PUERTO RICO

Laura E. Adams[1], Liliana Sanchez-Gonzalez[1], Robert Rodriguez Gonzalez[2], Kyle Ryff[1], Dania M. Rodriguez[1], Chelsea Major[1], Emma M. Little[1], Olga Lorenzi[1], Mark Delorey[1], Freddy A. Medina[1], Manuela Beltran[1], Jorge L. Muñoz-Jordán[1], Stephen H. Waterman[1], Marianyoly Ortiz[3], Vanessa Rivera-Amill[2], Gabriela Paz-Bailey[1]

[1]*Division of Vector-borne Diseases, Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*Ponce Health Sciences University, Ponce, PR, United States,* [3]*Puerto Rico Vector Control Unit, San Juan, PR, United States*

Communities Organized for the Prevention of Arboviruses (COPA) is a cohort study in southern Puerto Rico, initiated in 2018 to measure arboviral disease risk and provide a platform to evaluate interventions. Chikungunya virus (CHIKV) caused a large outbreak in Puerto Rico in 2014, followed by a Zika virus (ZIKV) outbreak in 2016. To identify risk factors for infection, we assessed previous CHIKV infection and recent ZIKV infection among COPA participants. Participants aged 1-50 years

(y) were recruited from randomly selected households in study clusters. Each participant completed an interview and provided a blood specimen, which was tested by anti-ZIKV IgM MAC-ELISA assay and anti-CHIKV IgG ELISA assay. We assessed individual, household, and community factors associated with a positive result for CHIKV or ZIKV after adjusting for confounders. Preliminary results are presented. During 2018-2019, 3,422 participants were enrolled; 59% were female and median age was 30y (IQR: 18-42). Among 3,176 participants tested for ZIKV, 462 (15%) had evidence of recent infection. Infection risk increased with older age, from 7% among 1-10y olds up to 18% among 41-50y olds (OR 3.1; 95% CI 1.9-5.1). Males had an increased risk of Zika infection compared with females (OR 1.4; 95% CI 1.1- 1.7). Participants with home screens (OR 0.6; 95% CI 0.5-0.8) and air conditioning (OR 0.6; 95% CI 0.6-0.9) were at lower risk for ZIKV infection compared to those without. ZIKV infection also decreased with higher income and later recruitment dates. Among 1,542 participants tested for CHIKV, 491 (32%) had evidence of previous infection. CHIKV infection did not differ by age or sex. Lower CHIKV infection was associated with home screens (OR 0.5; 95% CI 0.3-0.6) and air conditioning (OR 0.5; 95% CI 0.4-0.6). CHIKV infection also varied by study cluster of residence, employment status, and insurance type. Different infection patterns were observed for recent ZIKV infection and previous CHIKV infection by age, sex, time of specimen collection, and study cluster. However, the presence of screens and air conditioners in the home decreased infection risk from both viruses by as much as 50%.

59

SAFETY AND IMMUNOGENICITY OF A REPLICATION DEFICIENT SIMIAN ADENOVIRAL VECTORED CHIKUNGUNYA VACCINE: A PHASE I, FIRST-IN-HUMAN, DOSE ESCALATION TRIAL

P**edro M. Folegatti**[1], Kate Harrison[1], Fernando Ramos Lopez[1], Mark W. Tilley[1], Cesar Lopez-Camacho[1], Young C. Kim[1], Lorena Preciado-Llanes[1], Shannan L. Rossi[2], Ian Poulton[1], Daniel Jenkin[1], Mehreen Datoo[1], Yrene Themistocleous[1], Alison Lawrie[1], Rachel Roberts[1], Katie Ewer[1], Eleanor Berrie[1], Adrian Hill[1], Arturo Reyes-Sandoval[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*University of Texas Medical Branch, Galveston, TX, United States*

Chikungunya virus (CHIKV) is an alphavirus, transmitted by *Aedes* mosquitoes. The disease consists of an acute illness characterized by fever, rash, myalgia and generally symmetrical, peripheral, and often incapacitating polyarthralgia and/or polyarthritis, which lasts weeks to months. The most cost-effective means of controlling the spread of CHIKV is by vaccination. There is currently no licensed vaccine against CHIKV and although there are several candidates in pre-clinical development, only a few have entered clinical trials. Replication deficient vectors, as opposed to recombinant replication competent live attenuated viruses, avoid the risks of disseminated disease in immunocompromised hosts while maintaining the advantages of native antigen presentation, elicitation of T cell immunity and the ability to express multiple antigens. We developed a chimpanzee adenoviral vector expressing the structural polyprotein cassette of CHIKV (ChAdOx1 Chik) and conducted a Phase I, first-in-human, clinical trial. Twenty-four participants received a single intramuscular injection at 3 escalating doses in Oxford, UK. The trial is now fully recruited and is expected to be completed within 6 months. Preliminary data shows that ChAdOx1 Chik was well tolerated at all doses with no serious adverse events related to the vaccine reported to date. All local and systemic adverse events were self-limiting and short-lived, although higher reactogenicity has been observed at the top dose. A single unadjuvanted dose of ChAdOx1 Chik was immunogenic, eliciting high neutralising antibody titres and an increase in antibody levels was seen at D28 and D56 post vaccination compared to D0 in all groups. Responses against the structural CHIKV antigens show significant increases in IFN-γ levels, peaking at D14 post vaccination and there were no significant differences in ELISpot responses between dosage groups. The safety, cellular and humoral immunogenicity results up to 6 months post vaccination will be presented at the meeting. The results of this trial support a subsequent Phase Ib clinical trial of ChAdOx1 Chik in an endemic region.

60

ROBUST IMMUNOGENICITY OF 1- AND 2-DOSE SERIES OF AN ADJUVANTED VLP-BASED CHIKUNGUNYA VACCINE

Sean R. Bennett[1], Jason Mendy[2], Lisa Bedell[1], Kelly L. Warfield[3], Paul Shabram[2], Paul-Andre deLame[3]

[1]*Emergent BioSolutions, Inc., Redwood City, CA, United States,* [2]*Emergent BioSolutions, Inc., San Diego, CA, United States,* [3]*Emergent BioSolutions, Inc., Gaithersburg, MD, United States*

Rapid and durable protection against Chikungunya is highly desired, but currently no licensed vaccine exists. An unadjuvanted Chikungunya virus-like particle (CHKV-VLP) candidate vaccine has previously demonstrated a good safety profile and robust immunogenicity in phase 1 and 2 trials in both CHKV-naive and CHKV-exposed adults. Here we report interim results of a phase 2 trial of alum-adjuvanted CHKV-VLP. Healthy US adults 18 to 45 years of age (n=415) were given a 1- or 2-dose series at doses of 6 to 40 mcg over a 2- or 4-week period. Serum neutralizing antibody (SNA) was assessed by a luciferase-based assay and 80% neutralization titers (NT80s) were calculated. All regimens were well-tolerated, with mostly mild or moderate injection site pain in 21% to 49% of subjects, and no vaccine-related severe (Grade 3 or higher) or serious adverse events or discontinuations. Seroconversion occurred in 74% to 98% of subjects by 7 days after 1 dose, and in all subjects by 28 days after the last dose (the primary endpoint). Peak NT80s were similar to those seen after natural CHKV infection. The immune response was persistent, with mean NT80s of 196 to 457 through 6 months. Long-term follow-up is ongoing. These findings strongly support the potential utility and continued development of an alum-adjuvanted VLP-based vaccine for the prevention of CHKV infection, including future studies in a wider age range.

61

CHIKUNGUNYA: PHASE 1 CLINICAL DEVELOPMENT OF A SINGLE-SHOT LIVE-ATTENUATED VACCINE

Nina Wressnigg, Romana Hochreiter, Andrea Fritzer, Robert Schlegel, Andreas Meinke

Valneva, Vienna, Austria

VLA1553 is a live-attenuated Chikungunya virus (CHIKV) vaccine candidate designed for active immunization of the general population living in endemic regions, as well as serving as a prophylactic measure for travelers to endemic areas or areas at risk for an upcoming outbreak. The replicating CHIKV vaccine comprises a deletion of 60 amino acids in the nsP3 gene encoding the non-structural replicase complex protein leading to attenuation of the virus *in vivo*. VLA1553 is based on the La Reunion strain of the East Central South African genotype, is produced in Vero cells and purified by centrifugation, ultrafiltration, batch-chromatography and sucrose gradient centrifugation. In preclinical models of CHIKV disease (mice and non-human primates) the vaccine candidate demonstrated to be highly attenuated as evidenced by the absence of clinical manifestations typically associated with wild-type CHIKV infections in addition to strongly reduced viremia and inflammatory cytokine levels. Moreover, VLA1553 was highly immunogenic, induced a strong and long-lasting as well as protective neutralizing antibody response against a high dose wild-type CHIKV challenge. A blinded, randomized phase 1 clinical study investigating the safety and immunogenicity of three dose levels administered intramuscularly as a single-shot immunization designed to elicit long-term immunological memory is currently ongoing (NCT: NCT03382964). Initial blinded data across all dose groups up to Day 28 after a single immunization showed an excellent immunogenicity profile with 100% seroconversion rates (proportion of subjects achieving a CHIKV-specific neutralizing antibody titer of NT50≥20) and a high GMT of 648. The safety profile is acceptable supporting further development: no serious adverse events or adverse events of special interest occurred

up to Day 28. Further analysis of safety and immunogenicity up to Month 6 including a homologous challenge will elucidate the optimal dose for further development of the vaccine and at the same time provide an early indication of efficacy paving the way to late stage clinical development.

62

VACCINATION WITH A *TRYPANOSOMA CRUZI* CYCLOPHILIN 19-DELETION MUTANT CONFERS COMPLETE PROTECTION AGAINST ACUTE CHAGAS DISEASE IN MICE

B**ijay Kumar Jha**[1], Sanjay Varikuti[1], Nicholas Bishop[1], Gregory Pedroso dos Santos[2], Manjusha Kulkarni[1], Sergio Schenkman[3], Abhay Satoskar[1], Bradford Scott McGwire[1]

[1]*The Ohio State University, Columbus, OH, United States,* [2]*Universidade Federal de São Paulo, Estado de Sao Paulo, Brazil,* [3]*Universidade Federal de São Paulo, São Paulo, Brazil*

Human infection with *Trypanosoma cruzi* causes Chagas disease, a major public health problem that affects up to 8 million people world-wide and causes 10,000 deaths per year. Chronic infection with this parasite is the leading cause of heart failure in Latin America. There are no therapeutic or prophylactic vaccines for this infection, however they are urgently needed since the incidence of Chagas is increasing and there are only two drugs approved for treatment of the infection. Previously, we described that *T. cruzi* the cis-prolyl isomerase, cyclophilin 19 (Cyp19), binds to and neutralizes antimicrobial peptides promoting parasite survival and potentiating parasite infectivity. We have also recently found that Cyp19 is expressed in and secreted by all parasite stages. In order to understand the potential role of this protein in parasite virulence we generated a double allelic deletion mutant in Cyp19. This mutant replicates slowly *in vitro* and generates more abundant metacyclic trypomastigotes than wildtype parasites. Mutant metacyclics are complement-resistant as are wildtype parasites, but fail to infect host cells *in vitro* and cannot establish a productive infection. High-dose inoculation of mutant parasites does not produce infection in either immunocompetent A/J mice or in immunocompromised STAT-1$^{-/-}$ and STAT-4$^{-/-}$ mice which are normally hyper-susceptible to *T. cruzi* infection. Importantly, despite the inability to replicate in mice repeated inoculation produces escalating anti-parasite immunity. These immunized mice are protected from developing tissue parasitization, clinical infection and death from high-dose wildtype parasite challenge. These results indicate that *T. cruzi* Cyp19 is important for the infectivity of *T. cruzi* and that this mutant parasite line is a promising potential vaccine candidate for Chagas disease.

63

CHEMICAL CARTOGRAPHY OF HOST-PARASITE-MICROBIOME INTERACTIONS REVEALS NEW MECHANISMS OF DISEASE TOLERANCE IN AMERICAN TRYPANOSOMIASIS

Ekram Hossain[1], Chaoyi Wu[1], Sharmily Khanam[1], Danya A. Dean[1], Adwaita Parab[1], Shelley Kane[1], Karina Flores[1], Sharon Lostracco-Johnson[2], Diane Thomas[2], Danyang Li[3], Christine Woelfel-Monsivais[1], Mitchelle Katemauswa[1], Camil Gosmanov[1], Krithivasan Sankaranarayanan[1], **Laura-Isobel McCall**[1]

[1]*University of Oklahoma, Norman, OK, United States,* [2]*University of California San Diego, La Jolla, CA, United States,* [3]*Beijing Normal University, Beijing, China*

American trypanosomiasis (Chagas disease) is a neglected parasitic infection caused by *Trypanosoma cruzi* parasites. Over 7 million people worldwide are *T. cruzi*-positive, but only 30-40% of infected individuals will develop cardiomyopathy, megaoesophagus and/or megacolon. The mechanisms leading to symptomatic Chagas disease are still poorly understood, particularly for gastrointestinal (GI) manifestations. To address this issue, we implemented a novel integration of 3D mapping, analytical chemistry (liquid chromatography-tandem mass spectrometry), microbiome science and big data analytics ("chemical cartography") to systematically determine the local impact of *T. cruzi* infection throughout the GI tract, in a mouse model of Chagas disease. Increases in the magnitude of disturbance in local tissue metabolism were observed in the oesophagus and in the colon during the transition from acute to chronic stage, while only limited impact on chronic-stage metabolism was observed in the stomach and small intestine, sites not associated with GI Chagas symptoms. Infection also altered local microbiota composition and diversity. Major infection-associated chemical differences include increases in specific members of the acylcarnitine family of molecules, and changes in short-chain acylcarnitine distribution. Based on these observations and our prior data on acylcarnitine alterations in cardiac Chagas disease, we then directly investigated the role of carnitine in Chagas disease pathogenesis. Strikingly, treatment of infected mice with 1.3% carnitine in drinking water prevented infection-related mortality with no changes in parasite burden, indicating that carnitine supplementation induces disease tolerance in Chagas disease. Overall, these results significantly expand our understanding of Chagas disease pathogenesis, with potential for the development of new therapeutic regimens. More broadly, these results highlight the ability of chemical cartography approaches to provide insight into tropical disease pathogenesis, with translational applications for infectious disease drug development.

64

THE ROLE OF MONOCYTE MOBILIZATION IN RESPONSE TO SAND FLY BITES IN INCREASED TRANSMISSION FROM MAMMALIAN HOSTS TO THE SAND FLY VECTOR

Andrea Paun, Joanna G. Valverde, David L. Sacks

National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Mammalian hosts in areas endemic for leishmaniasis can be exposed to multiple and repeated bites from sand flies over a short period of time. Previous work in our laboratory has shown that prior exposure of *Leishmania donovani*-infected hamsters to sand fly bites results in an increased rate of parasite transmission during subsequent exposure to a new set of sand sand flies after 24 hours. By artificial feeding, parasites could be picked up from both the skin and the blood, though only transmissions from the blood correlated with the direct feeding. Building upon these observations we describe a mobilization of monocytes, including parasite-infected cells, neutrophils and lymphocytes into the blood in the 24 hours following the fly bite. The magnitude and cellular composition of this systemic response was found to differ between naïve and *L. donovani*-infected animals; monocytes being preferentially mobilized in infected animals compared with a greater neutrophil response in naïve animals. These differences suggest that there is a host-vector-parasite interaction that the parasite takes advantage of in order to increase its potential transmission to new hosts. We are now utilizing *L. donovani*-infected mice in order to gain a better understanding of the mechanisms underlying the host response to vector bites that promotes the cyclical transmission of *Leishmania*.

65

MYELOID AND LYMPHOID IMMUNE EXHAUSTION PROFILE DURING MURINE VISCERAL LEISHMANIASIS

Diogo Valadares[1], Richard E. Davis[2], Ellen Kiser[1], Mary Wilson[1]

[1]*University of Iowa, Iowa City, IA, United States,* [2]*University of Utah, Salt Lake City, UT, United States*

Leishmaniasis is a chronic disease of reticuloendothelial organs that is usually controlled by TH1-type CD4 T cells. Anti-microbicidal responses are ineffective during disease progression, and it is reported that T cells in humans and dogs with visceral leishmaniasis (VL) express markers of cell exhaustion. We hypothesized that myeloid cells provide counter-receptors for inhibitory receptors on T cells at the local sites of *Leishmania infantum* infection, inhibiting T cell effector functions. We therefore examined inhibitory receptors PD1, LAG3, CTLA4 and TIM3 on lymphoid cells, and counter-receptors PDL1, MHCII, CD80 and Galectin 9 on myeloid cells using flow cytometry, throughout 5 weeks of infection. In livers and spleens of infected BALB/c mice, significant increases in myeloid cells

expressing PD-L1 and lymphoid cells expressing PD-1 were detected. In livers of infected mice, an increased population of neutrophils with DC features (CD11c+MHCII+) expressing PD-L1 and IL-10 was also observed. In all infected samples an increased DC subset with a reduced expression of MHCII, CD80 and CCR7 and higher expression of Galectin 9, PD-L1 and IL-10, suggesting a pro- T cell exhaustion profile of those cells in infected mice. Furthermore, CD4 and CD8 T cells in infected mice expressed higher levels of the exhaustion markers LAG3, CTLA4 and PD-1 than uninfected mice, with greater proportions of CD8 than CD4 T cells in livers and blood demonstrating the exhausted phenotype. Using PEPCK tetramers and CD11b and CD49d surface markers to identify antigen (Ag)-experienced cells, the majority of Ag-experienced CD8 T PD-1+ cells in liver and spleens produced IL-10, whereas most Ag-experienced CD4 T PD-1+ cells produced IFN-γ at the time points studied[MW1] . The data suggest either CD8 T cells assume an exhausted phenotype earlier than CD4 T cells, or CD8 T cells are the predominant exhausted T cells. Data also suggest that both DC and neutrophil subsets could represent a pro-exhaustion myeloid population that may influence the unresponsiveness of lymphocytes in infected tissues during visceral leishmaniasis.

66

SUBNATIONAL MAPPING OF UNDER-FIVE MORTALITY AND ITS DETERMINANTS IN KENYA SINCE 1965

P**eter M. Macharia**[1], Emanuele Giorgi[2], Pamela Thuranira[1], Noel K. Joseph[1], Benn Sartorius[3], Robert W. Snow[4], Emelda Okiro[1]

[1]*Kenya Medical Research Institute-Wellcome Trust Research Programme, Nairobi, Kenya,* [2]*Lancaster Medical School, Lancaster University, Lancaster, United Kingdom,* [3]*Public Health Medicine, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa,* [4]*Kenya Medical Research Institute-Wellcome Trust Research Programme, Nairobi, Kenya and Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom*

Kenya did not achieve MDG 4 despite substantial health funding and significant declines in under five mortality (U5M) since 2000. The launch of SDG 3 to ensure healthy lives and wellbeing calls for approaches to quantify variations in U5M and its determinants at sub national units of decision making to inform *what* interventions are needed and *where* needed. We characterized spatio-temporal variation and inequalities in child survival and its determinants since 1965 at decentralized health planning units (counties) in Kenya. Household surveys and census data undertaken between 1989 and 2015 were assembled and aligned to the respective counties. Demographic techniques and Bayesian spatio-temporal Gaussian process regression were applied to estimate annual U5M per county between 1965 and 2015. Bayesian spatio-temporal areal level models were applied to produce reliable estimates for key determinants between 1990 and 2015 due to insufficient sample size at county level. 23 surveys and 3 censuses were assembled between 1989 and 2015. Nationally, U5M reduced by 62%, from 1965 to 2015. However, the decline was heterogeneous ranging between 19% and 80% across the counties. Inequalities in child survival declined significantly between counties but, disparities continued to persist by 2015. The progress towards meeting international developments goals on U5M was sub-optimal. The coverage of most the health interventions such as household bed net coverage and Vitamin A supplementation increased over time while major risk factors e.g. malaria risk declined. Changes observed in intervention coverage and risk factors were uneven across time and between counties with varying rates of change. These results form a baseline for tracking local (county) targets under the decentralized governance and the SDGs. In spite of significant progress made in child survival, U5M in Kenya remains high and heterogeneous across counties. Focused allocation and targeted interventions are required to achieve further improvement in child survival and continued decline in subnational inequalities.

67

A SYSTEMATIC REVIEW OF PROACTIVE CASE DETECTION BY COMMUNITY HEALTH WORKERS FOR THE MANAGEMENT OF COMMON CHILDHOOD ILLNESSES

Caroline Whidden[1], Julie Thwing[2], Julie Gutman[2], Clemence Leyrat[1], Kassoum Kayentao[3], Ari Johnson[4], Brian Greenwood[1], Daniel Chandramohan[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*University of Sciences Techniques and Technologies of Bamako, Bamako, Mali,* [4]*University of California San Francisco, San Francisco, CA, United States*

Many low- and middle-income countries (LMICs) are scaling Community Health Worker (CHW) programs as an evidence-based strategy to improve child health, but the expected benefits are often not realized. Proactive case detection by CHWs may overcome barriers to access seen with a conventional community-based approach. We systematically reviewed the evidence of the effects of proactive case detection by CHWs in LMICs on access to care, morbidity, and mortality for common childhood illnesses. Published studies were identified via electronic health databases from 1978 to 2017. We included randomized, non-randomized, controlled before-after, and interrupted time series studies and assessed their quality for risk of bias. We reported measures of effect the same way study investigators reported them and synthesized by outcomes of mortality, disease prevalence, hospitalization, and access to treatment. We calculated risk ratios (RRs) as a principal summary measure, with confidence intervals adjusted for cluster design effect. We identified 14 studies of 11 interventions from nine LMICs that met inclusion criteria. There was considerable diversity in intervention design and implementation, comparison, choice of outcomes, and study quality, which precluded meta-analysis. There was low quality evidence that proactive case detection may reduce neonatal (RR 0.43 - 1.07) and infant mortality (RR 0.52 - 0.94), increase access to effective treatment (RR 1.24 - 1.29), and have little or no effect on wasting and stunting (RR 0.61 - 1.16). There was very low quality evidence that it may reduce mortality among children under five years of age (RR 0.04 - 0.80), prevalence of infectious diseases (RR 0.06 - 1.02), hospitalization (RR 0.38 - 1.26), and increase access to prompt treatment (RR 1.00 - 2.39). Proactive case detection by CHWs may reduce child mortality, morbidity, and improve access to care. This review calls for more rigorous study designs in operational research around proactive case detection, standardization of outcomes and their measurement, and more complete reporting of complex intervention design and implementation.

68

USING PARTNERSHIPS AND EXISTING SYSTEMS TO IMPROVE THE QUALITY OF INTEGRATED SERVICES FOR SICK CHILDREN AT PATENT AND PROPRIETARY MEDICINE VENDORS IN TWO STATES IN NIGERIA

Kate E. Gilroy[1], Abimbola Olayemi[2], Adedeji Onayade[3], Olujide Arije[3], Miranda Gyang[2], Felix Ogaga[4], Chinwe Nweze[5], Olusegun Afolabi[3], Abimbola Phillips[3], Michel Pacqué[1]

[1]*MCSP/JSI, Washington, DC, United States,* [2]*MCSP/JSI, Abuja, Nigeria,* [3]*Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria,* [4]*MCSP/JSI, Lokoja, Nigeria,* [5]*MCSP/JSI, Abakaliki, Nigeria*

Patent and Proprietary Medicine Vendors (PPMVs) are a significant source of treatment for sick children in Nigeria, although concerns are widespread that PPMVs provide poor quality services. The Maternal and Child Survival Program, in close coordination with public and private state and national-level stakeholders, worked to improve and assess the quality of integrated community case management (iCCM) services for childhood malaria, diarrhea, and pneumonia that PPMVs provide. The "Enhancing Quality iCCM through PPMVs and Partnerships (EQuiPP)" approach supported 833 registered PPMVs to improve service quality in 4 local government areas in Kogi and Ebonyi states through initial iCCM training and joint supervision, with supervisors from the public primary health centers and peers within

PPMV associations. Coordination with suppliers, wholesalers, and PPMV associations aimed to improve the availability of essential commodities at PPMV shops through market forces. To evaluate EQuiPP, we conducted baseline (March 2018), midline (July 2018), and endline (November 2018) assessments that included direct observation of services provided to sick children with clinical re-examination and inventory audits at a sample of 176 PPMV shops in each assessment. EQuiPP improved the availability of essential stocks: 30% of PPMV shops had rapid diagnostic tests (RDTs) on the day of the survey at baseline, improving to 83% at endline. The availability of artemisinin combination therapies, zinc and amoxicillin dispersible tablets (AmoxDT) at PPMV shops improved by 20%, 52% and 91% points between baseline and endline, respectively. Among febrile children, only 11% received an RDT to confirm malaria status at baseline, compared to 95% at endline. The percent of children with cough and fast breathing (suspected pneumonia) given AmoxDT was 0% at baseline and 66% at endline. EQuiPP is a promising approach to improve availability of medications and quality of child health services at PPMV shops. Improvements observed after initial training were sustained at endline; future studies should examine trends in the quality of PPMV services over longer periods.

69

PREVALENCE OF CHILD MARRIAGE IN RURAL BANGLADESH AND ASSOCIATIONS WITH ADVERSE PREGNANCY OUTCOMES

Kyu Han Lee[1], Atique I. Chowdhury[2], Qazi S. Rahman[2], Sanwarul Bari[2], Shams El Arifeen[2], Emily S. Gurley[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*ICDDRB, Dhaka, Bangladesh*

Bangladesh has one of the highest rates of child marriage in the world with an estimated 18% of girls married by the age of 15 and 52% married by the age of 18. Early marriage is a primary health concern as they lead to pregnancies in children and are associated with adverse pregnancy outcomes and poor health outcomes among newborns. We used data from an ongoing demographic surveillance system to examine annual trends in child marriage in Baliakandi, a rural subdistrict of Bangladesh. Further, we examined whether early pregnancies were associated with adverse pregnancy outcomes. Starting in September 2017, we visited households in Baliakandi every 2-4 months to identify pregnancy outcomes and child deaths. Married women provided their marriage date through a pregnancy history questionnaire. We used generalized estimating equation models to examine the association between maternal age and 1) stillbirths or spontaneous abortions and 2) neonatal mortality among singleton pregnancies. We identified a total of 49,794 marriages from 1990-2018. During this period, the proportion of marriages to children decreased from 72% to 54%, largely due to a reduction among girls <13 years (23% to <1%). However, marriages among older adolescents (16-17 years) became more prevalent (17% to 29%) and a notable increase was observed from 2017-2018 among girls 13-15 years (19% to 24%). In girls <16 years, 13% of singleton pregnancies resulted in stillbirths compared to 8% in older adolescents and 6% in adults 18-34 years. Neonatal deaths occurred in 1% of live births in girls <16 years compared to 6% in older adolescents and 2% in adults. The odds of stillbirth and spontaneous miscarriage were significantly higher among older adolescents (OR: 2.3, 95% CI: 1.3-4.2) and the odds of neonatal death were significantly higher among girls 16-17 (OR: 2.5, 95% CI: 1.5-4.2). Although child marriage has become less common among young girls, it remains common practice for older adolescents. Given the associated risks with child health and the Bangladesh government's commitment to ending child marriage, regular assessments should be made on legal protections against child marriage.

70

FACTORS ASSOCIATED WITH CONSENT FOR MINIMALLY INVASIVE TISSUE SAMPLING (MITS) TO IDENTIFY THE CAUSE OF DEATH FOR STILLBIRTHS AND CHILDREN UNDER THE AGE 5 IN BANGLADESH

S**hahana Parveen**[1], Farzana Islam[1], M. Saiful Islam[1], Hossain M. Sazzad[1], Farhana Hasnat Khan[1], Md. Al-Mamun[1], Mahadi Hasan[1], Safiur Rahman[1], Salim Reza[1], Sazzad Hossain Khan[1], Tonmoy Sarkar[1], Kamal Ibne Chowdhury[1], Dalia Yeasmin[1], Kyu Han Lee[2], Sanwarul Bari[1], Shams El Arifeen[1], Emily S. Gurley[2]

[1]*International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*John Hopkins University, Baltimore, MD, United States*

Minimally invasive tissue sampling (MITS), is a post-mortem procedure, can be used to determine the etiology of death, especially in countries with sparse data. However, people often confuse MITS with full autopsies, and many families may not provide consent for the procedure. Our objective was to identify factors associated with MITS consent in the Child Health and Mortality Prevention Surveillance study. From September 2017 to March 2019, we identified stillbirths and deaths among children <5 years in Baliakandi, a sub-district, Bangladesh, and approached their parents for MITS consent. Consent rates were compared by family involvement and maternal factors using χ^2. P-values <0.1 were considered meaningful differences. Later, we conducted in-depth interviews with 39 families to explore the factors that contributed their decisions about MITS. We approached to the parents of 78 stillbirths and child deaths for MITS consent and 35 (45%) of them agreed. Consent was more common among parents when the deceased child was multiparous (56% vs. 39%, p=0.080) and among parents who had a history of miscarriage and/or child loss (67% vs 40%, p=0.051). We also found death type (57% stillbirth vs 40% child death, p=0.18) and mother or maternal relatives' involvement in decision making (54% vs 36%, p=0.11) had some influence on consent. In-depth interviews, all respondents mentioned that they consented for MITS for the benefit of the society. Nearly two-thirds of families who consented to MITS did so to learn the cause of death and how to prevent it. The reported reasons for disagreement were an aversion to needle punctures in the body (39%), a lack of attentiveness to listen the content due to emotional vulnerability (30%) and a desire to hurry home to begin burial activities (30%). Findings suggest that prior child loss experience drove parents' interest in learning why their child died and for future prevention. Encouraging the mother and maternal relatives to participate in the decision-making, psychological support and sharing MITS result to families with further prevention measure may promote MITS acceptance and thereby prevent further child mortality.

71

INVOLVING COMMUNITY VOLUNTEERS FOR REAL-TIME IDENTIFICATION OF STILLBIRTHS AND UNDER-5 CHILD DEATHS IN A CHILD HEALTH PROGRAM IN RURAL BANGLADESH

A**bdullah Al Masud**[1], Shahana Parveen[1], Saiful Islam[1], Faruqe Hussain[1], John Blevins[2], Ahoua Kone[2], Kyu Han Lee[3], Qazi Sadeq-ur Rahman[1], Palash Mutsuddi[1], Sanwarul Bari[1], Shams El Arifeen[1], Emily S. Gurley[3]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [3]*John Hopkins University, Baltimore, MD, United States*

The Child Health and Mortality Prevention Surveillance (CHAMPS) Network is ongoing at sites in 7 countries in Africa and south Asia, including in Baliakandi, a rural sub-district in Bangladesh (population 220,000), that aims to identify specific causes of stillbirths and deaths among children under 5. CHAMPS uses minimally invasive tissue sampling (MITS) to provide information about the cause of death. MITS must be conducted shortly after death, before burial. Since many children die at home, receiving death notifications in a timely manner is a major challenge.

Therefore, 870 male and female community volunteers from 261 villages within the CHAMPS surveillance site were recruited to quickly identify stillbirths and deaths occurring in the community. Community volunteers were oriented to CHAMPS activities, including information about MITS procedure and were asked to report stillbirths and deaths of children under 5 to CHAMPS hotline number. Community volunteers were paid different amounts of incentives range from 0.5 to 2.3 USD for the each notification they provided within 4 hours to 1 month. The amount of which was inversely proportional to the elapsed time from the time of death to notification. We analyzed data from the first 8 months of the community volunteer engagement (August 2018 to March 2019) to understand the effectiveness of involving community volunteers to notify stillbirths and deaths of children under 5 in the community within a timeframe to allow for MITS consent. Among the 254 death notifications received by CHAMPS during the 8 month period, 98 (39%) notifications were received from community volunteers. Among those, 37% were received within 3 hours of death, 21% were received within more than 3 to 8 hours of death and 42% were received more than 8 hours after death. Death or stillbirth occurred at home was 37%, occurred at heath facilities was 54% and occurred on the way to the hospital was 9%. The use of community volunteers to notify deaths in real-time was shown to be a valuable approach that could be used in other public health programs through proper motivation, incentives, periodical performance monitoring and refresher training.

72

MINIMALLY INVASIVE TISSUE SAMPLING AMONG HOSPITAL-BASED CHILD DEATHS IN BLANTYRE, MALAWI: THE ROLE OF SOCIAL RELATIONSHIPS AND POWER DYNAMICS

D**ave Mankhokwe Namusanya**[1], Sarah Lawrence[2], Andrew Hamuza[1], Cornelius Huwa[3], Maureen Kelley[4], Sassy Molyneux[4], Wieger Voskuijl[5], Donna Denno[2], Nicola Desmond[1], Dennis Chasweka[3]

[1]*Malawi Liverpool Wellcome Trust, Blantyre, Malawi,* [2]*University of Washington, Seattle, WA, United States,* [3]*University of Malawi, College of Medicine, Blantyre, Malawi,* [4]*University of Oxford, Oxford, United Kingdom,* [5]*Global Health Child Group, Amsterdam University Medical Care, University of Amsterdam, Amsterdam, Netherlands*

Undernutrition is an underlying cause in almost 45% of child deaths globally. Case fatality among children hospitalized with acute malnutrition remains high even with guideline-based care. Cause of death (CoD) is often unknown. Minimally invasive tissue sampling (MITS) using needles to obtain post-mortem samples for histopathological and microbiologic investigation is increasingly being utilized to improve child and adult CoD attribution. "MITS in Malawi" employs standard MITS and a novel post-mortem endoscopic intestinal sampling approach to better understand CoD among children with acute illness and/or malnutrition enrolled in a parent study who die during hospitalization. This formative study aimed to understand sociocultural factors that may impact MITS acceptability in this setting in Malawi. We conducted eight focus group discussions with various hospital cadres and community members to explore attitudes towards MITS and to inform consent process design. We used thematic content analysis drawing on a conceptual framework developed from prior MITS acceptability work. Social relationships at hospital, as well as at community and individual levels, emerged as important factors likely to inform MITS acceptability. Feelings of power over decision-making, trust in healthcare systems facilitated by active involvement of caretakers in child's treatment, and content of communication about MITS were also important dimensions informing acceptability. Other facilitating factors included the potential for MITS to add CoD information to aid in sense-making of death, to reduce witchcraft accusations in communities, and to contribute to medical knowledge and new interventions. The findings informed how we developed an appropriate and contextualised consent approach within a hospital-based cohort study. They further highlight that understanding social relationships and power dynamics within healthcare systems, community social and cultural contexts, and how MITS is placed within existing parent studies are critical components to consider in consent processes and acceptability of MITS in facility-based settings.

73

H-IPSE, A PATHOGEN-SECRETED HOST NUCLEUS-INFILTRATING PROTEIN (INFILTRIN), HAS A LIMITED RANGE OF TARGET CELLS

Olivia Lamanna[1], Evaristus Mbanefo[2], Kenji Ishida[2], Luke Pennington[3], Theodore Jardetzky[3], Franco Falcone[4], Michael Hsieh[1]

[1]*Children's National Medical Center, Washington, DC, United States,* [2]*Biomedical Research Institute, Rockville, MD, United States,* [3]*Stanford University, Stanford, CA, United States,* [4]*The University of Nottingham, Nottingham, United Kingdom*

IPSE (IL-4 Inducing Principle from *Schistosoma mansoni* Eggs) is one of the most abundant egg secreted proteins of the *Schistosoma* parasite. IPSE has been shown to interact with IgE on the surface of basophils to induce IL-4 secretion, trigger Breg cell activation, and, as an infiltrin, translocate into host cell nuclei to alter host transcription. This suggests that IPSE is an important immunomodulator. IPSE binds to DC-SIGN and the mannose receptor, suggesting it may have specific cellular receptors. Our objective was to determine extracellular versus intracellular localization of H-IPSE (a *Schistosoma haematobium* ortholog of IPSE) in various cell types. H-IPSE variants H03 and H06 were conjugated to Alexa-488 fluorophore and incubated with urothelial, endothelial, immature dendritic (iDC), hepatocyte, and neuronal cells. Flow cytometry was used to quantify cells that were IPSE positive. Trypan blue (TB) quenching of extracellular Alexa-488 was used to measure intracellular H-IPSE signal. When urothelial, endothelial, and iDC cell lines were incubated with 2ug/mL of H03 for 24 hrs, the percentage of cells positive for intra/extracellular H03 was 69, 46, and 90%, respectively. Upon TB quenching, intracellular signal was 65, 42, and 84%, respectively. H03 was internalized by hepatocytes at a reduced rate and was not internalized by neurons. These results were similar for both H03 and H06 regarding urothelial cells and iDCs. However, H06 was less efficient than H03 in extra/intracellular localization with endothelial cells. H-IPSE's minimal internalization by hepatocytes suggests that IPSE may not be hepatotoxic, contradictory to previous findings. Our unpublished data showing that IPSE alleviates capsaicin receptor-mediated pain, led us to postulate that IPSE acts through the capsaicin receptor on nociceptive neurons. However, our findings indicate that IPSE does not interact with neurons and is inducing its effects through an alternative pathway. Additional research is underway on IPSE's internalization mechanisms to provide further insights into its therapeutic potential and role in the pathogenesis of schistosomiasis.

74

VACCINATION WITH CATHEPSIN B USING A YS1646 *SALMONELLA ENTERICA* TYPHIMURIUM VECTOR PROTECTS MICE AGAINST *SCHISTOSOMA MANSONI* CHALLENGE

Adam Hassan, Nicholas H. Zelt, Dilhan J. Perera, Brian J. Ward, Momar Ndao

Research Institute of the McGill University Health Centre, Montreal, QC, Canada

Schistosomiasis is among the most important neglected tropical diseases and *Schistosoma mansoni* threatens hundreds of millions of people in >50 countries worldwide. *S. mansoni* resides adjacent to mucosal tissues as adult worms. Several candidate vaccines are in pre-clinical and clinical development, but none is designed to elicit a mucosal response. We have repurposed an attenuated *Salmonella enterica* Typhimurium strain (YS1646) to produce such a vaccine. We have initially targeted cathepsin B (catB), a digestive enzyme important for both juvenile and adult worms. Using a series of plasmid constructs that exploit the type 3 secretory system of *Salmonella*, the expression of catB was screened in monomicrobial culture and in RAW 264.7 cells. Two strains bearing promising promoter-secretory signal pairings were selected for *in vivo*

evaluation (nirB_SspH1 and SspH1_SspH1). Female C57BL/6 mice were immunized twice, 3 weeks apart. Serologic (IgG) responses to catB were monitored by ELISA and, three weeks after the second dose, mice were challenged with 150 *S. mansoni* cercariae. Mice were sacrificed 7 weeks later to assess adult worm and egg burden (liver and intestinal tissue), granuloma size and egg morphology. Responses were highest in the animals immunized with the nirB_SspH1 YS1646 followed by catB IM. All vaccinated animals had reduced worm and egg burden in both the intestines and liver. In the nirB_SspH1 + catB IM group, the worm and intestine/liver egg burden were reduced 93.1% and 79.5%/90.3%. Granuloma size was reduced most significantly in the nirB_SspH1+ catB IM group (34.74 ± 3.35 μm^2 versus 62.22 ± 6.08 μm^2 in the saline controls. Many of the eggs in the granulomas of vaccinated animals had abnormal morphology. Furthermore, preliminary data shows elevated cathepsin B-specific IgA levels in mice immunized with the nirB_SspH1 strain. Targeting the digestive enzyme catB using a multimodality approach elicits both systemic and mucosal immune responses and provides protection against *S. mansoni* challenge. These novel YS1646-vectored candidate vaccines show considerable promise to address the need for a *S. mansoni* vaccine.

75

USE OF BODIPY LABELLED ATP ANALOGUE IN THE DEVELOPMENT AND VALIDATION OF A KINASE BINDING ASSAY FOR SCREENING OF KINASE INHIBITORS

Bernardo Pereira Moreira[1], Tom Armstrong[2], Izabella Cristina Batista[1], Naiara Clemente Tavares[1], Camilla Valente Pires[1], Marina de Moraes Mourão[1], Franco Falcone[3], Lodewijk Dekker[4]

[1]*Rene Rachou Research Centre/CPqRR - FIOCRUZ, Belo Horizonte, Brazil,* [2]*School of Chemistry, University of Nottingham, Nottingham, United Kingdom,* [3]*School of Pharmacy, Division of Molecular Therapeutics and Formulation, University of Nottingham, Nottingham, United Kingdom,* [4]*School of Pharmacy, Division of Biomolecular Science and Medicinal Chemistry, University of Nottingham, Nottingham, United Kingdom*

Human schistosomiasis is a neglected tropical disease that affects more than 230 million people worldwide. It is caused by a parasitic trematode that can survive for years or decades in the mammalian host. As a key strategy to survive in the host, *Schistosoma* integrates specific extracellular signals to generate an appropriate cellular response in order to inhibit or modulate host immune responses. One of the families of proteins involved in such processes are the eukaryotic Protein Kinases (ePK), which regulate tissue-specific biological activities such as cell proliferation, differentiation and survival. The primary catalytic function of PKs is based on the binding of ATP and phosphorylation of specific substrates. Hence, the ATP binding pocket is considered one of the main focuses for inhibitor design, with an increasing number of inhibitors now approved as drugs. Based on that information and considering the need for simple single-tool assay technologies with which one could assess 'all' kinases, we developed a fluorescence polarization-based assay to screen PKs against a library of small molecules predicted to block its ATP binding site. We used BODIPY ATP-y-S as probe to measure the shift in the polarization of a light beam when passed through the sample. We were able to optimize the assay using commercial Protein Kinase A (PKA) and H7 efficiently inhibited the binding of the probe when added to the reaction. Furthermore, we were able to employ the assay to validate in silico screening of a library of more than 80000 small-molecule compounds predicted to dock into the ATP binding site of PKA. Additionally, the assay was also employed to assess the binding activity of heterologous *S. mansoni* c-jun N-terminal kinase (JNK). Thus, this polarization assay is suitable to assess the binding capabilities of PKs as well as to screen larger libraries of compounds that may target PKs by blocking the ATP binding site.

76

HEADS OR TAILS? DIFFERENTIAL TRANSLATIONAL REGULATION IN CERCARIAL HEADS AND TAILS OF SCHISTOSOME WORMS

James R. Hagerty, Emmitt R. Jolly

Case Western Reserve University, Cleveland, OH, United States

Schistosomes are responsible for over 218 million cases of human schistosomiasis in 78 countries. Infection occurs when free-swimming cercariae penetrate host skin and initiate developmental progression into adult worms. Transcriptomic studies of cercariae reveal abundant mRNAs associated with energy metabolism and host invasion. However, the cercaria is mostly transcriptionally quiescent, suggesting that most mRNAs are primed prior to cercarial escape from the snail host. The use of transcriptomics to understand protein functionality presumes that transcription and translation are functionally coupled and the cercarial stage has categorically been treated as a single unit for -omic analysis. Per contra, the relationship between transcription and translation in infectious cercariae has not been described. To understand the correlation between transcription and translation in cercariae, we separately measured nascent translation levels in cercarial heads, cercarial tails and in the developing schistosomula, the next life cycle stage. The loss of the cercarial tail is essential for the transformation from a cercaria to a schistosomulum. We observed that translation increases during the first 72-hours after tail loss and that translational inhibitors such as anisomycin had little effect on the viability of the cercaria though some showed behavioral changes. When we tested nascent translation in cercarial heads, cercarial tails, and whole cercariae we found that translation is significantly upregulated in the cercarial tail when compared to the cercarial head and that translation was undetectable in heads using immunofluorescent image quantification ($p=.0005$). These data represent a major shift in how we understand the cercarial stage. The cercarial head is mostly transcriptionally and translationally quiescent while being sufficient for progression into a schistosomulum. In addition, transcription and translation are not linked in S. mansoni cercaria. Thus, our current conceptual approach of treating the cercaria as a single functional unit for -omic studies may be insufficient to understand cercarial development.

77

COMPARING CATHEPSIN B VACCINE FORMULATIONS IN A PRE-CLINICAL SCHISTOSOMIASIS MODEL

Dilhan J. Perera[1], Adam Hassan[1], Yimei Jia[2], Michael McCluskie[2], Risini Weeratna[2], Momar Ndao[1]

[1]*Research Institute McGill University Health Center, Montreal, QC, Canada,* [2]*National Research Council Canada; Human Health Therapeutics Research Center, Ottawa, ON, Canada*

Schistosomiasis (Schisto) is one of the most important helminthic parasitic diseases in the world with >700 million people at risk, and >200 million infected. Liver cirrhosis can also occur, due to egg deposition and granuloma formation in hepatic tissues. Our lab has shown effective vaccine candidates using *Schistosoma mansoni* cathepsin B (SmCB) as an antigen (Ag) in combination with either CpG dinucleotides (CpG) or Montanide ISA VG 720 (Montanide), reducing parasite burden up to 70% in initial formulations. The goal of this project is to screen other vaccine adjuvants and test their ability to protect from a *S mansoni* infection. Seven vaccine formulations were tested in a mouse model of schisto, each consisting of 20 ug of recombinant SmCB protein with adjuvant. The adjuvants tested included Montanide, aluminum hydroxide (alum), alum/CpG, sulfated lactosyl archaeol archaeosomes (SLA), and alum/monophosphoryl lipid A (alum/MPL). After immunization, vaccines formulated with Montanide, and alum/CpG demonstrated the highest Ag-specific total IgG titers as well highest IgG2c antibodies. Vaccine groups SLA, and alum/MPL produced the highest Ag-specific IgG1 antibodies. Previous data suggests that mixed antibody response, IgG1 and IgG2c, results in higher parasite reduction upon challenge. After challenge, adult worm reductions of 78.9%, 68.5%, and 68.5% were found in the

Montanide, SLA, and Alum/MPL groups respectively. Hepatic eggs were reduced the greatest by Montanide (75.5%), SLA (59.2%), and Alum/MPL (78.7%), and intestinal eggs were reduced the greatest by Montanide (78.9%), SLA (77.1%), and Alum/MPL (77.7%). The data obtained suggests that a vaccine formulated with several of the adjuvants tested could surpass the WHO's minimum threshold of 40% for an effective vaccine.

78

THE HUMAN TELOMERASE REVERSE TRANSCRIPTASE, HTERT IS ABSENT IN *SCHISTOSOMA MANSONI*: EFFECT OF INFECTION AND DRUG TREATMENT ON GENE EXPRESSION OF THE *BIOMPHALARIA GLABRATA* ORTHOLOG IN THE HOST-PATHOGEN RELATIONSHIP

Nana Adjoa Pels, Swara Yadav, Olayemi Akinyele, Freddie Dixon, Carolyn Cousin, Matty Knight

University of the District of Columbia, Washington, DC, United States

The human telomerase reverse transcriptase, commonly known as hTERT, is the vital catalytic sub-unit of telomerase. Together with telomerase RNA, the enzyme complex plays a significant role in the maintenance of telomeres at the ends of chromosomes. Regulation of hTERT is closely linked to cell growth states governing either malignancy or senescence. For this reason, hTERT is a target in developing anti-cancer therapy to block elevated hTERT activity in order to inhibit cell proliferation in cancer. We recently hypothesized that malignant cancer is a parasite in its host and, therefore, the snail host/parasite relationship serves as a good animal model to examine the regulation of cancer related transcripts, including the snail homolog of hTERT in the *Biomphalaria glabrata/Schistosoma mansoni* animal model. To test this hypothesis, we utilized an *in silico* approach to identify the snail hTERT ortholog. The human hTERT amino acid sequence was identified and utilized to interrogate the *B. glabrata* genome in NCBI. Results revealed a significant match with a *B. glabrata* hTERT ortholog (E-value of $2e^{-86}$). Further searches using the compatible parasite *Schistosoma mansoni* of *B. glabrata* as the query showed that unlike the snail host, the parasite lacks an hTERT ortholog. To determine the regulation of the snail hTERT ortholog in relation to the host-pathogen relationship, Gene Specific Primers were utilized for end- point PCR analysis. Results revealed the amplification of a 400bp amplicon at 2hr post- exposure of *B. glabrata* to *S. mansoni* miracidia. In addition, from real time qPCR analysis, results showed that the transcript encoding the snail hTERT ortholog was upregulated in *B. glabrata* at 2hr post-exposure to *S. mansoni.* To further validate whether hTERT plays a role in the snail host:schistosome relationship an anti-telomerase drug BPPA was utilized to block schistosomiasis in the snail host. Other anti-telomerase drugs are being evaluated in the snail-schistosome interaction to test the hypothesis that this host-pathogen relationship is a good model to study malignant cancer as a parasitic disease.

79

DUAL TRANSCRIPTOMICS PROFILING OF THE MOUSE BLADDER WALL INJECTION MODEL OF *SCHISTOSOMA HAEMATOBIUM* INFECTION

Kenji Ishida[1], Evaristus Mbanefo[1], Nirad Banskota[1], James Cody[1], Loc Le[1], Neil Young[2], Michael Hsieh[1]

[1]*Biomedical Research Institute, Rockville, MD, United States,* [2]*The University of Melbourne, Victoria, Australia*

Schistosoma haematobium parasitic flatworms have been estimated to infect over 100 million people. Symptoms include bloody urine, bladder pain, and other bladder dysfunction, and the infection is also associated with the development of bladder cancer. Additionally, animal models to study bladder pathology associated with *Schistosoma haematobium* infection are limited. Here, we use the mouse bladder wall injection model to profile the transcriptome of both the host bladder and parasite eggs to better understand the genes and pathways associated with the infection. We injected 6000 *S. haematobium* eggs into the bladder wall of BALB/c mice and performed RNA-seq on the egg-injected bladders after 4 days. In conclusion, we found 325 differentially expressed host mouse genes ($p < 0.05$) involved in 82 regulatory/signaling pathways when comparing egg-injected bladders with vehicle-injected control bladders. Top pathways include cell migration and fibrosis. Further experiments are planned to identify differences in the transcription profile of the parasite eggs.

80

THE INTERRUPTION OF TRANSMISSION OF ONCHOCERCIASIS BY AN ANNUAL MASS DRUG ADMINISTRATION (MDA) PROGRAM IN PLATEAU AND NASARAWA STATES, NIGERIA

Frank O. Richards[1], Abel Eigege[2], John Umaru[2], Barminas Kahansim[2], Solomon Adelamo[2], Jonathan Kadimbo[3], Jacob Danboyi[4], Hayward Mafuyai[5], Yisa Saka[6], Chukwuma Anyaike[6], Michael Igbe[6], Lindsay Rakers[1], Emily Griswold[1], Thomas Unnasch[7], Bertram E. Nwoke[8], Emmanuel Miri[2]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*The Carter Center, Jos, Nigeria,* [3]*Plateau State Ministry of Health, Jos, Nigeria,* [4]*Nasarawa State Ministry of Health, Lafia, Nigeria,* [5]*University of Jos, Jos, Nigeria,* [6]*Federal Ministry of Health, Abuja, Nigeria,* [7]*University of South Florida, Tampa, FL, United States,* [8]*Imo State University, Owerri, Nigeria*

The 30 districts (Local Government Areas-LGAs) of Plateau and Nasarawa states, located in central Nigeria, have received annual mass drug administration (MDA) with ivermectin for onchocerciasis for a period of 8-26 years. Depending on the LGA, during 8- 11 of these years ivermectin was combined with albendazole as treatment for lymphatic filariasis. Serial assessments during this time in hypermesoendemic sentinel villages showed reduction of onchocerciasis prevalence to near zero. In 2017 serological and entomological assessments were undertaken to determine if MDA could be stopped in accord to World Health Organization onchocerciasis elimination guidelines. Bistate evaluations were conducted in 39 sites located in 22 of the 30 LGAs. The sites selected for assessments were 'first line villages' locate near rivers and having conditions favoring *Simulium* vector breeding. IgG4 response to the OV16 antigen was determined by ELISA in 5-<10 year old resident children. *Simulium damnosum* s.l. collected in or around those same villages either by human landing capture or by Esperanza trap were tested by PCR for determination of the presence of infective *Onchocerca volvulus* L3 larvae. Only 2 (0.03%) of 6,262 children tested were positive. A total of 19,056 vectors heads were tested in pools of 100 flies and were PCR negative. Both states met WHO guidelines of an infection rate in children <0.1% (with 95% confidence) and the rate of infective black flies of <1/2000 (with 95% confidence). The Federal Ministry of Health concluded that transmission of onchocerciasis has been interrupted and approved halting 2.2 million ivermectin treatments in 2018. Post Treatment Surveillance was immediately launched with an emphasis on entomological collections near borders with other onchocerciasis endemic states still being treated. This was the first stop MDA decision for onchocerciasis in Nigeria and among the largest reported to date. The apparent positive impact of LF treatments on hypoendemic onchocerciasis areas will be discussed.

81

RESURGENT LYMPHATIC FILARIASIS IN THE SAMOAN ISLANDS: TIME FOR CHANGE IN SURVEILLANCE STRATEGIES AND THRESHOLDS FOR VALIDATION OF ELIMINATION?

C**olleen L. Lau**[1], Sarah Sheridan[2], Therese Kearns[3], Take Naseri[4], Robert Thomsen[4], Saipale Fuimaono[5], Tautala Mauala[6], Helen Mayfield[1], Brady McPherson[1], Kelley Meder[1], Gabriela Willis[1], Benjamin Dickson[1], Meru Sheel[1], Kimberly Won[7], Katherine Gass[8], Patricia Graves[9]

[1]*Australian National University, Canberra, Australia,* [2]*University of New South Wales, Sydney, Australia,* [3]*Menzies School of Health Research, Darwin, Australia,* [4]*Samoa Ministry of Health, Apia, Samoa,* [5]*American Samoa Department of Health, Pago Pago, American Samoa,* [6]*Samoa Red Cross, Apia, Samoa,* [7]*Centers for Disease Control and Prevention, Division*

of Parasitic Diseases and Malaria, Atlanta, GA, United States, [8]Task Force for Global Health, Atlanta, GA, United States, [9]James Cook University, Cairns, Australia

Samoa and American Samoa, two adjacent South Pacific island groups, had high circulating filarial antigen (Ag) prevalence (4.5% and 16.5% respectively) in 1999, when baseline surveys were conducted through the Pacific Programme to Eliminate Lymphatic Filariasis (LF). After 6-7 rounds of mass drug administration (MDA) with diethylcarbamazine (DEC) and albendazole from 1999-2006 with reported adequate population coverage, Ag prevalence in all ages reduced to 1-2%. American Samoa passed two Transmission Assessment Surveys (TAS) of 6-7 year-old children in 2011 & 2015. Samoa conducted four additional MDA rounds during 2008-2017, and passed TAS in two of three evaluation units (EUs) in 2013, but failed TAS in all EUs in 2017. Population representative household surveys found that overall Ag prevalence had risen to 6.2% (95% CI 4.5-8.6%) in American Samoa in 2016, and 4.9% (95% CI 4.0-5.9%) in Samoa in 2018. Ag prevalence in 6-7 year-old children was 0.7% (95% CI 0.3-1.8%) and 1.5% (95% CI 1.0-2.1%) respectively, indicating ongoing transmission. Overall, 15-25% of Ag-positive (Ag+) persons were microfilaraemic, including children as young as 5 years. Ag+ persons were identified throughout American Samoa (2016) and Samoa (2018) in all EUs, but village-level Ag prevalence varied significantly, ranging from 0% to 45%. Significant household clustering of Ag+ persons was found in both Samoas. The results indicate that neither Samoa nor American Samoa had interrupted transmission by 2011-2013 despite passing TAS thresholds, and both are now experiencing resurgence. In areas with highly efficient vectors (e.g. Polynesia), programmatic guidelines may need to be revised to ensure interruption of transmission is sustained. Potential strategies include: 1) reducing Ag prevalence thresholds for stopping MDA and validating elimination; 2) because Ag prevalence increases with age, increasing the target age groups for surveillance to improve the sensitivity for detecting any residual transmission; and 3) screening household members of Ag+ persons. Both Samoas are implementing nationwide triple drug MDA (DEC, albendazole, ivermectin) in 2018-2019.

82

ANNUAL VERSUS SEMI-ANNUAL MASS DRUG ADMINISTRATION WITH DIETHYLCARBAMAZINE PLUS ALBENDAZOLE FOR ELIMINATION OF LYMPHATIC FILARIASIS IN EAST SEPIK PROVINCE, PAPUA NEW GUINEA

Michael C. Payne[1], Philip Lus[2], Nelly Sanuku[2], Brooke Mancuso[1], James Suamani[2], Delma Beaso[2], Gary J. Weil[3], Peter U. Fischer[3], Moses Laman[4], Leanne J. Robinson[5], Daniel J. Tisch[1], Christopher L. King[1]

[1]*Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute of Medical Research, Maprik, Papua New Guinea, [3]Washington University School of Medicine, St. Louis, MO, United States, [4]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea, [5]Burnet Institute, Melbourne, Australia*

Papua New Guinea (PNG) has over 5.4 million people at risk of lymphatic filariasis (LF) with some of the highest infection rates in the world. Modeling studies based on data from Ghana and India suggest that semi-annual mass drug administration (MDA) could accelerate LF elimination. The aim of this study was to compare the impact of annual and semi-annual MDA for clearance of microfilaremia (Mf) and circulating filarial antigen (CFA) in highly endemic areas in PNG. Repeated annual cross-sectional surveys were conducted in 8 sentinel villages (~300-400 individuals/site) in the Dreikikir District, East Sepik Province; 4 sentinel sites in communities received semi-annual MDA with DEC plus albendazole (ALB) at 6 month intervals for 3 years (total of 5 rounds), and 4 sentinel sites received 3 rounds of annual MDA with the same medications. Filarial test strips (FTS) evaluated the presence and levels of CFA and Mf was assessed by microscopic examination of 60 µl night blood smears. Approximately 1,400 persons were surveyed for each treatment area each year. Baseline Mf and CFA prevalence were similar in annual and semi-annual MDA communities (26% Mf+ and 51% CFA+ vs. 26% Mf+ and 53% CFA+). Clearance of Mf and CFA was assessed annually for 3 years after baseline. Mf prevalence decreased dramatically over 3 years from 26% to 0% after annual MDA and from 26% to 0.3% after semi-annual MDA. Declines in CFA prevalence were also comparable (from 51% to 29% after annual MDA, and 53% to 35% after semi-annual MDA). In conclusion, MDA with DEC + ALB was highly effective for clearing microfilaremia from communities but less effective for clearing CFA. Additional work is needed to understand the significance of persistently high CFA prevalence in these communities. Semi-annual MDA was not superior to annual MDA. We recommend that LF elimination programs conserve resources by focusing on delivery of a single round of high quality MDA per year.

83

ONCHOCERCIASIS ELIMINATION IN LOW-ENDEMIC SETTINGS: MATHEMATICAL MODELLING TO ASSESS THE REQUIRED DURATION OF MASS DRUG ADMINISTRATION OF IVERMECTIN

Wilma A. Stolk, Anneke S. De Vos, David J. Blok, Luc E. Coffeng, Sake J. De Vlas

Erasmus MC, Rotterdam, Netherlands

Onchocerciasis is targeted for elimination in Africa by mass drug administration (MDA) of ivermectin. Many low-endemic areas have not yet been treated, as MDA programmes historically focussed only on meso- and hyperendemic areas for morbidity control. We used the ONCHOSIM simulation model to assess how many rounds of MDA are needed to eliminate onchocerciasis from low-endemic areas. We simulated annual MDA for settings with 0%-40% baseline microfilaria (mf) prevalence and varying local transmission conditions (annual biting rate, inter-individual variation in exposure to flies, and rate of infection importation from other areas). The number of MDA rounds to bring mf prevalence below 1.4% (the assumed critical threshold) depends on baseline endemicity, coverage, trends in infection importation, and the stability of transmission without importation. If importation rates remain constant until the start of MDA, it would take 5, 11, 14 and 15 annual MDA rounds, respectively, to reduce mf prevalence to <1.4% in areas with 0-10%, 10-20%, 20-30% and 30-40% baseline mf prevalence. Shorter durations are expected if the importation rates start declining before introducing MDA, thanks to treatment in surrounding areas. In some areas, infection transmission can only persist thanks to importation from surrounding higher-endemic areas. Treating the surrounding areas - leading to reduced importation - is then sufficient for elimination, although elimination can be accelerated by also treating the low-endemic area. In conclusion, the number of MDA rounds required to eliminate onchocerciasis from hypo-endemic areas varies strongly. Under some conditions as many as 15 annual MDA rounds may be required to bring mf prevalence below the assumed <1.4% threshold, and acceleration strategies may have to be considered (e.g. treating biannually). The assumed 1.4% threshold is subject to uncertainty. The actual threshold depends on local transmission conditions, and further work is needed to understand where more lenient threshold can be used or more stringent threshold are needed.

84

FEASIBILITY OF ONCHOCERCIASIS ELIMINATION USING A "TEST-AND-NOT-TREAT" STRATEGY IN *LOA LOA* CO-ENDEMIC AREAS

David J. Blok[1], Joseph Kamgno[2], Sebastien D. Pion[3], Hughes C. Nana-Djeunga[2], Yannick Niamsi-Emalio[2], Cedric B. Chesnais[3], Charles D. MacKenzie[4], Amy D. Klion[5], Daniel A. Fletcher[6], Thomas B. Nutman[5], Sake J. de Vlas[1], Michel Boussinesq[3], Wilma A. Stolk[1]

[1]*Department of Public Health, Erasmus MC, University Medical Center, Rotterdam, Netherlands, [2]Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaoundé, Cameroon, [3]IRD UMI [233]-INSERM U[1175]-Montpellier University, Montpellier, France, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [5]Laboratory of Parasitic*

Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [6]Department of Bioengineering and the Biophysics Program, University of California, Berkeley, CA, United States

Mass drug administration (MDA) with ivermectin is the main strategy for onchocerciasis elimination. Ivermectin is generally safe, but has been associated with serious adverse events in persons with high microfilarial densities (MFD) of *Loa loa.* Therefore, ivermectin MDA is not recommended in areas where onchocerciasis is hypoendemic and *L. loa* is co-endemic. To eliminate onchocerciasis in those areas, a test-and-not-treat (TaNT) strategy has been proposed. Using the Loascope, a mobile video-microscope, people with high *L. loa* MFD can be identified and excluded from ivermectin treatment. While TaNT was successfully piloted in Cameroon, it remains unclear whether onchocerciasis elimination is possible using this strategy. We used the established individual-based model ONCHOSIM to assess whether onchocerciasis can be eliminated using TaNT in *L. loa* co-endemic areas and what the required duration until elimination would entail in comparison to MDA. We simulated pre-control onchocerciasis microfilarial prevalence (MFP) levels ranging from 1-50%. The impact of TaNT was simulated under varying levels of participation rate, systematic non-participation and exclusion from ivermectin due to high *L. loa* MFD. We predict that in areas with a pre-control MFP of 30-40% it normally takes around 10 and 14 years to bring onchocerciasis MFP below 1.4% using MDA, if the participation rate is 80% and 65%, respectively. These durations would increase with about 1 year, if 2.5% of the population is randomly excluded from ivermectin treatment due to TaNT. This increase can be up to about 5 years if systematic non-participation is assumed, and if participation rates are lower and pre-control MFP higher. Although the chosen elimination threshold is provisional, our model predicts a high probability of achieving true elimination after reaching this threshold. In conclusion, onchocerciasis can be eliminated using TaNT in areas co-endemic for *L. loa*. The required treatment duration of TaNT until elimination is only slightly longer than in areas with MDA, if participation is good.

85

ENVIRONMENTAL FACTORS ASSOCIATED WITH CONTRASTING GEOGRAPHICAL DISTRIBUTIONS AND HOTSPOTS OF ONCHOCERCIASIS AND LOIASIS IN KONGO-CENTRAL, DEMOCRATIC REPUBLIC OF CONGO

Xavier Badia-Rius, Hannah Betts, **Louise A. Kelly-Hope**

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Onchocerciasis and loiasis are important filarial diseases in the Democratic Republic of Congo (DRC), and the Kongo-Central region has shown to have contrasting geographical distributions, which may be driven by the ecological niche of the *Simulium* and *Chrysops* vectors. To better understand environmental factors associated with geographical patterns and potential hotspots, REMO and RAPLOA survey data from 335 villages available from the ESPEN portal were examined. Maps were developed using ArcGIS 10.5.1 and prevalence distributions examined for spatial clustering using the Getis Ord Gi* statistic. Environmental factors including annual mean temperature (°C), annual precipitation (mm), elevation (m), tree canopy coverage (%) and tree canopy height (m) were obtained from publicly available sources, and data extracted for each village and analysed using statistical methods with a significance level of $P < 0.05$. Overall, mean environmental measures for both diseases were similar (temperature 31.6-32.1°C; precipitation 1287-1290mm, elevation 23.5-23.7m, canopy coverage 30.2-32.7%, canopy height 7.3-11.3m). Prevalences were found to be positively correlated with temperature, and negatively with precipitation. However, onchocerciasis prevalence was significantly negatively correlated with canopy coverage (r=-0.30) and canopy height (r=-0.16), whereas loiasis was significantly positively correlated with coverage (r=0.50) and height (r=0.26). For onchocerciasis, 86 villages were identified as hotspots and clustered near the Congo River with mean temperature 28.2°C; precipitation 1184mm, elevation 22.0m, canopy coverage 16.4%, and canopy height 5.6m. In contrast for loiasis, 57 villages were identified as hotspots and clustered in the forested region with mean temperature 24.7°C; precipitation 1165mm, elevation 18.5m, canopy coverage 63.6%, and height 13.4m. This study provides insights into the environmental parameters of transmission, which may help to delineate high and low risk, and target intervention strategies for filariasis control and elimination.

86

EVALUATION OF RESPONDENT-DRIVEN SAMPLING TO ESTIMATE LYMPHATIC FILARIASIS MORBIDITY BURDEN IN HAITI

Alexia Couture[1], Luccene Desir[1], Ernest Jean Romuald[1], Madsen Beau De Rochars[2], Brittany Eddy[1], Karen E. Hamre[3], Michelle A. Chang[3], Katherine M. Gass[4], Caitlin M. Worrell[3], Jean Frantz Lemoine[5], **Gregory S. Noland**[1]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*Department of Health Services Research, Management and Policy, College of Public Health and Health Professions, University of Florida, Gainesville, FL, United States,* [3]*Division for Parasitic Diseases and Malaria, Center for Global Health, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Neglected Tropical Diseases Support Center, Task Force for Global Health, Decatur, GA, United States,* [5]*Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti*

More than 36 million people suffer from lymphatic filariasis (LF)-related limb swelling (lymphedema) or urogenital swelling (hydrocele). The World Health Organization requires LF-endemic countries to document the number of lymphedema and hydrocele cases in all historically endemic districts, yet there is no agreed methodology for assessing morbidity burden. Case reporting is hindered by the social stigma and isolation associated with LF. Respondent driven sampling (RDS) is a method developed for accessing hidden populations through recruitment of a limited number of starting 'seeds' followed by successive waves of participant referral. We evaluated the feasibility of RDS for estimating LF morbidity burden in four districts in Haiti classified as high, medium or low burden based on baseline antigen prevalence at mapping in 2001 (range: 1%—44%). We compared RDS burden estimates with those derived from recent cross-sectional household surveys (HHS) conducted in the same areas. From July 2018-January 2019, approximately 20-25 individuals per condition (lymphedema or hydrocele) identified from local health facility records were enrolled as seeds and asked to refer others in the community with the same condition. A total of 179 confirmed lymphedema cases (52% male) and 238 hydrocele cases (100% male) were enrolled through RDS across four districts. The number of referral waves directly correlated with antigen prevalence classification for both lymphedema (r^2=0.88) and hydrocele (r^2=0.97). Surprisingly, a greater number of waves were achieved for hydrocele than for lymphedema in every district. District-level RDS prevalence estimates calculated with successive sampling - population size estimation (SS-PSE) and a flat prior ranged from 0.2% (95% CI: 0.15%—0.21%) to 1.4% (95% CI: 0.4%—2.1%) for lymphedema and from 0.4% (95% CI: 0.20%—0.50) to 1.5% (95% CI: 0.7%—1.9%) for hydrocele. The 95% confidence intervals for RDS and HHS overlapped in three of four districts for hydrocele, but only one or four districts for lymphedema. Results suggest that RDS may offer a viable option to efficiently obtain LF morbidity burden data.

87

HEALTH CARE PROVIDERS AND CAREGIVERS' VIEWS ON THE FEASIBILITY, USABILITY AND ACCEPTABILITY OF LUNG ULTRASOUND FOR DIAGNOSING PEDIATRIC PNEUMONIA IN MANHIÇA DISTRICT, MOZAMBIQUE

Olga Cambaco

Manhica Health Research Centre, Vila da Manhica, Mozambique

In Mozambique, pneumonia is a leading cause of death among children under 5 years of age, yet its diagnosis remains a challenge. Although chest radiography (CXR) remains the recommended diagnostic method, existing evidence from other suggests that CXR it is often unavailable, costly and

exposes children to hazardous radiation. Lung ultrasonography (LUS) is a promising diagnostic method due to its high sensitivity in detecting diseases such as pneumonia. This is the first study to date to explore the anticipated acceptability and usability of LUS for diagnosing pediatric pneumonia among health care providers and caregivers in Mozambique. We conducted a qualitative study in the Manhiça district Hospital (MDH), in southern Mozambique, from April to May 2018. Semi-structured interviews were conducted among health care providers (10) and caregivers (10) combined with direct observations of ultrasound utilization. Data were analysed following a combination of content and thematic analysis. This study showed a high level of acceptability of LUS among caregivers and health care providers in our setting. We identified the following acceptability factors: LUS is perceived to be precise to diagnose, portable, easy to use, and non-invasive. Despite this, participants expressed some reservations. Caregivers were concerned with battery life limitation and device's susceptibility to dropping and breaking down. On the other hand, health care providers were concerned about the potential cost of the devices, poor conservation and management and the language of the software. These findings anticipate potential reasons for acceptability and advances recommendations to overcome the perceived limitations that are important to take into account prior to any future implementation of LUS for pneumonia diagnosis.

88

AWARENESS AND WILLINGNESS TO USE PREP AMONG FEMALE SEX WORKERS IN DAR ES SALAAM

Diana Faini[1], Mucho Mizinduko[1], Samuel Likindikoki[1], Alexander Mwijage[1], Melkizedeck Leshabari[1], Neema Makyao[2], Kåre Moen[3], Germana H. Leyna[1], Claudia Hanson[4], Patricia Munseri[1], Elia J. Mbagga[1]

[1]*Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [2]*National AIDS Control Program, Dar es Salaam, United Republic of Tanzania,* [3]*University of Oslo, Oslo, Norway,* [4]*Karolinska Institutet, Stockholm, Sweden*

Tanzania is implementing a demonstration project on pre-exposure prophylaxis (PrEP) to female sex workers (FSW) whose HIV prevalence is five times higher than the general population. While PrEP has demonstrated to be an effective biomedical intervention in reducing HIV incidence, no data is available on the extent of PrEP awareness and willingness to use among FSW in Tanzania. Understanding the level of awareness, the extent of willingness to use PrEP and identifying factors associated with PrEP awareness could inform the ongoing country efforts to roll-out PrEP. FSWs were recruited in the integrated bio-behavioral surveillance in December 2017 using responded-driven sampling method. Face-to- face interviews were conducted to collect information on awareness and willingness to use PrEP as well as factors associated with PrEP awareness. Weighted bivariate and multivariable logistic regression models were used to estimate factors associated with PrEP awareness. We recruited 958 FSW, their median age was 26 (IQR 22-32) years. Among self- reported HIV negatives(n=751), 228 (31%) reported having heard of PrEP. PrEP awareness was higher among those aged 25 years or more compared to 18-24 years (33% vs 26%, P=0.02), but did not differ by education levels [none/primary vs secondary/tertiary (24% vs 30%, P=0.26)]. Contact with peer educator in the past 12 months (aOR 1.48,95%CI 1.06-2.06), HIV testing at a health facility (aOR 1.65, 95%CI 1.03-2.66) or research project (aOR 3.41, 95%CI 1.74-6.68) compared to testing in the community were strongly associated with higher PrEP awareness.Substance use (aOR 0.64, 95%CI 0.41-1.00) and having >10 partners in the past month (aOR 0.65, 95%CI 0.43-0.96) were associated with lower PrEP awareness. Nearly all of the self-reported HIV negative women 96% (n= 710/751) were willing to use PrEP. Despite the low PrEP awareness, there is a great willingness to use PrEP among FSW in Dar es Salaam. PrEP implementation programs should focus on increasing PrEP knowledge among younger FSW. Peer educators and HIV testing opportunities could be seized to raise PrEP awareness.

89

A BASELINE ASSESSMENT EVIDENCE INFORMED DECISION MAKING NETWORK IN THE HEALTH SECTOR IN MALAWI USING SOCIAL NETWORK ANALYSIS AND A PROSPECTIVE CASE STUDY

Melody Sakala[1], Kate Gooding[1], Jenny Hill[2], Linda Nyondo Mipando[3], Bertie Squire[2]

[1]*Malawi Liverpool Wellcome Trust, Blantyre, Malawi,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*College of Medicine, Blantyre, Malawi*

Despite the steady progress in health-related research only a limited number of that research translates into policy and impact. Global advocacy for evidence-based decision making is recognized both nationally and internationally and there has been recent global initiatives to ensure use of research findings in decision making processes. To further support research evidence use in Malawi, all the main actors in the space of knowledge translation and evidence use have joined efforts to establish a formal Network for Evidence-Informed Decision-Making in Health Policy & Practice in Malawi (EvIDeNt).The overall objective of this study is to conduct a baseline assessment of key indicators and processes of EvIDeNt in supporting use of research outcomes and to inform the evaluation of the network at a later stage. In achieving this, the baseline will assess the connectivity of the networks in terms of membership and structure, its health in terms of resources and internal systems. Social network analysis will be used to examine the networks existing ties among members and to assess network efficiency and a case study on use of findings from a malaria trial, to trace specific effects of EvIDeNt and identify aspects of the research and policy context that affect research uptake. Methods for data collection will employ a desk review analysis to capture the overall structure, systems and resources of the network. Key informant, interviews will be used to clarify on the mapped intended outcomes. The study intended outcomes aims to support evidence informed health policy by strengthening EvIDeNt and advancing understanding of factors influencing research uptake.

90

GEOSPATIAL MAPPING OF TIMELY ACCESS TO COMPREHENSIVE EMERGENCY OBSTETRIC CARE IN KENYA

Paul Ouma, Robert Snow, Mike English, Emelda Okiro

KEMRI-Wellcome Trust, Nairobi, Kenya

Ensuring access to comprehensive emergency obstetric care (CEmOC) can reduce maternal mortality by 85%. In many African countries, access to these services is poor, partly due to inadequate hospital capacity and long distances to the few hospitals that can provide care. This study aimed to assess readiness to provide CEmOC services and combine with population data to define county level geographic access to CEmOC hospitals in Kenya. We assessed hospital readiness to provide caesarean deliveries and blood transfusion which are the two key indicators needed to provide CEmOC services, using data from nationwide service availability assessments. This included collecting information on availability of operating theatres, blood transfusion, medical doctors, nurses and basic surgical equipment, all which are needed to provide safe CEmOC. We then assembled road networks, elevation and land use at high spatial resolution to formulate a cost friction surface. This surface was then combined with location of CEmOC hospitals to estimate population living more than two hours from the nearest CEmOC hospital at county level. In 2018, 512 public and private hospitals were assessed and indicator availability were; operating theatres (n=295), blood transfusion (n=318), medical doctor availability (n=443), nurse availability (n=512) and surgical equipment (n=290). There were 228 hospitals that provide CEmOC services, seven of which could perform caesarean deliveries without blood transfusion. Approximately 19% of Kenya's expectant women in 2018 were living more than 2 hours from the nearest CEmOC hospital, with significant variations at county level. These ranged from as high as 75% in Turkana to less than 5% in Nairobi, Kisii, Kiambu, Kisumu, Nyamira and Vihiga

counties. The results show a substantial variation in access to CEmOC services at county levels, highlighting the need for targeted investments to bridge the inequities.

91

COST OF DENGUE ILLNESS IN INDONESIA

Oliver J. Brady[1], Lauren Carrington[2], Emilie Hendrickx[1], Dinar D. Kharisma[3], Ida S. Laksanawati[4], Kathleen O'Reilly[1], Donald S. Shepard[5], Cynthia Tschampl[3], **Nandyan N. Wilastonegoro**[6], Laith Yakob[1], Wu Zeng[3]

[1]*London School of Tropical Medicine & Hygiene, London, United Kingdom,* [2]*Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam,* [3]*Brandeis University, Waltham, MA, United States,* [4]*Dr. Sardjito General Hospital, Yogyakarta, Indonesia,* [5]*Heller School for Social Policy and Management, Waltham, MA, United States,* [6]*Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia*

There has been a shift in the global burden of disease from communicable non-communicable disease. Interestingly, global dengue cases have been increasing over the decades. Prognosis of dengue varied from asymptomatic to fatal cases with outbreaks often occurred. As a consequence, the economic burden due to dengue is a threat to endemic countries. We estimated the cost of dengue illness in Indonesia using mix approach of macro- and micro-costing. We gathered financial and utilization data of health facilities (hospitals and primary cares) and interviewed respondents' cases for hospital, ambulatory and non-medical cares in Yogyakarta City. We analyzed the cost per patient and the portion of that paid by households and other paying mechanisms. To obtain the estimation of dengue cases and cost in Indonesia, part of our study produced consensus estimates using statistical and mathematical modeling. We summed the total cost of dengue illness and multiplied to our estimation of dengue cases to obtain the estimated national cost. Dengue cost per case for hospital, ambulatory and non-medical treatments were US$ 318.36 (95%UI 243.93-392.79), US$ 23.03 (95%UI 14.45-31.62) and US$ 7.53 (95%UI 2.37-12.68), respectively. There was a total of 7.535 million (95%UI 1.319-16.513 million) dengue cases in 2017 and our national estimate suggests that the economic cost of dengue in Indonesia was US$ 666.83 million (95%UI 531.57-802.08 million). To this figure, about 37%, 20%, 25% and 18% paid by the households, social contributors, Indonesian National Health Insurance Program (*Jaminan Kesehatan Nasional*/JKN) and other parties, respectively. In conclusion, the cost of dengue illness will be useful in Indonesia and in other countries. This economic analysis is informing out-of-pocket expenditures and direct and indirect costs within three different treatment settings. Hence there are needs for refinement of dengue policy options to existing and promising interventions e.g. vaccine and Wolbachia technologies which are attributed to lowering economic burden.

92

HEALTH CARE OPTIONS AND FACTORS INFLUENCING HEALTH SEEKING BEHAVIOR IN A RURAL NIGERIAN COMMUNITY

Paul W. Okojie

Liberty University, Lynchburg, VA, United States

There is an urban and rural disparity in health care infrastructure in Nigeria. With over 60% of its population living in rural communities, there is an opportunity for addressing the root causes of the poor health status of rural populations. The paper aimed to analyze sociodemographic factors influencing health-seeking practices in a rural community setting. A cross-sectional study of 380 rural dwellers in Utese, Southern, Nigeria was done using interviewer-administered questionnaires. Data were analyzed with SPSS version 25 software. Chi-square test was used to find the association between sociodemographic characteristics and health-seeking practices. Comparable proportions (43.4%, 42.9%) of the respondents fell within the younger age categories of 10-29 and 30-49 years, respectively. Self-reported factors influencing the choice of health care were: Promptness of care (41.8%), cost (22.4%), professionalism (16.8%), distance (15.8%), and cultural belief (3.2%). Patent medicine store was the most utilized source of health care (42.1%). 140 (36.8%) respondents sought health care in the hospital. The hospital was utilized by 41.8% of respondents with secondary education; 34.9% with tertiary education; 31.7% primary and 26.1% with no formal education, respectively. Females tended to seek healthcare from hospitals (40.2%) and patent medicine store (43.7%) compared to males (33.3% and 41.0%). There was a significant association between educational attainment and healthcare options utilized ($P<0.05$). Less than half of rural dwellers utilize minimum standard health care mainly due to cost and timeliness of health care. Education is the key to influencing health-seeking practices in rural communities.

93

MATERNITY WAITING HOMES IN LIBERIA: RESULTS OF A 5-YEAR COUNTRY-WIDE MULTI-SECTOR SCALE-UP

Alphonso W. Kofa[1], Joseph E. Perosky[2], Aloysius Nyanplu[1], Cheryl A. Moyer[3], Jody R. Lori[4]

[1]*Liberia Ministry of Health, Phebe, Liberia,* [2]*Michigan State University College of Human Medicine, East Lansing, MI, United States,* [3]*University of Michigan Medical School, Ann Arbor, MI, United States,* [4]*University of Michigan School of Nursing, Ann Arbor, MI, United States*

Maternity waiting homes (MWHs) are small structures built adjacent to a healthcare facility where women can stay prior to giving birth. In 2010, a cohort study of 10 rural primary health facilities (5 with and 5 without a MWH) was conducted in Bong County, Liberia to evaluate their impact on maternal and newborn outcomes. Results showed a decrease in maternal and perinatal mortality in communities with a MWH compared to those without one. Following this study, the Liberian Ministry of Health identified MWHs as one component of continuing health system strengthening efforts to improve maternal and newborn outcomes. In the ensuing eight years, an additional 114 MWHs have been constructed in 14 of the 15 counties in Liberia for a total of 119 MWHs by an array of funders and implementers. Of these 119, 54 (45.4%) are open and functional, 8 (6.7%) are currently under construction, 35 (29.4%) were started but construction ceased prior to opening, 15 (12.6%) have been repurposed mainly for staff quarters, and 7 (5.9%) MWHs were opened and later abandoned. There were various funders and implementers involved in the scale up of MWHs including 72 (60.5%) by NGOs, 35 (29.4%) by the local community, 6 (5.0%) by the United Nations H6 consortium, 4 (3.4%) by Individuals, and 2 (1.7%) by the local Liberian government. One hundred fifteen focus groups were conducted with community members (Chiefs, Community Leaders, Women of Reproductive Age, Traditional Birth Attendants (TBAs), Women currently staying at a MWH, and Male Partners) as well as 113 interviews with health care providers providing services at the health care facilities associated with a MWH. Nearly all communities hold the following three beliefs: MWHs 1) reduce home deliveries; 2) reduce maternal deaths; and 3) improve relationships between health facility staff, TBAs, and communities. The data also revealed the following challenges and barriers to sustainability and scalability of MWHs in Liberia: 1) lack of initial community engagement; 2) lack of community awareness; 3) food security; 4) inadequate capacity; 5) lack of development of a self-governance model; and 6) lack of on-going support/resources.

94

PRECISION PUBLIC HEALTH AND PANDEMIC PREPAREDNESS: QUANTIFYING TRAVEL TIME TO HEALTH CARE AND LOCATIONS AT RISK FOR PATHOGEN TRANSMISSION

E**rin Hulland**[1], Kirsten Wiens[1], Shreya Shirude[1], Beth Bell[2], Peter Rabinowitz[3], Judith Wasserheit[2], Daniel Weiss[4], Simon Hay[1], David Pigott[1]

[1]*Institute for Health Metrics and Evaluation, Seattle, WA, United States,* [2]*Department of Global Health, University of Washington, Seattle, WA,*

United States, [3]Department of Global Health and School of Public Health, University of Washington, Seattle, WA, United States, [4]Big Data Institute, Nuffield Department of Medicine, Oxford, United Kingdom

Infectious disease threats exist even in places without evidence of past events, and without clear preparedness plans to prevent, detect, and respond to such threats, outbreaks can easily overwhelm fragile health systems. Recent emphases on developing National Action Plans have required subnational evaluations of vulnerable communities, and precision public health using geospatial resources have provided a method to quantify observed heterogeneities. In early stages of an outbreak, proper diagnosis and treatment are essential for containment and care, and proximal access to a health facility is vital, but just as the risk of infectious disease is not homogeneous across the world, neither is health care accessibility, leaving gaps in coverage. Moreover, many of those regions at higher risk are often those that also lack accessibility to care. Here we mobilized large, open-source geospatial resources including a travel-time friction surface, geolocated health facility information, and pathogen environmental suitability maps to quantify health facility accessibility and pathogen suitability transmission to any location within a given country. Noting significant cross-border migration and previous disease transmission stemming from such movements, such as the detection of Ebola cases at the Uganda border with the Democratic Republic of Congo (DRC), we considered the pathogen suitability of neighboring countries and calculated the time it would take to travel to any point in-country. For Uganda, much of the border with DRC was within five hours of locations with known Ebola suitability. Finally, we developed methods to identify areas where additional resources directed to health facility development would be most impactful. Together, these maps highlight existing preparedness gaps and sub-national risk of infectious disease and demonstrate the potential for precision public health resources to inform preparedness activities. With the development of comprehensive, geolocated health facility lists, these maps can be used as one tool among a set of many in-country to ensure preparedness for the next public health threat.

95

BURDEN OF NON-COMMUNICABLE DISEASES IN A KENYAN CASUALTY DEPARTMENT

Gladys Wambua[1], Mugane Mutua[1], **Daniel Rafiki Owambo**[2], Morgan Muchemi[3], Thomas Kedera[4], Kipkoech Rop[5], Benjamin Wachira[6], Christine Ngaruiya[7]

[1]*Kenyatta National Hospital, Nairobi, Kenya,* [2]*Narok County Referral Hospital, Kenya, Narok, Kenya,* [3]*PCEA Chogoria Mission Hospital, Chogoria, Kenya,* [4]*Kakamega County Referral Hospital, Kakamega, Kenya,* [5]*Kilifi County Referral Hospital, Kilifi, Kenya,* [6]*Aga Khan University Hospital, Nairobi, Kenya,* [7]*Yale School of Medicine, New Haven, CT, United States*

Non-communicable diseases (NCDs) account for 41 million deaths annually. Low and Middle-Income countries (LMICs) suffer 75% of this global NCD death burden. In Kenya, these deaths have risen from 35% in 2003 to 45% in 2010. However, limited primary data exists on this burden. Resource-limited health systems unable to capture data describing patients presenting for acute care impede early detection and treatment of NCDs. This study assesses NCDs and associated risk factors in a Kenyan Casualty Department to provide knowledge to inform development of protocols, policies and educational interventions on their management. Data was collected using the WHO STEPwise approach and PHQ-9 questionnaires. We targeted patients at the Kenyatta National Hospital Casualty Department aged 18-69. Sample size was 2400; 10% of the total estimated presentations. Majority (53.6%) of respondents were male, with a mean age of 35y. 11% of respondents smoke tobacco with 8% doing so daily. The mean starting age for smoking was 20y. 36% of participants are exposed to second-hand smoke from their homes and work-places. 51% of participants reported engaging in alcohol use while less than 5% have attempted to stop drinking upon medical recommendation. 21% of respondents are hypertensive but only 11% are on medication. 10% of respondents reported symptoms of angina and 7% reported elevated blood sugar. A high burden of NCDs exists in the Casualty Department, yet treatment is minimal. Overlooking this risks loss of productivity and escalation of healthcare costs. This emphasizes the need for interventions unique to this population.

96

TRASH TO TREASURE: COLLECTING TRASH FOR PROFIT TO REDUCE VECTOR BREEDING SITES IN KWALE COUNTY, KENYA

Gathenji B. Njoroge

University of California Berkeley School of Public Health, Berkeley, CA, United States

Plastic pollution harms the environment, promotes zoonoses, and promotes vector-borne diseases (VBD) like dengue, chikungunya, and Zika which are transmitted by *Aedes aegypti*. Community-based methods to control this vector rely on a continuous stream of external funding and are rarely sustainable. Some of the most productive *Aedes aegypti* habitats worldwide and in Kwale County, Kenya (Kwale) are synthetic containers, such as recyclable plastic containers, tires, and trash. Recognizing individuals may not be incentivized to clean up their environment to improve health if basic needs are not met due to poverty, we aim to assess the potential for community-based recycling that engages aspiring entrepreneurs to repurpose trash for profit in Kwale. Our goals are to improve health by reducing VBD and to alleviate poverty by generating income. We will empower and train aspiring entrepreneurs to repurpose trash for profit in Kwale (9.1% unemployment). We hypothesize profitable businesses which motivate community members to remove trash from the community will reduce vector breeding containers. We plan to measure pre- and post-intervention: vector abundance (ovitraps), and visual presence of trash; and during the process: volumes of trash collected, and profits generated. We are currently engaging local entrepreneurs, policy makers, community leaders and NGO's. We have identified 14 target communities, are 7 engaging community leaders and 2 NGOs to mobilize entrepreneurs, and have mapped the landscape of trash volumes and market opportunities through key-informant interviews and 10 spatial video geonarratives. Interest from Kenyan entrepreneurs exists to reuse plastics and some programs are already collecting and sorting trash and making items such as building materials and art. Creating demand will be a challenge. Our next step is to pilot the entrepreneur incubator program to remove trash from the environment by inviting interested individuals to apply to participate in a social entrepreneurship program to create and execute business plans.

97

HEALTHCARE UTILIZATION IN PATIENTS WITH SUSPECTED ENTERIC FEVER

Alexander T. Yu[1], Rajani Shakya[2], Caryn Bern[3], Bikram Adhikari[2], Dipesh Tamrakar[2], Krista Vaidya[2], Caitlin Barkume[4], Denise Garrett[4], Stephen Luby[1], Isaac Bogoch[5], Jason Andrews[1]

[1]*Stanford University, San Francisco, CA, United States,* [2]*Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Nepal,* [3]*University of California San Francisco, San Francisco, CA, United States,* [4]*Sabin Institute, Washington, DC, United States,* [5]*University of Toronto, Toronto, ON, Canada*

We used a hybrid approach, combining population-based, age-structured health care utilization data with facility-based typhoid surveillance, to produce age-adjusted burden estimates in an urban and peri-urban setting in Nepal. We conducted a randomized cluster-based healthcare utilization survey in the catchment areas of Kathmandu Medical College Hospital (urban Kathmandu) and Dhulikhel Hospital (peri-urban areas of Kavrepalanchok district), where prospective typhoid surveillance has been ongoing since September 2016. Data on healthcare seeking behavior, socioeconomic status and symptom severity were collected for each household member with an episode of fever ≥ 3 days in the previous 8 weeks. From January 2017 to October 2018, we enrolled 24,515

households, representing 81,369 individuals (47,198 in Kathmandu, 34,171 in Kavrepalanchok) with a median age of 27 years (IQR 18-40). Household period prevalence of fever ≥ 3 days was lower in Kathmandu (5.7% vs 15.4%, p<0.01). Children <5 had the highest period prevalence (13.9%) while adults 22-45 had the lowest (2.1%). The period prevalence of fever was lower in the rainy season compared to drier winter months (4.9% vs 6.4%, p<0.01). Higher SES and education was associated with lower fever prevalence. The majority with fever sought medical care (87.7%), most commonly from pharmacies. Only a small proportion presented to the surveillance facility (6.2% in Kathmandu, 12.6% in Kavre). Fever lasted a median 4 days (IQR 3-7) and kept participants from activities for a median 3 days (IQR 1-5). Those taken to a facility had a longer mean duration of fever (5.8 vs 4.7 days, p<0.001) and spent more time in bed on the worst day of illness (13.1 vs 11.7 hrs, p<0.001). In conclusion, more than 85% of individuals with suspected enteric fever sought medical care, most frequently at pharmacies. Although our surveillance facilities are major medical centers, only a small proportion of those with febrile illness presented to them, highlighting the importance of adjusting for healthcare utilization to produce accurate estimates of disease burden.

98

ESTABLISHING AN URBAN HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN A SEMI-INFORMAL SETTLEMENT, INITIAL EXPERIENCES AND FINDINGS OF FIRST TWO YEARS, MANYATTA KISUMU

Thomas Misore, Maurice Ombok, David Obor, Peter Otieno, Stephen Liech, Leonard Oyuga, Janet Agaya

Kenya Medical Research Institute (KEMRI), Kisumu, Kenya

Health and demographic surveillance systems (HDSS) are important sources for health planning and policy implementation in many low and middle income countries. They provide longitudinal data regarding health and vital statistics in countries where vital registration systems perform poorly. Most HDSS sites are set up in rural settings. Through the support of the Child Health and Mortality Prevention Surveillance network, the Kenya Medical Research Institute set up an urban HDSS in Manyatta informal settlement area of Kisumu town, western Kenya. The HDSS was set up primarily to assist in identifying child deaths, provide denominators for cause specific mortality as well as follow up on public health interventions. We present experiences and findings of the first two years of setting up and operation. Manyatta site was mapped and numbered into 121 villages. Consent was obtained from household heads before the enumeration exercise. . Household data was collected by trained Community Interviewers and Community Health Volunteers. Supervisors conducted random, repeat interviews in 5% of households for quality checks. Half yearly cohort follow-ups were conducted to record information on vital events. Flexible working hours allowed for households visits. Data analysis was conducted using Stata 14. Baseline enumeration registered a population of 77,061, distributed in 31,000 households. Females made up 39,221 (50.9%).Sex ratios were 96 males per 100 females. The average household size was 3. There were 11,699 (15%) under five years children. Refusal rate stood at 0.03% and revisits 11% .Observing working time flexibility, security, respect for diverse cultures and religion is key. In conclusion, findings demonstrate feasibility of setting up and operating a HDSS and to collect accurate and reliable data in an informal urban setting such as Manyatta. The urban setting has unique characteristics and challenges that require flexibility and modification of strategies that ordinarily apply in rural settings.

99

INTERRATER RELIABILITY OF AN ADAPTED AND TRANSLATED VERSION OF THE MULLEN SCALES OF EARLY LEARNING (MSEL) IN RURAL GUATEMALA

Alison M. Colbert[1], Molly M. Lamb[1], Desirée Bauer[2], Sara Hernández[2], Maria Alejandra Martínez[2], Paola Arroyave[2], Alejandra Paniagua-Avila[2], Daniel Olson[1], Mirella Calvimontes[2], Guillermo A. Bolaños[2], Hana M. El Sahly[3], Flor M. Muñoz[3], Edwin J. Asturias[1], Amy K. Connery[1]

[1]*University of Colorado School of Medicine, Aurora, CO, United States,* [2]*Center for Human Development, Fundación para la Salud Integral de los Guatemaltecos, Retalhuleu, Guatemala,* [3]*Baylor College of Medicine, Houston, TX, United States*

Prenatal Zika virus (ZIKV) and chikungunya virus (CHIKV) infections have been associated with long-term neurodevelopmental sequelae, though the impact of early post-natal infection on neurodevelopment in children in low- and middle-income countries (LMICs) is less clear. This paucity of knowledge is exacerbated by the lack of standardized, performance-based neurodevelopmental assessment tools for LMICs. We evaluated the reliability of an adapted and translated version of the Mullen Scales of Early Learning (MSEL) in a rural, resource-limited region of Guatemala highly endemic for arboviruses. MSEL administration interrater reliability was calculated from an audit conducted during a prospective cohort study of the effects of postnatal ZIKV infection. Guatemalan psychologists were trained by Spanish-speaking US neuropsychologists to administer the MSEL. Twenty consecutive MSEL administrations by trained Guatemalan psychologists were independently live double-scored by US neuropsychologists (*mean*child age = 21 months; *range*= 6-62 months). Interrater reliability for each MSEL subscale (gross and fine motor, visual-reception, receptive and expressive language) and composite score were excellent (*ICC* = 0.99, *p*< .001). Additionally, 32 MSEL administrations (*mean*child age = 20 months; *range*= 6-63 months) by trained Guatemalan psychologists were independently live double-scored by another trained, Guatemalan psychologist. Interrater reliability was excellent for each MSEL subscale (*ICC* = 0.99, *p*< .001) and composite score (*ICC* = 0.98, *p*< .001). These results suggest our training model was effective, administration instructions were clear, and child performance was similarly interpreted by examiners. This, along with previous work supporting the construct validity of this version of the MSEL, provides support for MSEL use in this resource-limited population in Guatemala. Our findings indicate that rigorous neurodevelopment assessment tools with strong psychometric properties can be applied in LMICs when carefully translated, adapted and applied.

100

ENGAGING YOUNG PEOPLE AS AGENTS OF CHANGE: A SCHOOL-BASED INTERVENTION TO REDUCE ARBOVIRUS TRANSMISSION – ONE YEAR FOLLOW-UP INTERVIEWS

Arielle M. Kempinsky[1], Jenna E. Forsyth[2], Emilia J. Ling[1], Catharina J. Alberts[3], Francis Mutuku[4], Lydiah Kibe[5], A. Desiree LaBeaud[6]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*Emmett Interdisciplinary Program on Environment and Resources, Stanford University, Stanford, CA, United States,* [3]*International Agency for Research on Cancer, World Health Organization, Geneva, Switzerland,* [4]*Technical University of Mombasa, Mombasa, Kenya,* [5]*KEMRI-Wellcome Trust Programme, Kilifi, Kenya,* [6]*Lucille Packard Children's Hospital at Stanford University School of Medicine, Stanford, CA, United States*

Creating sustainable behavior change is a necessary step in reducing the burden of *Aedes aegypti* mosquito-borne diseases in rural Kenya. Without the ability to cure these diseases or to implement insecticide-based control, we must focus on source reduction. Intentions and perceptions directly impact the degree to which households adopt source reduction. This study aims to better understand the impetus for or against behavior change following a school-based educational intervention. The goal of the curriculum was to provide targeted behavioral recommendations based

on container types, including covering any with standing water, removing old containers, and turning over unused containers. From a sample size of 500, 17 adopter households (those who decreased the number of improperly managed containers) and 17 non-adopters were randomly selected for inclusion. Field research assistants conducted structured interviews with mothers of children in the intervention to synthesize why households did or did not change their behavior, along with the effect of these choices. Using images to rank their mosquito related priorities, respondents showed that container behaviors ranked second of five after bed nets. We found nine adopters ranked container management in their top two, as compared with five non-adopters. Respondents prioritized covering cooking and drinking water, but seemed less inclined to cover laundry water. The interviews also revealed barriers in the non-adopters. The most common extrinsic obstacle that mothers faced was the behavior of other family members, while the most common intrinsic barrier was lack of time. Questions about project efficacy in both groups showed us the most common knowledge increase surrounded maintaining a trash-free, clean compound. Since children used old containers and tires as toys, respondents in both categories reported difficulty discarding these items, even when water tended to pool inside of them. Long-term behavior change may require empowering local community members to create structural changes from within.

101

UNDERSTANDING THE CONTEXT OF DEATH OF UNDER-FIVE CHILDREN IN RURAL GHANA USING VERBAL POST MORTEM NARRATIVES

Samuel Afari-Asiedu, Charles Zandoh, Edward Anane Apraku, Mahama Abukari, Felix Boakye Oppong, Wisdom Adeapena, Samuel Harrison, Kwaku Poku Asante

Kintampo Health Research Centre/Ghana Health Service, Kintampo, Ghana

Globally, under-five deaths have decreased by 58% but still high in Africa. Compared to global estimate of 39 deaths, under-five deaths in Ghana was 49 per 1000 live births in 2017. Verbal Post Mortem (VPM) is vital for understanding the context of death at the community level. This paper reports on the context and health seeking behavior for under-five deaths in rural Ghana. Fifty (50) VPM narratives, 5 from each year (2006 to 2015) were randomly selected. VPM transcripts were imported into Nvivo 12 for coding and analysis. The multidimensional approach to health was used as a framework to develop themes of interest and their relationships using a mind map. Data were presented as narratives with quotes to substantiate the findings. Narratives of under-five deaths were based on symptoms experienced prior to death including fever, diarrhea, anal sore, convulsion, swollen stomach and feet and anaemia. Some carers treated their children with orthodox medicine and others used both orthodox and traditional/herbal medicine. Carers who used orthodox medicine initiated treatment by obtaining medicine from over-the-counter sellers. Children were sent to the community-based health planning and services compound/heath centers in their communities and subsequently to the district hospital when the illness did not resolve. There was no clear sequence of health seeking for carers who used both orthodox and traditional/herbal medicine. It was observed that some carers initiated treatment with traditional/herbal medicines at home and continued with orthodox medicine when the illness did not resolve. Others started with orthodox and later with traditional/herbal medicine. It emerged that some carers believe certain illnesses including convulsion and body swellings are not "hospital illnesses" and requires traditional/herbal medicine. There was no clear trajectory for the management of under-five illness. Cultural beliefs influences health seeking behaviour for under-five illnesses. Continues public health education is needed to encourage early care seeking at health facilities and to discourage self-medication to reduce under-five deaths.

102

QUALITY OF CARE FOR FEBRILE CHILDREN: AN ANALYSIS OF NATIONAL HEALTH FACILITY SURVEYS FROM MALAWI AND TANZANIA

Cameron Taylor, Jehan Ahmed, Wenjuan Wang

ICF, Rockville, MD, United States

The WHO Global Malaria Program's T3: Test. Treat. Track initiative recommends that every suspected malaria case is tested, every confirmed case is treated, and the disease is tracked by surveillance systems. To investigate the quality of care for febrile children and adherence to the "test" component of the T3 initiative, we examined data from the observation of sick child consultations and the exit interview of caretakers from the 2013-14 Malawi Service Provision Assessment (SPA) and the 2014-15 Tanzania SPA which are nationally representative health facility surveys. We identified essential elements of febrile clinical care for sick children recommended by the WHO that are also available in the SPA surveys. These included 1) the provider asking about fever, 2) child was felt for temperature, had their temperature taken with a thermometer, or checked for pallor by looking at palms, and 3) provider instructed child to see another provider or laboratory for a finger or heel stick for blood testing. As a proxy for severe malaria, children were excluded from the analysis if they presented in hospitals or were referred/admitted at end of the consultation. Among children who had a fever in the past two days according to the caretaker, 25% (N=2,698) in Tanzania and 43% (N=1,368) in Malawi received all three essential elements of febrile clinical care. We assessed facility, provider, and patient factors that might explain variations in febrile patient quality of care, using random-effects logit regressions. In both Malawi and Tanzania the results showed that facility readiness for malaria services including availability of mRDTs and microscopy supplies, were significantly associated with children receiving all three essential elements of febrile clinical care ($p<0.10$). Provider and patient factors were not significant except child's age. In both countries, these findings show the importance of malaria service readiness for providing high quality of care for febrile children. Having facilities with diagnostic capabilities will help ensure that providers adhere to the WHO T3 initiative.

103

SMS FOR LIFE, A DIGITAL SOLUTION TO IMPROVE THE AVAILABILITY OF ESSENTIAL MEDICINES IN NORTHERN ZAMBIA

Marcel Braun, Nadine Schecker, Viviam Patricia Canon

Novartis Social Business, Basel, Switzerland

By 2017, the population of Zambia was 17.4 m, life expectancy at birth was 66.3 y for females and 60.4 y for males. Despite the advances in diagnostic and treatments and the 63% reduction in mortality compared to 2007, HIV/AIDS is still the first cause of death. Tuberculosis and malaria mortality increased compared to 2007 and represented the 4th and 7th cause of death respectively. Timely and sustained availability of diagnostic tools and first-line treatment for those diseases is crucial. Interruption of HIV or TB treatments increases the risk of failure and selection of resistance. Lack of availability of rapid diagnostic test (RDT) and/or malaria treatment increase the risk of adverse outcomes mostly in young children. SMS for Life in Zambia is a Novartis initiative started in 2018 as a public-private partnership with the MoH, Right to Care/EQUIP and Vodacom. Objectives: 1) demonstrate that visibility of weekly stock levels of essential commodities will promote actions to reduce or eliminate stock-outs, 2) Demonstrate that simple customized digital solutions may be successfully implemented in remote areas and 3) demonstrate the benefit of public-private partnerships in public health. The items surveilled included RDT (13), malaria treatments (4), HIV medicines (4 ARV, 1 prophylaxis), and TB (1 first-line treatment, 1 prophylaxis). One hundred eighty-six (186) health care facilities across three provinces of Zambia (Luapula, Muchinga and Northern) were included, covering approx. 3.2 million people. SMS for life works with SIM card equipped tablets, smartphones, feature phones

and a widely available telecommunications technology. Health care workers are trained and enabled to track stock levels of essential tests/medicines and send notifications to district officers every week. A report on the availability of commodities is generated as needed. A public-private partnership allows SMS for Life to provide visibility of stock levels of essential medicines using simple technology. The system has the potential to facilitate the provision of stock and alleviate the restricted availability of drugs in rural or under-resourced areas.

104

SOCIAL MEDIA USE IN EMERGENCY RESPONSE TO NATURAL DISASTERS: A SYSTEMATIC REVIEW WITH A PUBLIC HEALTH PERSPECTIVE

Kamalich Muniz-Rodriguez[1], Sylvia K. Ofori[1], Kadiatou Diallo[1], Manyun Liu[1], Jessica S. Schwind[1], Gerardo Chowell[2], Isaac Chun-Hai Fung[1]

[1]*Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA, United States,* [2]*School of Public Health, Georgia State University, Atlanta, GA, United States*

Natural disasters affect the lives of thousands of people every year. The damages caused by these events call for a multiagency response, where effective communication and coordination are essential. Previous research has explored the usefulness of social media analysis during a natural disaster as a tool to guide response efforts with a focus on geography and computational methods. The purpose of our research is to study the implications of social media use during natural disaster specifically focusing on the public health implications in the response phase of the emergency. We conducted a systematic literature review on natural disasters and social media use during the emergency response phase. Three databases, PubMed, IEEE Xplore, and Web of Science were searched in September 2018 using three different keyword combinations. Articles that complied with our inclusion criteria were independently reviewed, and their findings were analyzed to answer our research questions. A total of 47 full-text articles were included in our study. Analyses of social media data were performed at a wide range of spatial and time scales ranging from countries, states, or cities, and measured from minutes, hours, and days. Twitter was the most analyzed platform, followed by Facebook. Social media was used as a tool to identify areas in need of relief operations by using self-reported location. Researchers identified individuals in need of medical assistance, areas without water supply and electricity. One of the most common uses of social media data is map development for location, needs, direction of relief operations, and disaster intensity. Twitter was mostly identified as a broadcasting tool, and platforms like Facebook were recognized as a two-way communication tool. The identification of social media platforms as a broadcasting tool presents an opportunity for public health agencies to address rumor control during a natural disaster. In retrospective analyses, social media analysis shows promise as an opportunity to reduce the time of response and to identify the individuals' location. However, further research is needed to evaluate the use in real-time.

105

THE VALUE-ADD OF PARTICIPATORY RESEARCH TECHNIQUES TO INFORM ZIKA PREVENTION PROGRAMS: A QUALITATIVE STUDY IN THE DOMINICAN REPUBLIC

Tilly Gurman, Anne Ballard, Gabrielle Hunter

Johns Hopkins University, Baltimore, MD, United States

A challenge in Zika prevention efforts is the need to promote multiple behaviors (e.g. using repellent/condoms during pregnancy, cleaning water storage containers, removing standing water). While epidemiological data and quantitative surveys offer important information for Zika-related programs aiming to foster behavior change, data elicited from novel qualitative participatory techniques (e.g. free listing, pile sorting) can inform even more comprehensive programs. The current qualitative study (n=88) used three participatory techniques in focus group discussions (FGDs) and in-depth interviews (IDIs) to explore Zika-related perceptions among pregnant and non-pregnant women (ages 18-30) and male partners of pregnant women in the Dominican Republic. First, using free listing, individuals met with an interviewer and listed, top-of-mind, the behaviors that people in their community perform to prevent Zika. Second, FGDs employed pile sorting to categorize and prioritize Zika-related behaviors according to perceived effectiveness/feasibility. Finally, participants simulating the cleaning of water storage containers via drawings and physical demonstrations spurred group discussion about common cleaning practices. Data analysis also included a participatory process with local stakeholders that yielded contextually relevant themes. Free listing findings indicated that no single behavior was mentioned by a majority of respondents. From pile sorting activities, multiple insights surfaced. Participants associated health/well-being with cleanliness—the concern for Zika only existing during outbreaks or when given attention by media/government. Participants further noted that responsibility for vector-control behaviors fell to community groups/local authorities. The cleaning simulation activities found that people valued the use of bleach, although the amount and technique varied. The current study contributes insights about perceptions of Zika-related behaviors and highlights the value-add of using participatory research techniques to inform behavior change programs for Zika or other arboviruses.

106

DETERMINANTS OF VACCINE COVERAGE IN A COHORT OF CHILDREN IN OSHIKHANDASS, A NORTHERN PAKISTANI VILLAGE

Alexandra Jamison[1], Elizabeth Thomas[1], Ejaz Hussain[1], Iqbal Azam[2], Wasiat Shah[1], Benjamin McCormick[1], Zeba Rasmussen[1]

[1]*Fogarty International Center, National Institutes of Health, Bethesda, MD, United States,* [2]*Aga Khan University, Karachi, Pakistan*

Pakistan initiated its Expanded Programme on Immunization (EPI) in 1978, but the incidence of vaccine preventable disease remains among the highest in the world. We describe vaccine completeness, timeliness and determinants of coverage from a cohort study (2012-2014) in a rural northern Pakistani village. Health workers compiled vaccination history using EPI vaccine cards along with maternal report and Lady Health Worker registries. Vaccination was complete if all doses were received according to the EPI schedule and timely if doses were not ≤3 days early or > 28 days late. Univariate chi-squared tests and a multivariable logistic regression were used to examine factors associated with completeness; variables included parental education and age, household income and assets, distance from the vaccine dispensary and the total number of children in the family. Of 1104 children followed to 12 months, 73% (802) had full vaccination histories: of these, 514 (64.1%) were fully vaccinated, 245 (30.5%) partially vaccinated and 43 (5.4%) unvaccinated. Coverage was highest for the BCG vaccine (94.1%) and lowest for measles (74.7%). Timeliness declined with subsequent doses, for example, 80.8% received their first dose of OPV on time, but only 42.6% received a fourth dose on schedule. Overall, only 18.5% of those fully vaccinated received all doses on schedule. The child's birth order, total number of children in the family, parental education and the year of family's migration to the village were all significant in univariate analysis of coverage. Adjusting for other variables, only the total number of children in the family remained statistically significant (OR=1.45, 95%CI=1.12, 1.88). Our results highlight three key points: (i) coverage decreases for vaccines with multiple or later doses. (ii) Drivers of vaccine coverage are multifactorial, but after adjusting for child and family factors, the more children in the family the more likely that a child is fully vaccinated. (iii) Despite implementing the EPI with greater coverage than the national average, disappointingly few vaccines were timely, potentially undermining their effectiveness.

107

COLLABORATIVE IMPROVEMENT APPLIED TO MALARIA SURVEILLANCE DATA QUALITY IN UGANDA: A COMPARISON OF OUTCOMES REPORTED BY INTERNAL QUALITY IMPROVEMENT TEAMS VERSUS AN INDEPENDENT EVALUATION IN UGANDA

Nelli Westercamp[1], Sarah Staedke[2], Catherine Maiteki-Sebuguzi[3], Simon Kigozi[3], John Michael Okiring[3], Alexander K. Rowe[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Infectious Disease Research Collaboration, Kampala, Uganda*

Transforming malaria surveillance into a core intervention is a pillar of the Global Technical Strategy for Malaria but ensuring high quality of routinely collected data remains a challenge. Collaborative improvement (CI) is an innovative quality improvement method that encourages shared learning and continuous assessment of performance indicators by health facility (HF) based teams. Evidence suggests CI is promising; however CI effectiveness has been evaluated primarily through data self-reported by internal CI teams, which may be subject to bias. In 2015-16, we evaluated whether CI could improve the quality and accuracy of malaria surveillance data in 5 HFs in Kayunga, Uganda. Data accuracy was measured by comparing the number of individual malaria cases recorded monthly in the outpatient register to aggregated numbers reported in monthly summary reports. The relative difference in these numbers (the accuracy indicator) was determined by internal CI teams and an external team of independent evaluators. Comparisons were made with Pearson's correlation and mean differences. Overall, Pearson's correlation was 0.43 for the accuracy indicator across all 5 sites, ranging from -0.06 to 0.99 for each individual HF. Mean differences in the self-reported accuracy by CI teams compared to the evaluation data were -2 %-points for all sites, ranging from -15 %-points to -3 %-points for higher level (larger, complex) HFs and 4-6 %-points for lower level HFs. Negative values here indicate that CI teams found greater reductions in discrepancies between the data sources, and thus a greater improvement in accuracy of data, compared to evaluation results. The correlation between the CI team and evaluation data was moderate, with considerable variations across different HFs. Our results suggest that in higher level HFs, internal CI teams may have overestimated the impact of CI on data accuracy. Improving quality of malaria surveillance data is a high priority. Additional research of the effectiveness of CI on improving data quality is needed. Future studies of CI should include an independent evaluation to ensure the impact evaluation is valid and unbiased.

108

THE INFLUENCE OF TRAINING ATTRIBUTES ON THE EFFECTIVENESS OF TRAINING TO IMPROVE HEALTH WORKER PRACTICES IN LOW- AND MIDDLE-INCOME COUNTRIES: A SYSTEMATIC REVIEW

Alexander Rowe[1], Samantha Rowe[1], David Peters[2], Kathleen Holloway[3], Dennis Ross-Degnan[4]

[1]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Institute of Development Studies, University of Sussex, Brighton, United Kingdom,* [4]*Harvard Medical School, Boston, MA, United States*

Health worker (HW) performance in low- and middle-income countries (LMICs) is often inadequate, and in-service training is a common strategy for improving performance. To explore how training attributes influence training effectiveness (and thus identify ways to increase training impact), we identified training studies with HW practice outcomes expressed as a percentage (e.g., % of patients treated correctly) from a systematic review on improving HW performance in LMICs. Effect sizes were calculated as adjusted risk differences. To estimate the effect of training attributes, we examined head-to-head studies that directly compared different training approaches, and we used random-effects linear regression modeling on studies of training with different approaches versus a control group. We searched 52 databases for published studies and 58 document inventories for unpublished studies up to 2016. We screened 216,477 citations and identified 179 eligible studies for this analysis. Most studies (105/179 or 58.7%) had a high risk of bias. Training tended to be more effective when it was at least partly conducted where HWs routinely work (by 9.4–10.9 %-points), tailored to a HW's stage of readiness to change (23.3 %-points; only 1 study), taught a protocol (versus allowing HWs to use their discretion, by 8.4 %-points; only 1 study), or used clinical practice (6.7–7.5 %-points). The mean effect of training without supervision tended to decrease over time after training (0.8–1.0 %-point decrease per month), but this decay was not seen when training was combined with supervision. Attributes such as training duration, training with computers or interactive lectures, training via live video interactive sessions, and pedagogical background of trainers had little influence (effects of 4.3 %-points or less). The effect of training group size and having trainers with content expertise was unclear because of conflicting results from different analyses. In conclusion, although many knowledge gaps remain, there is promising evidence that several approaches might increase the effectiveness of training courses to improve HW practices in LMICs.

109

EXPANDED FEVER SURVEILLANCE AMONG PROVINCIAL HOSPITALS IN THE LAO PEOPLE'S DEMOCRATIC REPUBLIC

Jose A. Garcia[1], Vilada Chansamouth[2], Matthew Robinson[2], Paul Newton[2]

[1]*US Navy, Phnom Penh, Cambodia,* [2]*Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic*

With declining *Plasmodium falciparum* malaria in the Lao PDR (Laos), there is an urgent need to determine the causes of fever in order to guide optimal empirical therapy and to determine key diseases for enhanced surveillance. Presently, there remain gaps in information from Southeast Asia to provide a comprehensive assessment. An ongoing two-year study is currently being conducted in three hospitals in the Luang Nam Tha, Xieng Khuang and Salavan provinces located in northern, central and southern Laos, respectively, to obtain evidence on the causes of fever and address this information gap. From August 2017 until February 2019, a total of 2,899 subjects who met the minimum case definition of body temperature ≥37.5°C were enrolled. The majority of the enrolled subjects were adults (>15 years), with 1,740 (60%) being within the median age of 23 (4-50) while 1,508 (52%) were male. Preliminary data shows that of the 2,899 subjects, 125 (4.3%) were confirmed to have dengue fever, followed by bacteremia (84, 2.9%) and Japanese encephalitis virus infection (9, 0.3%). This study to date has demonstrated important differences in the aetiology of fever among the northern, central and southern regions of Laos with there being 48% and 71% more febrile cases detected in the southern region (121/218, 55.5%) when compared against the northern (63/218, 28.8%) and central regions (35/218, 16%) respectively, with dengue fever being the most prevalent. Thus paving the way to initiate more in-depth resistance profiling surveys to address if differences exist in the presence of antimicrobial resistance among both viral and bacterial pathogens within these areas.

110

PILOTING A NON-VERBAL COGNITIVE ASSESSMENT BATTERY, THE LEITER INTERNATIONAL PERFORMANCE SCALE, THIRD EDITION (LEITER-3), IN HEALTHY UGANDAN CHILDREN AND ADULTS

Erika S. Phelps Nishiguchi[1], Shubaya K. Naggayi[2], Mary Nyakato[2], Jacqueline Nakitende[3], Megan S. McHenry[4], Robert O. Opoka[3], Paul Bangirana[3], Chandy C. John[4]

[1]*University of Washington, Seattle, WA, United States,* [2]*Global Health Uganda, Kampala, Uganda,* [3]*Makerere University, Kampala, Uganda,* [4]*Indiana University, Indianapolis, IN, United States*

As global pediatric health priorities shift from surviving to thriving, there is need for globally relevant language-independent developmental assessment tools. The Leiter International Performance Scale, 3rd Edition (Leiter-3) evaluates nonverbal cognitive abilities through ten sub-tests explained using pantomimed instructions. It is promoted as a culturally fair, language-neutral assessment. To assess feasibility, validity, and acceptability of the Leiter-3 in the Ugandan population, a pilot study will be conducted in two phases: 1) training and 2) psychometric evaluation and acceptability. Three experienced Ugandan psychology research assistants (RAs) were trained to administer the Leiter-3. The training, clarification, and adaptation process took place over 2 months. This included roleplaying and field testing with volunteer children and adults. The RAs worked with a trainer and a representative from the test's publisher to clarify instructions, develop responses to participants' behaviors, and adapt pantomimes for poorly understood sub-tests. Qualitative feedback was sought from RAs and volunteers. Administration consistently required 90-120 minutes, longer than the published 20-45 minute duration. The RAs reported initial discomfort with the manual's instructions, with administering the test in silence, and with managing challenging behaviors. The next phase is to pilot the Leiter-3 in 40 healthy Ugandan children and young adults. The pilot will include administration of previously validated verbal tools, the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) and Ugandan Neuropsychology Battery (UNPB), to enable psychometric evaluation of the Leiter-3. Face validity and acceptability will be assessed through semi-structured interviews of pilot participants. Inter-rater reliability will be calculated, and RAs will be interviewed at the beginning and end of the pilot period to assess acceptability of test administration. We expect these efforts to provide a comprehensive understanding of the strengths and limitations of using the Leiter-3 in the Ugandan setting.

111

LESSONS LEARNED IN HEALTH INFORMATION SYSTEM STRENGTHENING: WHAT WORKED FOR THE DEMOCRATIC REPUBLIC OF THE CONGO

Lavanya Gupta[1], Scott McKeown[1], Johanna Karemere[2], Olivier Kakesa[2], Ramine Bahrambegi[3]

[1]*MEASURE Evaluation, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*MEASURE Evaluation, ICF, Kinshasa, Democratic Republic of the Congo,* [3]*MEASURE Evaluation, ICF, Rockville, MD, United States*

Since 2014, MEASURE Evaluation has helped the National Malaria Control Program (NMCP) of the Democratic Republic of the Congo (DRC) to streamline and improve routine malaria data collection, reporting, management, and use at all levels of the health system to facilitate intervention targets. We have supported the rollout of the electronic health information platform—DHIS 2—in all 178 health zones and 77 health facilities in 9 PMI-targeted provinces; the integration of malaria indicators in DHIS 2; the training of more than 400 staff in monitoring and evaluation, data collection, analysis, and use; the development of health information system (HIS) management resources; and the implementation of data review and data quality check mechanisms. We sought to document the outcomes of our work with the NMCP and identify effective HIS strengthening interventions for health data including malaria, by conducting in-depth interviews with staff members at the national, provincial, and health facility levels. Interviews included questions about engagement with MEASURE Evaluation, any trainings attended, and perceived facilitators of and barriers to a strong HIS. From the audio-recorded interviews we developed in-depth notes and analyzed them with thematic coding. The data analysis yielded three primary themes: access to DHIS 2 supported capacity building in data collection and reporting; the capacity to oversee and participate in data quality assurance practices facilitated more timely and complete routine data; and the establishment of data review mechanisms at all levels of the health system provided opportunities for stakeholders to make key data-informed decisions. Each of these contributions has improved data quality and data use, thus improving overall HIS performance in the DRC. Although respondents noted that some challenges remain, these observations demonstrate the interventions' effectiveness. Our approaches represent best practices in HIS strengthening that could be adapted and implemented in other disease and country contexts.

112

DESCRIPTIVE ANALYSIS OF VACCINE-PREVENTABLE DISEASES IN THE DOMINICAN REPUBLIC: IS PUBLIC MEDIA COUNTERACTING THE PUBLIC HEALTH EFFORTS?

Priscilla M. Abate[1], Jose A. Duran[2], Paola Peña[3], **Leandro Tapia**[2], Robert Paulino-Ramirez[2]

[1]*Centro Medico Otorrino, Santo Domingo, Dominican Republic,* [2]*Institute for Tropical Medicine and Global Health - Universidad Iberoamericana, Santo Domingo, Dominican Republic,* [3]*Centro de Gastroenterologia Avanzada, Santo Domingo, Dominican Republic*

The Anti-vaccination movement has dominated media; meanwhile public health policies are being conducted to counteract declining vaccination rates. Despite the efforts made, in recent years there has been an increase in vaccine preventable diseases, especially in tropical and sub-tropical regions. These diseases have caused outbreaks in the Caribbean and low-income countries. The aim of this study is to characterize vaccine preventable diseases in the Dominican Republic from 2012-2018. Data of cases of Vaccine Preventable Diseases included in the Governmental Immunization Program and their demographic characteristics were extracted from the Ministry of Health Weekly Reports (digepi.com.do) in order to establish prevalence and describe demographic patterns. A total of 777 cases were analyzed, of which *B. pertussis*, represented a total of 411 cases, 48% females, and a, mean age of 128 days (3 days - 16 years); *C. tetani*, a total of 329 cases, 88% males with a, mean age of 41 yo. On the neurological related preventive illnesses, those caused by *S. pneumoniae*, a total of 27 cases, 59% males and 41% females, with a mean age of 8 years (73 days - 76 years). Mortality rates related to those illnesses accounted for 41% related to meningitis, 27% for tetanus, and 5% related to pertussis. In animal-transmitted preventable vaccination Rabies accounted for a total of 10 cases, in the majority affecting males (70%), and a mortality rate of a 100% in mid-age individuals (mean age 12 yo). Vaccine preventable diseases seem to have a major impact in public health, especially *B. pertussis*. Mortality by vaccine preventable diseases constitutes an important part of reported cases. Counteracting measures to increase intake of vaccines and reinforcement of vaccine coverage are still needed, many questions remains from the local perspective, are this lower coverage induced by lack of education, or the negative impact of media, case-control and infectious disease modeling are needed to estimate the real impact of communicable diseases prevented by current interventions in the DR.

113

CHARACTERIZATION OF SIERRA LEONEAN VILLAGES USING SATELLITE IMAGERY AND OBJECT IMAGE RECOGNITION TECHNOLOGIES

Jeffrey G. Shaffer[1], Katherine L. McKeon[2], Christian J. Geneus[2], Seydou O. Doumbia[3], Frances J. Mather[2]

[1]*Tulane University, Harahan, LA, United States,* [2]*Tulane University, New Orleans, LA, United States,* [3]*University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali*

Field research in Africa often necessitates the characterization of community-level attributes such as populations, household sizes, population densities, and proximity to water bodies. Field-based characterization approaches such as door-to-door censuses and data capture with global positions system devices is commonly impractical due to resource limitations. This work focuses on remote characterization techniques through feature extraction from satellite imagery through the joint use of the ENVI (Broomfield, CO) image analysis application and the ArcGIS geographic information system (Redlands, CA). Prior efforts in Africa utilizing satellite imagery in this sense have focused on (through alternative approaches) capturing census data and developing sampling frames in Ethiopia and Malawi, respectively. Here we detail an approach for characterizing Sierra Leonean villages according to population size, household size, and household distances to roads and water bodies. Supervised classification methods were applied using the ENVI Feature Extraction Module classifying features according to village housetops, roads, and water bodies. Vector-based shapefiles were generated these features and were summarized using the ArcGIS application. Summary statistics were generated for the number of households in each village, and village populations were determined by calculating the number of housetops and multiplying by a factor proportional to housetop sizes. Average distances between households to streams or roads were determined using spatial joins. Village population densities were estimated using the ArcGIS Nearest Neighbor Tool. Summary population statistics were generated for an entire Sierra Leonean chiefdom and compared with the Sierra Leone 2015 Population and Housing Census. Village characterization using the ENVI/ArcGIS approach described here has great utility for carrying out research in remote areas and may provide a viable alternative or complementary approach to more costly field-based methods.

114

SOCIAL DETERMINANTS OF HEALTH ASSOCIATED WITH LEPTOSPIROSIS IN A RURAL POPULATION IN CÓRDOBA-COLOMBIA

Virginia C. Rodríguez, Ana M. Castro, Alfonso Calderón, Maria F. Yasnot, Isabel Arcila, Luis F. Urango

Universidad de Córdoba, Monteria, Colombia

Leptospirosis is a zoonotic bacterial disease of worldwide importance, caused by pathogenic species of the genus *Leptospira*. Despite the great negative impact that it have on public health, little is known about the environmental and social conditions that are important for its development in Alto Sinú in Córdoba (Colombia). A cross-sectional descriptive study was carried out from May to October 2018. By a randomized sampling 324 individuals from the localities of Crucito and Frasquillo of the municipality of Tierralta-Córdoba were included in the study. The informed consent and the epidemiological record with articulated questions about the structural and intermediate social determinants that could be related to the disease were completed by the participants. A microagglutination test (MAT) was performed on blood samples, and the seroprevalence was determined. A univariate analysis of the information, associated tests and statistical significance were performed. The majority of the population was male adults residing in scattered, rural areas of low socioeconomic status without fixed income and under the legal monthly minimum wages. The largerst part of the population (95.5%) have not waste disposal or solid waste management services and only 9.6% had an aqueduct service. The existence of the disease was 58.3% and 27.47% had titers greater than 1:400. A risk association was determined for the presence of leptospirosis in the waters of the dam in which the population engage in recreational activities, bathe or fish. The environment where the population developed has characteristics that act as coadjutants in the development of leptospirosis. For example, inadequate basic sanitation conditions, close coexistence with the animals, low level of schooling, difficult access to the services of health and consumption of untreated water are all factors that can increase the risk of contracting leptospirosis. The contact with water from the dam is a risk factor that can actively participate in the dissemination or development of contagious leptospirosis in the analyzed population.

115

GENETIC DIVERSITY OF *BARTONELLA* SPP. IN VAMPIRE BATS IN BRAZIL

Marcos Rogério André[1], Ricardo Gutierrez[2], Priscila Ikeda[1], Renan Amaral[1], Keyla Sousa[2], Yaarit Nachum-Biala[2], Luciana Lima[3], Marta Teixeira[3], Rosangela Machado[1], Shimon Harrus[2]

[1]*Universidade Estadual Paulista (UNESP), Jaboticabal, Brazil,* [2]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel,* [3]*Universidade de São Paulo (USP), São Paulo, Brazil*

Recently, an increasing number of *Bartonella* species have emerged to cause human diseases. Among animal reservoirs for *Bartonella* spp., bats stand out due to their high mobility, wide distribution, social behavior, and long-life span. Although studies on the role of vampire bats in the epidemiology of rabies have been extensively investigated in Latin America, information on the circulation and genetic diversity of *Bartonella* species in these bat species is scarce. In the present work, 208 vampire bats, namely *Desmodus rotundus* (the common vampire bat; n=167), *Diphylla ecaudata* (the hairy-legged vampire bat; n=32) and *Diaemus youngii* (the white-winged vampire bat; n=9) from 15 different states in Brazil were sampled. DNA was extracted from liver tissue samples and submitted to real-time PCR and conventional PCR assays for *Bartonella* spp. targeting five genetic *loci*, followed by phylogenetic and genotype network analyses. Fifty-one out of 208 liver samples (24.51%) were positive for *Bartonella* DNA in the ITS real-time PCR assay: 40 (78.43%) of them were from *D. rotundus* from 11 states, and 11 (21.57%) samples from *D. ecaudata* from three states. Eleven genotypes were found among the obtained *gltA* and *rpoB* sequences. Several ITS sequences detected in the present study clustered within the lineage that includes *B. bacilliformis* and *B. ancachensis*. The Bayesian phylogenetic inference based on the *gltA* gene positioned the obtained sequences in six different clades, closely related to *Bartonella* genotypes previously detected in *D. rotundus* and associated ectoparasites sampled in Latin America. On the other hand, the *Bartonella rpoB* genotypes clustered together with the ruminant species, *B. schoenbuchensis* and *B. chomelii*. The present study describes for the first time the molecular detection of *Bartonella* spp. in *D. ecaudata* bats. It also indicates that *Bartonella* spp. of vampire bats are genetically diverse and geographically widespread in Brazil.

116

INVESTIGATION OF THE EMERGENCE OF TYPHUS GROUP *RICKETTSIA* IN CENTRAL TEXAS

Leigh E. Preston[1], Rebecca Fischer[1], Kristy Murray[2], Sarah Hamer[3], John Midturi[4], Rodion Gorchakov[2], Bonnie Gulas-Wroblewski[5], Jennifer Horney[1]

[1]*Texas A&M University School of Public Health, College Station, TX, United States,* [2]*Baylor College of Medicine, Houston, TX, United States,* [3]*Texas A&M University College of Veterinary Medicine, College Station, TX, United States,* [4]*Baylor Scott & White Health, Temple, TX, United States,* [5]*Texas A&M University Department of Wildlife and Fisheries Sciences, College Station, TX, United States*

Typhus group rickettsiosis (TGR) is a vector-borne disease caused by *Rickettsia typhi* or *Rickettsia prowazekii*, transmitted via flea and thought

to circulate among small mammals. TGR may present as non-specific and resemble other febrile illnesses. Little is known about TGR in Texas, but evidence of its recent emergence and spread highlights knowledge gaps about its clinical and epidemiologic features. This study sought to describe TGR clinical disease and risk factors, as well as identify animal hosts in central Texas. Medical record abstractions were done on patients referred for TGR testing at Baylor Scott & White (BSW) hospitals and clinics from Jan 2012 to Aug 2018. Odds ratios (OR; 95% CI) are reported for factors associated with laboratory confirmed TGR. PCR for *R. typhi* was performed on banked animal specimens collected in central Texas from Apr 2012 to May 2018. Of 384 patients referred for testing, 16 (4%) had TGR, 69 (18%) had Rocky Mountain spotted fever (RMSF), and 299 (78%) had neither. The number of TGR cases increased from 0 in 2012 to 5 in 2018 ($p<0.001$). Most TGR patients were febrile (87.5%) and suffered acute liver injury (68.8%). Compared to RMSF patients, TGR patients were older (OR: 1.06, per year; 1.01-1.10) and more likely to have liver signs (OR: 8.60; 2.06-35.96) and systemic inflammation (OR: 6.58; 1.21-35.89). RMSF patients more often had heart disease (OR: 0.13; 0.02-0.77). TGR patients lived on a farm, ranch, or wooded area (OR: 31.05; 4.01-240.17) more often than patients without rickettsiosis. *R. typhi* was not detected in animals (238 skunks, 196 canines, 7 opossums, 6 rodents, 2 raccoons). This study confirms TGR as an emerging zoonosis in central Texas, making clinician education on rickettsial diseases a critical next step. While no specific animal was found to harbor *R. typhi*, it remains vital to explore what species are involved in maintenance of transmission cycles in the region. This study also revealed important discrepancies in diagnostic criteria for clinical, surveillance, and research purposes, which likely contributes to under-reporting and missed diagnoses, ultimately to an underestimation of disease.

117

"STOPPING THE ITCH": MASS DRUG ADMINISTRATION FOR SCABIES OUTBREAK CONTROL FOR OVER 15 MILLION PEOPLE IN THE AMHARA REGION OF ETHIOPIA

Wendemagegn Embiale Yeshanehe[1], Tariku Baynie[2], Ashenafi Ayalew[3], TekileHaimanot Gebrehiwot[3], Tesfa Getanew[3], Alie Ayal[3], Rony Zachariah[4]

[1]*Bahir Dar University, Bahir Dar, Ethiopia,* [2]*Amhara Health Bureau, Bahir Dar, Ethiopia,* [3]*Amhara Public Health Institution, Bahir Dar, Ethiopia,* [4]*UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization, Geneva, Switzerland*

Scabies is a Neglected Tropical Disease (NTD) of public health importance. The Amhara region in Ethiopia is one of the scabies endemic area with public health intervention since 2015. In June 2018 following screening of more than 15 million people in house to house census intervention was planned. This study was ubdertaken to describe the implementation of mass drug administration campaign for scabies outbreak control and community prevalence in the Amhara region of Ethiopia. Cross sectional descriptive study using routine monitoring data from the Mass Drug Administration (MDA) campaign. The study has included all individuals screened for scabies during the MDA campaign in the region. The study looked in to the numbers screened and identified with scabies (cases and contacts); the proportion of detected scabies stratified by treatment sub-groups. We used descriptive statistics to present the finding. From May 14 to June 20 on the campaign planning 15701907 residents were screened which has registered 718597 cases and 956017 contacts in 142 administrative districts. While the follow up treatment campaign from July 2 to August 26, 2018, has treated 815065 scabies cases and 1055071 contact. The regional mean scabies prevalence of 9 % (ranging 0.1 to 40.4%). 55.2% of cases are under 18 years old and 3.7 % of those are below 2 years old. While Ivermectin was used to treat 93.7% of scabies cases and 95.2% of contact the rest treated by Permethrin. There were about 202 health workers per 100,000 population involved in the MDA campaign and in each district the campaign has taken in average 6 days. In conclusion, the campaign has screened over 15 million people, delivering an Ivermectin based MDA for 1.87 million scabies cases and their contact successfully. The experience will contribute to the WHO initiative of development of scabies control framework.

118

THE POSSIBLE POLYMICROBIAL ETIOLOGY OF ALZHEIMER'S AND RELATED DEMENTIA

Remi L. Landry[1], Shiva K. Gadila[2], Monica E. Embers[3]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Tulane University National Primate Research Center, Covington, LA, United States,* [3]*Tulane University School of Medicine, New Orleans, LA, United States*

Observational and pathological studies have generated evidence for the complexity and possible polymicrobial causality of dementia-inducing diseases. It is thought that microbes and pathogens can act as triggers and interact with genetic factors to initiate neuronal loss, progressive synaptic dysfunction, the accumulation of amyloid-β peptide parenchymal plaques, tau protein neurofibrillary tangles, and inflammation in the brain. There is parallel concern regarding the role of persistent pathogens in the development of chronic Lyme neuroborreliosis. Conversely, review articles have suggested that Alzheimer's disease (AD) could be a neurospirochetosis. Evidence indicates that *Borrelia burgdorferi, Chlamydophila pneumoniae,* and *Candida albicans* are able to infect and evade the immune system and consequently are prevalent in the AD brain. To examine the possibility that microbes are contributing to Lyme and AD neuropathology, we will probe post-mortem brain tissues from patients with a history of neurologic Lyme disease, Alzheimer's disease, and non-dementia controls. This broad approach will include immunofluorescent staining with two separate antibodies and fluorochromes per pathogen, RNA *in situ* hybridization to identify targets and assess their viability, PCR with degenerate primers, and DNA and RNA next-generation sequencing. Samples of the hippocampus, frontal and parietal cortices, and the inferior temporal cortex will be sectioned for molecular detection and pathogen-specific staining. We will employ a target enrichment approach by utilizing oligonucleotides containing conserved species-specific sequences prior to sequencing. Pathology in the form of inflammatory lesions and AD plaques will be used as a road map for pathogen detection. Non-human primate samples spiked with pathogen or free of infection will serve as positive and negative controls, respectively. By completing this study, we expect to have a comprehensive understanding of the roles of persisting pathogens in both AD and Lyme disease.

119

SYSTEMATIC REVIEW OF SCRUB TYPHUS DRUG EFFICACY STUDY LANDSCAPE

Kartika Saraswati[1], Brittany Maguire[2], Sauman Singh[2], Nicholas P. Day[3], Philippe J. Guérin[2]

[1]*Eijkman-Oxford Clinical Research Unit, Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [2]*Infectious Diseases Data Observatory (IDDO), Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [3]*Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

One billion people are at risk of scrub typhus. Considering the magnitude of this public health problem, the evidence available to optimise treatments and disease control is sparse. Existing data collected from past clinical trials and longitudinal observational studies could be a source of information to address research priorities and knowledge gaps. Meta-analysis using an individual participant-level data (IPD) approach can produce a more representative secondary analysis because it facilitates the use of data from observational studies as well. We conducted a systematic review to assess the characteristics of scrub typhus clinical studies, the strength of evidence supporting current recommendations and to explore the feasibility of developing a scrub typhus IPD platform. Six databases and two clinical trial registries were searched for clinical trials and longitudinal

observational studies conducted between 1998 and 2018. Variables for extraction include treatment tested, patient characteristics, diagnostic methods, geographical location, outcome measures, and statistical methodology. The literature searches identified 5,163 citations, of which 2,647 unique articles were independently screened by two reviewers. A total of 95 studies (7 clinical trials and 88 observational studies) met the pre-specified inclusion criteria. The studies have been conducted in 11 countries and enrolled a total of 9,010 patients. Although there were only a few scrub typhus clinical trials found, there are substantially more data available from observational studies. Understanding the landscape of scrub typhus treatment studies allows assessment of the feasibility of addressing research questions using IPD meta-analysis method as well as research gap analysis.

120

MOLECULAR CHARACTERIZATION OF *BARTONELLA* SPECIES IN ECTOPARASITES COLLECTED FROM DOMESTIC ANIMALS, CUZCO, PERU

Carmen Flores-Mendoza[1], Steev Loyola[1], Ju Jiang[2], Mariza Lozano[1], Michael Fisher[1], Allen L. Richards[3]

[1]*U.S. Naval Medical Research Unit No. 6, Callao, Peru,* [2]*Medical Research Center, Silver Spring, MD, United States,* [3]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States*

Rickettsiae and bartonellae are Gram-negative bacteria that cause zoonotic and human diseases and are vectored by hematophagous arthropods such as mites, ticks, lice, sandflies, and kissing bugs. In the Americas, rickettsioses and bartonelloses have reemerged as a significant public health threats. A total of 222 domestic animals representing 8 different species of domestic animals (donkeys, horses, cattle, pigs, goats, guinea pigs, sheep and llamas) were sampled. Ectoparasites (n=1,697) collected from 122 of the animals were identified: 1657 lice, 39 ticks, and 1 flea. A total of 600 (35%) samples were DNA extracted for qPCR from the following ectoparasite species: *Tunga penetrans* (1), *Bovicola caprae* (3), *Haematopinus eurysternus* (3), *Bovicola equinus,* (8), *Bovicola bovis* (8), *Linognathus stenopsis* (15), *Haematopinus suis* (22), *Otobius megnini* (35), and *Melophagus ovinus* (505). All samples tested were negative for rickettsial DNA. A total of 173 (28.8%) DNA samples were screened for *Bartonella* by a genus-specific qPCR assay (StepOne Plus). *Bartonella* positive samples (n=91) were identified with threshold (Ct) values ranging 14.7 to 29.8 (mean: 21.4). For *Bartonella* species identification, PCR primers targeting *gltA*, *rpoB*, *ftsZ*, *rrs* and *groEL* genes fragments were used. *Bartonella bacilliformis* served as the PCR positive control. Seven PCR products were sequenced using Big Dye terminator kit and 3130XL genetic analyzer sequencing. According to Mega Analysis Software (version 7.O.21) and multilocus sequence typing analysis (MLST) *gltA*, *rpoB*, *ftsZ*, *rrs* and *groEL* gene sequences were found to matched to two *Bartonella* species: *Bartonella* melophagi and and an *Bartonella bovis*-like agent. *Bartonella* species are associated with disease in humans and animals. *B. melophagi* has been found in human blood but we do not know the roll of *Bartonella* bovis-like agent in human disease. Human and veterinary clinicians should be aware of the potential for louse-borne disease due to these *Bartonella* species. Research is on-going to determine the identity of the *Bartonella bovis*-like agent.

121

INVESTIGATING THE PREVALENCE OF PREVIOUS SPOTTED FEVER GROUP RICKETTSIA EXPOSURE ALONG THE SOUTHERN BORDER OF MONGOLIA

Michael E. von Fricken[1], Matthew A. Voorhees[2], Carmen Asbun[1], Brandon Lam[3], Paul Kuehnert[2], Jeffrey W. Koehler[2], Barbara Qurollo[4], Dulamjav Jamsransuren[5], Dolgorkhand Adiyadorj[5], Uyanaga Baasandagwa[5], Battsetseg Jigjav[5], Randal J. Schoepp[2]

[1]*George Mason University, Fairfax, VA, United States,* [2]*United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, MD, United States,* [3]*Johns Hopkins School of Medicine, Baltimore, MD, United States,* [4]*North Carolina State University, Raleigh, NC, United States,* [5]*National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia*

Ticks are second only to mosquitoes as vectors of disease. Human tick-borne disease occurs through the bite of an infected tick, thus outdoor occupations in tick habitats are put humans at a higher risk of disease exposure. In Mongolia, 26% of the population relies on nomadic herding, which places them in contact with ticks and tick-borne diseases like rickettsia. This study was undertaken to investigate the extent of rickettsia exposure occurring in the nomadic population a total of 1,926 human serum samples were collected from southern Mongolia in 2013-2014, across five aimags (provinces) including Bayankhongor, Dornogovi, Govi-Altai, Khovd, and Ömnögovi. Immunofluorescence assays (IFA) were used at an initial screen of 1:100 to detect previous rickettsia exposure compared to *R. rickettsii* and *R. conorii* controls. Of the 506 slides screened thus far, 114 (22%) had antibody evidence of previous exposure. Additional analysis pending to determine hotspots of disease and the full extent of previous exposure. Rickettsia exposures are occurring along the southern border of Mongolia at a high rate based on antibody prevalence. Known species of spotted fever group rickettsia detected in ticks collected in Mongolia include *R. raoultii*, *R. sibirica*, and *R. sibirica mongoloitimonae*, with *Candidatus R. tarasevichiae* endemic in the north. These preliminary findings align with previous work in other parts of Mongolia that found ~20% seroprevalence, suggesting exposure occurs frequently and across a wide geographic range.

122

IMPROVING THE MOLECULAR DIAGNOSIS OF SCABIES USING A PCR ASSAY TARGETING HIGH COPY NUMBER REPEATS IDENTIFIED IN THE PARASITE GENOME

Cielo Pasay[1], Lena Chng[1], Deborah Holt[2], Katja Fischer[1], Matt Field[3], Josh Francis[4], Dev Tilakaratne[5], Zuleima Pava-Imitola[1], Kate Mounsey[6], Asha Bowen[7], Tony Papenfuss[8], Bart Currie[2], James McCarthy[1]

[1]*QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia,* [2]*Menzies School of Health Research, RDH Campus, Darwin, NT, Australia,* [3]*James Cook University, Cairns, Queensland, Australia,* [4]*Royal Darwin Hospital, Tiwi, NT, Australia,* [5]*Darwin Dermatology Clinic, Tiwi, NT, Australia,* [6]*University of Sunshine Coast, Sippy Downs, Queensland, Australia,* [7]*Perth Children's Hospital, Nedlands, WA, Australia,* [8]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia*

In recent years, considerable advances have been made in molecular diagnostic methods for many pathogens. This escalation in interest has occurred in parallel with data indicating inaccuracy of scabies diagnostics using currently available methods. With the recent availability of the scabies genome, we have developed two pilot diagnostic PCR assays targeting abundant repetitive DNA elements. These two abundant repetitive sequences were identified from Next Generation Sequencing (NGS) of scabies mite genomes: a >400bp satellite sequence and a 606bp LTR sequence. These targets generated > 200 and 300 BLAST hits respectively, across datasets assessed. The sensitivity of these new assays is being compared against a published reference assay targeting the Cox1 gene. To enhance assay performance, a mite DNA extraction method is also being optimised using a non-invasive (flocked swabs) sample collection methodology and will be tested against the standard skin scraping method. Results of assay optimisation and of qPCR sensitivity and specificity will be presented. A more sensitive and specific diagnostic assay for scabies would greatly assist clinical management, epidemiological studies and ease the conduct of trials to assess new acaricides.

123

SCRUB TYPHUS-AN EMERGING ETIOLOGY OF ACUTE FEVER WITH JAUNDICE IN ADULTS PRESENTING TO A TERTIARY CARE HOSPITAL IN NORTH INDIA

Vikas Suri, Mandeep Singh, Ajay Duseja, Manisha Biswal, Mini P Singh, Kamran Zaman, Ashok k Pannu, Kapil Goyal, Ashish Bhalla, Rk Ratho, Sanjay Jain, Savita Kumari

PGIMER, Chandigarh, India

Fever with jaundice or tropical jaundice is a common presenting feature of the patients visiting for emergency medical care.140 adult patients (more than or equal to 14 years of age) with acute fever (body temperature > 101°F of 14 days or less in duration) without any localized source of infection on initial clinical evaluation accompanied with jaundice (hyperbilirubinemia ≥ 1.5 mg/dl or elevation of aspartate aminotransferase or alanine aminotransferase more than three times upper limit of the reference range) were enrolled. All these patients with fever and jaundice were evaluated on the basis of a standard proforma and were evaluated for malaria (peripheral smears/rapid diagnostic kits), scrub typhus(PCR / IgM ELISA),leptospirosis(IgM ELISA), enteric fever by blood cultures and dengue by dengue (NS1 antigen test and IgM ELISA),Hepatitis(IgM ELISA of EBV/HSV, IgM ELISA of HAV/HEV and HBsAg with IgM HBc ELISA if HBsAg positive)59.5% were males and 40.5% were females. The mean duration of fever before the presentation was 7.75 ± 3.58 days.7 patients (5%) died. 133 patients (95%) improved with treatment and were discharged. Scrub typhus 30 (21.4%), Hepatitis E 18 (12.8%), malaria 9 (6.4%), dengue fever, enteric fever, hepatitis A and leptospirosis in 12 (8.5%), 2 (1.4%), 4 (2.2%) patients and 1 (0.7%) patient respectively were the prominent etiology a patient presenting with fever and jaundice . Probable sepsis (Fulfilling SIRS criteria with a negative culture) accounted for 40 patients. Conjunctival suffusion (OR=22.17), severe anemia (OR=5.5), respiratory crepitations (OR=5.27), thrombocytopenia (OR=1.14), hepatomegaly (OR=1.04), normal INR (OR=0.29) and altered mentation (OR=0.25) were significant predictors of a diagnosis of scrub typhus in patients with fever and jaundice. Severe anemia (Hb<8), Hypoalbuminemia, severe thrombocytopenia (Platelet count <50,000) and a near normal INR at admission were predictors of a malarial vs a viral etiology of Tropical jaundice. Co-infection with scrub typhus and malaria was seen in 4 patients (vivax-3 and falciparum-1) and viral hepatitis A & E was observed in 3 patients.

124

MOLECULAR DETECTION OF SELECT MOSQUITO- AND TICK-BORNE DISEASES IN BELIZE

Lynne Sloan[1], Elitza Theel[1], Dane Granger[1], Gerhaldine Morazan[2], John W. Wilson[1], Nicole Achee[3], John Grieco[3], Bobbi Pritt[1]

[1]*Mayo Clinic, Rochester, MN, United States,* [2]*Central Medical Laboratory, Belize City, Belize,* [3]*University of Notre Dame, Notre Dame, IN, United States*

The prevalence of vector-borne diseases (VBDs) is not routinely monitored in resource-limited countries such as Belize. As a component of a collaborative effort with the Mayo Clinic, University of Notre Dame and Belize Ministry of Health, we aimed to determine the value of PCR testing for 4 mosquito- and 11 tick-borne pathogens in conjunction with serology in acutely-ill patients suspected of having a VBD. Sera were collected from Belizean patients with suspected acute VBD (1-5 days post-symptom onset) during 3/2017-12/2018. Nucleic acid was extracted (Roche MagNAPure) and PCR testing was performed (Roche LC instruments) for 15 VBD pathogens: *Anaplasma phagocytophilum, Ehrlichia* chaffeensis, *Ehrlichia ewingii*, *Ehrlichia muris, Babesia microti, Babesia divergens, Babesia duncanii, Borrelia miyamotoi, Borrelia hermsii, Borrelia parkeri, Borrelia turicatae,* West Nile virus (WNV), Dengue virus (DENV), Zika virus (ZIKV), and Chikungunya virus (CHIKV). PCR for 15 VBD pathogens was performed on sera from 77 patients (1155 analyses). Patients comprised 42 women, 31 men, 4 unknown; median age was 27 years (range 1-59). Among PCR tests for mosquito-borne pathogens, only 1 specimen was DENV PCR positive, while the remaining 76 were negative. In comparison, serologic testing performed on 42 of the 77 specimens revealed 2 specimens with possible past DENV infection (IgM-/IgG+/NS1 Ag-), 11 presumptive and 1 possible positive ZIKV IgM positive and 1 Flavivirus, NOS positive results. No specimens were positive for antibodies to WNV and CHIKV. All PCR tests for tick-borne pathogens were negative. In comparison, serology performed on 44 of the 77 specimens showed seropositivity rates of 14% for *B. microti*, 14% for *E. chaffeensis,* and 9% for *A. phagocytophilum*. Molecular testing has been previously shown to play an important but limited role in detection of acute VBD. Our results support this finding, with the detection of only a single positive PCR result (DENV) among 77 patients with suspected acute VBD. These results may be used to shape VBD testing programs in Central American countries such as Belize where testing resources are limited.

125

NUCLEIC ACID-BASED ASSAYS FOR DETECTION OF TICK-BORNE *ANAPLASMA PHAGOCYTOPHILUM* AND *BORRELIA MIYAMOTOI*

Scott Meredith, Hong Zheng, Victoria Majam, Sanjai Kumar

Food and Drug Administration, Silver Spring, MD, United States

Tick-borne pathogens present a significant public health problem and a risk to blood safety in the United States and elsewhere. Two such pathogens, *Anaplasma phagocytophilum* and *Borrelia miyamotoi*, are rapidly emerging and represent a matter of concern for U.S. blood safety; both pathogens are transmitted by the black-legged tick species (*Ixodes scapularis* and *I. pacificus*) and have been observed to survive in blood products. *A. phagocytophilum* has caused at least 10 cases of transfusion-transmitted human granulocytic anaplasmosis (HGA), while *B. miyamotoi*, a causative agent of tick-borne relapsing fever (TBRF), has been observed to be transmissible by blood in animal models. Despite this, no FDA-licensed tests for diagnostic or donor screening exist for either agent. To support the development of these assays, we are developing nucleic acid amplification test (NAT) for both pathogens. Protocols for culturing both bacteria have been designed based on methods from the published literature, which allows careful development of the NAT reference panel at low bacteria densities. Methods for purification, amplification, and detection of pathogen nucleic acids are currently under development. Primer sequences for amplification of an appropriate target gene have been designed for both pathogens. These target genes, the 16S rRNA sequence or *GlpQ* in *B. miyamotoi* and *msp2* in *A. phagocytophilum*, have been previously identified and evaluated in the literature. Throughout the development of the NAT assays, other promising amplification targets may be identified by analysis of purified gDNA or cDNA; in this case, new primers may be developed and evaluated for their effectiveness in diagnostic and detection assays. The NAT reference panel will be evaluated by qPCR with SYBR Green I fluorescent dye. Sequence-specific probes may be designed for newly identified amplification targets throughout the course of assay development. The results of NAT assays for *A. phagocytophilum* and *B. miyamotoi* will be presented.

126

MOLECULAR DETECTION OF KNOWN < POTENTIALLY NEW *BARTONELLA* SPECIES IN ARTHROPOD VECTORS CIRCULATING IN PERU

Giovanna Mendoza, Victor Alberto Jimenez- Vasquez, Yanina Zarate-Sulca

Instituto Nacional de Salud-Perú, Lima, Peru

Nine out of 32 recognized species of *Bartonella* genus, are known as human diseases agents transmited by different arthropod vectors. However the transmission of unidentified Bartonella sp is still poorly understood, especially in rural areas of peruvian andean valleys. The aim of the study was the molecular identification of *Bartonella* sp in arthropod vectors from endemic localities in Peru. We collected ticks, pet fleas, and sheep flies from cats, dogs, guinea-pigs, in 10 sites. We extracted DNA from 677

arthropod pools and designed nested PCR primers to amplified ITS region. DNA sequences obtained by Sanger sequencing, were aligned with other Gen Bank sequences and a maximum likelihood (ML) tree was stimated. We successfully detected *Bartonella* in 29.5% of samples. Our ML tree generated clades according with all species included in the alignment with supported nodes. The identified species were: *B.henselae, B.clarridgeiae, B. rochalimae* and *B. vinsonii*. However, 11 sequences amplified from *M. ovinus* and *R. sanguineus* sampled in sheeps and dogs presented 100% similarity with 2 unidentified *Bartonella* deposited in GenBank (accessions AF415209 and EU189218), while 1 sequence amplified from *P. irritans* found in guinea-pig presented 99.61% similarity with another undescribed *Bartonella* species (accession AF415210). Our nested specific PCR primers detected 4 known species and probably 2 new undescribed *Bartonella* species. Our research detected 29.5% of positive samples in contrast with other studies carried out by Parola 2002 and Nelder 2009, they detected less 10% and found these two unidentified Bartonella in sheeps and gray squirrel ectoparasites, respectively. We cannot discard the fact that some clinical manifestations could be associated with transmission of these still uncharacterized and uncultured *Bartonella* species. We suggest complete the research with methodologies like MLST or metagenomic sequencing. This study demonstrates the circulation of undescribed Bartonella species in Peru highlights the necessity of carry out new and more complex studies in order to understand present and future bartonellosis outbreaks

127

PROFILING OF MALARIA INFECTION IN ASYMPTOMATIC POPULATION IN KISUMU COUNTY, WESTERN KENYA

Benjamin Humphrey Opot, Hosea Akala, Raphael Okoth, Gladys Chemwor, Charles Okello, Redeptah Yeda, Edwin Mwakio, Jackline Juma, Agnes Cheruiyot, Dennis Juma, Ben Andagalu, Jim Ray Managbanag

United States Army Medical Research Directorate - Kenya, Nairobi, Kenya

Countries in malaria-endemic zones aim to enter pre-elimination phase. There is scanty information on the burden of asymptomatic malaria and its contribution in sustaining transmission in malaria holoendemic regions. This study aims to determine the burden of malaria parasite among asymptomatic cases. 480 individuals from Kisumu County were grouped into 29 clusters. Each cluster containing 4 households was followed up monthly between July 2015 and June 2016 and was tested for malaria by real-time reverse transcription PCR (rt RT-PCR). Malaria positive cases were treated with artemether-lumefantrine and further screened for *Plasmodium falciparum* 16 (Pf16) early, 25 (Pf25) late gametocytes stages and *Plasmodium* species. Prevalence of malaria was established at baseline diagnosis and incidence per house hold and per cluster across the study period. Fifty-six percent (2375/4214) of the tested participants were positive for malaria at multiple follow-up time-points. A case study of one of the clusters showed subsequent positive malaria detection of 72.2±21% infected during visit 5 to visit 9. Of these malaria positive samples, 68% harboured gametocytes comprising 48% both Pf16 and Pf25 stages, 19% Pf16 only and 1% Pf25 only. 70% were positive for *P. falciparum* only, 6.7% had mixed-infections of *P. falciparum* and *P. malariae*, 10% had *P. falciparum*, *P. malariae* and *P. ovale* while 1 was *P. falciparum* and *P. ovale mixed* infection. The Pf16 positive samples comprised 11 *P. falciparum* mono infections and 1 *P. falciparum* and *P. ovale.* Only 15% of samples that had no gametocytes were positive for *P. falciparum* single species infections. Gametocyte prevalence remained comparable despite artemisinin-based combination therapy (ACT) administration upon positive diagnosis suggesting that asymptomatic cases are involved in transmission of malaria. High rate of gametocytemia among mixed species infections than those comprising *P. falciparum* single species infections suggest that other species are involved in modulating *Pf* transmission. Analyses are underway to determine if recurrent gametocytemia is due to recrudescence or new infections.

128

SPATIAL DISTRIBUTION AND ALLELE FREQUENCIES OF THIOESTER CONTAINING PROTEIN 1 (TEP1) GENE, A KEY IMMUNE GENE AGAINST *PLASMODIUM* INFECTION IN *ANOPHELES GAMBIAE* MOSQUITOES IN KENYA

Shirley Akinyi Onyango[1], Andrew K. Githeko[2], Yaw A. Afrane[3], Kevin O. Ochwedo[4], Harrysone Atieli[5], James W. Kazura[6], Guiyan Yan[7]

[1]*Kenyatta University, Nairobi, Kenya,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*University of Ghana, Ghana, Ghana,* [4]*International Center of Excellence for Malaria Research, Homa bay, Kenya,* [5]*International Center of Excellence for Malaria Research, Homa Bay, Kenya,* [6]*Case Western Reserve University, Cleaveland, OH, United States,* [7]*University of Carlifonia, Irvine, Irvine, CA, United States*

Anopheles gambiae mosquitoes are efficient vectors for malaria in sub-Saharan Africa. Implemented control interventions, climate change, and environmental modifications enhance the local mosquito densities promoting selection of efficient malaria vectors either resistant or susceptible to *Plasmodium* and insecticides. In *An. gambiae*, the thioester-containing protein 1 (TEP1) is a highly polymorphic gene crucial in the mosquito's immune system and exhibits defenses against *Plasmodium* parasites. The TEP1 displays allelic variations associated with distinct genotypes in its resistance to malaria infections. Interactions between *Anopheles* and *Plasmodium* parasites are a potential target for blocking transmission. Therefore, genotyping local populations is critical for monitoring changes in mosquito densities that may explain variations in malaria prevalence within different settings. Female *An. gambiae* s.l. Mosquitoes were collected from Homabay, Vihiga, and Kakamega in western Kenya, and further identified to species using PCR. PCR-RFLP method was used to determine the TEP1 allelic classes. Mosquitoes collected from Homa Bay were all *An. arabiensis,* and they exhibited R1 (11%), S1 (68%), S2 (20%) and R2 (1%) alleles whereas Vihiga had R1 (22%), S1 (50%), S2 (6%) and R2 (22%). Kakamega had R1 (25%), S1 (56%), and S2 (19%), but no R2 allele was detected. The frequencies of TEP1 allele classes in *Anopheles* species varied significantly among locations, suggesting spatial heterogeneity in vector competence to malaria parasites.

129

MASSIVE BEHAVIORAL AND GENOMIC VARIATION IN *AEDES AEGYPTI* ACROSS SUB-SAHARAN AFRICA

Noah H. Rose[1], Massamba Sylla[2], Diego Ayala[3], Joel Lutomiah[4], Athanase Badolo[5], Jewelna Akorli[6], Ogechukwu Aribodor[7], Nnenna Ibe[1], John-Paul Mutebi[8], Rosemary Sang[4], Jacob E. Crawford[9], Bradley J. White[9], Carolyn S. McBride[1]

[1]*Princeton University, Princeton, NJ, United States,* [2]*Université Cheikh Anta Diop, Dakar, Senegal,* [3]*Centre International de Recherches Médicales de Franceville, Franceville, Gabon,* [4]*Kenya Medical Research Institute, Nairobi, Kenya,* [5]*University of Ouagadougou, Ouagadougou, Burkina Faso,* [6]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [7]*Nnamdi Azikiwe University, Awka, Nigeria,* [8]*Centers for Disease Control and Prevention, Fort Collins, CO, United States,* [9]*Verily, Mountain View, CA, United States*

The recent evolution of a strong preference for human hosts in the globally invasive form of *Aedes aegypti* promotes the transmission of the Zika, dengue, chikungunya, and yellow fever viruses. Recent evidence suggests that this form of *Ae. aegypti* may have originated in West Africa about 10,000 years ago, but the geographical distribution, ecological correlates, and genetic determinants of preference for human hosts in ancestral African populations are largely unknown. Although African populations are often thought to be primarily animal-biting or opportunistic, they occupy diverse habitats ranging from forests where they encounter few human hosts to highly urbanized cities where humans are by far the most common potential host. We characterized behavioral and genomic variation in 26 forest, rural, and urban populations from seven countries

across sub-Saharan Africa. Behavior varied more or less continuously across the continent, ranging from strong preference for animal odors to strong preference for human odor in our assay. Both climatic variables and human population densities predicted behavior, with preference for humans appearing in relatively densely populated areas with high seasonal variation in precipitation in a band across the southern Sahel region. One population in Northern Senegal showed nearly as great a preference for human hosts as a laboratory strains derived from the globally invasive human specialist form. We also use whole genome sequence data to identify genomic *loci* and evolutionary processes that contribute to behavioral divergence between populations.

130

COMPUTATIONAL KARYOTYPING OF POLYMORPHIC CHROMOSOMAL INVERSIONS IN THE MAJOR MALARIA VECTORS *ANOPHELES COLUZZII* AND *AN. GAMBIAE*

R. Rebecca Love[1], Seth Redmond[2], Marco Pombi[3], Beniamino Caputo[3], Vincenzo Petrarca[3], Alessandra della Torre[3], The Anopheles gambiae 1000 Genomes Consortium[4], Nora J. Besansky[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Institute Vector Borne Disease, Monash University, Melbourne, Australia,* [3]*Dipartimento di Sanità Pubblica e Malattie Infettive, Laboratory affiliated to Istituto Pasteur Italia - Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome, Italy*

Chromosomal inversions are a type of genomic structural variant that arises when a segment of DNA breaks, rotates 180 degrees, and reattaches. Because inversions can protect sets of alleles from being separated through recombination, they have wide-ranging and long-studied consequences. In the *Anopheles gambiae* complex, which includes the major malaria vectors *An. arabiensis*, *An. coluzzii*, and *An. gambiae*, paracentric chromosomal inversion polymorphism can complicate both genomic analysis and vector control; in these species, chromosomal inversions are associated with variation in traits as diverse as indoor-outdoor resting behavior, aridity tolerance, and *Plasmodium* infection rates. However, nearly all common chromosomal inversions in these species are currently identified cytogenetically, through the labor-intensive preparation and examination of slides of stained chromosomes. This process requires not only extensive expert knowledge, but also the ovaries of half-gravid female mosquitoes, severely limiting the contexts in which the roles of chromosomal inversions can be explored. By leveraging the genomic resources of the *Anopheles gambiae* 1000 Genomes Project (Ag1000G), as well as known cytogenetic karyotypes, we developed and validated an innovative computational method for accurately genotyping chromosomal inversions based on single nucleotide polymorphism (SNP) data. In *An. gambiae* and, where applicable, *An. coluzzii*, our methods can predict the genotype of inversions 2L*a*, 2R*j*, 2R*b*, 2R*c*, 2R*d*, and 2R*u* with accuracy well above 90%. Our results suggest this method is also capable of accurately genotyping the same chromosomal inversions in mosquitoes sequenced to lower coverage than is typical of the specimens included in Ag1000G.

131

METAGENOMIC PROFILING FROM *ANOPHELES GAMBIAE* INDIVIDUAL GENOME SHOTGUN SEQUENCING DATA IN AG1000G PROJECT

Jiannong Xu, Dong Pei, Jinjin Jiang, Yuxin Yan, Jiandong Wang, Ashmita Pandey

New Mexico State University, Las Cruces, NM, United States

Anopheles gambiae Ag1000G project aims to profile natural genetic variation using individual genome shotgun sequencing data from wild specimens in Africa. In the sequencing output, there are reads derived from non-mosquito sources, largely from associated microbiota and blood source. We developed a pipeline to profile metagenomic composition and genetic capacity. The reads were mapped against *An. gambiae* reference genome to extract non-mosquito reads. The non-mosquito reads were used for further characterization. Two approaches were used. (1) The non-mosquito reads were mapped against Microbial Reference Database to get a peek of possible taxa, and (2) the non-mosquito reads were assembled into contigs for taxonomic and functional annotation. Reference reads mapping revealed taxa of fungi, bacteria, viruses and protists, but only <2% reads have hits. Assembled contigs contain more sequence information for annotation. In some cases, the size of contigs is longer than 10kb. In individuals that had taken blood, sequences of human and *Plasmodium* were discovered. Enteric bacteria, *Acinetobacter* and *Elizabethkingia* were often found, but abundance was not high. Quite portion of identified fungal taxa belong to Ascomycota. Mosquito-associated viruses like Mononegavirus were found in some individuals. At nucleotide level, usually less than 20% contigs have homologues with >70% identity. In most cases, >50% contigs do not have good nucleotide hit to any entries in the database. Apparently these contigs were derived from unidentified taxa. In these novel taxa, some predicted protein sequences show a similarity to fungal taxa. The data indicate that bacteria may not be predominant in the microbiota, fungi are more abundant than what we thought. There are large number of novel taxa that are associated with wild *An. gambiae.* Based on annotated genes, metabolic pathways can be predicted. Chemical ecology may be similar in different microbial communities in which taxonomic composition may vary. The shotgun sequencing data are a valuable source to characterize mosquito microbiome comprehensively.

132

SEX-SPECIFIC TRANSCRIPTIONAL PROFILING IN THE MOSQUITO, *CULEX PIPIENS*

Patricia Kamanda, Cheolho Sim

Baylor University, Waco, TX, United States

The autoregulatory pathway controlling sex determination is highly variable and has been identified in only a few non-mammalian model species. Therefore, sex determination in mosquitoes is largely unknown. Previous research suggests that a dominant male-determining factor, depending on the mosquito species, is the primary signal that initiates controls sex determination and initiates male development. In this study, we identify sex-specific transcripts indicative of sex-determining function in the *Culex pipiens* complex mosquitoes that transmit many human pathogens such as West Nile virus and filarial nematodes. We investigated sex-specific transcripts in the mosquito, *Cx. pipiens*. We used Illumina RNA-Seq to simultaneously identify and quantify differences in transcript abundance between male and female in two developmental stages, pupae and adult. We found 76 genes with differences in transcript abundance between male and female in the pupal stage and 214 genes in the adult stage. In addition, we also uncovered the sex-specific morphological key in pupal stage of *Cx. pipiens*. A deeper understanding of the genetic basis of sex-specific traits may provide a novel method of control for these disease vectors including sterile insect technology.

133

OXIDOREDUCTIN-LIKE PROTEIN AND PDZ ENHANCE THE COLD TOLERANCE IN THE OVERWINTERING DIAPAUSE OF THE MOSQUITO *CULEX PIPIENS*

Bryan P. King

Baylor University, Waco, TX, United States

Stress tolerance and low temperature survival are a few key features of the diapause program for the mosquito *Culex pipiens* to have a successful overwintering period. Here, we suggest two enzymes; oxidoreductin-like protein and PDZ are involved with these diapausing characteristics for overwintering survival. The gene that encodes oxidoreductin-like protein was suppressed by RNAi, and the proportion of degenerating follicles were assessed. Consequently, suppression of the oxidoreductin-like protein with RNAi significantly increased the proportion of degenerating follicles in diapausing mosquitoes. Similarly, the gene that encodes PDZ

was also suppressed by RNAi, and F-actin polymerization was assessed in the midgut and ovaries of *Cx. pipiens.* Inhibition of oxidoreductin-like protein and PDZ significantly reduce the survivability of diapausing female mosquitos, which indicates that these enzymes play key roles in protecting multiple tissues during early diapause.

134

MOLECULAR KARYOTYPING OF CHROMOSOMAL INVERSION 2RB IN *ANOPHELES GAMBIAE AND AN. COLUZZII*

Raquel Montanez Gonzalez[1], R. Rebecca Love[1], Verena Pichler[2], Maria Calzetta[2], Marco Pombi[2], Beniamino Caputo[2], Alessandra Dellatorre[2], Nora J. Besansky[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Sapienza University of Rome, Rome, Italy*

The Afrotropical mosquitoes *Anopheles gambiae* and *An. coluzzii* are the deadliest vectors of human malaria. Types of structural chromosomal mutations known as chromosomal inversions are very common in these species and they appear to play important roles in ecological adaptation, influencing mosquito behavior and physiology in ways relevant to malaria epidemiology and control. Inversion 2Rb is the only pan-African *An. gambiae* inversion lacking a cheap and reliable molecular genotyping assay. Current methods of microscopical karyotyping are time consuming, prone to human error, and feasible only for female mosquitoes at one particular developmental stage—and only if they are properly preserved. Failure to account for inversion status is a barrier to further knowledge of important vector traits (e.g. biting and resting behavior, seasonality, aridity tolerance and *Plasmodium* infection rates), which can be influenced by inversions. To overcome this limitation, we exploited genome sequences of cytogenetically karyotyped specimens to identify tag SNPs (Single Nucleotide Polymorphisms) highly predictive of inversion status. Using these SNPs, three low cost PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) diagnostic assays were developed and then validated on cytogenetically karyotyped population samples field collected from eight different African countries (Benin, Burkina Faso, Cameroon, The Gambia, Guinea Bissau, Mali, Senegal, Tanzania). One assay showed a high concordance rate of 95% between PCR-RFLP and cytogenetic approaches in both *An. gambiae* (N=437) and *An. coluzzii* (N=133), and the other two assays agreed well (93%-97%) on either one or the other species only. The combination of the two most specific assays provides a useful diagnostic for inversion 2Rb in *An. gambiae* and *An. coluzzii* with a concordance rate of >96% with the 'gold standard' cytogenetic karyotyping method and opens the way to extensive assessment of the adaptive role of this inversion.

135

CONSTRUCTING A MODEL OF GENE EXPRESSION REGULATION IN *ANOPHELES* SALIVARY GLANDS

Michael B. Wells, Deborah J. Andrew

Johns Hopkins University, Baltimore, MD, United States

Mosquito-borne diseases remain among the deadliest threats to human health. Despite substantial diversity among the pathogens that mosquitoes spread, one commonality among them is that viruses and microbes must invade the mosquito salivary glands (SGs) and enter the salivary duct to be transmitted to a new host, during probing prior to the next blood meal. SGs are a high-priority target for new interventions for blocking transmission. Mosquito SGs display remarkable sexual dimorphism and functional distinctions. Female SGs are composed of two lateral lobes (with proximal and distal portions) flanking a medial lobe. An open ended duct runs most of the length of the lateral lobes and a short distance into the medial lobe. Each region adds different protein components to the saliva, which is thought to contribute to both sugar and blood feeding. The male SG, which contributes to sugar feeding, is made up of a single lobe (most similar to the female proximal lateral lobe) with a closed salivary duct running nearly the entire length of the SG. We propose that at least three types of factors coordinately regulate mosquito SG gene expression. SG transcription factors, including Fork head, Sage, Senseless, and CrebA likely provide SG identity, developmental progression, homeostatic maintenance, and secretory cargo identity. Sex determination cues may guide male versus female developmental, structural, and secretory fates. Finally, ecdysone hormone signaling is likely to play a role in stage-specific gene expression during developmental transitions. We discovered that both female and male SGs contain male-biased characteristics in *Anopheles gambiae* and female-biased characteristics in *Anopheles stephensi*. We find that gene expression may be regulated differently between SG secretory cells and duct-associated cells just outside the secretory portion of the SG. Here, we present our model, preliminary findings, and plans to interrogate this phenomenon. We will genetically, or molecularly, reduce female SG function, imposing additional barriers to pathogen invasion and reducing production of saliva proteins critical for pathogen transmission.

136

DEVELOPMENT OF MOLECULAR KARYOTYPING ASSAYS FOR COMMON INVERSIONS IN *ANOPHEL FUNESTUS*

Martin Lukindu[1], Rachel R. Love[1], Scott T. Small[1], Guelbeogo M. Wamdaogo[2], N'Fale Sagnon[2], Nora J. Besansky[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso*

Anopheles funestus is a proficient malaria vector in sub-Saharan Africa with a wide distribution across heterogeneous ecological settings, but its population structure is less studied relative to that of *An. gambiae* with which it is broadly co-distributed. In a situation reminiscent of *An. gambiae* and its sister species *An. coluzzii*, sympatric but assortatively mating *An. funestus* populations from Burkina Faso, West Africa differ in seasonality, larval habitat, adult resting behavior, and chromosomal inversion frequencies. The Kiribina form is largely devoid of inversion polymorphism and carries mostly standard arrangements, while Folonzo is highly polymorphic for inversions 3Ra, 3Rb and 2Ra. Unfortunately, there are no known molecular markers to guide taxonomic identification of these otherwise morphologically indistinguishable units. Their identification depends upon classical cytotaxonomy. Because cytogenetic karyotyping procedures are laborious, time consuming and require extensive expertise, this approach is impractical for large-scale field studies. Exploiting the new chromosome-based genome assembly for *An. funestus* (AfunF3) and more than 170 fully sequenced and cytologically karyotyped Folonzo and Kiribina specimens, we have been developing and validating molecular karyotyping approaches based on computationally identified tag-SNPs with high SNP-karyotype concordance in these samples. Our results based on array hybridization and amplicon sequencing will be discussed.

137

COMMUNITY ACCEPTANCE OF YEAST INTERFERING RNA LARVICIDE TECHNOLOGY FOR CONTROL OF *AEDES* MOSQUITOES IN TRINIDAD

Akilah Stewart[1], Nikhella Winter[1], Azad Mohammed[1], Limb K. Hapairai[2], Jessica Igiede[3], David W. Severson[3], Molly Duman-Scheel[2]

[1]*The University of the West Indies at St. Augustine, St. Augustine, Trinidad and Tobago,* [2]*Indiana University School of Medicine, South Bend, IN, United States,* [3]*The University of Notre Dame, Notre Dame, IN, United States*

RNA interference (RNAi), a technique used to study gene function in mosquitoes and other insects, is attracting attention in agricultural pest control communities but is a largely unexplored new approach for mosquito control. We recently began to engineer *Saccharomyces cerevisiae* (baker's yeast) to produce interfering RNA that silences genes required for mosquito survival, but which does not match genes in humans or other non-target organisms. These larvicides, which facilitate cost-effective production and delivery of interfering RNA to larvae that

consume the yeast, effectively kill mosquito larvae in laboratory and semi-field trials. Prior to pursuing field evaluation of larvicides targeting *Aedes* species in Trinidad, a Caribbean island with endemic diseases resulting from pathogens transmitted by *Aedes* mosquitoes, we engaged adult residents living in prospective trial site communities of Tamana, St. Augustine and Caroni. Paper surveys and open community forums were used to assess the potential acceptability, sustainability, and societal desirability of yeast interfering RNA larvicides. Respondents have good working knowledge of mosquitoes and mosquito-borne diseases. A majority of respondents practice some means of larval mosquito control and agree that they would use a new larvicide if it were shown to be safe and effective. During community engagement forums, participants were educated about mosquito-borne illnesses and the new yeast larvicides. When invited to provide feedback, forum attendees voiced strong support for the new technology, raised very few concerns, and offered advice regarding optimal larvicide formulations and prices. The results of these activities suggest that participants are open to the potential use of yeast interfering RNA larvicides and that the communities assessed are viable field sites.

138

MOLECULAR BASIS OF DDT AND PERMETHRIN RESISTANCE IN AN *ANOPHELES FUNESTUS* FROM BENIN

Genevieve Tchigossou

University of Abomey-Calavi, Benin, Abomey-Calavi, Benin

Insecticide resistance in *Anopheles* mosquitoes is threatening the success of malaria control programmes. To implement suitable insecticides control strategies, we investigated the molecular basis of permethrin and DDT resistance in an *Anopheles funestus* population from Benin. *GSTe2* gene was the most upregulated detoxification gene in both DDT- [fold-change (FC: 16.0)] and permethrin-resistant (FC: 18.1) mosquitoes. *CYP6P9a* and *CYP6P9b* genes were also significantly overexpressed with FC 5.4 and 4.8, respectively, in a permethrin resistant population. The absence of the *L1014F* or *L1014S kdr* mutations in the voltage-gated sodium channel gene coupled with the lack of directional selection at the gene further supported that knockdown resistance plays little role in this resistance. In conclusion, the role played by metabolic resistance to pyrethroids in *An. funestus* population in Benin suggests that using novel control tools combining the synergist such as PBO-based bednets, could help manage the growing pyrethroid resistance in Benin.

139

INSIGHT INTO GENOMIC AND CHROMOSOMAL DIFFERENTIATION BETWEEN *CULEX PIPIENS PIPIENS* AND *CULEX PIPIENS MOLESTUS*

Reem A. Masri[1], Andrey A. Yurchenko[2], Jeremy Jenrette[1], Natalia V. Khrabrova[3], Anuarbek K. Sibataev[3], Megan L. Fritz[4], Maria V. Sharakhova[1]

[1]*Virginia Tech, Blacksburg, VA, United States,* [2]*Gustave Roussy Cancer Center, Paris, France,* [3]*Tomsk State University, Tomsk, Russian Federation,* [4]*University of Maryland, Maryland, MD, United States*

The members of the *Culex pipiens* complex are globally distributed and represent competent vectors of West Nile virus and Eastern equine encephalitis virus transmitted to both birds and mammals. Two members of the complex, *Cx. p. pipiens* and *Cx. p. molestus*, exhibit important behavioral and physiological differences. *Cx. p. pipiens* mates in open spaces, feeds on birds, and requires a blood meal for oviposition. *Cx. p. molestus*, in contrast, mates in confined spaces, feeds on mammals, can lay eggs without a blood meal and is well adapted to human environment. However, the taxonomic status of these two members of the *Cx. pipiens* complex is under debate. We used whole-genome resequencing analysis of two laboratory colonies derived from Chicago, USA and field collections from Eurasia (Belarus and Kyrgyzstan). A cytogenetic analysis was performed on the Chicago colonies. The whole-genome resequencing analysis revealed strikingly different levels of genomic diversity within the genomes of *Cx. p. pipiens* and *Cx. p. molestus*. The diversity was low in the *Cx. p. molestus* genome but was high and distributed along the chromosomes in the *Cx. p. pipiens* genome. The comparison between the two members revealed high level of genomic divergence between them (mean Fst ≈ 0.3) which was more or less uniformly distributed along the chromosomes. Phylogenomic and ADMIXTURE analyses clustered *Cx. p. pipiens* from North America and Eurasia together but clustered *Cx. p. molestus* from both continents into two separate clades. Furthermore, mitotic chromosome analyses revealed significant length difference for chromosome 3 and distinct banding patterns in Hoechst 33342 staining between *Cx. p. pipiens* and *Cx. p. molestus*. High level of the genomic divergence and chromosome differentiation between *Cx. p. molestus* and *Cx. p. pipiens* suggest a long history of genetic isolation between them and support the idea that they may represent two distinct species.

140

STRUCTURE AND FUNCTION OF THE Y-CHROMOSOME IN *ANOPHELES ALBIMANUS*

Austin Compton[1], Varvara Lukyanchikova[2], Zhijian (Jake) Tu[1], Victor Llaca[3], Stephane Deschamps[3], Chujia Chen[4], Chunhong Mao[5], Igor Sharakhov[2]

[1]*Department of Biochemistry, Virginia Tech; Fralin Life Sciences Institute of Virginia Tech, Blacksburg, VA, United States,* [2]*Department of Entomology, Virginia Tech, Blacksburg, VA, United States,* [3]*Corteva Agriscience™, Agriculture Division of DowDuPont™, Johnston, IA, United States,* [4]*Department of Biochemistry, Virginia Tech; Fralin Life Sciences Institute of Virginia Tech; and Interdisciplinary PhD Program in Genetics, Bioinformatics, and Computational Biology, Virginia Tech, Blacksburg, VA, United States,* [5]*Biocomplexity Institute and Initiative, University of Virginia, Charlottesville, VA, United States*

Female mosquitoes are responsible for the transmission of harmful human pathogens, while Y chromosome-bearing males are not. Yet much remains unknown regarding the elusive mosquito Y chromosome, both regarding its structure and function. To date, no Y chromosome has been assembled in mosquitoes. With a growing interest in Y chromosomes and its importance in male biology, it is crucial to make strides to better resolve this critical gap in knowledge. These efforts have the potential to reveal novel methods for mosquito control as the Y chromosomes harbor the male-determining factor and can regulate dosage compensation. For example, when the Y chromosome gene Guy1 is expressed in female *Anopheles stephensi*, it confers 100% female lethality, making it salient targets for genetic control strategies and for improving sex separation techniques. However, despite the apparent significance of Y chromosome genes, the molecular mechanisms underlying their biological function remains unknown. Here we report the assembly of the first mosquito Y chromosome and describe our efforts to determine the function and molecular mechanism of a novel candidate for a male-determining factor, *albimanus Y chromosome gene 1* (*albY1*) in the New World malaria mosquito *Anopheles albimanus*.

141

IMPROVED GENE EDITING EFFICIENCY OF RECEPTOR-MEDIATED OVARY TRANSDUCTION OF CARGO -REMOT CONTROL- IN *AEDES AEGYPTI*

Duverney D. Chaverra-Rodriguez[1], Chan C. Heu[1], Donghum Kim[1], Vanessa Macias[1], Jason L. Rasgon[2]

[1]*Pennsylvania State University, State College, PA, United States,* [2]*Pennsylvania State University, University Park, PA, United States*

Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control) is a technology that uses a peptide ligand (P2C) to deliver Cas9 ribonucleoprotein (RNP) complex from the female hemolymph to the developing oocytes. Heritable gene editing of the injected female's offspring is achieved at efficiencies as high as 0.3 mutants per injected mosquito. Here, we discuss experiments testing factors that affect the efficiency of ReMOT Control in the mosquito *Ae. aegypti*. First, we tested

the effect of using the P2C-derived peptides P2C1, P2C2 and P2C3 on Cas9 RNP transduction and gene editing efficiency. Second, we tested several concentrations of ribonucleoprotein and endosomal escape reagents for gene editing. Results confirmed that smaller P2C derivatives transduced Cas9 into the ovaries and that efficiency of ReMOT control could be improved using conditions that facilitate the transduction/release of Cas9 RNP into the oocytes.

142

THE GENETIC BASIS FOR INCREASED OUTDOOR HOST-SEEKING IN *ANOPHELES COLUZZII* DURING THE BIOKO ISLAND MALARIA CONTROL PROJECT

Jacob I. Meyers[1], Godwin Fuseini[2], Guillermo Garcia[2], Abrahan Matias[2], Hans Overgaard[3], Christopher Schwabe[4], Jo Lines[5], Immo Kleinschmidt[5], Michel A. Slotman[1]

[1]*Texas A&M University, College Station, TX, United States,* [2]*Medical Care Development International, Malabo, Equatorial Guinea,* [3]*Norwegian University of Life Sciences, As, Norway,* [4]*Medical Care Development International, Silver Spring, MD, United States,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Malaria vector control programs largely rely on indoor application of insecticides to target host-seeking adult mosquitoes. However, the efficacy of these control tools is threatened by behavioral resistance. For example, mosquitoes can avoid insecticides applied indoors by increasing outdoor host-seeking. One location where increased outdoor host-seeking has been documented is Bioko Island, Equatorial Guinea, where the Bioko Island Malaria Control Project (BIMCP) has conducted indoor residual spraying since 2004. We examined the genetic basis of increased outdoor host seeking of *Anopheles coluzzii* on Bioko Island. Using a pool-seq approach with large sample sizes (34-268 mosquitoes/pool) and high sequence coverage (average 491-894x/pool), we screened the genomes of *An. coluzzii* collected in 2009, and 2013/2014 for genetic variation linked to indoor vs. outdoor host-seeking. We also compared pre-intervention *An. coluzzii* collected in 2004 to these post-intervention samples to identify *loci* under selection during the BIMCP. We simulated the sampling effects of pool size and coverage level on *FST* estimates to determine significant cut-offs. Additionally, we used the Cochran-Mantel-Haenszel test followed by a Benjamini & Hochberg correction (CMH+BH) to identify significantly differentiated SNPs. Thirty-five SNPs significantly differentiated indoor and outdoor pools from 2009 according to both methods, five of which are located within genes and are non-synonymous. In the 2013/2014 data, a 1MB region on 2R differentiates indoor from outdoor feeding mosquitoes. This Fst peak contains 10 genes with differentiated SNPs. In addition, several genomic regions are differentiated between samples collected over the course of the BIMCP. Two of these regions include SNPs associated with insecticide resistance (IR) including *kdr* and possibly ABC-transporters. In summary, we have identified sites and genes linked to outdoor host-seeking, although the function of these genes does not currently point to a specific mechanism.

143

SNP-BASED HERITABILITY ESTIMATION FOR HOST PREFERENCE IN *CULEX TARSALIS*

Bradley J. Main, Fatima Tuqan, Tara C. Thiemann, Christopher M. Barker

University of California Davis, Davis, CA, United States

In the western United States, *Culex tarsalis* is an important vector of arboviruses, including West Nile, western equine encephalomyelitis, and St. Louis encephalitis viruses. *Cx. tarsalis* feeds frequently on birds, although it is opportunistic and host selection among bird species is non-random with respect to host availability. There is also a well-characterized increase in the frequency of mammalian blood meals starting in July and peaking in September, which is attributed at least in part to changes in host availability due to fledging chicks. In this study, we considered the question: do opportunistic versus strictly ornithophilic subpopulations of *Cx. tarsalis* occur in sympatry, and if so, are opportunistic genotypes over-represented among mammal-fed individuals? To test this, we resequenced 13 mammal-fed and 23 bird-fed *Cx. tarsalis* from a single site with known host availability in Yolo county, California. Using 262K high quality SNPs, we estimated the "SNP heritability" of host choice to be $H^2 = 0.08$, SE=2.247, indicating that there is no evidence for a substantial genetic component underlying host preference among these samples. However, genetic analysis of another population(N=24) from a single site in Sutter County, California using AmpliconSeq revealed an association between specific genotypes at the *prophenoloxidase* gene and cattle-feeding. Thus, an improved assessment of population structure across geography and season is needed to properly characterize functional genetic variation in this system. Future studies will involve genotyping more *loci* from larger mosquito populations across CA. In addition, we will sequence *Cx. tarsalis* populations early and late in the season to assess whether distinct genotypes contribute to the increase in mammal-feeding behavior.

144

REVERSE GENETIC ANALYSIS OF *AEDES AEGYPTI* SALIVARY PROTEIN GENES USING CRISPR/CAS9

Bianca B. Kojin, Zachary N. Adelman

Texas A&M, College Station, TX, United States

Mosquito saliva is composed of a complex cocktail of proteins that provide anti-hemostatic, vasodilatory, imunomodulatory and sugar digestion functions. While the sialomes of major disease vector species have been characterized, the majority of proteins identified on those studies have no known function. With advances in genome engineering tools, particularly CRISPR/Cas9 tool, reverse genetic experiments have become tractable in disease vector mosquitoes. We used CRISPR/Cas9 to generate loss-of-function knock out strains for four different salivary gland proteins. Target proteins were selected based on expression levels in female salivary glands of the vector *Aedes aegypti,* including both prior proteomic and transcriptomic analyses. For each loss of function mutant, we analyzed mosquito probing time and duration of blood acquisition, as well as impact on fecundity and fertility.

145

VECTORBASE.ORG: NEW OMICS AND POPULATION BIOLOGY DATASETS FOR INVERTEBRATE VECTORS OF HUMAN PATHOGENS

Gloria I. Giraldo-Calderón[1], Daniel Lawson[2], Scott J. Emrich[3], Mary Ann McDowell[1], VectorBase consortium[4]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Imperial College London, London, United Kingdom,* [3]*University of Tennessee, Knoxville, TN, United States,* [4]*EMBL-EBI, Hinxton, United Kingdom*

VectorBase.org is a free online resource to all users, that started 15 years ago with the genome of *Anopheles gambiae* PEST. Currently, it hosts 40 genomes including vector mosquitoes, kissing bugs, tsetse flies, ticks, sand flies, body lice, non-vector species, and a snail intermediate host. A summary of our data sets, including major changes since April 2018 is provided here. The new *Aedes aegypti* Liverpool AGWG genome (AaegL5) is now available. Transcriptomes and proteomes, under different experimental conditions, are available for 37 and 2 species, respectively. Variation data sets, with >50 million SNP calls for *A. gambiae* (mostly from the Ag1000g), and between 0.29 to 12.9 million SNP calls, in other 15 species are available via the new Search (tool) capabilities. The population biology (PopBio) data comes from ~400 species of mostly field origin, and is divided in different map views including >20k and >13k insecticide resistance phenotypes and genotypes assays, respectively, >28 million population abundance mosquitoes records, >48k pathogen infection status assays. The most recent addition is >49k blood meal source assays. New Map views are also in development, including one for 'barcodes'. One of the largest to date (April 2019) PopBio datasets, is the one from Iowa (USA), for which we display more than 40 years of population abundance data going back to 1969. The PopBio map is used

for visualization, search, analysis and also for raw data download. For all data submitted to VectorBase the user will obtain the proper identifier, a project ID for PopBio data, VBPxxxxxxx or similar for other data types. For all data hosted by VectorBase, the source including scientific papers or, DOI numbers for unpublished datasets are provided. This latter case is more common for PopBio population abundance records, that mostly come from mosquito and vector control districts, and not from research projects. Interested users can request private or public webinars.

146

ADDITIONAL BLOOD-FEEDING REVEALS DIFFERENCES IN OOCYST SURVIVAL AND GROWTH BETWEEN *PLASMODIUM* SPECIES IN *ANOPHELES GAMBIAE*

Hyeogsun Kwon[1], **Rebekah Reynolds**[1], Maria L. Simões[2], George Dimopoulos[2], Ryan Smith[1]

[1]*Iowa State University, Ames, IA, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Blood-feeding is an integral determinant of mosquito-borne disease transmission in which the mosquito must take at least two blood meals during its lifespan to acquire, as well as transmit the pathogen. The vast majority of studies assessing mosquito vector competence simply examine pathogen infection as a single endpoint, without consideration of the influence of additional blood meals during the extrinsic incubation period (EIP) on the pathogen once initially acquired. To determine the role of an additional blood meal on malaria parasite development in *Anopheles gambiae*, we examined rodent and human malaria parasites with and without an additional non-infected blood meal. Blood-feeding has no effect on mature oocysts that have begun sporogony, yet significantly influence immature oocyst development revealing important differences in between *Plasmodium berghei* and *P. falciparum* adaptation to their mosquito host. An additional blood meal following an infection with the laboratory model *P. berghei* resulted in a significant reduction in immature oocyst numbers. A similar phenotype resulted when mosquitoes were fed on an artificial protein meal, suggesting that this reduction in parasite numbers is not due to physiological effects of blood feeding, but instead due to the distension of the mosquito midgut enabling complement recognition of immature oocysts. In contrast, an additional blood meal did not reduce *P. falciparum* oocyst survival, suggesting that these parasites evolved a mechanism of complement immune evasion. Moreover, *P. falciparum* parasites were significantly larger following a second blood or protein meal, a phenotype not seen with *P. berghei*. This argues that human malaria parasites developed mechanisms to evade immune recognition as well as benefit from the additional nutrients provided with a second mosquito feeding. Together, these data demonstrate additional mechanisms that determine mosquito vector competence and provide support that human malaria parasites utilize nutrients provided by a second blood meal to enhance oocyst growth and potentially decrease the EIP required for malaria parasite transmission.

147

AEDES SPECIES COMPOSITION AND ABUNDANCE IN NORTHEASTERN STATE OF ADAMAWA, NIGERIA

Iliya Shehu Ndams, Ibrahim Isa, Maryam Aminu, Thaddeus T. Gbem, Gloria Chetchet

Ahmadu Bello University Zaria, Zaria, Nigeria

The study focused on the occurrence, distribution and abundance of Aedes species in communities displaced by lingering upheaval in the north-eastern region of Nigeria. *Aedes* species were sampled from three locations namely; Mubi, Yola and Numan towns from July to October, 2017. They were collected using human bait methods and identified to species level using microscopy and coloured morphological identification keys of Rueda. The abundance of each species of *Aedes* identified was expressed in percentages while diversity was revealed using Shannon-Wiener diversity index. A total of 768 mosquitoes were examined, higher abundance of *Ae. aegypti* species were observed in Numan (53.3%;176/330), while lower species occurrence was recorded in Mubi (12.7%; 42/330). The abundance of *Ae. africanus* was highest and lowest in Yola (66.4%;146/220) and Numan (12:7%; 28/220) respectively. *Aedes hensili* showed higher abundance in Yola (43.1%; 88/204) while lower number (16.7%; 34/204) was recorded in Mubi. Also, higher occurrence of *Ae. triseriatus* (77.8%:7/9) was seeing in Numan whereas it was absent in Mubi. There was higher occurrence of *Ae. albopictus* (40%:2/5) in Mubi and Yola, whereas lower species abundance was recorded in Numan (20%:1/5). However, Shannon index showed higher diversity and evenness (H′= 0.06; e^H/S = 0.96) of *Ae. albopictus*, followed by *Ae. hensili* (H′=1.03; e^H/S=0.93) and Ae. aegypti (H′=0.96; e^H/S=0.87) while lower abundance of *Ae. triseriatus* (H′= 0.53) was observed in the three locations. This study indicates that *Ae. aegypti, Ae. africanus Ae. hensili, Ae. triseriatus* and *Ae. albopictus* are abundant and well diverse. The large distribution and evenness of *Ae. albopictus* observed may result from the increase in international travel and urbanization. This posed a potential danger of spreading infections like dengue, yellow fever, chikungunya and zika viruses

148

QUANTIFYING THE RISK OF CHIKUNGUNYA TRANSMISSION IN AUSTRALIA UNDER CLIMATE CHANGE

B.M.C. Randika Wimalasiri-Yapa[1], Liesel Stassen[1], Xiaodong Huang[1], Gregor J. Devine[2], Francesca D. Frentiu[1]

[1]*Queensland University of Technology, Brisbane, Australia,* [2]*Queensland Institute of Medical Research, Brisbane, Australia*

The recent worldwide epidemics of Chikungunya virus (CHIKV) represent a global public health threat. The geographical expansion of this mosquito-borne virus in Southeast Asia and the Pacific region necessitates the accurate forecasting and prediction of transmission risk in Australia. The vector competence of *Aedes* (*Ae.*) *aegypti*, the primary vector of CHIKV, is shaped by the interaction of vector and virus genotype with environmental temperature. Here, we investigate the vector competence of local *Ae. aegypti* for CHIKV strains from Asian and East/Central/South Africa (ECSA) lineage. Specifically, we evaluate the impact of environmental temperature and virus genotype on CHIKV transmission and the extrinsic incubation period. *Ae. aegypti* mosquitoes were orally challenged with a CHIKV strain from either Asian or ECSA lineage (10^7 pfu/ml), and maintained at a constant temperature of either 18°C, 28°C or 32°C. At 3, 5- and 7-days post-infection (dpi), CHIKV RNA copies were quantified in mosquito bodies, and wings and legs using qRT-PCR, while virus in saliva was amplified in cell culture and cytopathic effect observed in Vero cells. Overall, *Ae. aegypti* displayed high susceptibility and rapid viral dissemination of both CHIKV genotypes across all three temperatures. However, viral transmission was temperature and strain-dependent, with higher temperatures (28ºC and 32ºC) generally translating into more infectious mosquitoes and a shorter extrinsic incubation period (EIP) for both genotypes. There were significant treatment effects of temperature, genotype, and the interaction of temperature by day post-infection (dpi) on body and disseminated viral titres. Our results suggest a high risk of local transmission of the Asian genotype of CHIKV if introduced into North Queensland where *Ae. aegypti* are present. Based on our empirical data, we present models of transmission probabilities for this virus under different climate change scenarios and geographic areas in Australia.

149

MULTIPLE BLOOD FEEDING: A FORCE MULTIPLIER FOR MALARIA TRANSMISSION

Riley E. Tedrow[1], Ernest Chan[1], Tovonahary A. Rakotomanga[2], Thiery Nepomichene[3], Rosalind Howes[4], Jocelyn Ratovonjato[2], Arséne C. Ratsimbasoa[2], Gavin J. Svenson[5], Karen C. Abbott[1], Peter Zimmerman[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*National Malaria Control Program, Antananarivo, Madagascar,* [3]*Institut Pasteur*

Madagascar, Antananarivo, Madagascar, [4]The Foundation for Innovative New Diagnostics, Geneva, Switzerland, [5]Cleveland Museum of Natural History, Cleveland, OH, United States

Modeling serves as a tool for disease elimination by helping us to understand the complicated processes that dictate vector-borne disease transmission. Malaria transmission models rely on several entomological parameters extrapolated from field-collected data. When data from the field is sparse or difficult to interpret, it is crucial to evaluate how model predictions rely on unknown quantities or uncertain assumptions. One of the classic entomological parameters for malaria transmission, the human feeding rate (*a*), is calculated as the product of the proportion of bites taken on a human (*Q*) and the number of bites a mosquito takes per gonotrophic cycle (*f*). However, *f* is ubiquitously taken to equal 1, meaning the number of bloodmeals taken by a mosquito is equivalent to the number of gonotrophic cycles it has undergone. Numerous field studies, including several entomological surveys we have recently conducted in Madagascar and Papua New Guinea, indicate a violation of this assumption. Altering this parameter can significantly change the conclusions derived from transmission models, leading to different recommendations for disease control. Our analysis highlights how violating this assumption, a phenomenon known as multiple blood feeding (MBF), impacts malaria transmission models. To assess the impact of MBF, we show the effect of raising the number of bites per gonotrophic cycle on several important metrics, including the entomological inoculation rate, the vectorial capacity, and the proportion of living, infectious vectors in a population. We consider the effects of MBF in low, moderate, or high transmission environments (by altering values for other parameters that determine overall transmission intensity). Our data suggeststhat MBF causes a substantial increase in infection risk and warrants further investigation to determine the significance of this behavior for malaria control.

150

IDENTIFYING THE MOST SUITABLE SPATIAL SCALE FOR MICROGEOGRAPHIC MALARIA VECTOR STUDIES

Edgar Manrique[1], Gabriel Carrasco-Escobar[2], Jorge Ruiz-Cabrejos[1], Joseph M. Vinetz[3], Dionicia Gamboa[4], Manuela Herrera-Varela[1], Jan E. Conn[5]

[1]*Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Division of Infectious Diseases, Medicine School, University of California San Diego, San Diego, CA, United States, [3]Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT, United States, [4]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [5]Wadsworth Center, New York State Department of Health, Albany, NY, United States*

Malaria transmission in the Peruvian Amazon has been characterized by high temporal and spatial heterogeneity, and associated with the presence of water bodies, deforested areas and other environmental variables that allow mosquito population to thrive. For this reason, remote sensing is widely used to detect landscape composition. Recently, the use of drones was explored as a tool to map malaria vector breeding habitats in a range of landscapes. While this presents many advantages compared with satellite data, including better spatial resolution, this does not necessarily translate into better spatiotemporal modeling. Here we aim to distinguish the most appropriate spatial resolution of drone imagery to analyze landscape characteristics with malaria vector abundance in the Peruvian Amazon. Data collection was conducted during June and July, 2018 in six villages in the Mazan district of Loreto, Peru. Anopheline mosquitoes were collected using human landing catch in 28 locations distributed across peri-domestic areas. Villages were mapped using a drone equipped with a multispectral camera. In order to determine the most suitable spatial resolution, first we calculated normalized difference vegetation and red edge index (NDVI and NDRE) from the imagery, and distances to water bodies and forest from manually digitized areas. The outputs were resampled from 0.5 m to 10 m, at 0.5 m intervals. We then extracted the average values of these parameters using a 50 m buffer from each mosquito collection point for each spatial resolution. A generalized linear model (GLM) was fitted with the mosquito abundance as the response variable. We found that images with 9 m spatial resolution provided the best fit to the model based on the Akaike information criteria. On average, the surroundings of the collection points (50 m buffer) at 9 m resolution had low values of NDVI (mean = 0.2, CI= 0.17-0.23) and NDRE (mean = 0.06, CI= 0.04-0.07), and a distance to water bodies of 39.23 m (CI= 28.58 - 49.88 m). This methodology will allow researchers to fine-tune regression and classification models to determine the relationship of the landscape and malaria vector abundance.

151

MALARIA IN VENEZUELA: ENTOMOLOGICAL SURVEILLANCE IN A CHALLENGING OPERATING ENVIRONMENT

Yasmin Rubio[1], Melfran Herrera[2], Jorge Moreno[3], Darjaniva Molina de Fernandez[4], Luisa Figueroa[4], Leopoldo Villegas[5]

[1]*Universidad de Carabobo, Maracay, Bolivarian Republic of Venezuela, [2]Fundasalud Sucre, Cumana, Bolivarian Republic of Venezuela, [3]Centro de Investigacion de Campo Dr. Francesco Vitanza, Tumeremo, Bolivarian Republic of Venezuela, [4]Instituto de Altos Estudios "Dr. Arnoldo Gabaldon", Maracay, Bolivarian Republic of Venezuela, [5]Asociacion Civil Impacto Social (ASOCIS), Tumeremo, Bolivarian Republic of Venezuela*

Malaria in Venezuela has increased exponentially in the past 10 years due to several technical, socioeconomic, and political reasons. Vector control activities have been reduced to sporadic and insufficient distribution of LLINs. Entomological surveillance (ES) at country level has been reduced to three teams with limited resources working in Bolivar and Sucre States, which reported 72% and 12% of the total malaria cases in the country in 2018. In Bolivar State (Sifontes Municipality) all night mosquito collections using mosquito magnet traps were conducted in 5 villages along the road that communicates Venezuela with Brazil (8 gold mining camps and 2 towns). The most abundant species were *Anopheles darlingi*, *An albitarsis* and *An nuneztovari* in villages along the road and in mining camps. These species are resistant to organophosphates insecticides but susceptible to carbamates and pyrethroids. *An darlingi* bites throughout the night, *An nuneztovari* has a biting peak between 1800 and 2200 hrs. More mosquitoes were caught outdoors. Unfed mosquitoes were collected indoors and outdoors, but no blood-fed mosquitoes were found. Larval habitats were identified and characterized. ES in remote Amerindian villages were conducted by local leaders in Sucre Municipality (Bolivar State). The most abundant species were *An darlingi*, *An nuneztovari*, and *An oswaldoi*; *An darlingi* showed a different biting pattern with a peak around midnight, *An nuneztovari* has a peak early in the evening and at sunrise and *An oswaldoi* has a peak between 1800 and 2200 hr. Larval habitats were identified and characterized. All night human landing catches indoors and outdoors were conducted in 23 villages of Sucre state. Only *An aquasalis* was collected, mainly outdoors; biting behavior varies locally but over 70% of biting occurs between 1800 and 1900 hrs. Parous rate fluctuated around 55%. In general malaria vectors in Venezuela bite mainly in the first 4 hours of the night outdoors, making the conventional vector control measures available IRS and LLINs less effective to reduce transmission. Integrated vector control strategies should be based on local entomological indicators.

152

AEDES POPULATION AND ENTOMOLOGICAL INDEX RISK OF ARBOVIRUS TRANSMISSION DURING A CHIKUNGUNYA OUTBREAK IN DR CONGO

Fabien Vulu Zimbombe[1], Gillon Ilombe[2], Placide Mbala[2], Pitshou Mampuya[1], Mitterrand Moyo[1], Veerle VanLeberghe[3], Seth Irish[4], Steve Ahuka[2], Thierry Bobanga[1]

[1]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [2]*National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo,* [3]*Institute of Tropical Medicine, Antwerp, Belgium,* [4]*Center For Disease Control and Prevention (CDC), Atlanta, GA, United States*

Chikungunya fever is an expanding viral disease. Kinshasa (DR Congo) has faced chikungunya fever outbreaks for about twenty years. The on-going outbreak seems to be larger than previous outbreaks. Probable and confirmed chikungunya cases have been found in many health areas of Kinshasa and Kongo Central provinces. Since vector control activities are essential in chikungunya control, this study aimed to determine *Aedes* populations, their breeding sites and entomological risk index of arbovirus transmission. We conducted *Aedes* adult catches and larvae collections in many sites in Kinshasa and Kongo Central provinces from 6 to 28 February 2019. Larvae were reared into adult stage and with adult wild caught mosquitoes were identified to species following Highton morphological key. The entomological risk index were determined according WHO criteria. We collected 1,172 *Aedes* (359 wild adults and 813 adults reared from larvae) which belonged to 3 species; *Ae. aegypti* (15.4%), *Ae. albopictus* (84.3%), and *Ae. vittatus* (0.17%). In all sites *Ae. albopictus* (62 - 100%) was the predominated species. Water storage containers (27.4 - 51.1%), discarded tires (14.2 - 30.1%), and discarded plastic containers (16.6 - 23.8%) were predominant breeding sites. House index, container index, and Breteau index ranged from 24 to 59%, 18 to 67%, and 22 to 59 positives containers/100 houses. *Aedes* densities found were sufficient to promote chikungunya outbreak. This study is relevant since *Aedes* were collected during chikungunya outbreak. Risk index found can be used as reference for *Aedes* surveillance activities. It would be useful to test *Aedes* mosquitoes of different species to determine which species are involved in transmission of chikungunya and other arboviruses.

153

EFFECT OF MIXED CROP IRRIGATION SYSTEM ON MALARIA VECTOR POPULATION ON WESTERN KENYA

Benyl Ondeto[1], Guofa Zhou[2], Ming-Chieh Lee[2], Harrysone Atieli[3], Simon Muriu[4], David Odongo[1], Horace Ochanda[1], Andrew K. Githeko[5], Guiyun Yan[2]

[1]*University of Nairobi, Nairobi, Kenya,* [2]*University of California, Irvine, CA, United States,* [3]*Maseno University, Kisumu, Kenya,* [4]*Pwani University, Mombasa, Kenya,* [5]*Kenya Medical Research Institute, Kisumu, Kenya*

Environmental degradation exacerbated by climate change has necessitated adaptation programs such as irrigation, in order to increase food security. However, irrigation may increase the risk of malaria. This study was undertaken in a semi-arid area, Homa Bay County, Western Kenya where mixed crop irrigation in ongoing. Vector control using long-lasting insecticidal nets and indoor residual spraying is underway. The study was conducted from January to December 2018. Indoor resting anopheline mosquitoes were collected using pyrethrum spray catches in two sites, irrigated and non-irrigated zones. Mosquitoes were morphotyped and analyzed for spatiotemporal density variation. *Anopheles gambiae* sibling species were identified by polymerase chain reaction (PCR). Of the 449 vector *anopheles* mosquitoes collected 94.9% were from the mixed irrigated zone while 5.1% were from the non-irrigated zones; they comprised of *An. gambiae* s.l. (89.8%) and *An. funestus* s.l. (10.2%). In the mixed irrigated (n=426) zone *An. gambiae* s.l. comprised 90.4% and *An. funestus* s.l. 9.6%. In the non-irrigated zone *Anopheles gambiae* s.l. was also dominant 78.3% compared to *An. funestus* s.l. 21.7 %. PCR analysis (n=79) indicated that all the *An. gambiae* s.l. were *An. arabiensis*. There was no significant variation in *An. arabiensis* densities between wet and dry season in either irrigated areas (t_7 = 0.1, p>0.05) or in non-irrigated areas (t_7 = 0.4, p>0.05). The irrigation systems increased vector density and the risk of malaria transmission. The application of IRS may have suppressed vector density seasonality. Previous studies in a similar ecosystem in the region have indicted that *An. arabiensis* is significantly zoophilic.

154

RESTING BEHAVIOR OF MALARIA VECTORS IN A HIGHLAND AND LOWLAND AREA OF WESTERN KENYA

Maxwell G. Machani[1], Eric Ochomo[2], Fred Amimo[3], Stephen Munga[1], Andrew K. Githeko[1], Guiyun Yan[4], Yaw Afrane[5]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Entomology Section, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [3]*School of Health Sciences, Jaramogi Oginga Odinga University, Kisumu, Kenya, Bondo, Kenya,* [4]*Program in Public Health, College of Health Sciences, University of California, Irvine, CA, United States,* [5]*Department of Medical Microbiology, College of Health Sciences, University of Ghana, Ghana, Ghana*

Major progress in malaria control has been achieved in sub-Saharan Africa in the last two decades. Despite demonstrated success in reducing human-mosquito interactions, continual transmission has been experienced posing a significant challenge to malaria elimination efforts. The aim of this study was to assess the resting behavior, host preference and infection with *Plasmodium falciparum* sporozoites by malaria vectors in the light of emerging insecticide resistance in western Kenya. Indoor and outdoor resting *Anopheline* mosquito were sampled during the dry and rainy seasons in Kisian (lowland site) and Bungoma (highland site), both in western Kenya. PCR was used for mosquito speciation, genotype for resistance mutations and for specific host blood meal origins.ELISA was used to determine sporozoite infections. *Anopheles gambiae* s.l was prevalent (74.7%) followed by *Anopheles funestus* s.l (25.3%.) Majority of *Anopheles funestus* (97 and 100%) and *Anopheles gambiae* s.l (85.8 and 58%) were resting indoors in Bungoma and Kisian respectively. *Anopheles gambiae s.s* was the dominant sibling species in Bungoma followed by *Anopheles arabiensis* 7.6%, whereas in Kisian, *An.arabiensis* was dominant 60.2% followed by *An. gambiae* s.s 38.9%. Vgsc-1014S was observed at high frequency in *An. gambiae s.s* than *An.arabiensis*. Vgsc-*1014F* and *Ace1 G119S* mutations were detected in *An. gambiae s.s* resting indoors in Bungoma but at very low frequencies (0.6 and 0.1% respectively). In Bungoma, human blood index (HBI) for *An. gambiae*, *An .arabiensis,* and *An. funestus* resting indoors were 64.7, 25 and 74% and (46.2, 37.5 and 75% respectively) for outdoor. For Kisian, the HBI for the three species resting indoors was 59.7, 7 and 82.4% respectively whilst outdoors was 100 and 6.25% for *An. gambiae* and *An. arabiensis* respectively. Overall, in Bungoma, the sporozoite rate for indoor resting mosquitoes was 8.6 and 4.2 for outdoors. In Kisian the sporozoite rate was 0.9% for indoor resting *An.gambiae.* The occurrence of *Vgsc-1014F* and *Ace1 G119S* mutation calls for further screening and monitoring of other resistance mutations in this population.

155

EVALUATION OF IVERMECTIN BASED ATTRACTIVE TOXIC SUGAR BAIT AGAINST *AEDES AEGYPTI* IN A CONTROLLED ENVIRONMENT

Frank S. Tenywa[1], Revocatus Musiba[2], Fredros Okumu[2], Marta Ferreira Maia[3]

[1]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [2]*Ifakara Health Institute, Ifakara, United Republic of Tanzania,* [3]*Kemri Wellcome Trust Research Program, CGMRC, Kilifi, Kenya*

Use of insecticides for *Aedes* mosquitoes control has been a main focus for eliminating mosquito- borne diseases such as dengue fever. However, emergence of insecticide resistance in the recent years and economic constrains are limiting this approach. This study aimed at determining the dose of ivermectin (IVM) in sugar solution and time needed for knock

down (KD) of 90% of the *Ae. aegypti.* This study also assessed the sugar feeding behaviour of *Ae. aegypti* when a blood host is available; and evaluated the efficacy of an attractive toxic sugar bait against *Ae. aegypti* in a semi field environment. Dose response experiments to determine KD90 of IVM against *Ae. aegypti* were done in laboratory conditions through a serial dilution of IVM in 10% sugar solution in separate cages. Mosquito mortality was observed at 4, 8, 24 and 48 hours post introduction of the treatments. *Ae. aegypti* feeding preference between sugar and blood meal was determined by releasing female *Ae. aegypti* into a cage in which both rabbit and sugar bait were deployed. Mosquito feeding status was detected after 24 hours. Ivermectin was toxic against male and female *Ae. aegypti* at 4‰ IVM dose. Over 90% of male and female *Ae. aegypti* were knocked down 48 hours post sugar feeding on sugar solutions containing at least 4‰ IVM respectively. Sugar bait significantly reduced *Ae. aegypti* blood feeding preference (OR=0.06; 95%CI [0.023-0.134]) within 24 hours. An ivermectin dose (0.04%) is enough to be incorporated into attractive sugar baits (ATSB) to achieve 90% *Ae. aegypti* mortality and would be effective against both sexes of *Ae. aegypti*. The presence of sugar bait will significantly inhibit *Ae. aegypti* blood seeking behaviour compared to if there was no sugar bait. Semi field experiments are ongoing to determine the efficacy of the sugar bait against *Ae. aegypti* in a controlled environment.

156

WHERE DO THE MALARIA VECTORS ACTUALLY REST INSIDE HOUSES?

Betwel John Msugupakulya, Emmanuel Kaindoa, Halfan Ngowo, Fredros Okumu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Countries have set ambitious goals towards malaria control, primarily by scaling up effective interventions such as long-lasting insecticide treated nets (LLINs), indoor residual spraying (IRS) and effective case management. LLINs and IRS particularly target indoor-biting and indoor-resting malaria transmitting mosquitoes. It is therefore important to understand resting behaviours of the major malaria vectors inside houses and how much they can be affected by indoor interventions. This would provide crucial information on where best to direct the interventions to effectively prevent malaria. We investigated the resting behaviors of mosquitoes inside common house types in rural south-eastern Tanzania to identify preferred resting surfaces for the two main malaria vectors, *Anopheles arabiensis* and *Anopheles funestus*. The study houses were selected based on the following inclusion criteria: i) thatched roofs and un-plastered mud walls, ii) thatched roofs and un-plastered brick walls, iii) corrugated iron roofs and un-plastered brick walls, and iv) corrugated iron roofs and plastered brick walls. In each of these houses, mosquitoes were collected from multiple surfaces (floors, walls, roof and ceilings, furniture and utensils, clothes and bed nets) using Prokopack aspirators. Preliminary findings suggest that the two vector species do not only rest on walls, where they could be targeted with IRS, but also on the underside of roofs and other surfaces, such as on bed nets, floors, furniture and utensils. We have detected differences between preferred resting sites between the two major vector species. Additionally, we have observed that different house designs influence the preferred resting surfaces for studied vector species. Particularly, *An. funestus.* are much more of generalists in terms of their resting surfaces compared to *An. arabiensis*, even though both species preferred thatched roofs over iron roofs. Besides, while *An. arabiensis* rest mostly on underside of roofs, *An. funestus* readily also rests on other surfaces as well, such as the walls and on bed nets.

157

MOSQUITO ABUNDANCES AND BEHAVIOR SUPPORT POTENTIAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN COLORADO

Daniel A. Hartman, Justin DeMaria, Lauren M. Rice, Erin M. Borland, Nicholas A. Bergren, Anna C. Fagre, Lucy L. Robb, Colleen T. Webb, Rebekah C. Kading

Colorado State University, Fort Collins, CO, United States

Rift Valley Fever Virus (RVFV) poses a major threat of introduction to several continents, including North America. Such an introduction could cause significant losses to the livestock industry, in addition to substantial human morbidity and mortality. Because of the opportunistic blood host selection of *Cx. tarsalis* mosquitoes in this area, we hypothesized that this species could be a locally important bridge vector of RVFV in the event of an introduction. We investigated the mosquito diversity community composition at livestock feedlots and surrounding natural and residential areas to determine differences in mosquito relative abundance and blood feeding patterns attributed to cattle feeding operations. Blood meals from engorged mosquitoes were sequenced to determine blood source, and these data were used to model host-selection behaviors by habitat type. Multivariate regression analyses revealed differences between mosquito community assemblages at feedlots and non-feedlot sites, with this effect driven largely by differential abundances of *Ae. vexans*. Mosquito diversity was lower on feedlots than surrounding areas for 3 out of 4 feedlots. *Cx. tarsalis* was abundant at both feedlots and nearby sites. Diverse vertebrate blood meals were detected in *Cx. tarsalis* at non-feedlot sites, with a shift towards feeding on cattle at feedlots. These data support a potential for *Cx. tarsalis* to serve as a bridge vector of RVFV between livestock and humans in Colorado.

158

A RETROSPECTIVE SURVEILLANCE OF THE OCCURRENCE OF FLAVIVIRUSES IN *AEDES* MOSQUITOES IN PHETCHABUN PROVINCE RELATED TO ENVIRONMENTAL FACTORS

Pornsawan Leaungwutiwong

Faculty of Tropical Medicine, Bangkok, Thailand

Mosquito-borne diseases are related to the medically important viruses in the Family *Flaviviridae*. Zika virus (ZIKV) and Dengue virus (DENV) are transmitted to humans by the bite of the infected mosquitoes. The most important vector is *Aedes* mosquito. Environmental factors such as temperature, relative humidity, and biting rate affect dengue virus infection. In addition, surveillance of field-caught mosquitoes is imperative for determining the natural vector and can provide an early warning sign at risk of transmission in an area. In this study, *Ae. aegypti* mosquitoes were collected in Phetchabun Province, Thailand in 2004-2005. The mosquitoes were collected in the rainy and dry season both indoor and outdoor. During mosquito's collection, the data of environmental factors were observed and recorded. Mosquitoes were pooled according to genus/species, and sampling location. Pools consisted of 10 mosquitoes. Ninety-eight pools of 939 *Aedes* mosquitoes were screened with PCR assay for Pan-flavivirus, ZIKV and DENV, respectively. Two pools were detected as DENV positive and no ZIKV was detected from the mosquito. To confirm individual infection for determining true infection rate, the mosquitoes which gave positive DENV infection were tested for dengue virus by RT-PCR method. Four individual *Ae. aegypti* mosquitoes were detected as DENV serotype 4. The infection rate of DENV in this study was 0.43%. Moreover, the probability to detect dengue virus in mosquitoes at the neighbor's houses was 1.25 times, especially where distances between neighboring houses and patient's houses were less than 50 meters. The relative humidity in dengue-infected villages with dengue-infected mosquitoes was significantly higher than villages that free from dengue-infected mosquitoes. Indoor biting rate of *Aedes aegypti* were 14.87 times higher than outdoor, and biting times of 09.00-10.00, 10.00-11.00, 11.00-12.00 yielded 1.77, 1.46, 0.68mosquitoes/man-hour, respectively.

These finding confirm environmental factors were related to Dengue infection in Thailand. Data obtained from this study will be useful for prevention and control of the diseases.

159

AN EPIDEMIOLOGIC SURVEY AND RISK FACTORS ASSOCIATED WITH LYMPHATIC FILARIASIS IN THE MIDDLE BELT OF GHANA

Dorcas Atibilla[1], David K. Dosoo[1], Joseph Ana-aro[1], Yussif Tawfiq[1], Love Ankrah[1], Gabriel Jakpa[1], Kingsley Kayan[1], Seth Owusu-Agyei[2], Kwaku Poku Asante[1], Felix B. Oppong[1], Michael D. Wilson[3]

[1]*Kintampo Health Research Centre, Kintampo-Ghana, Ghana,* [2]*University of Health & Allied Sciences PMB* [31]*, Ho, Ghana,* [3]*Noguchi Memorial Institute for Medical Research, Accra-Ghana, Ghana*

Lymphatic filariasis has been shown to be endemic in rural areas of Ghana with regional variations in prevalence and disease manifestations. A community based survey was conducted using Binax Now Immuno-Chromatographic Test (ICT) cards for screening participants. Pyrethrum spray collections (PSC) method for mosquito collections, dissection of mosquitoes as well as microscopy was also done. To assess the prevalence, 1320 participants aged between 2 and 80 years in 21 communities were screened during the day time using (ICT) cards. Participants positive with ICT were followed up for night-time finger-prick blood sampling (10 pm and 12 midnights). From a total of 1101 Anopheles spp. collected,500 mosquitoes were dissected for filarial worm examination. No filarial worm was present in the dissected mosquitoes, and hence the absence of active transmission of lymphatic filarasis in the study area. From the screening, a total of 82 (6.2%) out of the 1320 were antigen positive with the day-time samples using the ICT cards. Surprisingly, microscopy results later confirmed the filarial present as Mansonella perstans with a prevalence rate of 39.0% (32/82). There is an indication of a possible cross reactivity of the Binax Now ICT cards with Mansonella perstans since the ICT cards are mostly used for screening Lymphatic filariasis. From the survey, low ITN use,poor housing structure, type of roof and the absence of netting on windows were also some of the associated risk factors.

160

EFFECTS OF NECTAR PHYTOCHEMICALS ON LIFE-HISTORY TRAITS OF THE INVASIVE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*

Teresia Njoroge[1], May Berenbaum[2], Chris Stone[3]

[1]*University of Illinois at Urbana-Champaign, Urbana, IL, United States,* [2]*University of Illinois at Urbana-Champaign, URBANA, IL, United States,* [3]*Illinois Natural History Survey, Champaign, IL, United States*

Plant nectar is an essential component of adult mosquito diets. During nectar-feeding, mosquitoes ingest various phytochemicals, including phenolics, terpenoids, and alkaloids. Compared to other nectar-feeders (e.g., bees), the ecological significance of nectar phytochemicals for mosquitoes has not been extensively explored. The aim of this study was to conduct laboratory-based assays to evaluate the effects of nectar phytochemicals on the longevity, fecundity and sugar-feeding behavior of adult female *Aedes albopictus*. Longevity was assessed by subjecting newly emerged females to 10% sucrose solution containing quercetin, *p*-coumaric acid and caffeine and recording daily mortality. For fecundity assays, the mosquitoes were subjected to the same dietary phytochemicals for seven days and eggs counted following a single blood meal. The cold-anthrone test was used to quantify the amount of sugar consumed by mosquitoes exposed to the dietary phytochemicals. Dietary quercetin and *p*-coumaric acid were associated with lifespan extension (mean longevity= 69 and 67 days, respectively, compared to control =54 days). Although caffeine ingestion resulted in low sugar consumption (33.84 μg/μl) compared to the control group (67.84 μg/μl), none of the three phytochemicals affected the fecundity of gravid females. Our results demonstrate that dietary phytochemicals can affect mosquito longevity and sugar-feeding behavior and thereby may influence their fitness and vectorial capacity. We are currently conducting an experiment using a whole transcriptome-based approach to identify mosquito-nectar phytochemical interactions at the genomic level.

161

CLIMATE EFFECTS RELATED TO OUTBREAKS OF ACUTE CASES OF CARRION DISEASE IN PERU, 2000 - 2016

Gabriela Ulloa U.[1], César Munayco[2], Andree Valle[1], Andrés Lescano[1]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Centro de Control y Prevención de Enfermedades del Perú (CDC Perú), Lima, Peru*

Introduction Carrion disease (CD), present in Peru, Ecuador and Colombia, is considered an emerging disease due to its expansion to new areas. This is related to the migration of asymptomatic carriers and climate change. It has been demonstrated that climatic factors are associated with the incidence of vector-borne diseases. However, there is little information on the relationship of climatic factors associated with possible outbreaks of CD. Therefore, we analyze the relationship between climatic events and the distribution of acute cases of CD in order to develop future prevention and control plans that anticipate future events. Methods: Cases recorded by the CD surveillance system of the Centers for Disease Control and Prevention of Peru during the period 2000 - 2016 were analyzed. Epidemiological weekly data on environmental temperature (ºC) and rainfall (mm/day), obtained from NASA satellites and atmospheric models, were used. Possible outbreaks at district level were selected from the cumulative sum of acute cases of CD. The climatic variables of the weeks before the possible outbreaks were selected. Multiple regression with Poisson distribution, Stepwise Forward method and likelihood ratio test (LRT) were applied to select the variables that entered the final model, Stata statistical package and RStudio program were used. Results: Five of 11 departments had the highest number of possible CD outbreaks (84.9%) in Peru. Precipitations four and five weeks before the start of an "outbreak" presented significant association with the number of acute cases accumulated at the start of a possible outbreak of CD ($p<0.01$). However, the minimum ambient temperature was not significantly associated. The association observed between the increase in the number of cases and climatic variables could be related to the population dynamics of the vector. That is to say, the increase of the population density and variations in the transmission capacity of the etiological agent. Future studies should include the population density of the vector as a variable for a better explanation of climatic factors in the epidemiology of CD.

162

MOSQUITO DYNAMICS AND MALARIA IN ALULU-NIKE COMMUNITY, ENUGU-EAST LOCAL GOVERNMENT AREA, ENUGU STATE, NIGERIA

Pauline U. Umeanaeto[1], Angus E. Onyido[1], Martin O. Ifeanyichukwu[2], Joseph U. Anumba[3]

[1]*Nnamdi Azikiwe University, Awka, Nigeria,* [2]*Department of Medical Laboratory Sciences, Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi, Nigeria,* [3]*National Arbovirus and Vectors Research Centre, Federal Ministry of Health, Enugu, Enugu, Nigeria*

Mosquito dynamics and malaria were studied in Alulu-Nike Community, Enugu-East Local Government Area, Enugu State, Nigeria for a period of 12 months. Malaria was diagnosed using microscopy method. Mosquito collection was done using pyrethroid knockdown collection, human bait and larval collection methods. Mosquito identification was done using morphological characteristics and PCR. A total of 1440 people were screened for malaria and 476(32.4%) were infected. The overall mean malaria intensity was 545 parasites per microlitre of blood (p/μl). Nnegbune village had the highest malaria prevalence 86 (41.5%) which was significant (P&lt0.05) and the highest mean malaria intensity 741 p/μl which was not significant (P&gt0.05). Age 0-10 years had the highest malaria prevalence 229(44.7) and mean intensity 991 p/μl which was

statistically significant (P&lt0.05). Malaria prevalence and intensity were not dependent on sex and occupation of the participants (P&gt0.05) but on educational status (P&lt0.05). PCR characterization of indoor biting and resting adult mosquitoes revealed *Anopheles gambiae ss*and *A. arabiensis* and *A. funestus* asmalaria vectors which constituted 764 (77.1%) of the total mosquitoes collected while the Culicines accounted for 226(22.8%) of the total indoor collection. *A. gambiae sl*7(1.0%) was the only malaria vector collected while other species of Culicine mosquitoes accounted for 687(98.99%) of the outdoor collections. *A. gambiae*yielded 45(3.9%) while Culicine mosquitoes yielded 1,117(96.1%) of the total larval sampling. Mosquitoes were more abundant in wet months than in dry months. The distribution of indoor and outdoor mosquitoes and larvae in different villages and in different months showed significant results (P&lt0.05). Entomological study of mosquitoes is important for planning and optimizing malaria control in both time and space.

163

DISTRIBUTION OF MOSQUITO SPECIES IN KADUNA METROPOLIS, KADUNA STATE NORTHERN NIGERIA

Hosea Chigari Yayock[1], Iliya S. Ndams[2], Ezekiel Kogi[2], Ado Baba Ahmed[1], Jonathan B. George[3]

[1]*Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria,* [2]*Department of Zoology, Ahmadu Bello University Zaria, Zaria, Nigeria,* [3]*Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria*

Entomological survey was conducted to determine the diversity and abundance of mosquito species in relation to their malaria implication in the fast growing city of Kaduna located in Northern Guinea Savannah vegetation belt of Nigeria. The metropolis was sectioned into old and new settlement areas. Larval samples were used to identify 15 species of mosquito morphologically from 15 randomly selected settlement areas in Kaduna metropolis. *Culex pipiens* complex comprise 80.42% of the total catch (6,991 specimens). *Anopheles* species (0.06%) rarely occurred within Kaduna metropolis. Government Reserved Area; one of the old settlements had the highest number (8) of individual mosquito species. There were 4 species, which exclusively occurred in the old settlement areas and 2 species exclusively occurred at the new settlement areas. The mosquito species diversity (H′) and the similarity between the old and new settlement areas (H′ = 3.079; 3.128 old and new settlement areas respectively and Sorenson's coefficient was calculated (CC) = 0.667). There was no significant difference in the total mosquito species sampled between the two settlement areas (t = 0.436). This research identified *Culex pipiens quinquefasciatus* as the most abundant mosquito species within Kaduna Metropolis; thus further research should examine the implication for filariasis in this area.

164

EMERGENT VIRUSES AND THEIR INTERACTIONS IN *AEDES AEGYPTI*: MAYARO AND ZIKA VIRUS COINFECTED MOSQUITOES CAN SUCCESSFULLY TRANSMIT BOTH PATHOGENS

Marco Brustolin, Sujit Pujhari, Cory Henderson, Jason Rasgon

The Pennsylvania State University, University Park, PA, United States

The emergence of arboviral outbreaks represents one of the most challenging issues for human and animal health and can be complicated by the simultaneous circulation of multiple arbovirus in affected regions. However, little is known about the vector-pathogen interactions during a co-circulation scenario. In this study we explored the effect of simultaneous intake of Mayaro virus (*Togaviridae*) and Zika virus (*Flaviviridae*) on *Ae. aegypti* vector competence. After exposing *Ae. aegypti* mosquitoes to an infectious blood meal containing either one or both pathogens, we analyzed viral titers in body, legs and saliva using a focus forming assay at day 7 and 14 post feeding. Results demonstrate that *Ae. aegypti* can support both viruses concurrently and that coinfected mosquitoes can efficiently transmit both viruses at the same time. The data also suggest that these pathogens can interact in complex ways during the co-infection process.

165

IMPACT OF HOUSEHOLD CHARACTERISTICS ON *AEDES AEGYPTI* ABUNDANCE IN RURAL ECUADOR

Rachel Sippy[1], Froilan Heras[2], Anna Stewart Ibarra[1], Sadie Ryan[3], Erin Mordecai[4]

[1]*SUNY-Upstate Medical University, Syracuse, NY, United States,* [2]*Salud Comunitaria, Machala, Ecuador,* [3]*University of Florida, Gainesville, FL, United States,* [4]*Stanford University, Stanford, CA, United States*

Aedes aegypti is the principal vector for dengue, chikungunya and Zika. Both human cases and mosquito abundance measures are linked to the built environment but the relationship at household level is unclear. Using surveys and entomological data captured across the dengue transmission season (pre: July—December 2016, peak: January—May 2017, post: April—August 2018) in two cities (Zaruma and Portovelo) in Ecuador, we compare the effect of household environment on *Ae. aegypti* abundance. Data were analyzed using Poisson generalized linear mixed models (houses within clusters as random effects). Across seasons, 65 houses from Portovelo and 73 houses from Zaruma participated. In Portovelo, home ownership, cane /wooden housing and standing water presence had protective effects on the risk of capturing *Ae. aegypti* (40—55% decrease). Gaps in flooring, patio cleanliness and living near abandoned property were associated with increased risk (13—70%). Compared to homes with no patio, those with a sunny patio had an 18% decrease, mixed shade had a 44% increase, and shady patio had a 5% decrease in risk. Compared to homes with no water storage, using cisterns had a 17% decrease; other storage had a 32% risk increase. In Zaruma, gaps in flooring were protective against risk of capturing *Ae. aegypti* (63% decrease). Housing material, trash collection frequency, presence of standing water, crowding, and dog ownership were associated with increased risk (47—1517%). Compared to homes with no patio, sunny patios were protective (61% decrease); mixed shade and shady patios had a risk increase (83—128%). Compared to homes with no patio, those with a moderately clean patio had a 191% increase and those with an extremely clean patio had an 85% risk decrease. Compared to homes with no water storage, using cisterns had a 3% increase; other storage had an 81% risk decrease. Across locations, housing factor importance differed with some opposing directions of effect. This work demonstrates that the impact of built environment on *Ae. aegypti* abundance may vary according to location, suggesting that public health communication and control efforts should be place-specific.

166

CHIKUNGUNYA INFECTION AMONG SUSPECTED DENGUE PATIENTS IN SELECTED COMPOSTELA VALLEY PROVINCIAL HOSPITALS

Nestor Salcedo Arce Jr

Faculty of Clinical Tropical Medicine, Bangkok, Thailand

Controlling infectious diseases remains to be a dilemma and problem despite advancements in research and therapeutics in the past decades. An important area in the field of infection are cases of re-emerging diseases that have escalated in numbers after few years of hibernation, such as the Chikungunya infection which is caused by the same mosquito that carries dengue and zika infection. In the Philippines, dengue infection has been the more common infection among the illnesses caused by the mosquito vector than the other two infections, and the rapid diagnostic tests prepared and procured are commonly for dengue infection only. However, there are cases of Chikungunya outbreaks in the Philippines since the first case recorded in the 1950s. Clinical manifestation of chikungunya, dengue, and zika are almost the same, but in the Philippines, there is a limited resource for diagnoses that can differentiate the three illnesses. This report will discuss an ongoing prospective observational study for all

suspected clinical case of dengue infection of patients with acute febrile illness for seven days, with the aim to (1) determine prevalence of the disease, (2) identify clinical features of Chikungunya infections among suspected dengue patients, (3) determine Chikungunya and dengue co-infections, (4) evaluate significant differences in the laboratory features of chikungunya and dengue infection, and (5) identify the phylogenetic genomic characteristic of the chikungunya virus. Understanding the re-emergence of Chikungunya among closely-related vector-borne diseases presents an epidemiologic challenge that could lead to greater understanding of tropical infectious diseases.

167

FIELD TEST OF MINIATURIZED AUTOMATED WHOLE BLOOD CELLULAR ANALYSIS SYSTEM TO ASSESS IMMUNITY TO ARBOVIRUSES IN MSAMBWENI, KENYA

Amy R. Krystosik[1], Matthew Hale[2], Francis Mutuku[3], Sangeeta Kowli[4], Jael Sagina[5], Saidi Lipi[5], Phillip K. Chebii[5], Priscillah W. Maina[5], Elysse N. Grossi-Soyster[1], Holden Maecker[4], A. Desiree LaBeaud[1]

[1]*Stanford University, School of Medicine, Department of Pediatrics, Division of Infectious Disease, Stanford, CA, United States,* [2]*Smart Tube Inc., Menlo Park, CA, United States,* [3]*Technical University of Mombasa, Environment and Health Sciences Department, Mombasa, Kenya,* [4]*Stanford University, School of Medicine, Human Immune Monitoring Center, Stanford, CA, United States,* [5]*Vector Borne Disease Control Unit, Msambweni Laboratory, Kwale County, Kenya*

Our study aimed to evaluate the use of a novel miniaturized automated Smart Tube system for whole blood processing, that spares specimens, allows for immune stimulation and detailed immune function evaluation in remote regions of the world. We field tested—in Msambweni, Kenya in a pediatric cohort (median age, 8 years) known to be exposed to chikungunya virus (CHIKV) and/or dengue virus (DENV) by ELISA and PCR. In March 2018, 133 whole blood samples were processed in Kenya using the Smart Tube system for three conditions: no stimulation, stimulation with CHIKV or DENV peptide pools. Frozen samples were shipped to Stanford, where 121/133 samples will be tested by mass cytometry (CyTOF). We have tested 36/121 participant samples. Intracellular cytokine backgrounds were very low (<0.02% of CD4+ or CD8+ T cells). No IFNg+ TNFa+ and IL-2+ responses to CD4+ T cells were observed. There were occasional phenotypic differences suggestive of activated cell populations. MF1690, DENV exposed, showed a prominent CD16+CD14+ monocyte population, as well as a CD27+CD38+ plasmablast population, that were both much less visible in other subjects. Four donors showed CD8+IFNg+ and CD8+TNFa+ low-level responses to CHIKV and/or DENV peptide stimulation. MF1896 and MF1945, CHIKV exposed, showed a small IFNg+ response (0.05% and 0.02% of CD8+ T cells, respectively) only with DENV stimulation. MF0598, DENV exposed, showed a small population of DENV (0.02%) and CHIKV (0.02%) IFNg+ cells. MF1910, CHIKV exposed, showed a small IFNg+ and TNFa+ (0.04%, each) response to CHIKV and DENV stimulation (0.02% and 0.04% respectively). We will re-sample participants that responded to CHIKV and/or DENV, and test response using manual stimulation that will include, unstimulated, CHIKV and DENV stimulation (lysates and peptide pools), PMA+ ionomycin, and lipopolysaccharide, to confirm responses observed via automatic stimulation. The system has applications including vaccine trial monitoring, infectious disease treatment studies, and pathogenesis studies, and will enable new knowledge on the role of the cellular immune response in disease in pediatric populations.

168

INVESTIGATING THE PHYLOGENY, PHYLOGEOGRAPHY AND MOLECULAR EVOLUTIONARY HISTORY OF ROSS RIVER VIRUS THROUGH WHOLE GENOME SEQUENCING TECHNIQUES

Alice Michie[1], Michael Lindsay[2], John Mackenzie[3], David Smith[3], Allison Imrie[1]

[1]*University of Western Australia, Perth, Australia,* [2]*Department of Health, Western Australia, Perth, Australia,* [3]*PathWest Laboratory Medicine WA, Perth, Australia*

Ross River virus (RRV) infection causes Ross River fever, the most common arboviral disease in Australia, with an average of 5000 clinical cases reported each year nation-wide. Surveillance of RRV in Australia is based on isolation and identification of virus from homogenates of trapped and pooled mosquitoes. In Western Australia, mosquito-based arbovirus surveillance has been conducted routinely in the densely-populated south, and annually in the tropical and remote north, since the mid-1980s. Isolated viruses were identified using fixed-cell ELISA and virus-specific monoclonal antibodies and more recently, RT-PCR. Until now, phylogenetic studies of RRV have been based on analysis of partial gene sequences, typically 250nt long. We conducted whole genome sequence analysis of historical and contemporary mosquito- and human-derived RRV viruses to better understand their movement and evolution. Isolates were selected to cover a wide spatio-temporal range within Western Australia, including the regions of the Kimberley, Pilbara, Gascoyne, Mid-West, Goldfields, Perth Metropolitan, Peel and the South-West, sampled 1977-2014. Viruses isolated from individuals infected during the 1979-1980 RRV epidemic of the South Pacific Island region, as well as 13 published whole genome RRV sequences from humans and mosquitoes, were included in the analyses. Two of the three previously classified genotypes of RRV (G2-3) were identified within Western Australia during the study period, as well as a newly described fourth genotype (G4), which appears to be the contemporary lineage in circulation. The tMRCA and nucleotide substitution rate of RRV appears older and slower than previously estimated based on partial E2 gene sequence analysis. The biological implications of this observed genetic diversity, in terms of pathogenesis and antigenicity, are currently being investigated.

169

CHIKUNGUNYA INFECTION AMONG SUSPECTED DENGUE PATIENTS IN SELECTED HOSPITALS IN COMPOSTELA VALLEY PROVINCE

Nestor Salcedo Arce

Faculty of Clinical Tropical Medicine, Bangkok, Thailand

Controlling infectious diseases remains to be a dilemma and problem despite advancements in research and therapeutics in the past decades. An important area in the field of infection are cases of re-emerging diseases that have escalated in numbers after few years of hibernation, such as the Chikungunya infection which is caused by the same mosquito that carries dengue and zika infection. In the Philippines, dengue infection has been the more common infection among the illnesses caused by the mosquito vector than the other two infections, and the rapid diagnostic tests prepared and procured are commonly for dengue infection only. However, there are cases of Chikungunya outbreaks in the Philippines since the first case recorded in the 1950s. Clinical manifestation of chikungunya, dengue, and zika are almost the same, but in the Philippines, there is a limited resource for diagnoses that can differentiate the three illnesses. This report will discuss an ongoing prospective observational study for all suspected clinical case of dengue infection of patients with acute febrile illness for seven days, with the aim to (1) determine prevalence of the disease, (2) identify clinical features of Chikungunya infections among suspected dengue patients, (3) determine Chikungunya and dengue co-infections, (4) evaluate significant differences in the laboratory features of chikungunya and dengue infection, and (5) identify the phylogenetic genomic characteristic of the chikungunya virus. Understanding the

re-emergence of Chikungunya among closely-related vector-borne diseases presents an epidemiologic challenge that could lead to greater understanding of tropical infectious diseases.

170

ANOPHELES GAMBIAE DENSOVIRUS (AGDNV) INHIBITS MAYARO VIRUS IN CULTURED CELLS AND MOSQUITOES

Nadya Urakova, Marco Brustolin, Jason L. Rasgon

Pennsylvania State University, University Park, PA, United States

Recent studies demonstrate that insect-specific viruses can influence the ability of their mosquito hosts to become infected with and transmit arboviruses of medical and veterinary importance. The aim of this study was to evaluate the interactions between *Anopheles gambiae* densovirus (AgDNV) (Parvoviridae) and Mayaro virus (Togaviridae) in both insect cell culture and mosquitoes. AgDNV is a benign insect-specific virus that infects An. gambiae mosquitoes. Mayaro virus is an emerging human pathogen that can be transmitted by An. gambiae mosquitoes. Results demonstrate that Mayaro virus replication was reduced in An. gambiae cells infected by AgDNV. Similar results were observed in mosquitoes *in vivo*. These data suggest that insect-specific viruses can modulate transmission of epidemiologically important arboviruses, and that AgDNV has potential as an agent to suppress virus transmission in Anopheles mosquitoes.

171

SINGLE-DOSE ADMINISTRATION OF MV-CHIK INDUCES SUBSTANTIAL IGG3 RESPONSE AGAINST CHIKUNGUNYA VIRUS

Ruklanthi de Alwis[1], Lisa Henß[2], **Roland Tschismarov**[3], Barbara Schnierle[2], Raphael Zellweger[1], Erich Tauber[3], Katrin Ramsauer[3]

[1]*Duke-NUS Medical School, Singapore, Singapore,* [2]*Paul Ehrlich Institute, Langen, Germany,* [3]*Themis Bioscience GmbH, Vienna, Austria*

Chikungunya virus (CHIKV) is an emerging arbovirus, causing debilitating disease and posing an immense societal and financial burden in affected countries and throughout the world. Currently, no specific preventive measures or treatments are available against CHIKV infections. However, since effective immune responses against CHIKV largely depend on neutralizing antibodies and all CHIKV lineages appear to comprise a single serotype, vaccination remains a promising countermeasure. We have previously shown safety and immunogenicity of our Measles-vectored Chikungunya vaccine, MV-CHIK, in published phase 1 and 2 clinical trials. While a single immunization with the candidate vaccine induced substantial levels of neutralizing antibodies, a prime/boost regimen resulted in sustained high antibody levels. To better understand the nature of the immune response induced by MV-CHIK, we performed additional analyses on serum samples collected during phase 2 clinical studies. We examined potential cross-reactivity of these antibodies against other alphaviruses. We found that the antibodies raised by MV-CHIK cross-react with the closely related O'nyong-nyong Virus (ONNV), indicating that the candidate might also be protective against this tropical disease. In addition, we assessed the avidity and IgG subclass composition of the induced antibodies. Interestingly, we found that while prime/boost vaccination induced higher titers of neutralizing antibodies with increased avidity, the amount of IgG3 induced was only slightly higher than in participants receiving one immunization. Given the observation that IgG3 responses early during infection correlate with successful defense against Chikungunya, these data indicate that a single immunization with MV-CHIK might be suitable for a traveller's vaccine.

172

CLINICAL OUTCOMES OF 3-DAY COURSE OF ADJUNCTIVE ORAL IVERMECTIN FOR THE PATIENTS WITH CHIKUNGUNYA VIRAL INFECTION; A PRELIMINARY STUDY

Sarunyou Chusri[1], Pornapat Surasombatpattana[1], Thanaporn Hortiwakul[1], Boonsri Charernmak[1], Butsaya Thaisomboonsuk[2], Stefan Fernandez[2]

[1]*Prince of Songkla University, Hat yai , Songkhla, Thailand,* [2]*The Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

Chikungunya (CHIK) fever is characterized by abrupt onset of high grade fever, rash and debilitating joint pain. In recent 2018, this disease re-emerged in southern Thailand. Ivermectin has *in vitro* efficacy to inhibit replication of chikungunya virus (CHIKV), then clinical trial of this drug is needed. Forty patients with symptoms of CHIK fever with virological confirmation of CHIKV infection were enrolled and randomized (1:1) to receive 200 mcg/kg/day of oral ivermectin for 3 days as the adjunctive therapy to standard treatment. Daily physical examinations and blood tests including basic laboratories, polymerase chain reaction (PCR) for CHIKV, c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were performed in first weeks and every week for 12 weeks. Of 40 patients with CHIKV infection, 20 patients receiving only standard supportive treatment and 20 patients receiving adjunctive ivermectin. Clinical characteristics and basic laboratories of these 2 groups was indifferent. Time to disappearance of fever and joint pain among the patients receiving adjunctive ivermectin were significantly shorter than those receiving only supportive treatment [1.3 days VS. 2.2 days, $P = 0.002$ and 1.9 days VS. 2.8 days, $P < 0.001$, respectively]. Pain score in first 7 days and among those receiving adjunctive ivermectin was significantly lower than those receiving only supportive treatment [$P < 0.001$]. Persistent joint pain was observed in 2 patients receiving adjunctive ivermectin and 9 patients receiving only supportive treatment accounting to relative risk of 0.22(0.05-0.90), $P = 0.04$. Time to disappearance of CHIKV among the patients receiving adjunctive ivermectin were significantly shorter than those receiving only supportive treatment [1.9 days VS. 5.1 days, $P < 0.001$]. The level of CRP and ESR in first 7 days among those receiving adjunctive ivermectin was significantly lower than those receiving only supportive treatment [$P < 0.001$]. Adverse drug reactions were not different between these 2 groups. Favorable outcomes of 3-day course of adjunctive oral ivermectin for the patients with CHIKV infection was demonstrated.

173

CHARACTERIZING EXPOSURE TO CHIKUNGUNYA VIRUS INFECTION IN COASTAL KENYA

Doris Kemunto Nyamwaya[1], Donwilliams Omuoyo[1], Henry Karanja[1], John Gitonga[1], Barnes Kitsao[1], Daniel Wright[2], Rosemary Sang[3], Thumbi Mwangi[4], Charles Nyaigoti[1], George M. Warimwe[5]

[1]*KEMRI-Wellcome Trust, Kilifi, Kenya,* [2]*The Jenner Institute, University of Oxford, Oxford, United Kingdom,* [3]*KEMRI- Center for Virus Research, Nairobi, Kenya,* [4]*Washington State University, Seattle, WA, United States,* [5]*Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom*

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus first isolated in Tanzania in 1953 that has been a major cause of geographically widespread epidemics characterised by acute, often chronic, debilitating polyarthralgia and polyarthritis that can last for months or years. There is no licensed vaccine or specific treatment available. One of the largest chikungunya disease epidemics on record emerged in coastal Kenya in 2004 and spread along the coast to Mombasa and islands on the Indian Ocean, affecting hundreds of thousands of individuals, before spreading to India and subsequently to Southeast Asia and Europe. Descriptions of this and other more recent epidemics outside Africa have informed most of what we know about chikungunya disease. Very little is known regarding the burden, distribution and viral genetic diversity of CHIKV infections in the East Africa. To begin addressing these knowledge gaps we are characterising the epidemiology of CHIKV infections in coastal

Kenya, where recurrent chikungunya outbreaks have previously occurred. Specifically, we are utilising a unique biobank of samples from children and adults in coastal Kenya spanning 20 years (1998-2018) to estimate: 1) the annual prevalence, age profiles and incidence of seroconversion for antibodies to CHIKV, and 2) the burden of clinical CHIKV infections among acute inpatient and outpatient hospital visits within the same period. Results from these analyses will be presented at the meeting.

174

CHITIN SYNTHASE IS INVOLVED IN MAYARO VIRUS INFECTION IN *AEDES AEGYPTI*

Chan C. Heu[1], Scott Emrich[2], Jessica L. Gillmen[1], Jason L. Rasgon[1], Sujit K. Pujhari[1]

[1]*The Pennsylvania State University, State College, PA, United States,* [2]*The University of Tennessee Knoxville, Knoxville, TN, United States*

Mosquitoes transmit a variety of disease-causing agents. The vector competence of mosquitoes is affected by factors such as genetics, age, sex, pathogen titer, and pathogen species and strain. Chitin has been shown to promote the infection of some viruses in arthropods and may have a similar effect in virus infection of mosquitoes. In this study, we evaluated the effect of chitin synthase on Mayaro virus infection in *Ae. aegypti* using RNAi against *AaCHS1* and *AaCHS2*. Knockdown of chitin synthase genes resulted in significant reductions of viral titer and infection rate compared to GFP RNAi controls. These results show that chitin synthase is involved in viral infection phenotypes in *Ae. aegypti* and could be a target for novel disease control strategies.

175

EVALUATION OF EASTERN EQUINE ENCEPHALITIS VIRUS (EEEV) WINTER ACTIVITY IN FLORIDA

Kristi Miley[1], Joni Downs[1], Nathan Burkett-Cadena[2], Richard West[2], Brenda Hunt[3], Benjamin Brewer[4], Peter Brabant[4], George Deskins[5], Billy Kellner[5], Sandra Fisher-Grainger[6], Thomas R. Unnasch[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Florida Medical Entomology Lab, Vero Beach, FL, United States,* [3]*North Walton Mosquito Control, Defuniak Springs, FL, United States,* [4]*South Walton Mosquito Control, Santa Rosa Beach, FL, United States,* [5]*Citrus County Mosquito Control District, Lecanto, FL, United States,* [6]*Hernando County Mosquito Control, Brooksville, FL, United States*

Eastern Equine Encephalitis virus (EEEV) is a highly pathogenic arbovirus endemic primarily in the Eastern United States. Recent evidence suggests that Florida may serve as a primary reservoirto reintroduce the virus in the northeast. Florida is also the only state in which EEEV transmission occurs year-round. A risk index mapping and spatial optimization model for EEEV wintertime transmission was developed using sentinel chickens and horse case data. Risk-based surveillance was performed in Walton, Citrus, and Hernando counties. Results indicated that EEEV vector abundance was greater at high risk sites, with the primary enzootic vector *Culiseta melanura*as most abundant species collected. Bloodmeal analysisfrom blood fed mosquitoes collected during the winter months indicated that 73% were from avian hosts, 16% were from mammalian hosts and 11% were from reptiles. Wildlife cameras were deployed to evaluate fauna frequenting high and low risk sites. High risk sites had a greater overall abundance of wildlife, with *Procyon lotor*(racoon) as the predominant species at high risk sites. Although EEEV activity was high in the winter months of 2018, mosquito collections were quite low, suggesting the potential for non-vector borne pathways of transmission may exist. The results of this study may assist mosquito control districts in optimizing placement of surveillance sites as well as suggest modalities for interrupting EEEV transmission during the winter months in Florida, thereby protecting Florida residents from EEEV during peak transmission months and reducing the movement of EEEV from Florida to the northeastern states.

176

MOTOR NEURON DISEASE, PARKINSONISM AND OTHER NEUROLOGIC DISORDERS ASSOCIATED TO CHIKUNGUNYA AND ZIKA VIRUS INFECTION: A CASE SERIES

Roque P. Almeida, Phillip Nicolau G. Almeida, Juliana C. Alves, Camilla N. Santos, Philippe J. Macedo, Amelia R. de Jesus

Federal University of Sergipe, Aracaju-Sergipe, Brazil

Chikungunya (CHIKV) and Zika (ZIKV) virus has since 2014 been an emergent arbovirus in Brazil, transmitted by Aedes mosquitoes. Initial presentation is characterized by fever, headache, rash and myalgia and articular involvement is the hallmark of this infection. Neurological complications are common in severe cases and include encephalitis, myelopathy, Guillain-Barré syndrome and cranial neuropathies. Prognosis is poor in these cases and early recognition and adequate treatment may positively impact clinical outcomes. Due to the lack of clinical data in this area, mainly for motor neuron disease and parkinsonism, we here describe a case series of CHIKV and ZIKV patients who developed neurological disorders including these novel presentations.

177

SEROPREVALENCE OF DENGUE AND CHIKUNGUNYA VIRUS INFECTIONS IN FEBRILE PATIENTS FROM THREE SITES IN UGANDA

Elizabeth Namirembe[1], Andrew Walakira[1], Brenda Nakimuli[2], Joaniter Nankabirwa[1], Emmanue Arinaitwe[3], Moses Kamya[1], Sarah Stanley[4], Philip J. Rosenthal[5], Eva Harris[4], **Sam L. Nsobya**[1]

[1]*Makerere University, Kampala, Uganda,* [2]*MSH -Uganda, Kampala, Uganda,* [3]*IDRC- Uanda, Kampala, Uganda,* [4]*University of California Berkeley, Berkeley, CA, United States,* [5]*University of California San Francisco, Kampala, CA, United States*

Dengue virus (DENV) and chikungunya virus (CHIKV) infections are public health threats in sub-Saharan Africa. These infectionspresent with non-specific symptoms that are similar tothose of malaria. Wecharacterized associations between infections with these arboviruses and febrile illness in patients presenting with malaria-like illness in3 regions with different malaria transmission intensities in Uganda. Study subjects were participants aged 6months to 74 yearsfollowed in cohorts in Tororo, Jinja, and Kanungu Districts in Uganda. We studied 919 samples from study subjects that were collected at the time of presentation with fever(within 7 daysof onset of fever) irrespective of whether they had been diagnosed with malaria from 2011-2014.Temperature on the day of enrolment was measured to be >37.5°C.Thesamples were tested for malarial infection by microscopy of thick and thin blood smears and for DENVand CHIKVinfections using commercial rapid diagnostic tests (RDTs) for IgM for CHIKV (SD Bioline) and IgG/IgM/NS1for DENV (SD Bioline). Theoverallseroprevalence of DENVinfection was 2% and of CHIKV infection was 14%. Seroprevalence at the three sites for DENV and CHIKV was 3% and 27% in Tororo District, 2% and 10% in Jinja District, and 0.3% and 3% in Kanungu District, respectively. The prevalenceof *Plasmodium falciparum* infection in the same samplesfrom Tororo, Jinja and Kanungu districts was 34%,16% and42%, respectively. Thus,arbovirus seroprevalence did not correlate with relative intensity of malaria transmission, but it is noteworthy that, before recent implementation of malaria control measures, malaria transmission intensity at the 3 sites also followed the pattern Tororo>Jinja>Kanungu. In summary, we found serological evidence for relatively high prevalence of CHIKV infection and low prevalence of DENV infection in Uganda, with marked differences in seroprevalence across the country.Additional studies of the prevalence of arboviral infection in Uganda are needed.

178

INVESTIGATING THE ROLE OF EXOSOMES IN FLAVIVIRUS INFECTION OF *AEDES* MOSQUITOES

Alexander S. Gold, Sultan Asad, Fabiana Feitosa-Suntheimer, Tonya M. Colpitts

Boston University, Boston, MA, United States

Exosomes are a subset of small extracellular vesicles manufactured by most cell types and ranging in size from 40-150 nM in diameter. Originating from mutlivesicular bodies, the first step in exosome formation is the budding of these bodies, followed by the development and release of intra-luminal vesicles into the extracellular space by exocytosis. These vesicles have been detected in various bodily fluids and shown to mediate intercellular communication through the transfer of macromolecules, proteins, and genetic material. Apart from these natural functions, Ramakrishnaiah et al. (2013) demonstrated that exosomes from Hepatitis C virus (HCV)-infected human liver cells contained viral RNA and were infectious. Similarly, Bukong et al. (2014) reported that exosomes isolated from the sera of HCV-infected patients were infectious and contained viral RNA in complex with the protein Ago2 and micro RNA 122, a complex known to enhance viral infection. Whereas these findings support that exosomes are involved in cell-to-cell spread of HCV, the role of exosomes in other flavivirus infections has not yet been well-characterized. Vora et al. (2018) showed that exosomes derived from Dengue virus (DENV)-infected *Aedes* mosquito cells contained viral RNA and were infectious to naïve *Aedes* and mammalian cells, indicating a function of exosomes in the transmission of DENV from mosquito vector to human host. While the results of this study support the ability of DENV to use exosomes to enhance infectious output during transmission from vector to host, there has yet to be any evidence that exosomes serve a function in the opposite process: the transmission of DENV from human host to mosquito vector. By isolating exosomes from the sera of DENV-infected patients, confirming the presence of viral RNA in these exosomes, and demonstrating the ability of these exosomes to infect *Aedes* and *Aedes* cells, we have shown that DENV uses exosomes to mediate transmission from host to vector, further elucidating the role of exosomes in the viral lifecycle of DENV, as well as suggesting at the involvement of exosomes in other mosquito-borne flavivirus infections.

179

ARBOVIRUS SURVEILLANCE NEAR THE MEXICO-U.S. BORDER: CO-CIRCULATION OF DENGUE VIRUS SEROTYPES 1, 2, AND 3, WEST NILE VIRUS AND CHIKUNGUNYA VIRUS IN TAMAULIPAS, NORTHERN MEXICO

S. Viridiana Laredo-Tiscareño[1], Javier A. Garza-Hernandez[2], Ma Isabel Salazar[3], Erick J. De Luna-Santillana[4], Gloria L. Doria-Cobos[5], Julian E. Garcia-Rejon[6], Carlos Machain-Williams[6], Bradley J. Blitvich[7], Mario A. Rodríguez Pérez[4]

[1]*Centro de Biotecnología Genómica del Instituto Politécnico Nacional, Reynosa, Mexico,* [2]*Universidad Autónoma de Ciudad Juárez, Juarez, Mexico,* [3]*Instituto Politécnico Nacional, Mexico City, Mexico,* [4]*Instituto Politécnico Nacional, Reynosa, Mexico,* [5]*Secretaria de Salud, Reynosa, Mexico,* [6]*Universidad Autónoma de Yucatán, Merida, Mexico,* [7]*Iowa State University, Ames, IA, United States*

A clinical, serological, and molecular investigation was performed to determine the presence of dengue virus (DENV) and other mosquito-transmitted viruses among residents of the city of Reynosa, Tamaulipas on the Mexico-U.S. border in 2014 to 2016. The sample population consisted of 2355 patients with suspected dengue, in addition to 346 asymptomatic individuals recruited during a household-based epidemiological investigation designed to identify flavivirus seroconversions. Sera were collected from patients with suspected dengue in the acute phase of illness and from asymptomatic individuals at enrollment and every 5 to 7 months for 19 months. Sera from suspected dengue patients were tested for DENV antigen by enzyme-linked immunosorbent assay (ELISA) and select antigen-positive sera were further tested using a serotype-specific, quantitative reserve transcription-polymerase chain reaction. Sera from a subset of patients was also tested for chikungunya virus (CHIKV) RNA. A total of 418 (17.7%) patients with suspected dengue had laboratory-confirmed DENV infections, including 82 patients positive for DENV RNA. Three serotypes were detected (DENV-1, DENV-2, and DENV-3). CHIKV RNA was detected in 13 of 34 (38.2%) patients, including five who also contained DENV antigen. Sera from the household cohort were tested for flavivirus-reactive antibodies by immunoglobulin (Ig) M and IgG ELISAs using DENV antigen. A total of 217 (62.7%) household participants had flavivirus-reactive antibodies at enrollment and nine flavivirus-naïve individuals seroconverted. Sera from a subset of participants, including all those who seroconverted, were further tested by plaque reduction neutralization test, resulting in the detection of antibodies to DENV-1 and West Nile virus. In summary, we provide evidence for the co-circulation of five medically important arboviruses in Reynosa, Tamaulipas on the Mexico-U.S. border.

180

DENGUE: AN EMERGING DISEASE OF PUBLIC HEALTH CONCERN IN NEPAL

Sagun Paudel

Universitas Gadjah Mada, Yogyakarta, Indonesia

Dengue is fast emerging pandemic-prone vector-borne disease transmitted by the bite of a mosquito infected with dengue virus serotypes. The *Aedes aegypti* mosquito is the primary vector of dengue in Nepal. The first case of dengue was detected in 2005 then sporadic cases & outbreak was reported frequently. This paper aims to review dengue situation and existing challenges of dengue in Nepal. Information was gathered by reviewing articles, reports & government publications. Aedes aegipti was identified in five districts of Nepal which reflects that there was local transmission of dengue. The entomological survey shows that all four sub-types of the dengue virus were scattered in Nepal. 42 out of 77 districts were affected by dengue, spread of infection resembles that the disease is now going in tarai plains from west to east. In fiscal year 2015/16, total no. of 1527 dengue cases were reported from 42 districts. Integrated vector management strategies were conducted by government. However, frequent dengue outbreaks in the past couple of years & geographical expansion of dengue vector approves that, these measures are not sufficient. Dengue virus is rapidly expanding its range throughout the country with frequent major outbreaks. So, Early diagnosis and treatment, BCC, larva search and destroy, screening at border & strengthening early warning and reporting is recommended for prevention and control of dengue in Nepal.

181

DISCOVERY OF A TETRAHYDROTHIENOPYRIDINE DERIVATIVE, A DENGUE VIRUS NS4B INHIBITOR WITH POTENT ORAL *IN VIVO* EFFICACY

Fumiaki Yokokawa[1], Oliver Simon[2], Sandra Sim[2], Bin Zou[2], Mei Ding[2], Wai-Ling Chan[2], Cyrille S. Kounde[2], Hui-Quan Yeo[2], Gang Wang[2], Qing-Ying Wang[2], Kah Fei Wan[2], Hongping Dong[2], Ratna Karuna[2], Siew Pheng Lim[2], Suresh B. Lakshminarayana[2], Stephanie Moquin[1], Chang Bok Lee[1], Katherine Chan[1], Alex Chao[1], Christopher Sarko[1], Bryan K. S. Yeung[2], Feng Gu[1]

[1]*Novartis Institute for Tropical Diseases, Emeryville, CA, United States,* [2]*Novartis Institute for Tropical Diseases, Singapore, Singapore*

Dengue fever is the world's most prevalent mosquito-borne viral disease caused by the four serotypes of dengue viruses (DENV), which are widely spread throughout tropical and sub-tropical countries with 390 million infections and 40,500 deaths per year. Currently, the dengue vaccine, Dengvaxia® by Sanofi, has been approved in several countries for use in endemic areas, however recent analysis found that Dengvaxia® could cause more cases of severe disease for those who had never been infected by dengue virus. To date, no antiviral agents have been approved to treat dengue fever, therefore, there is an urgent need to develop effective and

safe antiviral therapeutics. We have identified tetrahydrothienopyridine derivatives from phenotypic screening as a first-in-class pan-serotype dengue virus inhibitor. Resistance analysis showed that mutations in the dengue viral NS4B sequence (nonenzymatic transmembrane protein and a component of the viral replication complex) conferred resistance to compound inhibition, suggesting that the NS4B protein is a molecular target of this scaffold. Extensive SAR studies of this scaffold led to the discovery of new analogs with improved potency in a nanomolar to submicromolar range against all four dengue serotypes. Optimization of physicochemical properties as well as oral *in vivo* pharmacokinetic profile led to a compound showing excellent oral efficacy in the DENV-2 infected AG129 mouse model with >one log viremia reduction when orally dosed at 30 mg/kg once daily for three days. Furthermore, this compound showed good selectivity against off-target safety and cytotoxicity panels. This presentation will discuss our dengue phenotypic screening campaign, medicinal chemistry strategy, SAR, *in vitro* and *in vivo* profile of the optimized compound.

182

DENGUE VIRUS INFECTION AND LEISHMANIASIS IN PATIENTS ATTENDING A MEDICAL HEALTH CENTER IN THE RURAL COMMUNITY OF ILARA-MOKIN, ONDO STATE, NIGERIA

Olayinka Osuolale, Tolulope Daramola, Olanike Alajo

Elizade University, Ilara-Mokin, Nigeria

Dengue and leishmaniasis are serious diseases that the World Health Organization (WHO) characterizes as lacking effective control measures. Transmitted by insect vectors and can result in epidemic outbreaks. Sustained control of the vectors are difficult for dengue and leishmaniasis because their high reproductive potential allows the vector populations to recover quickly after intervention wherever adequate breeding conditions exist. Because of their misdiagnosis or underdiagnosed, it is endemic in the tropical countries. Prevalence and epidemiology of these diseases is poorly understood and misdiagnosed in Nigeria, in most cases with malaria. Our study aims to investigate dengue virus and leishmaniasis co-infections in patients visiting a rural community medical center in Elizade University, Ilara Mokin, Ondo State. Blood samples were collected and analyzed for two months. SD Dengue Duo and Bio-rad IT Leish serological test kits was used for the samples analysis. This study examined 101 samples which were simultaneously tested for the target infections. In about 23.76% of the samples (24 samples) were positive for dengue infections. Gender wise, more males (79.17%) than females (20.83%) tested positive to the virus infection. 37.5% of the positive samples were primary infections, 91.6% were past or secondary infections and 33.3% of the samples show late primary infections and early secondary infections. Only 1 sample was positive for Leishmaniasis. The majority of the study population had no pre-knowledge of dengue infection and leishmaniasis as they are carriers of the diseases, providing new insights on both incidence and prevalence. It was discovered that those from villages especially the South-South of Nigeria tested positive the most as carriers of the diseases. Therefore, these areas need special attention for surveillance and treatment for preventive measures. In addition, this study recommends a concerted effort by all stakeholders to enlighten the people about dengue infection and leishmaniasis, and its prevention and eradication from such regions.

183

C-REACTIVE PROTEIN AS A POTENTIAL BIOMARKER FOR DISEASE PROGRESSION IN DENGUE: A MULTI-COUNTRY OBSERVATIONAL STUDY

Duyen Thi Le Huynh[1], Vuong Lam Nguyen[1], Lam Khanh Phung[1], Hoai Tam Thi Dong[2], Kinh Van Nguyen[3], Cameron Simmons[1], Ngoun Chanpheaktra[4], Lucy Lum See[5], Ernesto Pleités Sandoval[6], Kerstin Rosenberger[7], Vinh Chau Van Nguyen[8], Christine Halleux[9], Piero Olliaro[9], Thomas Janisch[7], Bridget Wills[1], Sophie Yacoub[1]

[1]*Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,* [2]*University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam,* [3]*National Hospital of Tropical Diseases, Ha Noi, Vietnam,* [4]*Angkor Hospital for Children, Siem Reap, Cambodia,* [5]*University of Malaya Medical Centre, Kuala Lumpur, Malaysia,* [6]*Hospital Nacional de Niños Benjamin Bloom, San Salvador, El Salvador,* [7]*Heidelberg University Hospital, Heidelberg, Germany,* [8]*Hospital of Tropical Diseases, Ho Chi Minh City, Vietnam,* [9]*TDR, WHO, Geneva, Switzerland*

Dengue infection can cause a wide spectrum of clinical outcomes. The severe clinical manifestations occur late in the disease course, during day 4-6 of illness, allowing a window of opportunity for risk stratification. Markers of inflammation may be useful biomarkers. We investigated the value of CRP measured early on illness days 1-3 to predict dengue disease outcome and the difference in CRP levels between dengue and other febrile illnesses (OFI). We performed a nested case-control study using the clinical data and samples collected from the IDAMS-consortium multi-country study. This was a prospective multi-center observational study that enrolled almost 8000 participants presenting with a dengue-like illness to outpatient facilities in 8 countries across Asia and Latin America. Predefined severity definitions of severe and intermediate dengue were used as the primary outcomes. 378 cases with severe/intermediate dengue were compared to 1134 uncomplicated dengue patients as controls (ratio 1:3), and also 400 patients with OFI. In patients with confirmed dengue, higher CRP levels in the first 3 days of illness were associated with a higher risk of severe outcome (OR 1.64, 95% CI 1.21-2.24), and longer fever clearance time (HR 0.87, 95% CI 0.77-0.99) but not with hospitalization. CRP levels showed a quadratic association between dengue and OFI diagnosis; with levels of approximately 30 mg/dL associated with the highest risk of having dengue. This risk decreased with lower and higher levels of CRPs, likely representing diagnoses with other viral infections with CRP levels <30mg/dL and bacterial infections >30 mg/dL. Antibiotics were given in 6.6% of dengue patients, and these patients had similar outcomes to those not given antibiotics, even those with CRP levels >40mg/dL. CRP had a positive correlation with total white cell count and neutrophils and negative correlation with lymphocytes, but did not correlate with liver transaminases, albumin, or platelet nadir. In summary, CRP measured in the first 3 days of illness is a useful biomarker for early dengue risk prediction and may assist differentiate dengue from other febrile illnesses.

184

MITIGATION AND PREVENTION OF DENGUE OUTBREAKS AND SUSTAINING LOW ENDEMICITY THROUGH A COMPREHENSIVE INTEGRATED APPROACH BASED ON BEST PRACTICES: SRI LANKA

Dompeyalage Shamali Anoja Fernando Dheerasinghe[1], Mizaya Cader[2], Prashila Samaraweera[1], Iroshini Abeysekara[1], W.m.i.p. Weerasinghe[1], O.b.w Rajapaksha[1], K.a.l.c Kodithuwakku[1], Nimalka Pannila Hetti[1], Hasitha Tissera[1]

[1]*National Dengue Control Unit, Ministry of Health, Colombo, Sri Lanka,* [2]*National Programme for Tuberculosis Control and Chest Diseases, Ministry of Health, Colombo, Sri Lanka*

Dengue is a leading public health problem in Sri Lanka where all ages are affected. The aims of the dengue control programme are to carry out proactive integrated vector management based on real-time Dengue surveillance (epidemiological & entomological) data and to strengthen human resource and infrastructure to improve clinical management of Dengue. There has been significant progress in epidemiological and entomological surveillance, clinical management, and active engagement of various ministries through the Presidential Task Force (PTF) on Dengue to enhance community participation. A guideline was developed for *Aedes* vector surveillance and control. Field health officers were trained on vector surveillance and vector control according to the guideline. Field Assistants Mosquito Control (1287) were recruited in a phased manner in 2017 to work at the community level to lead source reduction activities and other vector control and vector surveillance activities. Special Mosquito Control Campaigns (SMCCs) were conducted in 2017 and 2018 for source reduction, with the participation of stakeholders covering 2.7 million and

1.4 million premises respectively. The PTF was reactivated in 2017 and 24 PTF meetings have been conducted to date to assess the progress of control activities with the participation of relevant stakeholders. In-service training programmes were conducted (35 and 15 in 2017 and 2018 respectively) for medical doctors and other clinical staff by the local and foreign experts on clinical management. Moreover, the capacities of high dependency units were strengthened to manage Dengue patients efficiently. During SMCC's conducted in 2017 and 2018, health workers along with the Tri-Forces/Police found 20% and 21% potential breeding places and 1.98% and 2.29% positive breeding places in the respective years. Thus there was a reduction in the incidence of Dengue from 865.9 to 239.8 per 100,000 population (in 2017 and 2018 respectively) and case fatality rate has been halved from 0.24% to 0.11% from 2017 to 2018. An integrated approach is an effective method in sustaining low endemicity and curtailing Dengue outbreaks.

185

COMPARING THE COMPETENCE OF GHANAIAN AND VIETNAMESE *AEDES* MOSQUITOES AS VECTORS OF THE DENGUE VIRUS

Michael Amoa-Bosompem[1], Daisuke Kobayashi[2], Katsunori Murota[2], Astri Nur Faizah[3], Kentaro Itokawa[2], Mitsuko Ohashi[1], Samuel Dadzie[4], Kofi J. Bonney[4], Toshinori Sasaki[2], Haruhiko Isawa[2], Kyoko Sawabe[2], Shiroh Iwanaga[1]

[1]*Tokyo Medical and Dental University, Tokyo, Japan,* [2]*National Institute of Infectious Diseases, Tokyo, Japan,* [3]*The University of Tokyo, Tokyo, Japan,* [4]*Noguchi Memorial Institute for Medical Research, Accra, Ghana*

Dengue fever is an arboviral infection of public health concern caused by at least 4 antigenically different serotypes of the dengue virus. It is transmitted by the *Aedes* mosquito, infecting 390 million people annually with 25,000 deaths. The distribution, frequency and intensity of outbreaks have varied quite significantly over the years. In 2016, the Americas and Asia reported cases in the millions and hundreds of thousands respectively while Africa reported cases in the thousands. Within Africa, Ghana, unlike her neighboring countries, is yet to report a single outbreak despite an abundance of the *Aedes* mosquito vector. Serological reports have however suggested possible exposure to the dengue virus or a closely related flavivirus. While many factors have been hypothesized as the reason for the differences, determining the effect of vector competence is of utmost importance. The aim of this study was therefore to compare the vector competence of *Aedes* mosquitoes from Ghana and dengue endemic Vietnam. In this study, Mosquito larvae were collected from various parts of Ghana and Vietnam and established in the lab. The virome of the laboratory colonies were determined after which adult female mosquitoes were infected with the dengue virus. The Saliva, thorax/abdomen and carcass of individual mosquitoes were harvested and screened at 7 and 14 days post infection. The infection rate, dissemination rate and transmission rate were determined by qPCR and Focus assay. Our results show Vietnamese *Aedes* mosquitoes to be significantly more susceptible to Dengue virus colonization. Furthermore the dissemination rate of the virus in the Vietnamese mosquitoes was double that of the Ghanaian mosquitoes. Last but not least while there was no significant difference in the time viruses were detected in the saliva of infected mosquitoes, the concentration in the Vietnamese mosquitoes were significantly higher. In conclusion, *Aedes* mosquitoes from Vietnam are more efficient vectors of the Dengue virus than *Aedes* mosquitoes from Ghana and the difference in vector competence may play a significant role in distribution and intensity of outbreaks.

186

CONGENTITAL DENGUE: CD133+ AND CD34+ HEMATOPOETIC STEM CELLS IN UMBILICAL CORD BLOOD ARE INFECTABLE BY DENGUE VIRUS CONFERRING VERTICAL TRANSMISSION

Amrita Vats

National Cheng Kung University, Tainan, Taiwan

Dengue affects more than 100 million people worldwide annually. Numerous routes of transmission for dengue virus (DENV) have been documented. The cases of dengue resulting from stem cell transplantations and mother-to-infant vertical transmission are escalating in recent years. Consequences of mother-to-fetal transmission have been shown to be associated with miscarriage and stillbirth. DENV proteins and genomic RNA have been detected in placenta, serum and cord blood of baby with a fever and subclinical infection upon delivery. Umbilical cord blood (UCB) is the essential bridge connecting placenta and infant and is abundant with stem cells. We, therefore, hypothesize that DENV after passing through placenta may amplify further in stem cells within UCB. In this study, freshly obtained UCBs were utilized and fluorescence-activated cell sorting (FACS) was performed to analyze the stem cells after DENV infection. Viral titers in supernatants of infected UCB were performed by plaque assay. Results showed that cells in UCB were highly permissive to DENV, enhanced proliferation of hematopoietic stem and progenitor cells (HSPC) was observed, balance of transcriptional factors (GATA-1,GATA-2,GATA-3) were disturbed, DENV nonstructural protein 1 (NS1) was mainly associated with CD34+ and/or CD133+ cells analyzed by FACS and immunofluorescence staining, sorted CD133+ or CD34+ cells were infectable by DENV, and infectious DENV could be recovered from infected CD34+ and CD133+ cells upon co-culture . Furthermore, viral RNA in specific organelle was found after 30 days of infection, suggesting the longevity of stem cells in DENV infected UCB. Our cumulative results submit that CD133+ and/or CD34+ cells in UCB are not only permissive to DENV infection but also might serve as a reservoir for dissemination of the virus. The findings may indicate the unique property of DENV in stem cells contributing to DENV transmission from mother-to-infant, resulting in the clinical significances in newborn babies, especially in dengue-endemic regions.

187

DENGUE FEVER, *AEDES AEGYPTI* AND CLIMATE DYNAMICS FROM THE TEMPERATE CITY OF CÓRDOBA, ARGENTINA, DURING THE TIME SERIES OF 2009-2017

Elizabet L. Estallo[1], Rachel Sippy[2], Anna Stewart-Ibarra[2], Marta G. Grech[3], Francisco F. Ludueña-Almeida[1], Elisabet M. Benitez[1], Mariela Ainete[4], María Frías[4], Michael Robert[5], Moory Romero[2], Walter R. Almirón[1]

[1]*Instituto de Investigaciones Biológicas y Tecnológicas. Consejo Nacional de Investigaciones Científicas y Técnica. Centro de Investigaciones Entomológicas de Córdoba. Universidad Nacional de Córdoba, Argentina, Cordoba, Argentina,* [2]*Institute for Global Health and Translational Sciences, State University of New York. Upstate Medical University, Syracuse, NY, United States,* [3]*Centro de Investigación Esquel de Montaña y Estepa Patagónica. Consejo Nacional de Investigaciones Cientificas y Técnicas. Universidad Nacional de la Patagonia San Juan Bosco. Facultad de Ciencias Naturales, Esquel, Argentina,* [4]*Ministerio de Salud de la Provincia de Córdoba - Dirección de Epidemiología, Cordoba, Argentina,* [5]*Department of Mathematics, Statistics, and Physics. University of the Sciences, Philadelphia, PA, United States*

Argentina is located at the southern range of arboviral transmission by *Aedes aegypti* and has experienced a rapid increase in arbovirus transmission in recent years. The aim of this study was to present, for the first time, the methods and findings from a prospective long-term entomological surveillance study that began in 2009 in the city of Córdoba, following the first dengue virus (DENV) outbreak. We analyze the seasonal and interannual dynamics of DENV transmission in the city,

in relation to *Ae. aegypti* indices and local climate. Therefore, from 2009 to 2017, larval surveys were conducted monthly, from November to May, in 600 randomly selected households distributed across the city. From 2009 to 2013, ovitraps (n=177) were sampled weekly to monitor the oviposition activity of *Ae. aegypti*. Cross correlation analysis was used to identify significant lag periods between climate, entomologic and epidemiologic variables. Climate, entomologic and epidemiologic variables exhibited a strong seasonal pattern with a single peak within the year (climate, epidemiologic) or sampling period (entomologic). The largest correlation between autochthonous dengue and minimum temperature was at 9 weeks (positive), and there was a large positive correlation between autochthonous dengue and relative humidity (minimum, mean, maximum) at 4 weeks. There was a positive correlation with mean relative humidity at lag 4 in 2009, and a negative correlation between ovitrap positivity and minimum relative humidity at lag 2 in 2010. Ovitrap positivity was positively correlated with relative humidity (mean, maximum) and precipitation at a 5-week lag and negatively correlated with mean temperature in 2012. This prospective entomological surveillance study provides the first evidence that *Ae. aegypti* larval indices in this temperate region have increased over the last 9 years, a period when arboviral diseases have become epidemic for the first time. These findings suggest an increasing the risk of arbovirus emergence and sustained transmission at temperate southern latitudes, where these diseases were not previously reported.

188

UNCOVERING THE DENGUE-1 SPECIFIC MEMORY B CELL DERIVED ANTIBODY REPERTOIRE IN IMMUNE DONORS 1 TO 43 YEARS AFTER DENGUE INFECTION IN A NON-ENDEMIC COHORT

Zoe Lyski[1], Bettie Kareko[1], Brian Booty[2], Jana Mooster[1], William Messer[1]

[1]*Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, OR, United States,* [2]*Oregon Clinical and Translational Research Institute, Oregon Health and Science University, Portland, OR, United States*

Mosquito transmitted flaviviruses, including dengue virus (DENV) are among the most important vector-borne pathogens of humans worldwide, responsible for 100 million symptomatic cases and ~35,000 deaths *each* year. Upon DENV infection, DENV-specific host B-cells differentiate and proliferate. Some become long-lived antibody-secreting cells (long-lived plasma cells) while others become DENV-specific memory B cells (MBCs) that remain in circulation, poised to protect against future infections, forming a founder population that plays a critical role in establishing and maintaining long-term viral immunity. Despite this critical role, much remains incompletely described regarding lifespan, specificity, and the potency and breadth of MBCs and the antibodies they encode and secrete upon reinfection. This knowledge is vital for understanding long-term human immunity to flaviviruses as well as for rational vaccine design. Here we identify and quantify DENV-specific MBCs in humans with a single DENV infection. Using PBMCs from 15 primary DENV-1 donors with times since infection ranging from 1-43 years, we employed two complimentary experimental approaches to quantify DENV-specific MBCs. In our first approach, PBMCs were stimulated *in vitro* to become antibody-secreting cells and the resulting antibodies assessed for DENV-specificity by ELISA using whole DENV virus (DENV1-4) as well as non-structural protein NS1. The second method used flow cytometry to quantify human MBCs (CD3-CD14-CD19+CD27+IgD-) that bind fluorescently labeled DENV. Using these approaches, we were able to identify DENV-specific MBCs that remain in circulation decades after infection. MBC frequency was inversely correlated with time since infection. These experiments lay the foundation to functionally characterize DENV-specific MBCs and the antibodies they are programmed to secrete, including frequency of DENV-binding and neutralizing MBCs and the potency and breadth these antibodies have against antigenically diverse DENV. The results of this project will provide insight into the MBC founder population and the role it plays in broader DENV immunity.

189

CHARACTERIZING THE SPATIO-TEMPORAL DYNAMICS OF DENGUE IN BRAZIL

Saki Takahashi, Isabel Rodriguez-Barraquer

University of California San Francisco, San Francisco, CA, United States

Roughly half of the global population is at risk of dengue infection, predominantly in urban areas in Southeast Asia and Latin America. There are four main serotypes of dengue virus that co-exist, and infection confers long-term immunity against only the infecting serotype. Although dengue is classically considered a childhood disease, the age distribution of cases depends on local demography as well as how long serotypes have been present. In Brazil, where dengue was re-introduced in the 1980s and which now accounts for the majority of dengue cases reported in the Americas, the disease classically affected mainly adult populations. However, in 2007, the age distribution of cases drastically shifted towards younger age groups. Here, we investigate the causes and consequences of this punctuated drop in the age distribution of dengue cases, leveraging annual state-level counts of age-stratified, hospitalized dengue cases in Brazil between 1992 and 2017. We deploy a mathematical modeling framework to clarify spatio-temporal patterns of population-level susceptibility to dengue in Brazil, and explore the interplay between human demography, transmission epidemiology, and serotype-specific immunity on dengue burden more generally. With more countries prone to dengue transmission undergoing the demographic transition, the ability to project age-structured cases into the future will inform public health planning. Clarifying serotype-specific immunity and risk will also be important for countries considering introduction of childhood immunization with the dengue vaccine.

190

THE LABORATORY PROFILE OF DENGUE IN PATIENTS ADMITTED TO TEACHING HOSPITAL ANURADHAPURA

Shobha Sanjeeewani Gunathilaka[1], SAM Kularatne[2], Jayantha Rajapakse[3], Rohitha Muthugala[4]

[1]*Rajarata University of Sri Lanka, Faculty of Medicine and Allied Sciences, Anuradhapura, Sri Lanka,* [2]*Faculty of Medicine , University of Peradeniya., Peradeniya, Sri Lanka,* [3]*Faculty of Veterinary Medicine and Animal Sciences, Peradeniya, Sri Lanka,* [4]*Teaching Hospital Kandy, Kandy, Sri Lanka*

Dengue is an arthropod-borne viral disease caused by an RNA virus belonging to the genus Flaviviruses. Dengue has become a major public health problem in the world as well as in Sri Lanka. The clinical and laboratory profiles of dengue may vary depending on the age, immunity states and the involved serotype of the virus etc. Nevertheless, the differences of presentation, the timely diagnosis, triage, and proper monitoring plays a vital role in saving lives of dengue patients. For this, the knowledge on clinical presentations, the utility of the available national and international guidelines and the choice of ideal lab test/s for the confirmation, in the local context, should be available to the clinicians and other stakeholders. This study was aimed to assess the utility of specific laboratory tests for the correct diagnosis of dengue. The data on available investigation results and blood samples were collected from patients admitted to medical wards of Teaching Hospital Anuradhapura, with the probable diagnosis of dengue. The PCR testing was conducted in the PCR laboratory at the Department of Veterinary Pathobiology, University of Peradeniya, during one year starting from 1st December 2016. A total of 213 patients were recruited by convenient sampling. The mean age of the study group was 32.98 years (SD=13.1). Of them, the majority (81.7%) were males. Only 1.6 % of the patients have a maximum drop of more than 100 x 10^3 per mm^3 platelets per one day. RT PCR was positive only in 38 blood samples. The sensitivity and specificity of NS1 and/or Ig M dengue 44.7% and 65.7% respectively. The commonest serotype was DENV 2. The utility of serology, NS1 and, PCR and virus isolation tests in the diagnosis of the disease in the Sri Lankan context should be investigated further by researchers.

191

CHARACTERIZING EXPOSURE TO DENGUE VIRUS INFECTION IN COASTAL KENYA

Henry Kibe Karanja[1], John N. Gitonga[1], Doris Nyamwaya[1], Donwilliams Omwoyo[1], Everlyn Kamau[1], Barnes Kitsao[1], Rosemary Sang[2], Limbaso Konongoi[2], Daniel Wright[3], Charles Nyaigoti[1], George Warimwe[1]

[1]*KEMRI-Wellcome Trust, Kilifi, Kenya,* [2]*KEMRI-Centre for Virus Research, Nairobi, Kenya,* [3]*The Jenner Institute, University of Oxford, Oxford, United Kingdom*

Dengue virus (DENV) is the most widespread arbovirus globally, causing over 300 million infections every year. Typically, the disease occurs as outbreaks of a self-limiting febrile illness that can sometimes result in life-threatening complications including shock and bleeding diathesis. However, despite occurrence of dengue outbreaks in Africa, very little is known regarding the burden, distribution and viral genetic diversity of DENV infections in the continent. To begin addressing these knowledge gaps we are characterising the epidemiology of DENV infections in coastal Kenya, where recurrent dengue outbreaks have previously occurred. Specifically, we are utilising a unique biobank of samples from children and adults in coastal Kenya spanning 20 years (1998-2018) to estimate: 1) the annual prevalence, age profiles and incidence of seroconversion for antibodies to DENV, and 2) the burden of clinical DENV infections among acute inpatient and outpatient hospital visits within the same period. Results from these analyses will be presented at the meeting.

192

A NEW SET OF PRIMERS FOR IN-HOUSE LOOP-MEDIATED IS OTHERMAL AMPLIFICATION (LAMP) FOR DENGUE VIRUS DIFFERENTIAL DIAGNOSIS

Scarlett Barrientos Peña, Oscar Nolasco Cárdenas

Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

Early detection of infection is a key step in the management of dengue cases; both in the clinical management and the control of the expansion of the disease. However, serological tests, which are the most used tools for diagnosis in first-level health centers, need at least five days (from the beginning of the infection) to be useful. Even so, these tests are subject to problems of specificity. Loop mediated isothermal amplification (LAMP) tests are fast, simple and therefore attractive for environments where implements necessary for more complex tests do not exist, like real-time PCR. In this work, we performed the in-house design of LAMP primers for the differential diagnosis of dengue virus serotypes. To this end, dengue virus sequences were downloaded from GenBank and aligned in MEGA v7. Subsequently PrimerExplorer V5 was used to generate sets of candidate LAMP primers from the most conserved regions, for each serotype. Each set of primers was evaluated for the formation of secondary structures and specificity using OligoAnalyzer v3.1 and Primer Blast, respectively. The best sets were evaluated to determine their amplification conditions, using neutral red dye for visual detection, which shows a color change from yellow to fuchsia when DNA synthesis occurs. The limit of detection for the sets designed, using plasmids, was 664,99 copies/μL for DENV-1; 3 035,75 copies/μL for DENV-2; 19 6476.80 copies/μL for DENV-3 and 207,65 copies/μL for DENV-4. The average incubation time was 1 hour ± 10 minutes. The analytical specificity determined was 100%. Additionally, LAMP primers were evaluated in a RT-LAMP format using AMV enzyme for reverse transcription and RNA extracted from 106 samples obtained by active collection in peri-urban areas from Iquitos. Four samples were positive by DENV-2 LAMP and the results were compared with those obtained by real time PCR. The amplification region sequencing confirmed the presence of serotype 2 dengue virus in the samples, with a 99% identity to strains found in Peru according to GenBank (isolate DENV-2/PE/NFI1159/2010). The four sets of designed primers are shown as a promising alternative for use in LAMP tests.

193

CIRCULATION OF MULTIPLE DENGUE VIRUS SEROTYPES IN IBADAN NIGERIA

Anyebe B. Onoja[1], Mamoudou Maiga[2], Adekunle J. Adeniji[1], Georgina N. Odaibo[1], Robert L. Murphy[3], Olufemi D. Olaleye[1]

[1]*University of Ibadan, Ibadan, Nigeria,* [2]*Center for Innovation in Global Health Technologies, Northwestern University, Evanston Campus, IL, United States,* [3]*Centre for Global Health, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States*

Dengue is a rapidly spreading mosquito-borne illness in many parts of the world. In Africa, the first empirical evidence of dengue virus was from Ibadan Nigeria, where DENV-1 and -2 serotypes were identified during an epidemic between 1964 and 1968. In recent times, high rate of dengue has been reported in Ibadan as a result of increased breeding of *Aedes species*. However, specific strains responsible for dengue outbreaks after 1960s episodes has been unknown. We employed molecular techniques to identify currently circulating DENV strains among the general population across different parts of Ibadan Nigeria. A prospective study was carried out from July to August, 2018 involving 175 randomly selected febrile participants attending three big hospitals in Ibadan Nigeria. The participants tested negative for malaria parasite examination and typhoid fever. Viral RNA was extracted from plasma and prM/E junction of DENV was detected using conventional PCR. Heat-inactivated tissue culture-based DENV1-4 isolates used by the Global Dengue Laboratory Network were included as positive controls. Kruskal-Wallis test was used to compare positivity of each serotype with respect to participants' age, occupation, hospital, and clinical symptoms. The post hoc test was conducted using Mann-Whitney test to identify specific association. All tests were conducted at 0.05 level of statistical significance. A total of 40 (22.8%) participants were infected with at least one DENV serotype. Specific serotypes identified are DENV-1 2 (1.1%), DENV-2 19 (10.9%), DENV-3 11 (6.3%) and DENV-4 13 (7.4%). Five participants had dual infection with DENV-2 and other serotypes. Dual infectivity was not a function of age. A significant association was observed between symptoms of participants confirmed with DENV-3 (X^2 = 29.930, df = 17, p=0.027). In this study, DENV-3 and -4 serotypes were found, with very low incidence of DENV-1. Findings indicate a deviation from high DENV-1 and -2 activity previously reported in Ibadan. Phylogeny and evolutionary analysis is required to establish ancestry of these strains.

194

DIFFERENTIAL SUSCEPTIBILITIES AND IMMUNE RESPONSES OF *AEDES AEGYPTI* TO TWO DENGUE 4 VIRUS STRAINS

Caroline J. Stephenson, Seokyoung Kang, John A. Lednicky, Rhoel R. Dinglasan

University of Florida, Gainesville, FL, United States

Dengue virus (DENV) is responsible for the largest number of arbovirus infections among humans globally. There are four serotypes: DENV1, -2, -3 and -4. Little is known about how genetic differences between DENV serotypes and among strains can lead to differences in mosquito susceptibility. We compared mosquito susceptibilities and immune responses within the midgut and carcass of Orlando strain *Aedes aegypti* infected with two strains of DENV4 and one strain of DENV2. Mosquitoes were given infectious bloodmeals containing either DENV4 lab strain H241, a field isolate of DENV4 obtained from a child in Haiti in 2015, or DENV2 New Guinea C (NGC) strain. When *A. aegypti* ingest viremic blood, the blood passes into the midgut, and epithelial cells are a target for infection. If DENV passes the midgut barrier, it disseminates into the hemolymph and then throughout the body. In our work, mosquitoes were fed either naïve blood (no virus) or virus-spiked blood, then collected 7- or 10-days post-blood meal for plaque assay to quantify virus titer. The median virus titers were between 2-4 logs higher ($p<0.01$) within DENV4 Haiti- and DENV2 NGC-infected mosquito midgut and carcass samples than in DENV4 H241-infected samples. These results suggest that the DENV4 strains that were tested have different phenotypes. The mRNA

levels of transcription factor Rel 2 and its negative regulator, Caspar, of the Immune Deficiency (IMD) innate immune pathway of *A. aegypti* were measured via quantitative real-time RT-PCR, relative to an internal control gene transcript (RPS7). Rel 2 and Caspar were significantly different within DENV4- Haiti-infected mosquito pools from that of the naïve blood control group. No significant changes were noted between either DENV4 H241 or DENV2 NGC with the naïve blood control. These data further suggest that in addition to higher susceptibility of *A. aegypti* to DENV4 Haiti, there may be a concurrent negative effect on the immune response, but not for DENV4 H241 or for DENV2 NGC. Further research is needed to pinpoint the genetic difference(s) between the two DENV4 strains responsible for the different responses in Orlando *A. aegypti* after infection.

195

GENETIC DIVERSITY OF CIRCULATING DENGUE VIRUSES IN THE PHILIPPINES (2014 - 2017)

John Mark S. Velasco, Chonticha Klungthong, Ma. Theresa Valderama, Paula Corazon Diones, Piyawan Chinnawirotpisan, Wudtichai Manasatienkij, Yongyuth Poolpanichupatam, Khajohn Joonlasak, Damon Ellison, Stefan Fernandez, Louis Macareo

U.S. Army Medical Directorate-Armed Forces Institute of Medical Sciences, Bangkok, Thailand

The Philippines conducted a dengue vaccination campaign involving ≈ 830,000 population from Apr 2016 to Apr 2017. The Philippines is also part of the Phase 3 Takeda dengue vaccine clinical trial with the 1st and 2nd doses given from Sept 2016 to Jul 2017. Sieve analysis by Rabaa et al. (2017) on breakthrough dengue infections from the CYD14/15 trials showed absence of a direct relationship between vaccine efficacy and genetic similarity between Dengvaxia (CYD-TDV) strains and wild-type strains while Juraska et al. (2018) described this effect to be limited to DENV-4 and the 2-8 y.o. age group. To provide circulating DENV genetic diversity data during the mass vaccination, we obtained serum samples from dengue-diagnosed patients who were located in the general area where vaccinations occurred but who were not vaccinated with either Dengvaxia or the Takeda dengue vaccine candidate. DENV PCR-positive samples were inoculated onto C6/36 cell culture. We used Illumina MiSeq to sequence the complete genome of the DENV isolates. Quality control, de novo assembly, comparison to reference sequences, and mapping were done to generate consensus sequences. Per serotype genotypic analyses were performed by reconstructing rooted maximum likelihood trees from DENV, reference, and vaccine strain sequences (whole genome and envelope gene). 75 isolates were obtained from 100 DENV-PCR positive samples (15 DENV-1, 15 DENV-2, 35 DENV-3, and 10 DENV-4). Phylogenetic analyses of DENV sequences and Dengvaxia strains showed that wild-type (WT) DENV-1 isolates belonged to genotype IV, DENV-1 vaccine strain belonged to genotype I; WT DENV-2 isolates belonged to the cosmopolitan genotype, DENV-2 vaccine strain belonged to Asian I genotype; WT DENV-3 isolates belonged to genotype I, DENV-3 vaccine strain belonged to genotype II; WT DENV-4 isolates and DENV-4 Dengvaxia strain both belonged to genotype IIa. Analysis of the Takeda dengue vaccine candidate (TAK-003) strains and circulating dengue strains was also done. All WT dengue strains belonged to different genotypes versus the Takeda's dengue vaccine candidate strains.

196

EARLY GUILLAIN-BARRE' SYNDROME COMPLICATING DENGUE HEMORRHAGIC FEVER: TWO CASES FROM MYANMAR

Aye Mya Theingi Win[1], Khin Ko[1], Aye Win[1], May Zabe[1], Cho New[1], Mya Paing[1], Patricia F. Walker[2], Moe San[1]

[1]*University of Medicine ([1]), Yangon, Myanmar,* [2]*University of Minnesota, Minneapolis, MN, United States*

Dengue fever is a mosquito borne flavivirus which creates a heavy burden of clinical illness in tropical and subtropical countries, including Myanmar. Although it has very wide clinical presentations, neurological manifestations are quite rare. Here we report two cases of Guillain-Barre' syndrome complicating dengue hemorrhagic fever. In both cases Guillain-Barre' syndrome occurred in the first week of fever, which is unusual compared with other case reports and literature review. Case 1's motor weakness was severe and the patient had to undergo plasma exchange. Case 2 had mild motor weakness and needed only conservative treatment. Nerve conduction study of both cases revealed variant types of Guillain-Barre' syndrome which are more common in Asian countries, specifically acute motor axonal neuropathy (AMAN) and acute motor-sensory axonal neuropathy(AMSAN). Both cases ended with an excellent outcome. Awareness of early Guillain-Barre's syndrome in patients with dengue fever is important in order to potentially reduce morbidity and mortality from this serious complication.

197

PRIMARY DENGUE INFECTION MODULATES ZIKA PATHOGENESIS IN A TIME-DEPENDENT MANNER

Crisanta Serrano-Collazo[1], Petraleigh Pantoja[1], Lorna A. Cruz[1], Erick X. Perez[1], Idia V. Rodriguez[2], Teresa Arana[1], Melween Martinez[2], Mariah Hassert[3], Laura J. White[4], James D. Brien[3], Vida Hodara[5], Luis Giavedoni[5], Aravinda de Silva[4], Amelia Pinto[3], Carlos A. Sariol[1]

[1]*UPR-Medical Sciences Campus, San Juan, Puerto Rico,* [2]*Unit of Comparative Medicine, Caribbean Primate Research Center, UPR- Medical Sciences Campus, San Juan, Puerto Rico,* [3]*Department of Molecular Microbiology and Immunology, St. Louis University School of Medicine, St. Louis, MO, United States,* [4]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [5]*Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, TX, United States*

Zika virus (ZIKV) infection remains a public health concern with the recent epidemic infecting millions of people in the Americas. This flavivirus is transmitted primarily through the bite of *Aedes spp*. mosquitoes, providing the perfect vehicle to affect previously endemic dengue virus (DENV) areas with ZIKV. ZIKV and DENV share a homology in amino acid sequence of at least 50%, and studies have claimed that pre-existing immunity to ZIKV may enhance DENV pathogenesis. To address the role of prior flavivirus exposure on ZIKV associated to disease severity we recently showed that a previous DENV infection (> 2 years) does not result in enhanced ZIKV pathogenesis. For this study, we aimed to determine if the length of time between DENV and ZIKV infections has an impact on ZIKV pathogenesis. The experimental design is based on the infection of 16 rhesus macaques with ZIKV. Cohorts 1 (n=6) and 2 (n=4) had been exposed to DENV-2 one year and three months earlier, respectively, before infection with ZIKV. A third flavivirus-naive cohort (n=6) was included as a control group. qRT-PCR for measurement of viremia in serum, plaque reduction neutralization assays, ELISA tests, and cell phenotyping via flow cytometry were performed. qRT-PCR results show that viremia in serum was shorter in macaques that were exposed to DENV twelve months earlier in comparison to naïve and the cohort exposed to DENV three months earlier. We identified limited but significant differences in the magnitude of the early humoral immune response associated to a period of twelve months but not three months of DENV convalescence. However, the role of antibodies limiting ZIKV replication is not conclusive. Additionally, animals belonging to DENV 12M group display higher levels of activation of memory subsets of cellular immune response. Our results strongly suggest that there is a degree of cross-protection between DENV and ZIKV that is time-dependent. A one year time-lapse between DENV and ZIKV infections could positively modulate the immune response to induce protection.

198

AN RT-PCR PANEL FOR RAPID SEROTYPING OF DENGUE VIRUS SEROTYPES 1 TO 4 IN HUMAN SERUM AND MOSQUITO ON A FIELD-DEPLOYABLE PCR SYSTEM

Jih-Jin Tsai[1], Wei-Liang Liu[2], Ping-Chang Lin[1], Bo-Yi Huang[1], Ching-Yi Tsai[1], Pin-Hsing Chou[3], Fu-Chun Lee[3], Chia-Fong Ping[3], Pei-Yu Alison Lee[3], Li-Teh Liu[4], Chun-Hong Chen[5]

[1]*Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan,* [2]*National Mosquito-Borne Diseases Control Research Center, National Health Research Institutes, Zhunan, Taiwan,* [3]*GeneReach Biotechnology, Taichung, Taiwan,* [4]*Department of Medical Laboratory Science and Biotechnology, College of Medical Technology, Chung-Hwa University of Medical Technology, Tainan City, Taiwan,* [5]*National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Zhunan, Taiwan*

Dengue fever, a mosquito-borne disease, is caused by dengue virus (DENV) which includes four major serotypes (DENV-1, -2, -3, and -4). Some serotypes cause more severe diseases than the other; severe dengue is associated with secondary infections by a different serotype. Timely serotyping can provide early warning of dengue epidemics to improve management of patients and outbreaks. A mobile insulated isothermal PCR (iiPCR) system is available to allow molecular detection of pathogens near points of need. In this study, side-by-side comparison with the CDC DENV-1-4 Real Time RT-PCR (qRT-PCR) was performed to evaluate the performance of four singleplex DENV-1 - 4 serotyping reverse transcription-iiPCR (RT-iiPCR) reagents for DENV subtyping on the mobile PCR system. The four RT-iiPCRs did not react with Zika virus and chikungunya virus; tests with serial dilutions of the four DENV serotypes made in human serum showed they had detection endpoints comparable to those of the reference method, indicating great analytical sensitivity and specificity. Clinical performance of the RT-iiPCR reagents was evaluated by testing 40 serum samples each (around 20 target serotype-positive and 20 DENV-negative); all four reagents had high agreement (97.5 - 100%) with the reference qRT-PCR. Moreover, testing of mosquitoes separately infected experimentally with each serotype showed that the four reagents detected specifically their target DENV serotypes in mosquito. With analytical and clinical performance comparable to the reference qRT-PCR assay, the four index RT-iiPCR reagents on the field-deployable PCR system can serve as a useful tool for DENV detection near points of needs.

199

A PHASE I RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF A LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE (V181) IN FLAVIVIRUS-NAÏVE AND FLAVIVIRUS- EXPERIENCED HEALTHY ADULTS

Kevin Russell[1], Richard Rupp[2], Javier Morales-Ramirez[3], Clemente Diaz-Perez[4], Charles Andrews[5], Matthew Davis[6], Andrew Lee[1], Tyler Finn[1], Amy Falk Russell[1], Margaret Schaller[1], Jason Martin[1], Donna Hyatt[1], Sabrina Gozlan-Kelner[1], Jon Stek[1], Beth-Ann Coller[1]

[1]*Merck & Co., Inc., Kenilworth, NJ, United States,* [2]*University of TX Medical Branch, Galveston, TX, United States,* [3]*Ashford Presbyterian Community Hospital, San Juan, PR, United States,* [4]*University of Puerto Rico, San Juan, PR, United States,* [5]*Diagnostic Research Group, San Antonio, TX, United States,* [6]*Rochester Clinical Research, Rochester, NY, United States*

There is a large unmet medical need for dengue vaccines. A live-attenuated tetravalent vaccine (LATV) was developed by the National Institute of Allergy and Infectious Diseases, consisting of 4 viral components representing the 4 dengue serotypes (DENV1, DENV2, DENV3, and DENV4) and attenuated by deletion of 30 nucleotides in the 3' non-coding region of the genome (DENV3 has 2 deletions of 30 and 31 nucleotides). This Phase 1 study examined two LATV dengue vaccine admixtures of the 4 DENV serotypes (TV003 and TV005) and was conducted in the continental United States and Puerto Rico. TV005 is the same as TV003 except for a 10-fold higher DENV2 component. Two hundred participants (including flavivirus-naïve and -experienced) were randomized into the trial (2:2:1; TV003:TV005:Placebo) to receive a subcutaneous injection administered at Day 1 and Month 6. Vaccine safety (solicited and unsolicited adverse events (AEs); daily temperatures) was assessed using a Vaccination Report Card for 28 days following each vaccination. All participants were followed for serious AEs and deaths, from study entry through 1 year after last vaccination. Immunogenicity was assessed relative to vaccination using a Virus Reduction Neutralization Test at Day 1 (baseline); 28 days, 56 days, and 6 months Postdose 1; and 28 days Postdose 2. Since ~50% of participants were enrolled in dengue-endemic Puerto Rico, endpoints related to wild-type dengue infection were included. Participants were followed for dengue-related AEs (regardless of seriousness) including laboratory-confirmed dengue fever, dengue hemorrhagic fever, or dengue shock syndrome beginning at informed consent through 1 year after last vaccination. Virologically-confirmed dengue was defined as fever of ≥38.0°C (≥100.4°F) for ≥2 consecutive days with a positive qualitative and quantitative serotype-specific reverse transcription polymerase chain reaction assay. The results of the interim analysis at 28 days Postdose 1 demonstrate that the vaccine is generally well tolerated and immunogenic in these flavivirus-exposed and -unexposed participants. Data to 28 days Postdose 2 will be presented.

200

A DENGUE RT-PCR TEST PERFORMED DIRECTLY FROM BLOOD OR PLASMA FOR DIAGNOSIS IN LOW-RESOURCE SETTINGS

Ninad Mehta[1], Bastien Perrais[1], Kimberly Martin[1], Anil Kumar[1], Tom C. Hobman[1], Mary N. Cabalfin-Chua[2], Manuel E. Donaldo[3], Maria S. Panaiga[4], James Y. Gaite[4], Vanessa Tran[5], Kevin C. Kain[5], Michael T. Hawkes[1], **Stephanie K. Yanow**[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Chong Hua Hospital, Cebu, Philippines,* [3]*Cebu Institute of Medicine, Cebu City, Philippines,* [4]*Lebumfacil-Santa Ana Medicine Center, Cebu, Philippines,* [5]*University of Toronto, Toronto, ON, Canada*

Infection with dengue virus (DENV) is widespread across tropical regions and can result in severe disease. Early diagnosis is important both for patient management and to differentiate infections that present with similar symptoms, such as malaria, chikungunya, and Zika. Rapid diagnostic tests (RDT) that are used currently for point-of-care detection of DENV antigens lack the sensitivity of molecular diagnostics that detect viral RNA. However, no molecular diagnostic test for DENV is available for use in field settings. We developed and validated a RT-PCR for the detection of DENV adapted for use in resource-limited settings. RT-PCR was performed directly from patient blood or plasma samples without RNA extraction. The assay detected all four serotypes of DENV spiked into blood or plasma. The test performed equally well in a conventional lab qPCR instrument (Bio-Rad CFX) and the Open qPCR Thermocycler (Chai Biotechnologies Inc), a small, low-cost portable instrument that can be used in a field setting. The lower limit of detection for the assay was 1 x 10^4 genome copy equivalents/ml in blood. Finally, we validated our test using 126 archived patient samples. The sensitivity of our RT-PCR was 76.7% (95% CI: 65.8% to 87.9%) on the conventional instrument, and 78.3% (95% CI: 65.8% to 87.9%) on the field instrument, when compared with a commercial pan-DENV RT-PCR kit. The specificity was high, 93.9% (95% CI: 85.2% to 98.3%) on the conventional instrument and 90.9% (95% CI: 81.3% to 96.6%) on the field instrument. This molecular test is user-friendly, low-cost, and can be used in regions with limited laboratory capabilities.

201

INCREASED SEVERITY OF ILLNESS IN THE 2019 DENGUE FEVER EPIDEMIC IN RIBEIRAO PRETO, BRAZIL WITH CONSEQUENT INCREASING NUMBER OF HOSPITAL ADMISSIONS

Silvia N. Fonseca, Lude B. Silveira, Maria L. Freitas, Ivana C. Lucca, Ronaldo D. Marani, Deise R. Ustulin, Gabrielly R. Sarria, Franceliana B. Sgobi, Andre L. Fioravante, Rafael D. Duarte, Alexandre V. Celia, Felicia D. Maia, Ernani M. Martins, Viviane F. Veiga, Woe T. Chan

Hospital Sao Francisco, Ribeirao Preto, Brazil

Dengue fever (DF) is the most important arbovirus infection in the world. Our hospital emergency room (ER) located in an endemic DF region in Brazil has been dealing with large DF outbreaks since 2011. We established an ER suspected DF (SDF) surveillance, based upon the international classification of diseases codes, for ER patient visits and hospital admissions (HA); one patient was counted as SDF in his/her every ER visit for this reason. Yearly we calculated the ratio between #of SDF HA/SDF ER visits. In January 2019 and on, we noticed an increased number of DF patients with severe DF, requiring HA, even though we had not detected a large # of SDF ER patients compared to previous years. We trained all health care workers and followed the Brazilian Health Ministry Guidelines for DF patient care; the focus was prompt detection of warning signs, vigorous intravenous hydration for high hematocrit and low platelet count, oral hydration for every patient at the ER and daily or every other day returns based upon the patient risk to develop severe DF. All severe DF or DF with warning signs patient was considered for HA. We used NS1 and/or serology (IgM) to confirm DF diagnosis; the study period went from January 2011 to April 2019. The rates between # DF HA/ SDF ER visits in 2011, 2013 and 2016 were 112/29,349 (.38%), 54/21,842 (.25%) and 101/29,841 (.33%) respectively but in 2019, there was a 10-fold increase: 57/1,502 (3.8%). Of the 57 SDF HA, 45 patients had confirmed DF (31NS1+,14 IgM+); the patient median age was 58 y, the median length of stay (LS) was 4 days; 7 patients were admitted to the intensive care unit (median LS=4 days) and there were no deaths .The main causes for HA were low platelet count, hypotension, abdominal pain and spontaneous bleeding. A convenience sample was used to find out the circulating serotype by RT-qPCR; DENV-2 was detected in every sample. If this high proportion of HA for SDF continues in the next years, we will be facing difficult times ahead for available hospital beds if more patients are affected. To avoid this catastrophic event, control measures, aiming at reduction of vector population, should start before the next DF season.

202

DENGUE VACCINE INTRODUCTION ACCEPTABILITY AND FEASIBILITY IN BARRANQUILLA, COLOMBIA AND MERIDA, VENEZUELA

Elizabeth McMahon[1], Liliana Encinales[2], Carlos Navarro Encinales[3], Silvana Vielma[4], Nelly Pacheco[2], Lil Geraldine Avendaño Echavez[5], Sandra Acosta Rodríguez[6], Milena Calderon[6], Silvia Encinales Sanabria[6], Lorena Encinales Sanabria[6], Ericka Serrano Bernal[6], Andrés Gonzaléz Coba[6], Dennys Jiménez[6], Gary Simon[1], Aileen Y. Chang[1]

[1]*George Washington University School of Medicine and Health Sciences, Washington, DC, United States,* [2]*Allied Research Society, LLC, Barranquilla, Colombia,* [3]*Fundación Hospital Universitario Metropolitano, Barranquilla, Colombia,* [4]*Universidad de los Andes, Merida, Bolivarian Republic of Venezuela,* [5]*Universidad Simón Bolivar, Barranquilla, Colombia,* [6]*Clínica de La Costa LTDA, Barranquilla, Colombia*

Dengue fever is a major public health concern throughout much of the world. With one vaccine on the market and others in clinical trials, policy makers in endemic regions are faced with the decision of whether to introduce a dengue vaccine in their countries. The WHO recommends that before considering the introduction of a new vaccine, countries conduct individualized assessments that evaluate the disease in question and the strength of that country's current vaccination program. This study seeks to aid in that decision-making in the Americas by examining the acceptability and feasibility of dengue vaccine introduction in Colombia and Venezuela. The chosen study sites were Barranquilla, Colombia and Merida, Venezuela. Surveys were administered between February and June of 2018 for three target groups - patients (n=351), health professionals (n=197) and government officials (n=26). In Barranquilla, most respondents reported dengue to be a moderate-severe problem and that a dengue vaccine would be useful in their communities. In regards to subjective feasibility, most reported faith that their current vaccination programs can handle the addition of a new vaccine. In Venezuela, respondents were less likely to view dengue as a major concern and listed multiple barriers to not just dengue vaccine introduction, but to providing current vaccines as well. Further work is needed in Colombia to more objectively assess the country's readiness as a whole for a future dengue vaccine. As Venezuela continues to struggle with on-going political and social unrest, however, future studies and programs should focus on trust and capacity building.

203

DENGUE FOR FORECASTING: VIETNAM AS A CASE STUDY

Giang D. H. Dang[1], Thai Q. Pham[2], Thanh Ngo-Duc[3], Son Tong-Si[3], Tuong-Thuy Vu[4], Guy E. Thwaites[1], Marc Choisy[5], Hannah E. Clapham[1]

[1]*Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam,* [2]*National Institute of Hygiene and Epidemiology, Hanoi, Vietnam,* [3]*University of Science and Technology of Hanoi, Hanoi, Vietnam,* [4]*Hoa Sen University, Ho Chi Minh, Vietnam,* [5]*Oxford University Clinical Research Unit, Hanoi, Vietnam*

Dengue is a major disease in Vietnam. It is known that dengue has been persisting in the South and expanding to the North of the country since the 90s. Vietnam is an exciting study site as there is great variation geographically in meteorological variables that contribute to the difference in dengue incidence in each region. Dengue has multi-annual cycle in Vietnam. Therefore, one key question that the authorities ask us is how likely is it that a major epidemic will happen next year? The arrival of a trustworthy dengue forecasting system is of high demand in order to plan for hospital resources as well as for disease control. In this work, we study how changes in several climatic factors such as temperature, humidity and precipitation affect the dengue dynamics. In order to predict dengue cases across all provinces in Vietnam, we employ data over the period of 1994-2017 to train, test and validate prediction models using various techniques from simple regression to recent trend machine learning such as Gaussian Process. In addition, Vietnam has a rapidly-growing economy, and the land-use has been significantly altered over the last twenty years. We will explore the use of information from satellite images to investigate whether urbanisation has led to the recent rise of dengue cases, focusing first on the two major cities Hanoi and Ho Chi Minh city.

204

LABORATORY-ACQUIRED DENGUE IN A RESEARCHER WORKING WITH HIGH-TITER VIRUS, USA, 2018

Tyler M. Sharp[1], Teresa Fisher[2], Kristin Long[3], Garry Coulson[4], Freddy Medina[1], Carolyn Herzig[2], Mary B. Koza[4], Jorge L. Munoz-Jordan[1], Gabriela Paz-Bailey[1], Zachary Moore[2], Carl Williams[2]

[1]*Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*Division of Public Health, North Carolina Department of Health and Human Services, Raleigh, NC, United States,* [3]*State Laboratory of Public Health, North Carolina Department of Health and Human Services, Raleigh, NC, United States,* [4]*Environment, Health, and Safety, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Dengue is a common tropical illness caused by infection with any of four dengue viruses (DENV-1-4). Although the overwhelming majority of DENV infections result from the bite of an infected mosquito, other routes of transmission have been reported, including exposure while working with infectious DENV in the laboratory. In August 2018, the North Carolina

Department of Health and Human Services was notified of a dengue case in a laboratory researcher who had presented for care with a two-day history of fever, rash, body and eye pain, and lethargy. The patient tested positive for DENV infection by detection of NS1 antigen and anti-DENV IgM antibody, and also had a four-fold increase in anti-DENV IgG and neutralizing antibody titer. The case-patient was not hospitalized and recovered after a six-day illness. Interview of the case-patient confirmed absence of recent travel to an area with risk of DENV transmission. However, the case-patient did report having worked with large-scale preparation (~4 liters of supernatant from infected cells) and concentration (final viral titer of up to 10^{10} plaque-forming units per mL) of DENV-4 under biosafety level 2 conditions in the two weeks prior to illness onset. The case-patient reported that small splashes inside the biosafety cabinet were common while performing the virus purification protocol, and that gloves were only occasionally decontaminated while working in the biosafety cabinet and routinely changed when exiting the biosafety cabinet. Review of the case-patient's laboratory safety practices suggested improper technique for doffing of gloves. This in combination with a small open wound on the patient's finger may have resulted in cutaneous exposure. Nonetheless, we could not rule out other routes of exposure in the laboratory. This case demonstrates the importance of conducting ongoing risk assessments, which may warrant enhanced biosafety precautions while working with high-titer DENV. Current laboratory safety guidelines encourage use of enhanced personal protective equipment such as double gloving, especially if working with an open wound on the hand or finger.

205

AVIDITY AS ASSESSMENT OF ANTI-DENGUE VIRUS ANTIBODY RESPONSE ELICITED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE

Isamu Tsuji, David Dominguez, Christina DeMaso, Allan Parker, Sunil Palani, Nicole Messere, Melissa Zahralban-Steele, Sharma Mayuri, Ralph Braun, Hansi Dean, DEN 203 and 204 study groups

Vaccine Business Unit, Takeda Pharmaceutical Inc., Cambridge, MA, United States

The quantitative immune parameters commonly employed for functional measurement of immune responses to viral vaccines include antiviral antibodies and viral neutralization titers in post-vaccination serum. However, serum antibody avidity can be an important qualitative measure of the humoral response, and mechanism of protection. Takeda's live attenuated tetravalent dengue vaccine candidate (TAK-003) has structural proteins from each serotype on the dengue virus type 2 (DENV-2) genomic backbone. Here, we describe the development of an assay we used to measure the avidity of humoral responses to DENV-1, -2, -3 and -4 in eight dengue-naïve subjects, pre- (day 0), and up to one-year (day 360) post-vaccination with TAK-003. Microneutralization titers (MNT) were highest on day 28 post-vaccination and had declined by day 360. The strength of antibody binding increased post-vaccination: $k_{off,}$ the antibody dissociation rate, decreased over time. Therefore, the Avidity Index of the antibody response to all four serotypes increased at day 28 post-vaccination and was maintained until day 360, reflecting affinity maturation of the DENV-specific antibody responses. These data suggest that antibodies elicited by TAK-003 undergo affinity maturation and show a sustained increase in avidity for at least one-year post-vaccination. We are now working on combining measures of antibody avidity with anti-DENV antibody response and serotype-specific neutralization titers, to systematically characterize antibody responses to TAK-003 over time.

206

ESTIMATING FORCE OF INFECTION (FOI) OF ALL FOUR DENGUE SEROTYPES FROM SEROLOGICAL STUDIES IN TWO REGIONS OF VIETNAM

Huynh Thi Phuong[1], Nguyen Ha Thao Vy[1], Ha Minh Lam[1], Erwin de Bruin[2], Marion Koopmans[2], Maciej F. Boni[3], Nguyen Thi Le Thanh[1], Hannah E. Clapham[1]

[1]*Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam,* [2]*Department of Viroscience, Erasmus Medical Centre, Rotterdam, Netherlands,* [3]*Center for Infectious Disease Dynamics, Department of Biology, Pennsylvania State University, University Park, PA, United States*

Vietnam is endemic for dengue fever and it is a major public health concern. Studying the epidemiology of the disease bring about the benefit of improving quality of intervention and control. However, it is hard to estimate the true rate of dengue infection due to its high asymptomatic rate. All four serotypes of dengue (DENV 1-4) co-circulate year round in Vietnam and immune interactions between serotypes leads to complex transmission dynamics. Therefore, knowing about circulating serotypes plays a key role in understanding disease patterns and for carrying out effective vaccination campaigns. However, the Enzyme-linked immunoassay (ELISA) test, the most common use in cross-sectional sero-studies, cannot discriminate between DENV serotypes or infer how many times people have been infected. In this study, 958 population serum samples, 432 from Khanh Hoa (KH, central) and 526 from Ho Chi Minh (HCM, southern), in age groups 1-30 years old collected from 2013 to 2017 were tested using a flavivirus protein microarray assay. By applying a previously developed mathematical model to the antibody profiles generated from the assay, we are able to determine the proportions in the population that have been infected 0, 1 or more than 1 time, and infer the infecting serotypes in those infected once. With this data we are then able to use mathematical models to estimate time-varying and serotype-specific FoIs for all four DENV serotypes over the past 30 years to 2017 in KH and HCM Vietnam. We found that the annual FoI of DENV-1 had the most variation in both KH and HC and the FoI of all four serotypes was higher in KH than in HC. In the future, we try to understand whether the dominance of a serotype is a driver for an outbreak. Also, we will compare estimated FoIs to the cases from these regions over time to determine whether high FoI always means high case numbers, or if there are periods of "silent" transmission.

207

COMPREHENSIVE MUTAGENESIS OF DENGUE VIRUS ENVELOPE PROTEINS TO MAP ANTIBODY EPITOPES AND IDENTIFY RESIDES ESSENTIAL FOR FUNCTION

Edgar Davidson, Tabb Sullivan, Jen M. Pfaff, Srikar Reddy, Benjamin J. Doranz

Integral Molecular, Inc., Philadelphia, PA, United States

To characterize the immune response to dengue virus (DENV) infection, we have epitope mapped over 200 anti-DENV monoclonal antibodies (MAbs), using high-throughput, rapid screens of MAb binding to DENV prM/E comprehensive mutation libraries for all four DENV serotypes, 3,380 mutations in total. Each library of individual mutant expression plasmids was transfected into human cells to achieve native protein expression and folding, and immunoreactivity of MAbs to each individual prM/E variant was quantified by high-throughput flow cytometry. The epitopes obtained were both conformational (including quaternary) and non-conformational, were spread across prM and E domains I-III, and many have been correlated with their abilities to protect against DENV infection. A number of anti-DENV MAbs cross-reacted with ZIKV prM/E, predominantly within the fusion loop but also within Domain II of the E protein, identifying critical immunogenic residues shared by DENV and ZIKV. We have also produced DENV virions from all four DENV mutation libraries using a previously developed DENV reporter virus particle (RVP) system, allowing us to screen each individual DENV Env variant protein for DENV particle budding and infectivity. For DENV3, we identified residues whose mutation

eliminated virus infectivity but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants for use in vaccine design. To identify uncharacterized DENV cellular receptors we assayed wild-type DENV RVP infectivity in non-permissive cells expressing our membrane proteome array (MPA) of 5,300 unique human membrane proteins. This has identified candidate membrane proteins that enable DENV infectivity. We have identified neutralizing epitopes in DENV prM/E and specific sites that are critical for DENV infectivity, providing new targets and opportunities for vaccine development.

208

INTERCURRENT FLAVIVIRAL VIREMIA IN ILL RETURNED TRAVELERS WITH *PLASMODIUM VIVAX* MALARIA

Katherine Faith Tan[1], Ruwandi Kariyawasam[2], Rachel Lau[3], Filip Ralevski[3], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [3]*Public Health Ontario, Toronto, ON, Canada*

Similar epidemiology and clinical presentations of arboviral infections and malaria coupled with the typically sequential approach to diagnostic testing, where malaria is confirmed or excluded urgently in febrile returned travelers, may mask the true epidemiology of co-infections. Flaviviruses are known to trigger relapsing forms of malaria, including *Plasmodium vivax*, long after primary malaria infection, and this may delay the diagnosis of malaria. We aim to understand the incidence of intercurrent flaviviral infection in confirmed *Plasmodium vivax* infection. DNA and RNA from biobanked isolates of *P. vivax* detected in whole blood at the Public Health Ontario Laboratory between 2006 and 2018 were extracted and screened for intercurrent flaviviral infections using previously validated real-time PCR (qPCR) assays targeting multiple flaviviruses (pan-FLAV) and, specifically, dengue virus types 1-4 (DEN1, DEN2, DEN3, DEN4). Five hundred and two unique isolates of *P. vivax* were identified, of which 90 have been tested to date. Males accounted for 65.6% (n=59/90) of *P. vivax* cases, while females accounted for 32.2% (n=29/90), and sex was unassigned in 2.2% (2/90). Median age of *P. vivax* cases was 33.2 years (range 3.7 years - 85.8 years; IQR 23.4 - 45.9 years). Median parasitemia was 0.2% (range < 0.01% - 1.1%). Fifty-nine (65.6%) *P. vivax* cases had documented travel history exclusively to South Asia, with India as the most common source country (22/90 [16.7%]). Pan-FLAV assay yielded a 1.1% (1/90) positivity rate. Pan-DEN assay will be performed next. Intercurrent flaviviral viremia was noted in at least 1.1%, which may suggest that primary flaviviral infection triggered a relapse of *P. vivax*. Alternatively, such co-occurrence may suggest primary infection with both organisms known to cause fever in returning travelers. Consideration of flaviviral co-infection should be given to the *P. vivax* patient with deep thrombocytopenia, lymphopenia, and high-yield arboviral symptomatology such as rash and retro-orbital headache.

209

DETECTION AND SEQUENCING OF ZIKA VIRUS IN NORMOCEPHALIC NEWBORNS WITH CONGENITAL ZIKA IN FECTION

Breno L. de Almeida[1], Marta Giovanetti[1], João V. Oliveira[1], Tereza C. Xavier[2], Eduardo M. Figueiredo[3], Jaqueline J. Goes[1], Luiz C. Alcantara[1], Isadora C. de Siqueira[1]

[1]*Fundação Oswaldo Cruz-Fiocruz, Salvador, Brazil,* [2]*Maternidade de Referencia Prof José Maria de Maglhães Neto, Salvador, Brazil,* [3]*Maternidade de Referencia Prof José Maria de Magalhães Neto, Salvador, Brazil*

In 2015, Brazil has experienced an unprecedented Zika virus (ZIKV) outbreak and later that year, an unexpected outbreak of newborns with microcephaly occurred in major cities in northeastern Brazil, associated with Congenital Zika Infection (CZI). Most descriptions and publications regarding CZI focus on the clinical presentation of newborns and infants with microcephaly. Scarce information is available concerning CZI without microcephaly. During hospital surveillance for CZI in a reference maternity hospital, we identified 14 normocephalic newborns with confirmed CZI. Eight (57%) of the newborns were female and the mean gestational age at birth was 38.46 ± 1.90 weeks. The mean of head circumference was 38.57 ± 1.40cm. The transfontanel ultrasonography was performed in 13 (92.9%), and no alterations were observed in any of the cases. All newborns had a positive RT-PCR confirming the diagnosis of CZI, mostly in urine samples (57%). In two of the cases, ZIKV were detected in 2 distinct samples. ZIKV-specific RT-PCR amplification products have been obtained and NS5 gene fragments (426-bp) were obtained using Sanger sequencing. The phylogenetic analysis showed that the isolate belongs to the Asian genotype and clusters closely with strong bootstrap support (>90%) with sequences isolated in Northeast and Northern regions of Brazil. With this, we infer that CZI could present in a broad spectrum of clinical manifestation, including the asymptomatic presentation at birth. It is necessary careful surveillance to identify cases with few or no symptoms at birth and a close follow-up for early detection of clinical manifestations of CZI and timely intervention.

210

ZIKA VIRUS DETECTION IN PREVIOUSLY UNDIAGNOSABLE SAMPLES: OPTIMIZATION OF A QUANTITATIVE RT-PCR ASSAY FOR SAMPLES OF LOW VIRAL CONCENTRATION

Alejandra Garcia Glaessner[1], Patricia Barrera[2], Amy C. Morrison[3], Thomas W. Scott[4], Mariana Leguia[1]

[1]*Genomics Laboratory, Pontificia Universidad Católica del Peru, Lima, Peru,* [2]*Asociación Benéfica Prisma, Lima, Peru,* [3]*Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA, United States,* [4]*Department of Entomology and Nematology, University of California, Davis, CA, United States*

The Zika virus (ZIKV) outbreak of 2015 affected many countries in Latin America and highlighted a pressing need for sensitive molecular diagnostics to detect the presence of ZIKV in various sample types. At the time, only two RT-qPCR assays, developed by Faye et al. and Lanciotti et al., were available to diagnose human ZIKV infections. These assays used primer sets that target separate regions of the ZIKV genome with enough specificity to serve as diagnostic tools that could differentiate ZIKV from other related arboviruses, such as dengue and yellow fever. However, their sensitivity was low, resulting in false-negative diagnosis of many ZIKV cases, particularly when ZIKV was present at low levels (CT values around 30 or higher). We developed an improved RT-qPCR protocol that uses optimized primer sets and cycling conditions to detect the presence of ZIKV at levels previously considered undiagnosable (CT values up to 38). The parameters optimized include primer length, GC content and annealing temperature. We also created a positive control plasmid that includes both the NS5 and envelope regions of the genome, allowing for side-by-side comparisons of the amplification efficiency of old and new assays. Using this control plasmid, we estimated the limit of detection of our new assay at 31 copies of the viral genome. We used our assay to test a number of samples suspected of being false-negatives and found that about 10% of those re-tested were positive for ZIKV. Our results indicate that the improved ZIKV assay can help address the issue of false-negatives during ZIKV diagnosis and will be a useful tool for those interested in a fast, rapid, and sensitive diagnostic for ZIKV, particularly in samples of low viral concentration encountered during clinical evaluation or research.

211

SEROPREVALENCEOF ZIKA VIRUS (ZIKV) IN CEBU PROVINCE, PHILIPPINES

Cameron R. Adams[1], Ramesh Jadi[1], Michelle Ylade[2], Jedas Daag[2], Kristal An Agrupis[2], Jacqueline Deen[2], Aravinda de Silva[1], Premkumar Lakshmanane[1], Anna Lena Lopez[2]

[1]*University Of North Carolina, Chapel Hill, NC, United States,* [2]*Institute of Child Health and Human Development, University of the Philippines Manila, Manila, Philippines*

Zika virus (ZIKV) is an emerging mosquito-borne Flavivirus notable for severe teratogenic properties and Guillain-Barré syndrome seen during the 2015 epidemic in Latin America. While ZIKV infection is documented in different regions of Asia, the virus was not linked to large epidemics or severe clinical symptoms. Moreover, the incidence, prevalence and ecology of ZIKV in Asia is poorly understood. For monitoring ZIKV transmission at a regional/population level, serology is more useful than methods for detecting the transient viremia caused by ZIKV as ZIKV infection often presents with no overt clinical symptoms. ZIKV and dengue virus (DENV) co-circulate in the same populations. As DENV and ZIKV are closely related and share conserved epitopes, antibodies induced by DENV infections are cross-reactive with ZIKV antigens commonly used in diagnostic assays. Using recombinantly produced domain III of the ZIKV envelope protein (EDIII), we have developed an assay for specific detection of antibodies induced by ZIKV infection. In this study, we use the ZIKV-EDIII ELISA to determine the seroprevalence of ZIKV in children aged 9-14 in the Cebu province of the Philippines. In a sample of 64 children, 12 (18%) tested positive for ZIKV EDIII antibodies. By performing antibody depletions and neutralization assays with DENV1-4 and ZIKV, we have confirmed that 11/12 children testing positive in the ZIKV-EDIII assay have ZIKV type-specific neutralizing antibodies. We tested a cohort of 3,000 children with our ZIKV-EDIII assay and defined ZIKV seroprevalence of ZIKV type-specific antibodies.

212

PREGNANCY AND BIRTH OUTCOMES AMONG COLOMBIAN WOMEN WITH ZIKA VIRUS DISEASE IN THREE SURVEILLANCE SITES, PROYECTO VIGILANCIA DE EMBARAZADAS CON ZIKA

Van Tong[1], Marcela Mercado[2], Suzanne Gilboa[1], Diana Valencia[1], Marcela Daza[3], Romeo Galang[1], Christina Winfield[1], Shana Godfred-Cato[1], Monica Benavides[3], Julie Villanueva[1], Jonathan Daniels[1], Julu Bhatnaga[1], Jarad Schiffer[1], Sheryll Corchuelo[3], Sarah Tinker[1], Kayla Anderson[1], Johana Osorio[3], Veronica Burkel[4], Jacob Hojnacki[5], Maritza Gonzalez[2], Cynthia Moore[1], Margaret Honein[1], Martha Ospina[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*National Institute of Health, Bogota, Colombia,* [3]*Vysnova Partners, Bethesda, MD, United States,* [4]*Eagle Medical Services, LLC, San Antonio, TX, United States,* [5]*Oak Ridge Institutes for Science and Education, Oak Ridge, TN, United States*

Proyecto Vigilancia de Embarazadas con Zika (VEZ) was an intensified surveillance system built upon existing national surveillance of pregnant women with symptoms of Zika virus (ZIKV) disease and conducted in three Colombian cities with high prevalence of Zika. This analysis of data from VEZ estimates the risk of Zika-associated birth defects among pregnant women with symptoms of ZIKV disease, and among a subset with laboratory evidence of possible ZIKV infection during pregnancy. During April-November 2016, pregnant women were enrolled if they were reported to the surveillance system (Sivigila) or visited participating clinics with symptoms of ZIKV disease. Maternal and pediatric data were abstracted from prenatal care, ultrasound, and delivery records, as well as from pediatric or specialist visit records. Available maternal and infant specimens were tested for the presence of ZIKV RNA and/or anti-ZIKV immunoglobulin (IgM) antibodies. Of 1,223 women enrolled, 47.8% and 34.3% reported first or second trimester symptom onset, respectively. Of 381 pregnancies with maternal and/or infant specimens tested, 108 (29%) had laboratory evidence of possible ZIKV infection during pregnancy; half of these (53.3%) were positive for ZIKV RNA only, 37.4% for IgM antibodies only, and 9.3% for both. Of 1,190 of pregnancies with known outcome, 63 (5%) had Zika-associated brain or eye defects; among the subset with any laboratory evidence, 12 (11%) had Zika-associated brain or eye defects. The prevalence of Zika-associated brain or eye defects was 5.9% (35/593) and 4.5% (19/423) among pregnancies with symptom onset in the first and second trimester, respectively. Among pregnant women with symptoms of ZIKV disease enrolled during the height of the ZIKV epidemic in Colombia, prevalence of any Zika-associated brain or eye defect was 5%, with a higher prevalence among those with laboratory evidence of possible ZIKV infection. Rapid enhancements to Colombia's national surveillance enabled the estimation of the risk of birth defects associated with ZIKV disease in pregnancy.

213

PEDIATRIC ZIKA VARIES BY AGE AND IS OFTEN MISSED UNDER CURRENT CASE DEFINITIONS

Fausto A. Bustos Carrillo[1], Raquel Burger-Calderon[1], Lionel Gresh[2], Sergio Ojeda[2], Nery Sanchez[2], Miguel Plazaola[2], Leah Katzelnick[1], Brenda L. Mercado[2], Jairo C. Monterrey[2], Douglas Elizondo[2], Sonia Arguello[2], Andrea Nuñez[3], Aubree Gordon[4], Angel Balmaseda[3], Guillermina Kuan[5], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua,* [4]*Department of Epidemiology, University of Michigan, Ann Arbor, MI, United States,* [5]*Centro de Salud Sócrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua*

Pediatric Zika is an understudied aspect of the 2015-2016 Zika pandemic. The diagnostic performance of the World Health Organization (WHO) and Pan American Health Organization (PAHO) Zika case definitions have not been assessed in children. We prospectively followed a cohort of ~3,700 children aged 2-14 years old in Managua, Nicaragua, from January 2016 to February 2017. We tested participants with a broad set of clinical profiles using rRT-PCR and a machine learning algorithm based on 5 serological assays and analyzed acute clinical findings (signs, symptoms, and complete blood counts). We used generalized additive models to assess non-linear age trends in the prevalence of clinical findings and the sensitivity of the WHO and PAHO case definitions. Overall, 556 Zika and 548 non-Zika cases were analyzed. Rash and leukopenia were significantly more prevalent among Zika cases, whereas fever and monocytosis were more common among non-Zika cases. Among Zika cases, rash, fever, leukopenia, and headache were the most common clinical findings, which tended to peak within 3 days of illness onset. The most common presentation over the first week of illness included only two clinical findings, rash (79.1%) and leukopenia (48.0%). Only 31.7% and 19.6% of lab-confirmed Zika cases were captured by the WHO and PAHO case definitions, respectively. These sensitivity values increased with age, as the prevalence of most clinical findings (chiefly arthralgia) increased with age. Standard case definitions tended to capture cases that presented with a dengue-like clinical profile; the remaining two-thirds, characterized principally by undifferentiated fever or afebrile rash, were missed. Our results suggest that most pediatric Zika cases are undetected under the current case definitions and that Zika-related epidemiological findings, R_0 values for Zika, and transmission models may have been systematically biased in an age-dependent manner. Overall, our results and the literature suggest that Zika manifests more mildly in children than adults, presenting major challenges to diagnosis, surveillance, and efforts to control future Zika epidemics.

214

CLINICAL AND EPIDEMIOLOGICAL FEATURES OF THE ZIKA OUTBREAK IN NICARAGUA

Natalie M. Bowman[1], Filemon Bucardo[2], Matthew H. Collins[3], Yaoska Reyes[2], Edwing Centeno[2], Quique P. Guerra[1], Rebecca J. Rubinstein[1], Guei-Jiun Alice Liou[1], Aravinda de Silva[1], Becker-Dreps Sylvia[1]

[1]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Universidad Nacional Autonoma de Nicaragua-Leon, Leon, Nicaragua,* [3]*Emory University, Atlanta, GA, United States*

We conducted an observational study in León, Nicaragua between January 2016 and February 2018. Patients seeking care at the public hospital and health centers for reported fever, rash, and/or non-suppurative conjunctivitis of ≤7 day's duration were offered study participation. At enrollment, blood, saliva, and urine were collected and surveys recorded demographic information, clinical symptoms, and potential risk factors for ZIKV infection. All subjects were asked to provide a convalescent serum sample 2-4 weeks later. All PCR-positive samples at baseline were repeated in triplicate; if all three replicates were positive (Ct<38), the sample was considered a true positive and these subjects were considered acute ZIKV cases. Because of the short window of viral shedding in many ZIKV cases, we also considered those with negative PCR but serology (combined acute and convalescent dengue and Zika IgG and IgM) consistent with acute or recent ZIKV infection to be cases. 297 subjects were recruited, but 5 subjects could not be categorized due to missing or ambiguous serological and PCR test results and were excluded from further analyses. Mean age was 27.6 years (range 1-81, SD 17.3), and 187 (64%) were female. 55 subjects (19%) had acute ZIKV infection by PCR and/or serology, with the last case in June 2017. There was no difference in odds of ZIKV infection by age or sex. Pregnant women had higher odds of ZIKV infection than non-pregnant women of childbearing age (OR 10.66; 95%CI 2.19, 66.81). Conjunctivitis (OR 3.52; 95%CI 1.75, 7.39), rash (OR 8.39; 95%CI 3.18, 27.79), and sore throat were associated with increased odds of ZIKV infection while reported fever was associated with lower odds (OR 0.20; 95%CI 0.08, 0.53). ZIKV-infected subjects had significantly lower white blood cell, absolute neutrophil, and platelet counts. Multivariable modeling demonstrated that the clinical features rash, conjunctivitis, sore throat, and lack of fever were associated with increased odds of acute ZIKV infection in this cohort. These findings may help clinicians distinguish ZIKV from other acute febrile illnesses even where laboratory testing is not readily available.

215

DOSE SELECTION OF A PURIFIED IN ACTIVATED ZIKA VIRUS VACCINE (PIZV) CANDIDATE FOR FURTHER CLINICAL DEVELOPMENT

Htay Htay Han[1], the ZKV-101 Study Group[2]

[1]*Takeda Vaccines Inc., Cambridge, MA, United States*

We evaluated immune responses to a two-dose purified inactivated Zika virus vaccine (PIZV) candidate administered at three dosage levels (2, 5 or 10 μg) in a two-stage phase 1 study, first in flavivirus (FV)-naïve and second in FV-primed healthy adults. We enrolled 271 FV-naïve and -primed adults, aged 18–49 years, randomized into four groups (1:1:1:1) to receive two doses of either placebo or one of the three PIZV dose levels with 28 days intervals. Volunteers recorded solicited adverse events (AEs) for 7 days and unsolicited AEs for 28 days after each dose, and serious adverse events (SAEs) throughout the study period. Neutralizing antibody titers were measured before vaccination and 28 days after each dose using a plaque reduction neutralization test (PRNT) and an exploratory reporter virus particle (RVP) assay. We report the results from an interim analysis of safety and immunogenicity data leading to the decision of dose selection. This was based on the safety data of both FV-naïve and primed participants and the magnitude of the immune response in FV-naïve subjects, as measured by the pairwise ratios of geometric mean titer (GMT) of Zika virus neutralizing anti-antibodies and differences in seroconversion rates between the dosing groups. The safety data set included 271 FV-naïve and -primed participants, the per-protocol set for immunogenicity included 113 FV-naïve participants. Overall demographic characteristics were similar across the four study arms. The duration of safety follow-up at the time of interim analysis was until at least 28 days post-dose 2 for FV-naïve subjects and until at least 28 days post-dose 1 for FV-primed subjects. The PIZV vaccine was well tolerated with an acceptable safety profile at all dosages. The dose response effect was observed in FV-naïve subjects with increasingly higher GMTs with higher PIZV dosages. A pairwise comparison of GMTs (PRNT) confirmed the significance of the dose-ranging effect: i.e., the 10 μg group was significantly greater than in the 2 μg ($p<0.001$) and the 5 μg ($p<0.05$) groups. The RVP results showed a similar dose-ranging effect. The 10 μg PIZV candidate was selected for further development.

216

TGF-β AND TNF-α CYTOKINE GENE POLYMORPHISMS MAY INFLUENCE PREGNANCY OUTCOMES OF ZIKA VIRUS INFECTED WOMEN

Benedito A. Fonseca, Mayara R. Agostinho, Danillo L. Esposito, Vitor G. Floriano, Marcio J. Siconelli

School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Zika virus (ZIKV) infection and its association with Congenital Zika Syndrome (CZS) have recently become a great concern worldwide and several studies are investigating the mechanisms involved on the pathogenesis of fetal disease. During virus infection many cytokines are produced to induce a protective immunity, but some gene polymorphisms could interfere with cytokine expression, compromising the quality of the immune response. Cytokine gene polymorphisms were investigated on ZIKV-infected pregnant women (n=26) and correlated with their pregnancy outcome. TNF-α, IL-6, IFN-γ and TGF-β gene polymorphisms were evaluated in 12 pregnant women who had miscarriages or newborn malformations (study group) and in 14 who had healthy newborns (control group). Differences in TGF-β (*p=0.013*) and IFN-γ (*p=0.0161*) polymorphisms frequencies were statistically significant between the two groups. TGF-β polymorphisms (*low 10C/C 25G/C*; *intermediate 10T/C 25G/C*; *intermediate 10C/C 25G/G*; *high 10T/C 25G/G*) in the control group were 64.3%, 14.3% 14,3% and 7.1%, respectively, while (*high 10T/C 25G/G*; *low 10C/C 25G/C*) in the study group were 83.3% and 16.7%, respectively. IFN-γ polymorphisms (*high +874 T/T*; *intermediate +874 T/A*; *low +874 A/A*) in the control group were 50%, 14.3% and 35.7%, respectively, while in the study group (*low +874 A/A*; *intermediate +874 T/A*) were 66.7% and 33.3%, respectively. TGF-β inhibits the proliferation of endothelial, epithelial and hematopoietic cells, regulates the mesenchymal, epithelial, neuronal, immune cell differentiation and modulates their apoptotic response. IFN-γ is primarily involved in immune regulation and proinflammatory responses. Our data suggest that the increased frequency of *low +874 A/A* IFN-γ polymorphism and lower frequency of *high 10 T/C 25 G/G* TGF-β polymorphism could act synergistically in the causation of CZS following ZIKV infection as the IFN-γ polymorphism would downregulate the immune response against ZIKV and the increased inhibitory cell proliferation by the TGF-β polymorphism would interfere with fetal development, leading to miscarriages and fetal malformations.

217

CHARACTERIZATION OF A POTENTIAL CORRELATE OF PROTECTION PROVIDED BY TAKEDA'S PURIFIED INACTIVATED ZIKA VACCINE IN INDIAN RHESUS MACAQUES

Ginger Young[1], Stephanie Sonnberg[1], Hui-Ling Chen[1], Srisowmya Sanisetty[1], Melissa Zahralban-Steele[1], Tim Powell[1], Joseph Lee[1], Michael Johnson[1], Greg Hather[2], Lovkesh Karwal[1], Kelly Bohning[1], Lydia Andserson[1], Hetal Patel[1], Hansi Dean[1]

[1]*Takeda Vaccines, Inc., Cambridge, MA, United States,* [2]*Takeda Pharmaceuticals, Inc., Cambridge, MA, United States*

A substantial unmet medical need exists for a Zika vaccine which will help mitigate the effects of any future Zika epidemics. In this study we evaluated neutralizing antibody responses to Takeda's purified inactivated Zika vaccine (PIZV) and the efficacy against challenge with the Puerto Rico Zika virus (ZIKV) strain PRVABC59 in naïve Indian rhesus macaques. Macaques received one of five dose levels (0.08, 0.016, 0.4, 2.0, and 10 μg) of PIZV administered intramuscularly 28 days apart. Six weeks after the second immunization (Day 71), macaques were challenged with $1x10^4$ focus forming units of ZIKV PRVABC59 administered subcutaneously. Sera were collected prior to and after ZIKV challenge and assessed by a Zika reporter virus particle (RVP) assay for neutralizing antibody titers. Zika viral RNA (vRNA) load in the serum was measured using a quantitative real-time PCR assay. PIZV induced a dose-dependent immune response that was boosted by a second immunization. Importantly, after two immunizations at dose levels above 0.4 μg, the neutralizing antibody responses were similar in magnitude to the immune responses following ZIKV infection observed in the control group. Zika vRNA was detected in the serum of the control group for 5 consecutive days post-challenge. Complete protection of macaques, defined as the absence of quantified Zika vRNA in serum post-challenge, was achieved with doses of 0.4 μg, 2 μg and 10 μg. Only partial protection was achieved with the lower PIZV doses. Based on these data, a neutralizing antibody response above 3.67 $Log_{10}EC50$ was determined as the preliminary correlate of protection. In summary, Takeda's PIZV elicited a dose-dependent neutralizing antibody immune response, which correlated with lack of quantified Zika vRNA after ZIKV challenge.

218

RESEARCH OF ANTI-VIRUS ANTIBODIES OF MEASLES, RUBELLA, MUMPS AND T*OXOPLASMA GONDII* IN SALIVA OF SCHOOLS AND COLLEGES OF THE CITY OF SÃO PAULO

Barbara F. Sampaio

Institute of Tropical Medicine Sao Paulo, Sao Paulo, Brazil

Vaccines is a well-established public health intervention, with a major impact on the decline in the prevalence of infectious diseases, but outbreaks are occurring frequently due to primary and secondary. Serological control of the vaccination status population is essential but is based on invasive blood sampling, problematic for children and teenagers. Saliva can be as acceptable alternative IgG for children and other protected groups. We detect the prevalence of specific IgG response for measles, mumps, rubella and *T. gondii* in saliva samples, for evaluate vaccine efficiency and toxoplasmosis. For sampling, we collect 249 saliva samples from 7 to 13 years old students from São Paulo, Brazil. We developed and validated an IgG capture assay, with revealing of IgG specificity by the use of biotinylated recombinant measles, rubella, mumps and *T.gondii*. The assays had reproducibility greater than 98% and sensitivity and specificity> 95%, using sera. Saliva and sera of 47 university students were tested for paired comparison, without discordance. We detected in the saliva from elementary students, a prevalence of 8.5% for anti T.gondii IgG of, anti-measles IgG of 96.8%, anti-rubella IgG of 59.1% and anti-mumps IgG of 57.5%. The prevalence of antibodies against mumps and rubella was lower than measles, as described in other reports, but this approach shows the feasibility of saliva for sustained follow-up of vaccine immune status in teenagers for devising more adequate re-immunization protocols. Our approach was efficient in all aspects, from the hygiene exhibition for sampling, the use of saliva and the development of reliable tests for the determination of the IgG protection in students and the prevention of toxoplasmosis, in declining incidence. This approach allows cheaper follow-up for IgG detection of several diseases, including vaccine control. Appropriate public health measures, such as revaccination, can be properly planned and developed for avoiding outbreaks and upsurge of controlled infectious diseases.

219

ZIKA VIRUS COMPLICATIONS BEYOND CONGENITAL ZIKA SYNDROME: A SYSTEMATIC REVIEW

Leyla A. Hernandez-Donoso[1], Estelle Meroc[2], Laurence De Moerlooze[1]

[1]*Takeda Pharmaceuticals, Zurich, Switzerland,* [2]*P95 Pharmacovigilance and Epidemiology, Leuven, Belgium*

Zika virus (ZIKV) was considered a mild disease for decades until the outbreaks in French Polynesia and the Americas showed a link with severe complications: Congenital Zika Syndrome and Guillain-Barre Syndrome. We conducted a systematic review of the literature to quantify and characterize the scope of complications associated with the disease, independently of transmission mode and geography. We searched for articles published in English, Spanish, French, Dutch and Portuguese until July 2018 primarily in MEDLINE, EMBASE, LILACS, BIOSIS and SCIELO; complemented with manual searching from: WHO/PAHO, reports from Ministers of Health, Zika research associations and grey literature from academic theses and congress abstracts. Three researchers independently screened publications by title and abstract, only those relevant to the research questions were eligible for full text review. The quality of primary studies was assessed using Newcastle-Ottawa Scale (NOS) and NIH tools. We included a third reviewer to control for selection bias. The review followed PRISMA and is registered in PROSPERO. We identified 1535 articles and selected 396 for full-text review. 64 were included in the main analysis: 20 surveillance reports, 14 case-control, 24 cohort, 3 cross-sectional and 3 systematic reviews. Case reports and case series were described in a separate analysis. Beyond pregnancy outcomes and neurological disorders, other rare complications like cardiovascular, rheumatic, ophthalmic and cognitive were described. We quantified each outcome and summarized by type of study, geographical and temporal distribution, Zika confirmation, possible confounders and quality scores. This systematic review of ZIKV associated complications reveals new features of the disease that can be important to identify before a new outbreak occurs.

220

PROSPECTIVE STUDY OF HEPATITIS E VIRUS INFECTION AMONG PREGNANT WOMEN IN NIGERIA

Adeola Fowotade

University of Ibadan, Ibadan, Nigeria

Hepatitis E virus (HEV) is an emerging infectious agent causing acute viral hepatitis worldwide. HEV infection has a poor prognosis among pregnant women from high endemic countries. HEV infection during third trimester and especially with genotype 1 is associated with fulminant hepatitis. Varied HEV-prevalence and incidence among pregnant women has been reported in low-income settings. This prospective study was conducted to assess HEV infection among pregnant women attending antenatal care at the University College Hospital, Ibadan, Nigeria. Serum samples of 230 pregnant women were screened for the presence anti-HEV IgG and anti-HEV IgM using commercially available ELISA kits (DIA.PRO, Milan, Italy). All anti-HEV IgM positive samples were tested for HEV RNA using two independent reverse transcriptase polymerase chain reactions (RT-PCR) assays, targeting ORF2 and ORF3 of HEV genome. In addition, the PCR-positive samples were subjected to DNA sequencing to determine the prevalent HEV genotype. Socio-demographic variables and risk factors associated with HEV in these women, were analyzed using logistic regression to estimate statistical significance ($p<0.05$) and odd ratio. Of the 230 asymptomatic pregnant women, with a mean age of 32.1± 4.8

years, 11(4.8%) women had anti-HEV IgM, while 39(17.0%) women had anti-HEV IgG. All anti-HEV IgG positive samples were found to be negative for HEV RNA while 3 (1.3%) of the anti-HEV IgM positive samples were positive for HEV RNA. Sequencing data of all (100%) the HEV RNA positive samples identified genotype 1 HEV. HEV infection among pregnant women was statistically associated with age (p=0.044), educational status (p=0.005) and eating of food purchased from vendors (p=0.025). The HEV IgM and RNA prevalence rate in this pregnant population is on the lower part of the scale, compared with other Sub-Saharan African countries. However, the HEV IgG data provide indirect evidence of past contact with this virus and our findings also confirm the HEV genotype 1 as the most prevalent in West African sub region.

221

HEPATITIS E VIRUS INFECTIONS AMONG PATIENTS WITH ACUTE FEBRILE JAUNDICE IN BURKINA FASO, 2013-2016

Sylvie Zida[1], Chloé Di Méglio[2], Dramane Kania[3], Judith Mbombi Mantono[4], Thérèse Kagoné[3], Souleymane Tassembedo[3], Amadou Dicko[3], Bachirou Tinto[3], Seydou Yaro[3], Hervé Hien[3], Jérémi Rouamba[3], Brice Bicaba[5], Isaïe Medah[5], Nicolas Meda[5], Omar Traoré[6], Edouard Tuaillon[7], Florence Abravanel[2], Jacques Izopet[2]

[1]*Institut de Recherche en Sciences de la Santé, Ouagadougou, Burkina Faso,* [2]*CHU Toulouse, Hôpital Purpan, Laboratoire de virologie, Centre national de référence du virus de l'hépatite E, Toulouse, France,* [3]*Centre Muraz, Bobo Dioulasso, Burkina Faso,* [4]*Université Catholique d'Afrique de l'Ouest, Bobo Dioulasso, Burkina Faso,* [5]*Ministère de la Santé, Ouagadougou, Burkina Faso,* [6]*Agence nationale de biosécurité, Ouagadougou, Burkina Faso,* [7]*Pathogenesis and Control of Chronic Infections. INSERM, University of Montpellier, Etablissement Français du Sang, Montpellier, France*

Hepatitis E virus (HEV) infection is a significant public health problem in many parts of the world including Africa. Understanding HEV epidemiology in Africa will facilitate the implementation of evidence-based control policies designed to prevent the spread of this infectious agent. In Burkina Faso (West Africa), little is known about the epidemiology and the genotype causing hepatitis E. HEV infection symptoms include jaundice and fever. We have investigated the contribution of HEV to the cases of acute febrile jaundice reported in the Yellow Fever (YF) surveillance in Burkina Faso and identified the HEV genotype involved. We tested serum samples taken between 2013 and 2016 from 900 patients presenting febrile icterus. All samples were tested for anti-HEV antibodies. Wantai HEV IgG and IgM EIA kits (Wantai Biologic Pharmacy Enterprise, Beijing, China) were used as set out in the manufacturer's instructions. HEV RNA viral load was detected using the Procleix HEV RNA assay (Grifols, Barcelona Spain). HEV genotyping was conducted by sequencing a fragment of the ORF2 genome and by phylogenetic analysis based on the reference sequences. Demographic factors associated with exposure to HEV were evaluated using a bivariate analysis. For the 900 patients' samples tested, 23/900 (2.6%) contained markers of acute HEV infection (anti-HEV IgM and/or HEV RNA positive). Genotyping indicated that all the strains were HEV genotype 2b. There was an overall HEV IgG seroprevalence of 18.2% (164/900). HEV exposure was also higher in patients living in the arid, mainly Northern area (26.3%) than in the semi-arid and tropical areas (11.3%); OR = 2.8 (95% CI: 1.58-4.97, p<0.01). Age was the only variable linked to a recent HEV infection (OR = 1.02 (95% CI: 1-1.05), p<0.01). Patients with symptomatic acute hepatitis E infection were older than the other patients. Acute febrile icterus cases in Burkina Faso may include HEV genotype 2b (circulating only in humans) infections. Better access to safe water and sanitation and improved personal hygiene should improve the control of HEV infection in this country.

222

SEROPREVALENCE OF VIRAL INFECTIONS IN BLOOD DONORS IN YAOUNDE, CAMEROON

Diderot Fopa[1], Claude Tayou Tagny[1], Dora Mbanya[1], Daniel Candotti[2], Camille Doux[2], Syria Laperche[2], Edward L Murphy[3]

[1]*Yaounde University Teaching Hospital, Yaoundé, Cameroon,* [2]*National Institute of Blood Transfusion/INTS, Department of Blood Borne Agents, National Reference Center for Infectious Risks in Blood Transfusion, Paris, France,* [3]*University of California San Francisco and Vitalant Research Institute, San Francisco, CA, United States*

The high prevalence of transfusion-transmissible infections (TTIs) is the most important challenge of safe blood supply in Cameroon. The seroprevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV) was determined among prospective blood donors at blood bank Yaoundé University Teaching Hospital (YUTH), Yaoundé, Cameroon. Blood donors were consecutively screened for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). Additional HBV testing including anti-HBc (Monolisa Anti-HBc PLUS; BIO-RAD) was performed. HIV and HCV serology were confirmed with HIV BLOT 2.2 (Genelabs Diagnostic) and INNO-LIA HCV (Fujirebio), respectively. In total, 1.166 were serially included in the study. Screening for transfusion transmissible infections showed that 91 (7.80%) of total samples donations were reactive for HBsAg+, 14 (1.2%) for HIV+, 11 (0.94%) for HCV+, and 1 (0.08%) for HBsAg+ and /HIV+. 1.162 were screened for total Anti-HBc IgG+IgM and 613 (52.75%) were reactive. All the 91 samples HBsAg positive were also positive for HBcAb. In 1071 HBsAg negative participants, the prevalence of HBcAb was 48.7% (n=522). In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48.8% (n=511). Of 13 HIV and 9 HCV reactive samples, 4 and 3 were confirmed positive by western blot, respectively. This study clearly showed a high prevalence of viral infections among Cameroonian blood donors at the YUTH. Strategies to increase voluntary and regular donors should be intensified as such as medical selection of blood donors to reduce the frequency of TTIs in blood donors. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infection prevalences in Camerronian blood donors.

223

SEROPREVALENCE OF MOSQUITO-BORNE VIRUSES AMONG RESIDENTS FROM DIFFERENT CITIES OF NIGERIA

Garja S. Suner[1], Elysse N. Grossi-Soyster[1], Pius S. Ekong[2], Mabel K. Aworh[3], Yiltawe S. Wungak[2], Nanven A. Maurice[2], Michael J. Ekong[4], Bonto Faburay[5], A. Desiree LaBeaud[1]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*National Veterinary Research Institute, Vom, Nigeria,* [3]*Federal Ministry of*

Agriculture and Rural Development, Abuja, Nigeria, [4]University College Hospital, Ibadan, Nigeria, [5]Kansas State University, Manhattan, KS, United States

Arthropod-borne viruses (arboviruses), such as alphaviruses, flaviviruses, and phleboviruses are an important public health issue in many regions of the world. Nigeria, a West African country, has seen an emergence of these arboviruses over the past several decades, leading to concerns of a widespread endemic. The aim of this study is to determine the seroprevalence of alphaviruses, such as chikungunya virus (CHIKV) and o'nyong'nyong virus (ONNV), and flaviviruses, such as dengue virus (DENV) and Zika virus (ZIKV), and phleboviruses, such as Rift Valley fever virus (RVFV), in three areas of Nigeria. Human serum samples were collected from a wide age range (ages = <[1]-[89] years) of residents in three cities: Abuja, Ibadan and Jos. A total of [701] serum samples were collected between August [2011] and March [2018]. Data pertaining to occupation, animal exposure, and febrile illness was also collected during each visit in order to determine potential risk factors related to exposure to arboviruses. Testing for prior exposure to alphaviruses, flaviviruses, and phleboviruses was performed using indirect IgG ELISAs against CHIKV, DENV and RVFV antigen. Serologic results from this study confirmed that [142] ([20.3]%) were seropositive for prior alphavirus exposure, [230] ([32.8]%) were seropositive for prior flavivirus exposure, and [14] ([1.99]%) had prior phlebovirus infections. Additionally, seropositivity across children (ages <[1]-[14] years, n = [90]) was much higher for DENV ([56.7]%) than CHIKV ([4.4]%), indicating a recent flavivirus exposure. The high seropositivity across all age groups suggests that arboviral infections are prevalent in Nigeria and implies that further surveillance is required in order to determine the true burden of these infections.

224

OUTBREAK OF ENVIRONMENTALLY ISOLATED TYPE 2 CIRCULATING VACCINE-DERIVED POLIOVIRUS IN METROPOLITAN KANO, NORTHWESTERN NIGERIA. 2018

Usman L. Shehu

AFENET/National Stop Transmission of Polio Program, Kano, Nigeria

Globally, Nigeria remains one of the endemic countries with polio cases. With eradication of WPV in sight, continued focus is needed to eliminate immunity gaps through high-quality SIAs and strong routine immunization programs. In May, 2016, globally synchronized switch from tOPV to bivalent OPV occurred in all OPV-using countries, including Nigeria and a single dose of inactivated polio vaccine (IPV) was also introduced into routine immunization to interrupt transmission of type 2 poliovirus. Despite that, Kano has recorded outbreaks of vaccine-derived polioviruses (VDPVs) and polio compatibles. The last confirmed wild poliovirus (WPV) case in Kano had paralysis onset in July 2014.In December 2018, report of a cVDPV2 nucleotide isolate (ENV-NIE-KNS-TRN-HGL-18-005) was received. A state of emergency was declared immediately and an outbreak investigation and response team formed to investigate the source of the outbreak. Retroactive case search was done in 26 health facilities and community ACS in 13 settlements surrounding the site. OPV and IPV antigens were used for routine and supplemental Immunization coverage survey using sample size of 150 households around the environmental sample in 6 wards of the LGA. The routine administrative data was also reviewed. Of the 150 children surveyed, 59% were fully immunized for age while 20% were partially immunized and 21% were not immunized for OPV. For IPV, 59% were fully immunized for age while 14% were partially immunized and 27% were not immunized. For the last SIA and last three SIAs, OPV coverages were 97% and 93% for the respectively. Two missed AFP cases were found in the health facilities records while no case was found from the 4723 households searched. Routine immunization strengthening and quality mOPV and IPV SIA campaigns are necessary to minimize risks of cVDPV transmission. The missed AFP cases also strongly suggest surveillance gaps and calls for more AFP surveillance strengthening.

225

SPATIOTEMPORAL RESOURCES FOR PREEMPTIVE PREPAREDNESS AGAINST RIFT VALLEY FEVER

Austin Hardcastle, Joshua Osborne, Rebecca Ramshaw, Erin Hulland, Julia Morgan, Julia Hon, Lucas Earl, Shreya Shirude, Simon Hay, David Pigott

University of Washington, Seattle, WA, United States

Rift Valley Fever (RVF) is an arbovirus which has caused thousands of human cases, hundreds of human fatalities, and over 100,000 livestock deaths over the last 15 years. RVF has been reported from heterogeneous climates throughout Africa and the Middle East, but cases frequently occur in areas that have recently experienced an extended period of above-normal rainfall. Efforts are being made to use known environmental patterns to forecast areas suitable for outbreaks in real-time, but assessments of historic, consistent suitability are also necessary. Here we show synoptic maps of RVF suitability over its entire range at 5km-by-5km resolution for every month of the year. These resources can aid decisions regarding strategic, preemptive pandemic preparedness activities as opposed to immediate but necessary reactions to short-term forecasted risk. We conducted a systematic literature review to identify all reported cases of RVF from 1995-2018 and combined this data with records of RVF occurrence from the FAO's EMPRES-i database. We used boosted regression trees to analyze patterns in these occurrences across environmental covariates including precipitation, vegetation indices, and distances to floodplains. RVF suitability was found in known RVF hotspots such as Kenya and South Africa in addition to countries that have never reported cases. Some regions were suitable for the virus most or all months out of the year, while others only for short seasons. These findings can help policy makers prevent outbreaks by providing information regarding when and where to focus resources for preparedness such as health infrastructure, personnel training, and vaccine supplies for humans if and when they become available. Specifically, we show how our maps can be used to prioritize where a country or region should focus increased infrastructural resources for the surveillance of Rift Valley Fever. The temporal component of our maps can also advise about when during the year people in these identified geographies should be trained and re-trained in Rift Valley Fever surveillance to increase their awareness and hopefully prevent outbreaks.

226

CHARACTERIZING THE GENOMIC DIVERSITY, EVOLUTION AND PHYLOGEOGRAPHY OF RESPIRATORY SYNCYTIAL VIRUS GENOTYPE ON1 IN KENYA

James Richard Otieno[1], Everlyn M. Kamau[1], John W. Oketch[1], Joyce M. Ngoi[1], Alexander M. Gichuki[1], Špela Binter[2], Grieven P. Otieno[1], Mwanajuma Ngama[1], Charles N. Agoti[1], Patricia A. Cane[3], Paul Kellam[4], Matthew Cotten[5], Philippe Lemey[6], D. J. Nokes[7]

[1]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [2]Kymab Ltd., Cambridge, United Kingdom, [3]Public Health England, Salisbury, United Kingdom, [4]Imperial College London, London, United Kingdom, [5]Erasmus Medical Center, Rotterdam, Netherlands, [6]KU Leuven - University of Leuven, Leuven, Belgium, [7]University of Warwick, Coventry, United Kingdom

In December 2010, a new genotype of respiratory syncytial virus (RSV) with a 72-nucleotide duplication within the attachment (G) gene was identified in Ontario, Canada, and named ON1. Using the ON1 as a unique tag, this study aimed to understand; (1) how new RSV variants are introduced, spread and persist in communities, (2) the genomic signatures that define the emergent RSV variants and whether such substitutions may be associated with potential fitness advantages, and (3) the patterns of RSV spread across geographically defined regions (local and global). Partial G gene (n=483) and whole genome (n=184) sequence datasets collected between 2010 and 2016 were analyzed using genetic diversity, phylogenetics and statistical methods to understand the molecular epidemiology of RSV in Kilifi County, Coastal Kenya. Further, Kenyan

(partial G gene; n=2526) and global (full G gene, n=2238; whole genome, n=1194) sequence datasets collected between 1977 to 2016 were analysed in a Bayesian framework for the inference of the phylogeographic history of local and global RSV spread, respectively. Following initial detection genotype ON1 in Kilifi in 2012, there was rapid replacement of the previously circulating RSV group A genotype GA2 by ON1 in subsequent epidemics. While this suggests elevated fitness of ON1 viruses, there was no clear evidence of altered pathogenicity of ON1 relative to GA2 in Kilifi. Signature amino acid substitutions were identified between surface proteins (G, F), polymerase (L) and matrix M2-1 proteins of Kilifi ON1 and GA2 viruses, suggesting co-evolution amongst antigenic and non-antigenic genes of RSV variants. Genetic and phylogenetic analyses reaffirmed previous conclusions that each RSV epidemic is characterized by the frequent introduction of multiple variants, few of which persist across epidemics. Finally, the phylogeographic analyses predicted the northern hemisphere to be the major source population of RSV viruses into the tropics and the southern hemisphere and virus spread between locations in close proximity to be important for virus persistence within a country.

227

MAPPING THE ENVIRONMENTAL SUITABILITY FOR MIDDLE EASTERN RESPIRATORY SYNDROME CORONAVIRUS

Joshua C. Osborne, Rebecca E. Ramshaw, Ian D. Letourneau, Shreya Shirude, Simon I. Hay, David M. Pigott

University of Washington, Seattle, WA, United States

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a respiratory pathogen transmitted to humans from dromedary camels reported across the Arabian Peninsula with a case-fatality rate of 35% overall. Repeated infection in both the endemic and imported settings demonstrate the need for preemptive preparedness for detection and treatment of MERS-CoV. Serological detection of MERS-CoV antibodies in dromedary camels ranging beyond geographies with known occurrences of human infection suggests that broadscale mapping is warranted. To this end, we construct a Boosted Regression Tree model of the ecological niche suitable for MERS-CoV spillover into human populations. We use this model to show administrative level relative risk estimates that are weighted by human and camel populations. As a result, we define a much broader geography of spillover potential of MERS-CoV due to the environmental similarity of these regions. MERS-CoV is listed as a priority pathogen by the World Health Organization, and a primary candidate for vaccine development as identified by the Coalition for Epidemic Preparedness Innovations initiative. Moving forward, a geographically precise, broadscale understanding of MERS-CoV spillover potential will be vital to the formulation of active surveillance and investigation strategies and to inform the deployment and provisioning of future vaccines.

228

DEVELOPING ALTERNATIVE SURVEILLANCE METHODS, FROM THE NEAR INFRARED SPECTROSCOPY TO PREDICT ZIKA INFECTION ON *AEDES AEGYPTI*, TO METAGENOMICS TO DETECT THE INVASION OF NEW ARBOVIRUSES AND HAPLOTYPES

Marcio G. Pavan[1], Gabriela A. Garcia[1], Lilha M. Santos[1], Mariana R. David[1], Maggy T. Sikulu-Lord[2], Ademir J. Martins[1], Jeffrey R. Powell[3], Cristopher E. Mason[4], Rafael Maciel-de-Freitas[1]

[1]*FIOCRUZ, Rio de Janeiro, Brazil,* [2]*University of Queensland, St. Lucia, Australia,* [3]*Yale University, New Haven, CT, United States,* [4]*Weill Cornell Medicine, New York, NY, United States*

A significant expansion on the geographic distribution of arboviruses occurred in the last decades, making more than half of human population under risk of transmission. Global cities receive millions of tourists and goods from all over the world and thus it is likely that new mosquito genotypes can be introduced, as well as new viruses. Timely detection of emerging threats and outbreak depends on efficient risk assessment. In endemic regions, there is an urgent need to early diagnose DENV, CHIKV and ZIKV in native *Aedes aegypti* populations. In the other hand, mass gathering events raise the vulnerability of a global city to the introduction of new pathogens. Herein, we propose different surveillance methods - (i) for endemic areas, the use of Near Infrared Spectroscopy (NIRS) to detect arbovirus in *A. aegypti* for a rapid, cost-effective and accurate surveillance system; and (ii) for monitoring cities in mass gathering events, Next-Generation Sequencing methods to detect early introduction of new arboviruses/mosquito genotypes. We analyzed 400 *A. aegypti* mosquitoes for each group (infected with DENV, CHIKV or ZIKV) to develop calibration models for NIRS. Partial Least Square regression models were performed with leave-one-out cross validation. The ZIKV model had >95% specificity and 94.2-99.3% accuracy at 4 and 7dpi. Preliminary results of DENV at 7 and 14dpi showed 85% specificity and 76% accuracy. To accomplish the second aim, *A. aegypti* samples were collected 3-months before, 3-m after and 1-year after the Rio 2016 Olympics in 8 venues and also in a control area, where tourists were not expected (90 female mosquitoes/area). DNA was extracted individually and analyzed in SNP-chip containing ~30K SNPs of *A. aegypti*. These results will be compared to our SNP database of worldwide mosquito populations to identify its origin. Mosquito females also had the midgut dissected individually, and DNA and RNA extracted and prepared for metagenomics and RNA shotgun metagenomic sequencing (SMS) to determine if new arboviruses entered during the Olympics and if they recurrently infected mosquitoes after the games.

229

THE ARRESTING VERTICAL TRANSMISSION OF HEPATITIS B VIRUS (AVERT-HBV) STUDY IN THE DEMOCRATIC REPUBLIC OF THE CONGO: PRELIMINARY RESULTS

Peyton Thompson[1], Jonathan B. Parr[1], Kashamuka Mwandagalirwa[2], Noro L. Ravelomanana[2], Martine Tabala[2], Malongo Fathy[2], Patrick Ngimbi[2], Bienvenu Kawende[2], Charles Mbendi[2], Jérémie Muwonga[3], Ravi Jhaveri[4], Gavin Cloherty[5], Marcel Yotebieng[6], Steven R. Meshnick[7]

[1]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo,* [3]*National AIDS Control Laboratory, Kinshasa, Democratic Republic of the Congo,* [4]*Lurie Children's Hospital, Chicago, IL, United States,* [5]*Abbott Laboratories, Abbott Park, IL, United States,* [6]*Ohio State University, Columbus, OH, United States,* [7]*Gillings School of Global Public Health, Chapel Hill, NC, United States*

Despite the widespread availability of effective vaccination against hepatitis B virus (HBV), it remains endemic throughout sub-Saharan Africa. Mother-to-child transmission (MTCT) of HBV is preventable through birth dose vaccination and identification/treatment of "high-risk" pregnant women (those with high viral loads and/or HBV e antigen [HBeAg] positivity). The purpose of this study is to show the feasibility of adding HBV testing and treatment of pregnant women, and birth-dose vaccination, to the existing infrastructure of an HIV prevention of MTCT (PMTCT) program in the Democratic Republic of the Congo (DRC). We are enrolling pregnant women at two maternity centers in Kinshasa, DRC that have ongoing HIV PMTCT programs. We are screening women using point-of-care HBV surface antigen (HBsAg) testing (Abbott Alere Determine™, Abbott Park, IL). Women with positive HBsAg testing who present at <24 weeks' gestation are eligible for enrollment. Enrolled women are being evaluated for high-risk disease using HBV viral load (Abbott RealTi*m*e) and HBeAg (Abbott ARCHITECT) and for baseline liver and kidney function abnormalities. Pregnant women with high-risk disease are offered tenofovir disoproxil prophylaxis between 28- and 32-weeks' gestation and continued through 12 weeks' post-partum. Exposed infants are given a birth dose of monovalent HBV vaccine within 24 hours of life. Of 4,016 women screened, 109 (2.7%) were HBsAg-positive and 91 women have been enrolled. Among the 71 who have undergone additional testing thus far, nine (12.7%) of the enrolled women have high-risk disease; none of these women have baseline liver or kidney function abnormalities. Of the 19 infants who have been born to date, 14 (73.7%) have received birth dose vaccine within 24 hours. This ongoing study demonstrates the feasibility of adding HBV screening and treatment of pregnant women,

and infant birth-dose vaccination, to the existing HIV PMTCT platform in Kinshasa. Challenges exist in administering timely birth dose vaccination to Congolese infants; these implementation issues must be addressed prior to roll-out of universal birth dose vaccination in Africa.

230

SPATIO-TEMPORAL DYNAMICS OF MEASLES IN THE PROVINCE OF WESTERN KASAI IN DEMOCRATIC REPUBLIC OF CONGO FROM 2000 TO 2014

Dilubenzi Suami Divine, Bigirinama Rosine, Didier Bompague

URF/University of Kinshasa/INRB, Kinshasa, Democratic Republic of the Congo

Background: Despite immunization efforts since 2000, measles remains a major public health problem in the DRC. The upsurge of outbreaks throughout the country in general and in the province of the western Kasaï especially motivated the realization of this study. Aim: To start understanding of recurrence of these outbreaks. Methods: Measles cases and deaths reported in Kasai Occidental between 2000 and 2014 were used to calculate the attack rate and develop thematic maps for possible spatial heterogeneities. The outbreaks occurred during the period were analyzed together with an assessment of measles surveillance system. Results: A total of 33,126 cases (3.82% deaths) have been reported on all that ZS (Luebo, Mweka and Benaleka) were more at risk. Children <5 years unvaccinated (65.8%) were more affected and no difference in sex. Conclusion: The identification of the epicenter formed of the 3 ZS opens a perspective to lead the studies to the scale of health areas in order to search for the factors explaining these heterogeneities

231

SEROPREVALENCE OF HEPATITIS A AND HEPATITIS E VIRUSES AMONG PREGNANT WOMEN IN HAITI

Alexandra Tejada-Strop[1], Rania Tohme[1], Jocelyne Andre-Alboth[2], Lana Childs[1], Xin Ji[1], Vivianne de Oliveira Landgraf de Castro[3], Jacques Boncy[2], Saleem Kamili[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*National Public Health Laboratory, Port-au-Prince, Haiti,* [3]*Federal University of Mato Grosso do Sul, Campo Grande, Brazil*

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are mainly transmitted through contaminated food and water. HEV infection is associated with a high fatality rate among pregnant women, and gestational complications have been reported among pregnant women infected with HAV. Determine the seroprevalence of HAV and HEV infections among pregnant women in Haiti. We selected 1307 residual specimens from the 2012 Biannual Sentinel Serosurveys for HIV among Pregnant Women. We stratified the population into West (includes Metropolitan Port-au-Prince) and non-West regions (all other departments). Specimens were tested for total HAV antibody immunoglobulin (total anti-HAV), anti-HEV immunoglobulin M (Anti-HEV IgM), and anti-HEV immunoglobulin G (Anti-HEV IgG). We evaluated the overall prevalence and the prevalence in the West and non-West regions and the association between demographic and socioeconomic characteristics with HAV and HEV infections. Overall, 96.8% of tested pregnant women were positive for total anti-HAV, 10.3% for Anti-HEV IgG, and 0.3% for Anti-HEV IgM. The prevalence of Anti-HEV IgG in the non-West (12.3%) was significantly greater than in the West region (5.3%) ($p<0.0001$). Prevalence of total anti-HAV ($p=0.008$) and Anti-HEV IgG ($p\leq0.05$) was significantly associated with age. Total anti-HAV increased from 92% to 98%, and Anti-HEV IgG increased from 6% to 16% among women aged 15-19 years and those aged ≥35 years, respectively. The majority of pregnant women in Haiti had evidence of past exposure and immunity to HAV. HEV is circulating at a greater rate in the non-West region than the West region. Improvements in food and water safety such as the implementation of the Water, Sanitation, and Hygiene (WASH) program may help with prevention of these viral infections in Haiti.

232

ASSESSING SERUM ANTI-ROTAVIRUS IMMUNOGLOBULIN A AS A CORRELATE OF VACCINE-INDUCED PROTECTION AGAINST ROTAVIRUS GASTROENTERITIS IN HIGH AND LOW CHILD MORTALITY SETTINGS: ANALYSIS OF POOLED IN DIVIDUAL-LEVEL DATA FROM NINE CLINICAL TRIALS

Julia M. Baker[1], Jacqueline E. Tate[2], Juan Leon[1], Michael J. Haber[1], Benjamin A. Lopman[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

A correlate of protection would facilitate rapid and efficient assessment of modified rotavirus vaccine strategies and the next generation of rotavirus vaccines. We aimed to quantify a threshold of post-vaccine serum anti-rotavirus immunoglobulin A (IgA) antibody units that serves as an individual-level immune correlate of protection against rotavirus gastroenteritis among vaccinated infants across child mortality settings. Individual-level data on 5,074 infants enrolled in nine GlaxoSmithKline Rotarix phase II and III clinical trials from 16 countries were pooled. Cox proportional hazard models were fit to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) describing the relationship between a series of anti-rotavirus IgA thresholds and the occurrence of mild/moderate or severe rotavirus gastroenteritis up to 1 year of age. Seroconversion (serum anti-rotavirus IgA ≥20 U/mL) conferred substantial protection against mild/moderate and severe rotavirus gastroenteritis across settings. Among infants in low child mortality settings, seroconversion provided near perfect protection against severe rotavirus gastroenteritis (HR=0.04, 95% CI=0.01, 0.32). In high child mortality settings, seroconversion reduced the risk of severe rotavirus gastroenteritis by 52% (HR=0.48, 95% CI=0.26, 0.90). As anti-rotavirus IgA threshold increased, the HR comparing the rate of gastroenteritis among those above that threshold to seronegative infants generally decreased. A given anti-rotavirus IgA threshold typically provided higher protection in low child mortality settings compared to high child mortality settings and for more severe disease compared to mild/moderate disease. Serum anti-rotavirus IgA is a valuable, though imperfect, correlate of vaccine-induced protection against rotavirus gastroenteritis across settings. Serum anti-rotavirus IgA alone may be insufficient to accurately predict an infant's risk of rotavirus gastroenteritis, however, serum anti-rotavirus IgA seroconversion provides an informative threshold for assessing rotavirus vaccine performance.

233

MATERNAL SECRETOR STATUS AFFECTS ORAL ROTAVIRUS VACCINE RESPONSE IN BREASTFED INFANTS IN BANGLADESH

Frank B. Williams[1], Abdul Kader[2], Dorothy M. Dickson[1], Ross Colgate[1], Muhammad Muhammad Ikhtear[2], Salma Sharmin[2], Shaidul Islam[2], Taufiqur R. Bhuiyan[2], Masud Alam[2], Uma Nayak[3], Josyf C. Mychaleckyj[3], William A. Petri[3], Rashidul Haque[2], Firdausi Qadri[2], Beth D. Kirkpatrick[1], Benjamin Lee[1]

[1]*University of Vermont, Burlington, VT, United States,* [2]*International Center for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*University of Virginia, Charlottesville, VA, United States*

Rotavirus (RV) remains the leading global cause of infectious diarrhea among infants despite availability of live-attenuated oral vaccines, which underperform in low-income settings. Histoblood group antigen expression on mucosal surfaces and in exocrine secretions (i.e. Secretor status) has been associated with RV susceptibility, with non-Secretor infants protected against symptomatic RV infection. However, the contribution of maternal breast milk Secretor status on infant responses to oral RV vaccination remains unclear. We hypothesized that infant and breast milk Secretor-positive status would be associated with increased vaccine response. Therefore, we performed a sub-study within PROVIDE, a Rotarix vaccine efficacy trial conducted in Dhaka, Bangladesh, to determine if infant or maternal Secretor status was associated with infant post-vaccination RV-specific IgA seroconversion. Seroconversion

was defined as post-vaccination plasma RV-IgA ≥20 U/mL at 18 weeks of age following pre-vaccination concentration <20 U/mL. Infant and maternal Secretor status were determined by enzyme immunoassay of saliva and breast milk, respectively. Phenotype data were available for 246 maternal-infant dyads. Seventy-four mothers (30%) and 71 infants (29%) were non-Secretor. Seroconversion rates were significantly higher among infants born to maternal non-Secretors (37% vs 22%, P=0.01); this effect was strongest among infant Secretors (55% vs 24%, P<0.01). Maternal status was not associated with breast milk RV-IgA or infant pre-vaccination RV-IgG. In a model evaluating both maternal and infant status, maternal, but not infant, phenotype remained significantly associated with seroconversion (P<0.01). Maternal Secretor status significantly affected RV vaccine response in Bangladesh. One hypothesis for these findings suggests inhibition of vaccine response by Secretor-dependent antigens in breast milk, possibly by serving as decoy receptors or by modulating the infant microbiome. Further studies may yield insights into pathogenesis and host response, but breast feeding should not be withheld at vaccination.

234

ALLERGIES, OBESITY AND RISK OF HOSPITALIZATION FOR SUSPECTED ARBOVIRUS INFECTIONS

Anita Susana Hargrave[1], Rachel Sippy[2], Anna Stewart-Ibarra[3]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Institute for Global Health and Translational Science, SUNY Upstate Medical University, Syracuse, NY, United States,* [3]*Institute for Global Health and Translational Science, SUNY Upstate Medical University; Department of Medicine, Syracuse, NY, United States*

Dengue, chikungunya and Zika are mosquito-borne arboviruses that lead to over 390 million infections per year worldwide. They can manifest as a short flu-like illness or as life-threatening organ dysfunction. The risk factors for development of severe complications requiring hospitalization remain poorly understood. In the same communities afflicted by these diseases, there are growing rates of allergies and obesity. These conditions are linked to pro-inflammatory states and altered immunity. The objective of this study was to elucidate how allergies and obesity change the risk of hospitalization due to arboviruses in an endemic region in southern coastal Ecuador. From 2014 to 2018, subjects were enrolled after being clinically diagnosed with an arbovirus infection by a Ministry of Health physician in the city of Machala. Weight and height were measured to calculate Body Mass Index (BMI), and a survey of demographics, symptoms, and health history including self-report of allergies was conducted after enrollment. The relationships between hospitalization and three measures: (1) BMI, (2) history of allergies and (3) overweight/obese BMI or allergies, were analyzed using general liner models adjusting for potential confounders. Three hundred and thirty-seven adults were recruited with complete data; of these patients, 34 were hospitalized, 57 reported allergies and 203 patients had a BMI over 25, classifying them as overweight/obese. For every BMI unit above 25, there was a 9% decrease in odds of hospitalization (95% confidence interval, 0.83—0.99). Those with reported allergies had a 23% decrease in the odds of hospitalization (95% confidence interval, 0.25—1.99) compared to those without allergies. Patients with either a history of allergies or an overweight/obese BMI classification had a 44% decrease in the odds of hospitalization (95% confidence interval: 0.21—1.50). Having a BMI above normal or allergies exhibited protective effects against hospitalization among adults with clinically diagnosed arbovirus infection; additional research is necessary to better understand the mechanisms contributing to these associations.

235

LASSA FEVER IMMUNE RESPONSE CROSS REACTIVITY TO RECOMBINANT ANTIGEN BASED IMMUNOASSAYS

Megan Heinrich[1], Matthew Boisen[2], Luis Branco[1], Katherine Hastie[3], Diana Nelson[2], Duane Bush[2], Irina Aimukanova[2], Mambu Momoh[4], Francis Baimba[4], John Adeyemi[5], Testimony Olumade[6], Benevolence Ebo[5], Olusola Ogunsanya[7], MacDonald Onyechi[8], Johnson Etafo[8], Matthew Afam Eke[9], Philomena Eromon[6], Andrew Hoffmann[10], Brandon Beddingfield[10], Ikponmwosa Odia[5], Chinedu Ugwu[6], Onikepe Folarin[6], Augustine Goba[4], Pardis Sabeti[11], Christian Happi[6], Donald Grant[12], Erica Ollmann Saphire[3], John Schieffelin[10], Robert Garry[10]

[1]*Zalgen Labs LLC, Germantown, MD, United States,* [2]*Zalgen Labs LLC, Aurora, CO, United States,* [3]*La Jolla Institute for Immunology, La Jolla, CA, United States,* [4]*Kenema Government Hospital, Kenema, Sierra Leone,* [5]*Irrua Specialist Teaching Hospital, Irrua, Nigeria,* [6]*Redeemers University, Ede, Nigeria,* [7]*University of Ibadan, Ibadan, Nigeria,* [8]*FMC Owo, Owo, Nigeria,* [9]*FMC Abakaliki, Abakaliki, Nigeria,* [10]*Tulane University, New Orleans, LA, United States,* [11]*Broad Institute, Cambridge, MA, United States,* [12]*Ministry of Health and Sanitation, Freetown, Sierra Leone*

During the 2019 Nigerian Lassa fever outbreak, serologic studies were conducted at Redeemers University, Nigeria and Kenema Government Hospital, Sierra Leone to assess the level of Lassa-specific IgG and IgM cross-reactivity towards recombinant Lassa virus (LASV) antigens from the three most prevalent circulating strains: LASV lineages II and III (Nigeria) and Lineage IV (Sierra Leone, Liberia, Guinea). Long-term LF Survivor samples were screened using recombinant LASV antigen-based IgG/IgM ELISA kits for Lassa-specific reactivity to Nucleoprotein (NP), Glycoprotein (GP), and Z protein (ZP). Assay reagents included ELISA microwell plates that were coated with individual antigens or mixtures of all three lineages of a particular antigen (Pan-Lassa), stabilized and packaged for use in West Africa. For human sample screening a 1:100 dilution was used. Samples exhibiting Lassa-specific IgG or IgM reactivity were selected to be retested for antigen specific end-point titers. Correlation of NP and GP assay reactivity was low (R2 <0.5) highlighting a differential adaptive immune response to viral antigens in convalescence. Cross-reactivity of antibody response to multiple lineages was significant (R2 >0.850) for both NP and GP antigens however, ZP exhibited poor cross-reactivity (R2 <0.500) and specificity. Samples from both Nigeria and Sierra Leone demonstrated consistent cross-reactivity to all three lineages of NP and GP antigens. Endpoint dilution of NP and GP reactive samples further elucidated cross-reactivity with lineage specific endpoints within 4-fold dilutions. Median end-point dilution for NP antigen was 1:6400 (maximum 1:51,200) and GP antigen 1:1600 (maximum 1:12,800). For samples with anti-GP endpoint ≥1:400, positive agreement with the Lassa GP pseudovirus neutralization reference method was 97.4% (38/39; 95th% CI 86.5 - 99.9%). Detection of anti-NP and anti-GP antibodies are important biomarkers of Lassa fever humoral immune response. These results help will guide selection of recombinant LASV antigens used in LF surveillance and vaccine development.

236

EVALUATION OF THE PERFORMANCE OF ALERE Q FILOVIRUS DETECT TEST AND THE ALERE Q ANALYZER FOR DIAGNOSIS OF EBOLA COMPARED TO RT-PCR

Yanis Ben Amor[1], Zelda Moran[1], Maitreyi Oka[1], William Rodriguez[2], Cassandra Kelly[2], Mamdou A. Baldé[3], Moussa Moussa Condé[3], N'Faly Magassouba[3]

[1]*Columbia University, New York, NY, United States,* [2]*Foundation for Innovative New Diagnostics, Geneva, Switzerland,* [3]*Laboratoire des Fièvres Hémorragiques en Guinée, Conakry, Guinea*

Over the past five years, outbreaks of Ebolavirus have resulted in devastating morbidity and mortality in West and Central Africa and Ebola remains a significant public health threat in several countries. Access to molecular diagnostic testing is crucial for effective early outbreak

identification and response, but nucleic-acid based tests are primarily limited to large laboratories with advanced equipment and highly trained technicians. In 2017, we performed an analysis of the accuracy of the Alere q Filovirus Detect Test using whole blood samples collected in Guinea in 2014-15. We compared each test result from Alere q to its respective RT-PCR record from the time of collection. To account for uncertain sample transport and storage history between collection and investigative testing, whenever the Alere q test was discordant with the original result from 2014, a confirmatory RT-PCR was performed. Two separate statistical analyses were conducted, the first (primary) excluding any samples with differing RT-PCR results, and, to account for possible sample degradation, the second (secondary) including all samples but considering the 2017 RT-PCR as the true Ebola status. Sensitivity in primary analysis was 97.3% (95% CI: 90.7% - 99.7%), and 97.7% (95% CI: 91.85% - 99.7%) in secondary analysis. Specificity was lower, at 89.1% (95% CI: 81.4% to 94.4%) in both analyses. Limitations included uncertain sample transport and storage history of samples, including the transportation to the United States and treatment with gamma irradiation after testing by Alere q but prior to confirmatory RT-PCR testing. This may have impacted the accuracy of the 2017 RT-PCR test result. The Alere q Filovirus Detect Test demonstrated high sensitivity and moderate specificity when used on whole blood samples collected in Guinea during the 2014-15 Ebola outbreak. Significant challenges faced during this study are likely to apply in futures studies attempting to validate new diagnostics using banked samples, and should be considered during study design and results interpretation.

237

VIRAL HEMORRHAGIC FEVERS IN PREGNANCY: A SYSTEMATIC REVIEW AND META-ANALYSIS

Ribka Amsalu

University of California San Francisco, San Francisco, CA, United States

Driven by globalization, urbanization, changing human feeding habits, and change in land use, the risk for infectious disease epidemics and pandemics has increased over the years. In the last three decades, several infectious diseases have emerged as diseases of concern with epidemic or pandemic potential including Severe Acute Respiratory Syndrome (SARs), Influenza, Coronavirus infection (MERS-CoV), and Ebola Virus Disease (EVD). While, there are evidence-based interventions to tackle most infectious diseases, the evidence-base on the effect of viral hemorrhage fevers in pregnancy is limited. Viral hemorrhagic fevers (VHFs), is a term usually applied to disease caused by *Arenaviridae* (Lassa fever), *Bunyaviridae* (Rift Valley Fever), *Filoviridae* (Ebola and Marburg) and *Flaviviridae* (Yellow fever, Dengue) viruses. Globally, VHFs have accounted for nearly three fifth of infectious disease epidemic events in the last decade. We conducted a systematic review and meta-analysis to summarize the current evidence to determine whether VHF epidemics are associated with adverse pregnancy outcomes. Adverse pregnancy outcomes were defined as miscarriage, stillbirth, preterm birth, low-birth weight, and early neonatal death. Electronic literature search were conducted in PubMed, Embase, Web of Science and Cochrane library. The search was limited to English language. Common VHFs- dengue, yellow fever, lassa fever, rift valley fever, and ebola were included in the search. Medical subject heading, keyword search and the bibliographies and reference list of selected articles identified 1543 studies. Title and abstract screening was done by two authors using predesigned selection criteria. The eligible articles were then exported to Systematic Review Data Repository for data extraction. Most of the studies identified were observational studies, cohort and case control, and quality was assessed by the Newcastle-Ottawa Scale. Effect sizes were estimated for each adverse outcome and as composite indicator by disease by using Risk Ratio with corresponding 95% confidence interval. Results and conclusion will be presented at conference.

238

"THE CAT THAT KILLS PEOPLE": COMMUNITY BELIEFS ABOUT EBOLA ORIGINS AND IMPLICATIONS FOR DISEASE CONTROL IN EASTERN DEMOCRATIC REPUBLIC OF THE CONGO

Michael Hawkes[1], Kasereka Masumbuko Claude[2]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Université Catholique du Graben, Butembo, Democratic Republic of the Congo*

The current Ebola epidemic in Eastern Democratic Republic of Congo (DRC) has surpassed 1,000 cases and 600 deaths. Social resistance is a major barrier to control efforts, and invites an exploration of community beliefs around Ebola and its origins. Mixed-methods study, using focus group discussions (FGDs) with key community informants and a 19-item survey questionnaire broadly sampling the outbreak zone. Between 4 to 17 August, 2018, we conducted 4 FGDs (20 participants) and surveyed 286 community members across Eastern DRC. FGDs revealed a widespread rumour early in the epidemic of two twins bewitched by their aunt after eating her cat, who developed bleeding symptoms and triggered the epidemic. However, this myth appeared to dissipate as the epidemic progressed and biomedical transmission became generally accepted (medical syncretism). In our survey, 6% of respondents endorsed supernatural origins of Ebola. This subgroup did not differ from other respondents in terms of knowledge of biomedical modes of transmission or resistant attitudes toward control measures, but was more likely to believe that traditional healers could cure Ebola. Wild animals were recognized as sources of Ebola by 53%. Our findings suggest that skepticism and/or denial of the biomedical discourse, coupled with and mistrust and fear of ETUs may fuel "underground" transmission of Ebola outside western-style medical facilities, as patients seek care from traditional healers, who are ill-equipped to deal with a highly contagious biohazard. A deeper understanding of beliefs around Ebola origins may illuminate strategies to engage communities in control efforts.

239

A REVIEW OF THE GENETIC AND ANTIGENIC PROFILE OF INFLUENZA VIRUSES CIRCULATING IN CEBU CITY, PHILIPPINES, 2009-2017

Maria Theresa Payumo Alera[1], Louis Macareo[2], Stefan Fernandez[2], In-Kyu Yoon[3], John Mark Velasco[2], Wudtichai Manasatienkij[2], Catherine Lago[1], Chonticha Klungthong[2]

[1]*Philippines-Armed Forces Institute of Medical Sciences Virology Research Unit, Cebu City, Philippines,* [2]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [3]*International Vaccine Institute, Seoul, Republic of Korea*

Genetic changes of influenza viruses may occur and form novel subtypes resulting in pandemics. Influenza surveillance is critical to detect mutations to assess the potential effectiveness of seasonal influenza vaccines. The objective of the study was to identify the antigenic and molecular characteristics of circulating influenza viruses in Cebu City, Philippines. 2,491 nasal/throat swabs collected from volunteers aged 6 months old and above presenting with influenza-like illness in city health clinics from 2009 to 2017, were tested by influenza real-time RT-PCR. 627 of 2,491 (25%) samples with Ct value <27 were randomly selected for sequencing. Influenza A viruses accounted for 426 of the 627 (68%) and Influenza B viruses for 201 (32%). A breakdown of Influenza A subtype viruses were as follows: 236/426 (55%) Influenza A/H3 and 190/426 (45%) Influenza A(H1N1)pdm09. A phylogenetic analysis of influenza A/H3N2 using 41 gene sequences showed the following clades: 1 (2009), 3B (2011-2012), 3C.1 (2011), 3C.3 (2012-2015), and 3C.2a (2015 to 2017). Influenza A/H3 subtype showed close antigenic identity to Northern Hemisphere vaccine strain A/California/7/2004/ Vaccine and Southern Hemisphere vaccine strain A/Switzerland/8060/ 2017/Vaccine. A phylogenetic analysis using 23 Influenza A(H1N1)pdm09 gene sequences showed that the virus strain during the 2009 influenza season belonged to genetic clade 1. It was closely related to A/California /7/2009 vaccine strain. Clade 6 was

the dominant A(H1N1)pdm09 subtype virus circulating between 2013 and 2017. Phylogenetic analysis of influenza B using 44 gene sequences showed both B/Victoria and B/Yamagata lineages co-circulating between 2011 and 2014. Clades of Influenza B Victoria lineage belonged to 1A and 1B while Influenza B Yamagata lineage belonged to clade 3. The study emphasizes the importance of sustained epidemiologic and virologic influenza surveillance coupled with timely antigenic and genetic evolution analysis which can be used by health authorities to guide policy decision making.

240

DEVELOPMENT OF SUBSTITUTED GUANIDINO-OSELTAMIVIR AS POTENT AGAINST INFLUENZA VIRUS TARGETING NEURAMINIDASE ACTIVITY

Sumit Kumar[1], Sonu Kumar[2], Prem P. Sharma[2], Atul FNU[2], Prakasha Kempaiah[3], Brijesh Rathi[2], Poonam FNU[1]

[1]*Department of Chemistry, Miranda House, University of Delhi, New Delhi, India,* [2]*Laboratory for Translational Chemistry and Drug Discovery, Department of Chemistry, Hansraj College University Enclave, University of Delhi, New Delhi, India,* [3]*Department of Medicine, Loyola University Stritch School of Medicine, Chicago, IL, United States*

Influenza, is an inoculable viral infection, belongs to *orthomyxoviridae* family is caused by number of circulating influenza viruses classified as influenza A, B and C. The influenza strains, H1N1, H3N2, H2N2, H5N1 causes epidemic that influences the respiratory organs in human and animals. As per the WHO reports, influenza creates major threat for human population, killing > 2,50,000 - 5,00,000 people worldwide. Oseltamivir, orally approved drug inhibits the neuraminidase (transmembrane protein). Neuraminidase plays an important role in release of virus from infected cell by cleaving sialic acid present on the surface of cell membrane. Therefore, neuraminidase are considered as potential drug target for anti-influenza agents. The active form of oseltamivir, oseltamivir carboxylate (Tamiflu) formed by the action of endogenous esterase inside the cell membrane and targets the neuraminidase. The use of oseltamivir showed a substantial decrease in influenza related death over the time, however rapid mutations of the virus resulted in the resistance against oseltamivir and other antiviral agents. In view of the ongoing pandemic threat and the emergence of resistance to oseltamivir, the development of new effective therapeutics must be of high priority. Guanidino-oseltamivir (GO) is one of the pro-drugs of oseltamivir with high anti-influenza activity in culture, however its poor bioavailability needs to be improved. Therefore, we decided to develop new analogs of GO by implementing slight chemical modifications. The main objective of our investigation is to build N-alkyl amides substituted GO and O-alkyl ester substituted GO analogs as potent anti-influenza agent. Several new GO analogs were synthesized and assayed for biological testes. The initial screening results supported few of the compounds as highly potent with inhibitory concentration of ~20 nM in culture with minimal toxicity. All the interesting observation will be presented.

241

PREVALENCE OF HIGH-RISK HPV GENOTYPES RELATED TO CERVICAL CANCER IN WOMEN OF LIMA, PERU THROUGHOUT THE YEARS 2012 - 2017

Caddie Laberiano Fernández[1], Wilmer Silva-Caso[2], Javier Arias Stella[1], **Migue A. Aguilar-Luis**[3], Luis J. del Valle[4], Jorge Valverde-Ezeta[3], Denisse Champin[2], Graciela Risco de Dominguez[2], Juana del Valle-Mendoza[3]

[1]*Instituto de Patologia y Biología Molecular Arias Stella, Lima, Peru,* [2]*Facultad de Ciencias de la Salud - Universidad Tecnológica del Peru, Lima, Peru,* [3]*School of Medicine, Research and Innovation Centre of the Faculty of Health Sciences, Universidad Peruana de Ciencias Aplicadas, Lima, Peru,* [4]*Barcelona Research Center for Multiscale Science and Engineering, Department d' Enginyeria Quimica, EEBE, Universidad Politecnica de Catalunya (UPC), Barcelona, Spain*

Human Papilloma Virus (HPV) is the most important risk factor in the pathogenesis of cervical cancer and precancerous lesions of the cervix. Currently more than 170 types of HPV have been characterized and their distribution of genotypes varies geographically. In Peru, is reported that about 20.5% of cervical cells with normal morphology determined by Papanicolau test (PAP) were infected by HPV. However, data regarding HPV infection is still limited, therefore our study is of the utmost importance since we evaluated high-risk HPV frequencies over the span of 6 years (2012 - 2017) in women from Lima, Peru. A Retrospective study was conducted based on the statistical analysis performed on a database of a total of 6665 samples from asymptomatic women over 15 years of age from the Stella - Maris Clinic in Lima - Peru (2012 - 2017). All samples were subjected to the COBAS ® 4800 HPV test (PCR to amplification of and nucleic acid hybridization for the detection of 14 high-risk HPV types in a single analysis (HPV 16,18,31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). We found that 27.7 % (n = 1849) were positive for high risk HPV genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, while 9.8 % (n = 650) tested positive for the HPV16 genotype, lastly only 1.91% (n = 127) were positive only for the HPV genotype 18. The most frequent coinfection was between the HPV16 genotype and any of the other genotypes evaluated as high risk (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was seen in 4.29% (N = 286). In conclusion, our results demonstrate a significant percentage of positive infections by the HPV16 genotype, however the group that is considered as positive for the other high risk genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) except without characterizing them individually, represented a percentage of 27.74%, which indicates that in our study population the flow of viruses considered high risk is very heterogeneous.

242

THE ROLE OF *PLASMODIUM FALCIPARUM* DERIVED MICROVESICLES IN MALARIA RELATED ANEMIA

Florence Awamu[1], Kai Matuschewski[1], Faustin Kamena[2]

[1]*Humboldt University Berlin, Berlin, Germany,* [2]*Leipzig University, Leipzig, Germany*

Severe anemia represents one of the major complications in malaria and affects primarily young children. Red blood cell destruction by the causative agent of malaria, intra-erythrocytic *Plasmodium* parasites, is the main origin of anemia, but clinical data suggests that additional factors, including dyserythropoiesis and phagocytosis of erythrocytes, add to the pathology. The underlying mechanisms that explain the disproportional loss of erythrocytes when compared to the proportion of infected cells remain to be elucidated. One attractive hypothesis is coating and immune recognition leading to phagocytosis of non-infected erythrocytes with *Plasmodium* antigens, which are released into the blood stream during intra-erythrocytic replication through microvesicles. In this study, we investigated microvesicle-mediated transfer of parasitic material to uninfected erythrocytes and its contribution to phagocytic destruction. We purified microvesicles from *Plasmodium falciparum* cultures and characterized the protein content by mass spectrometry. We show that a signature microvesicle protein, ring-infected erythrocyte surface antigen (RESA), can be found on the surface of non-infected erythrocytes. Transwell co-culture experiments further substantiate our notion that coating of normal erythrocytes by parasite antigens occurs through microvesicle transfer. We finally tested whether this mechanism primes uninfected cells for phagocytosis *in vitro* by macrophages. Our findings show that microvesicles derived from *Plasmodium*-infected erythrocytes can deposit parasite antigens on uninfected red blood cells. Whether this mechanism contributes to the pathogenesis of malaria-related anemia can now be examined in clinical studies.

243

THE ASSOCIATION BETWEEN ABO BLOOD GROUPING AND MALARIA INFECTION WITHIN KENYAN ISOLATES

Redemptah Yeda

Kenya Medical Research Institute - Kenya, Kisumu, Kenya

The ABO blood groups consist of A, B and H carbohydrate antigens which can regulate protein activities. Investigations have been conducted to find out whether or not ABO blood groups antigens are associated with susceptibility of malaria infections in Kenyan. This study aims to assess the distribution of ABO blood groups and their relationship with malaria infection among Kenyan isolates. A total 138 positive, *Plasmodium species* whole blood samples were obtained from an ongoing malaria surveillance study microscopy was done on all samples. ABO blood group was determined by agglutination test using Anti ABD blood grouping, monoclonal antibodies. *Plasmodium* speciation was achieved using ssRNA real-time polymerase chain reaction. Data collected was analysed using R software version 3.4.2. Chi square was used to assess the difference between frequencies and ANOVA used to test the difference between parasitemia means. Out of a total of 140 samples collected, 130 (93%) were found to be infected with plasmodium parasites as determined by microscopy. All samples examined for malaria were also tested for ABO blood groups accordingly, 47.7%, 26.2%, 20.8% and 4.6% were found to be blood groups O, A, B and AB respectively. The highest proportion of individuals in all blood groups were infected with P.falciparum as compared with other species, 91(70%), 26(20%), 11(8%) and 2(2%) were positive for *Plasmodium falciparum*, *Plasmodium ovale wallikeri*, *Plasmodium malarae* and *Plasmodium ovale Curtis*. This study indicates that individuals of blood groups O, A and B are more susceptible to plasmodium falciparum infection compared to individuals with blood type AB.

244

PLASMODIUM FALCIPARUM GAMETOCYTE SEX RATIO IN AN ASYMPTOMATIC POPULATION: IMPACT ON MALARIA TRANSMISSION

Raphael O. Okoth[1], Benjamin Opot[1], Gladys Chemwor[1], Irene Onyango[1], Gladys Kerich[1], Dennis Juma[1], David Abuom[1], Hoseah Akala[1], Ben Andagalu[1], Edwin Kamau[2], Jessica Cowden[3], Jim Ray Managbanag[4]

[1]*US Army Medical Research Directorate - Africa (USAMRD-A, Kenya Medical Research Institute (KEMRI), Kisumu, Kenya,* [2]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [3]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [4]*US Army Medical Research Directorate - Africa, Kisumu, Kenya*

The burden of asymptomatic and sub-microscopic malaria infections in endemic settings is playing a role in sustaining transmission. *Plasmodium* gametocyte stage that is infectious to mosquito is central to sustained transmission. This argues for innovative transmission-blocking strategies in order to meet global elimination goals. Mosquito infection is mainly determined by gametocyte density and its sex ratio is crucial in ensuring fertilization. Conventional means of sex ratio quantification - microcopy - is imperfect and quantification has been improved by the introduction of molecular detection tools that allow detection at lower densities. It has been shown that adequate numbers of male and female gametocytes need to be generated during infection for successful fertilization. However, the range of the sex ratio that would allow transmission is unclear. Here, we aim to estimate a range of gametocyte sex ratio that would allow transmission in an asymptomatic population in a malaria holoendemic region. A total of 4214 blood samples collected from 29 clusters in a malaria transmission dynamics study in Kisumu between July 2015 and June 2016 were used in this study. Ribonucleic acid extraction was carried out followed by a DNase digest on all the samples. *Plasmodium* and gametocyte detection was then carried out using a reverse transcription quantitative real time polymerase chain reaction (RT-qPCR). Subsequently, *Plasmodium* species composition of each positive sample was determined. Participants who had gametocytes underwent either a membrane or direct landing feeding assay and on day 8, all the fed mosquitoes were dissected and diagnosed for the presence of oocyst - suggestive of successful infection. Gametocyte sex ratio analysis is being carried out as described by Schneider *et al.* with few modifications - replacing *Pfs230p* with *Pf*MGET as the male gametocyte marker. The findings of this study will describe a range of sex ratio fit for successful mosquito mid-gut infection and further give clue to whether a robust sex-specific quantification could replace mosquito feeding assays. Preliminary findings of the study will be presented.

245

URINARY METABOLITE CHANGES IN VOLUNTEERS CHALLENGED WITH *PLASMODIUM FALCIPARUM* SPOROZOÏTES

Madeleine Eunice Betouke Ongwe[1], Isabelle Kohler[2], Aswin Verhoeven[2], Jacqueline J. Janse[2], Yoanne D. Mouwenda[2], Peter G. Kremsner[3], Stephen L. Hoffman[4], Bertrand Lell[5], Akim A. Adegnika[5], Oleg A. Mayboroda[2], Maria Yazdanbakhsh[2]

[1]*LUMC/CERMEL, Leiden, Netherlands,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*Eberhard Karls University Of Tuebingen, Tuebingen, Germany,* [4]*Sanaria, Inc., Rockville, MD, United States,* [5]*Centre De Recherches Medicales De Lambarene, Lambarene, Gabon*

Immunity against malaria infection is being studied extensively but the underlying mechanisms of protection are not fully understood. Metabolomics is a post-genomic technology enabling a minimally invasive monitoring of the physiological responses to external and internal stimuli. Here, we present a longitudinal study of the urinary metabolic profiles of healthy individuals before and after intravenous administration of *Plasmodium falciparum* sporozoïtes, aiming at deciphering the metabolic changes observed during the course of malaria infection. Twenty healthy malaria-exposed Gabonese and 5 malaria-naïve Europeans were voluntary challenged with live *P. falciparum* sporozoïtes and followed up until they developed symptoms (Gabonese) and became thick blood smear positive (Europeans). Urine samples was collected before and after challenge at several times points until treatment. Samples were analysed in an untargeted approach using hydrophilic interaction chromatography-mass spectrometry (HILIC-MS). A combination of the univariate and multivariate data analysis approaches was used for dissecting the metabolic effects of a host response to the infection. Data analysis showed a clear difference in metabolomic profile between malaria-naïve and malaria-exposed subjects at baseline. Overall, 8 most important metabolites discriminate before and after the challenge and between parasitemic and non-parasitemic malaria-exposed Gabonese. One particular metabolite, glutamine conjugate phenylacetylglutamine, significantly distinguished malaria-exposed individuals who controlled their parasitemia even before the challenge from those who did not. This metabolomics study highlighted the changes in the urinary metabolite profiles related to *P. falciparum* challenge and identified potential urinary biomarkers of parasitemia.

246

INVESTIGATING A *PLASMODIUM FALCIPARUM* ERYTHROCYTE INVASION PHENOTYPE SWITCH AT THE WHOLE TRANSCRIPTOME LEVEL

Prince B. Nyarko

University of Ghana, Accra, Ghana

The central role that erythrocyte invasion plays in *Plasmodium falciparum* survival and reproduction makes this process an attractive target for therapeutic or vaccine development. However, multiple invasion-related genes with complementary and overlapping functions afford the parasite the plasticity to vary ligands used for invasion, leading to phenotypic variation and immune evasion. Overcoming the challenge posed by redundant ligands requires a deeper understanding of conditions that select for variant phenotypes and the molecular mediators. While host

factors including receptor heterogeneity and acquired immune responses may drive parasite phenotypic variation, we have previously shown that host-independent changes in invasion phenotype can be achieved by continuous culturing of the W2mef and Dd2 *P. falciparum* strains in moving suspension as opposed to static conditions. Here, we have used a highly biologically replicated whole transcriptome sequencing approach to identify the molecular signatures of variation associated with the phenotype switch. The data show increased expression of particular invasion-related genes in switched parasites, as well as a large number of genes encoding proteins that are either exported or form part of the export machinery. The genes with most markedly increased expression included members of the erythrocyte binding antigens (*eba*), reticulocyte binding homologues (*rh)*, surface-associated interspersed protein (*surfin)* gene families, exported protein family 1 (*epf1*) and the *Plasmodium* Helical Interspersed Sub-Telomeric (*phist*) gene family. The data indicate changes in expression of a repertoire of genes not previously associated with erythrocyte invasion phenotypes, suggesting the possibility that moving suspension culture may also select for other traits.

247

ENDOTHELIAL ACTIVATION IS ASSOCIATED WITH DISEASE SEVERITY ACUTE KIDNEY INJURY AND COGNITIVE IMPAIRMENT IN PEDIATRIC SEVERE MALARIA

Benson J. Ouma[1], John M. Ssenkusu[2], Estela Shabani[3], Dibyadyuti Datta[4], Robert O. Opoka[5], Richard Idro[5], Paul Bangirana[6], Gregory Park[4], Moses L. Joloba[1], Kevin C. Kain[7], Chandy C. John[4], Andrea L. Conroy[4]

[1]*Department of Medical Microbiology, College of Health Sciences, Makerere University, Kampala, Uganda,* [2]*Department of Epidemiology and Biostatistics, Makerere University School of Public Health, Kampala, Uganda,* [3]*Department of Pediatrics, University of Minnesota, Minneapolis, MN, United States,* [4]*Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States,* [5]*Department of Paediatrics and Child Health, Makerere University College of Health Sciences, Kampala, Uganda,* [6]*Department of Psychiatry, College of Health Sciences, Makerere University, Kampala, Uganda,* [7]*Department of Medicine, University of Toronto and University Health Network, Toronto, ON, Canada*

Endothelial activation is associated with disease severity in severe malaria (SM), but the relationship between endothelial activation and long-term cognitive outcomes in children surviving SM are unknown. Ugandan children with SM and community children (CC) were prospectively enrolled from 2008-2013 in Kampala, Uganda. Children underwent neurocognitive evaluation at enrollment (CC) or hospital discharge (SM) and at 6, 12, and 24 months follow-up using validated tools. Endothelial activation was measured on admission samples by ELISA (von Willebrand factor (VWF), angiopoietin (Angpt)-1 and-2) or Luminex (sICAM-1, sVCAM-1, sE-Selectin, P-Selectin). False discovery rate was used to adjust for multiple comparisons. SM was associated with widespread endothelial activation compared to CC (p&it0.0001 for all). Using multiple regression analyses to evaluate the relationship between severe malaria complications and endothelial activation, acute kidney injury (AKI) was an independent predictor of changes in VWF, sICAM-1, sE-Selectin, P-Selectin, Angpt-2 (p&it0.0001 for all). Using linear mixed effects modelling, increases in $\log_{10}$ levels of Angpt-2 were associated with worse cognition independent of disease severity (presence of coma, number of seizures, AKI), and sociodemographic factors (adjusted p&it0.05). Angpt-2 levels were associated with increases in sequestered parasite biomass, acidosis (lactate), markers of hemolysis (LDH, total bilirubin), inflammation (TNFα, IL-10), increased CSF-to-plasma albumin index, and markers of neuroinflammation/injury (TNFα, kynurenic acid, Tau) in children with CM (adjusted p&it0.05 for all). These data support Angpt-2 as an important mediator of disease severity in malaria that is a risk factor for long-term cognitive injury.

248

A *PLASMODIUM FALCIPARUM* NF54 REPORTER LINE EXPRESSING MCHERRY-LUCIFERASE IN GAMETOCYTES, SPOROZOITES AND LIVER-STAGES

Catherin Y. Marin-Mogollon[1], Ahmed M. Salman[2], Karin M. Koolen[3], Judith M. Bolscher[3], Fiona J. van Pul[1], Shinya Miyazaki[1], Takashi Imai[4], Ahmad Syibli Othman[5], Jai Ramesar[1], Geert-Jan van Gemert[6], Hans Kroeze[1], Severine Chevalley-Maurel[1], Blandine Franke-Fayard[1], Robert W. Sauerwein[6], Adrian V. Hill[2], Koen J. Dechering[3], Chris J. Janse[1], Shahid M. Khan[1]

[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*The Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [3]*TropIQ Health Sciences, Nijmegen, Netherlands,* [4]*Department of Infectious Diseases and Host Defense, Gunma University Graduate School of Medicine, Gunma, Japan,* [5]*Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Terengganu, Malaysia,* [6]*Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands*

Transgenic malaria parasites expressing fluorescent and bioluminescent proteins are valuable tools to interrogate malaria-parasite biology and to evaluate drugs and vaccines. Using CRISPR/Cas9 methodology a transgenic *Plasmodium falciparum* (Pf) NF54 line was generated that expresses a fusion of *mCherry* and *luciferase* genes under the control of the *Pf etramp10*.3 gene promoter (line mCherry-luc@etramp10.3). *Pf etramp10*.3 is related to rodent *Plasmodium uis4* and the *uis4* promoter has been used to drive high transgene expression in rodent parasite sporozoites and liver-stages. We examined transgene expression throughout the complete life cycle and compared this expression to transgenic lines expressing mCherry-luciferase and GFP-luciferase under control of the constitutive *gapdh* and *eef1a* promoters. The *mCherry-luc@etramp10.3* parasites express mCherry in gametocytes, sporozoites and liver-stages. While no mCherry signal was detected in asexual blood-stage parasites above background levels, luciferase expression was detected in asexual blood-stages, as well as in gametocytes, sporozoites and liver-stages, with the highest levels of reporter expression detected in stage III-V gametocytes and in sporozoites. The expression of mCherry and luciferase in gametocytes and sporozoites makes this transgenic parasite line suitable to use in *in vitro* assays that examine the effect of transmission blocking inhibitors and to analyse gametocyte and sporozoite biology.

249

CIRCULATING EXOSOMAL MICRORNAS LET-7I-5P AND MIR-451A LEVELS ARE DIFFERENTIALLY EXPRESSED IN SICKLE CELL TRAIT (HBAS), ANEMIA (HBSS) AND HBAA INDIVIDUALS

Keri Harp[1], Felix Botchway[2], Michael Wilson[3], Yvonne Dei-Adomakoh[2], Jonathan K. Stiles[1], Adel Driss[1]

[1]*Morehouse School of Medicine, Atlanta, GA, United States,* [2]*Korle-bu Teaching Hospital, Accra, Ghana,* [3]*Noguchi Memorial Institute for Medical Research, Accra, Ghana*

Sickle-cell disease (SCD) is a common genetic disorder in about 300,000 newborns globally each year and 50-80% will not survive to adulthood. The hemoglobin (Hb) allele variant S or C (HbS or HbC) causes sickle cell anemia when both alleles are inherited (HbSS or HbSC). Sickle cell trait (SCT) individuals carry one of the hemoglobin variants (HbAS or HbAC) and have been reported to have a reduced risk of developing severe malaria compared to HbAA controls. In 2017, there were 200-300 million cases and 435,000 malaria associated deaths mostly in Africa. Previous *in-vitro* studies determined that endogenous microRNAs (miRNAs), such as let-7i-5p, are differentially expressed in SCD erythrocytes compared to HbAA and were associated with reduced parasite proliferation. In this study, we tested the hypothesis that Hb genotypes govern susceptibility of erythrocytes to *Plasmodium* infection via exosomal let-7i-5p. Blood samples, with different Hb genotypes (HbAA, HbAS, HbAC, HbSS, HbSC, HbCC), with (+) and without (-) malaria, were collected from volunteers

in Accra, Ghana as part of NIH research projects (1K01TW010282, 1R01NS0916161 and 1R25TW009340). Exosomal let-7i-5p (using RT-qPCR), Complete Blood Counts (CBC) and other clinical data were compared between Hb genotypes with (+) or without (-) malaria. Exosomal let-7i-5p was significantly elevated in HbSS (-) compared to HbAA (-) and HbAS (-) individuals as well as HbAS (+) versus HbAS (-). In SCT (-) we found a correlation between exosomal let-7i-5p expression levels and some clinical characteristics. These results indicate that elevated levels of exosomal let-7i-5p in SCD and malaria mediate susceptibility to malaria and could be used as a predictive biomarker for severe malaria risk.

250

CEREBRAL MALARIA ASSOCIATED EXPRESSION OF NRG-1 IN HUMAN BRAIN

Juan Cespedes, Jonathan Stiles, Mingli Liu

Morehouse School of Medicine, Athens, GA, United States

Plasmodium falciparum malaria is associated with 500,000 deaths a year. Cerebral malaria (HCM) is a neurological manifestation of infection by *P. falciparum* and accounts for the majority of malaria associated deaths. CM is associated with increased expression of adhesion molecules, vascular endothelial dysfunction, sequestration of infected red blood cells, cerebral edema and disruption of blood brain barrier (BBB) integrity. Neuregulin-1 (NRG-1), a neurotrophic growth factor has been shown to attenuate several brain injuries caused by neurotoxin exposure, acute ischemic stroke, and most recently HCM. NRG-1 is mainly expressed in neurons, Schwann cells of the nervous system in humans and mice, human cornea epithelial and stroma cells, and in human/mouse vascular endothelial cells and cardiomyocytes. Previous assessments of circulating NRG-1 in serum of survivors and non-survivors of cerebral malaria indicated a significant decline in NRG-1 concentration compared to that in mild malaria. It was unclear whether this decline was a result of HCM induced inhibition or sequestration of NRG-1 at injury sites in brain. In this study, we assessed distribution of NRG-1 expression in post-mortem human brain tissues of individuals who died of HCM and compared results with those who died of non-malaria causes. Our hypothesis is that in HCM, NRG-1 expression is significantly increased in injured brain areas matching severity and mortality while its receptor phosphorylated ErbB4 (pErbB4) is decreased. Previous post mortem studies conducted in Ghana, yielded archived paraffin-embedded sections of brain tissues from cortex, cerebellum and brain stem. These samples were analyzed quantitatively for immunoreactive signals of NRG-1 and perbB4 antibody. Results indicate that NRG-1 expression levels in neurons and blood vessels increased significantly in HCM postmortem tissues compared to patients who died of non-malaria causes ($p<0.05$) suggesting that the observed depletion of circulating serum NRG-1 in severe disease may be due to sequestration of NRG-1 brain parenchyma. pErbB4 expression levels significantly decreased in brainstem neuronal cells

251

CENTRAL NERVOUS SYSTEM VIRUS INFECTION IN AFRICAN CHILDREN WITH CEREBRAL MALARIA

Douglas G. Postels[1], Lawrence Osei-Tutu[2], Karl B. Seydel[3], Qian Xu[3], Chenxi Li[3], Terrie E. Taylor[3], Chandy C. John[4], Macpherson Mallewa[5], Tom Solomon[6], Robert Opoka[7], Tsiri Agbenyega[8], Daniel Ansong[2], Lillian M. Khan[9], Kristoffer E. Leon[9], Joseph DeRisi[9], Charles Langelier[9], Michael R. Wilson[9]

[1]*Children's National Medical Center, Washington, DC, United States,* [2]*Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [3]*Michigan State University, East Lansing, MI, United States,* [4]*Indiana University, Indianapolis, IN, United States,* [5]*University of Malawi, Blantyre, Malawi,* [6]*University of Liverpool, Liverpool, United Kingdom,* [7]*Makerere University, Kampala, Uganda,* [8]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [9]*University of California, San Francisco, CA, United States*

Cerebral malaria (CM) is defined as an otherwise unexplained coma with malaria parasitemia. The condition predominantly affects African children and presents with coma, fever, and seizures. Two-thirds of African children with clinical CM have a malaria specific retinopathy. Based on autopsy studies, children with retinopathy-positive CM have a malaria infection exclusively responsible for their illness. Children dying of retinopathy-negative CM have non-malarial etiology of deaths. Children with retinopathy-negative CM were hypothesized to have a non-malarial etiology of acute illness coupled with asymptomatic malaria parasitemia, the latter reflecting residence in an area of high transmission intensity. As children with viral encephalitis have a similar phenotype to those with CM, our goal was to identify the contribution of central nervous system (CNS) viral infection to illness in children with retinopathy-negative CM. We collected cerebrospinal fluid (CSF) from 272 Ghanian, Ugandan, and Malawian children with CM, and selected CSF from 111 of these children (38 retinopathy-positive, 71 retinopathy-negative, 2 retinopathy-unknown) for analysis by metagenomic next-generation sequencing (mNGS). We found CSF viral co-infections in 7/38 (18.4%) retinopathy-positive children and in 18/71 (25.4%) retinopathy-negatives. Excluding HIV-1, human herpesviruses (HHV) represented 75% of viruses identified. CNS viral co-infection was equally likely in children who were retinopathy-positive and retinopathy-negative ($p= 0.1431$). Neither mortality nor neurological morbidity was associated with the presence of virus (OR=0.276, 95% CI: 0.056-1.363). Retinopathy-negative children with higher temperature, lower white blood cell count, or being dehydrated were more likely to have viral co-infection. Level of consciousness at admission was not associated with CNS viral co-infection in retinopathy-negative children. Viral CNS co-infection is unlikely to contribute to coma in children with CM. The herpesviruses other than HSV may represent incidental bystanders in CM, reactivating during acute malaria infection.

252

GLYCOSYLATION OF *PLASMODIUM FALCIPARUM* THROMBOSPONDIN REPEATS DRIVES MOSQUITO TRANSMISSION AND SPOROZOITE VIRULENCE

Sash Lopaticki[1], Charlie Jennison[1], Nichollas Scott[2], Alan John[1], Annie Yang[1], Matthew O'Neill[1], Norman Kneteman[3], Ethan Goddard-Borger[1], **Justin Boddey**[1]

[1]*Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia,* [2]*University of Melbourne, Melbourne, Australia,* [3]*University of Alberta, Edmonton, AB, Canada*

Apicomplexans are the only known unicellular organisms that express proteins bearing thrombospondin repeats (TSRs), ancient protein modules that are stabilized by O- and C-glycosylation in metazoans. However, other than the installation of GPI anchors, it was long believed that malaria parasites do not glycosylate their proteins. Recently, the presence of O- and C-glycosylation was identified on the TSRs of CSP and TRAP in *Plasmodium falciparum* and *P. vivax* sporozoites, which was a major advance for studying the glycobiology of malaria parasites and for vaccine design. We subsequently showed through genetic disruption of the O-glycosylation pathway in *P. falciparum* that this modification functionally stabilizes virulence proteins during infection of the mosquito midgut epithelium and human hepatocytes in humanized mice. However, the roles and glycosylation states of most parasite TSR proteins remain unknown, as does the function of C-glycosylation in the malaria lifecycle. Of the different types of protein glycosylation, C-glycosylation is unique because of the unusual C-C linkage formed between sugar (mannose) and target protein (tryptophan). Here, we identify new substrates that are O- and C-glycosylated in *P. falciparum* and demonstrate the functional importance of these modifications for sexual development, transmission to mosquitoes and virulence of sporozoites in humanized mice. By genetically disrupting the C-glycosylation pathway, we reveal hitherto unknown essential roles of tryptophan-mannosylation in the *P. falciparum* lifecycle. Our study shows that O- and C-glycosylation of TSR proteins comprises a protein quality control mechanism that has evolved to drive parasite-host interactions necessary for transmission and virulence of *P. falciparum*, the etiological agent of the most lethal malaria.

253

STUDY OF ANTI-MALARIAL DRUG RESISTANCE USING THE MUSE® RBC INVASION ASSAY

Kimvan Tran, **Kamala Tyagarajan**

Luminex Corporation, Hayward, CA, United States

The increasing resistance of malaria parasites to common anti-malarial drugs is a major concern for controlling the disease. The evolution of rapid, simple methods that can be used in diverse environments can help facilitate the discovery of new anti-malarial drugs. Current approaches include DNA-binding dyes and fluorometric approaches that result in loss of intact and parasitized cells. Flow cytometry can provide information on the percentage of parasitized cells; however, the cost and complexity of current instrumentation has been prohibitive for use in many environments. We have developed the research use only Muse® RBC Invasion Assay on the easy to use, low-cost Muse Cell Analyzer. The assay allows for the determination of the percentage of the RBCs invaded by *Plasmodium* parasites. The assay has been used for routine monitoring of RBCs infected by different *Plasmodium falciparum* strains, such as 3D7, D10, and Dd2 strains, as well as *in vitro* drug susceptibility studies. In this study, the *in vitro* impact of anti-malarial drugs on different *Plasmodium falciparum* strains was studied using the Muse RBC Invasion Assay. In particular, we looked at the dose response of chloroquinine and mefloquinine on 3D7 and Dd2 strains, and the impact on invaded RBC percentages. The IC50 for chloroquinine was 18.8 nM for 3D7 and 104 nM for Dd2 strains in this study, while mefloquine had IC50's in similar ranges for both strains. Our results demonstrate that the 3D7 strain showed susceptibility to chloroquine at low doses, while the Dd2 strain was resistant and only showed response at higher drug concentrations. Additional studies should help characterize the impact of more recent artemisinin drugs such as dihydroarteminisin on both strains. The studies performed demonstrate the importance of performing *in vitro* screening of antimalarial drugs on multiple strains, in order to truly characterize their action and utility. The Muse RBC Invasion Assay can be a useful and accessible method to characterize the *in vitro* impact of anti-malarial drugs, and can be a powerful drug discovery and development tool for malarial researchers.

254

ARTEMISININ RESISTANCE MARKER OF *PLASMODIUM FALCIPARUM* IN OSOGBO METROPOLIS, SOUTH WEST, NIGERIA

Sulaiman Adebayo Nassar

Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Artemisinin derivatives constitute a key component of the present-day treatment for *plasmodiumfalciparum* malaria and rsesistance with artemisinins is generally associated with S769N point mutation in the sarco-endoplasmic reticulum-dependant ATPase6 (SERCA ATPase6) gene of *Plasmodium falciparum*. However, few studies have been carried on the current level baseline and level of mutation of this drug in Nigeria most especially in Osun State. The present study determined the distribution of *plasmodiumfalciparum* and resistance marker for artmisinins drugs from the blood smears of 60 randomly sampled patients attending LAUTECH Teaching Hospital , Osogbo ,Osun state after obtaining ethical clearance from the relevant authority.The entire study period was divided into Pre-Treatment ,drug administration and post -Treatment phases .Blood smears of 70 consented participants were assessed microscopically using Giemsa ststaining technique for parasite identification and parasitaemia.. Samples found to have parasitaemia after drug administration were amplified and assessed for distribution of the *Pf*ATPase6 S769N mutation using Polymerase Chain Reaction (PCR) . 28 out of 30 samples subjected to PCR had successful amplification. However, none of the amplified samples harboured the *Pf*ATPase6 S769N mutation, suggesting 100% sensitivity of P. Falciparum population examined at the study area. There is therefore need for continuous surveillance for earlier detection of resistance as the use of ACT isbeing scaled up in the country.

255

SELECTION OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MARKERS POST-ACT INTRODUCTION AND A NEARLY COMPLETE REVERSION TO CHLOROQUINE SENSITIVITY

Kevin Wamae[1], Dorcas Okanda[1], Leonard Ndwiga[1], Victor Osoti[1], Kelvin Muteru[1], Abdirahman Abdi[2], Philip Bejon[3], Colin Sutherland[4], Lynette I. Ochola-Oyier[2]

[1]*KEMRI-Wellcome Trust Research Programme, CGMRC, Kilifi, Kenya,* [2]*KEMRI-Wellcome Trust Research Programme, CGMRC/Pwani University Bioscience Research Centre, Pwani University, Kilifi, Kenya,* [3]*KEMRI-Wellcome Trust Research Programme, CGMRC/Nuffield Department of Medicine, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, University of Oxford, United Kingdom, Kilifi, Kenya,* [4]*Department of Immunology and Infection, Faculty of Infectious Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom/PHE Malaria Reference Laboratory, London School of Hygiene & Tropical Medicine, London, United Kingdom, Kilifi, Kenya*

The emergence and spread of artemisinin resistance in South East Asia are of great concern as drug resistance renders drugs ineffective in the fight against malaria. Additionally, resistance to previous first-line treatments, including chloroquine (CQ) and sulfadoxine-pyrimethamine (SP), was imported to Africa from South East Asia. Genetic markers of resistance are important molecular epidemiological tools used for early detection and monitoring of the spread of drug-resistant parasites. We carried out a temporal analysis of changes in allele frequencies of 8 drug resistance markers and 4 putative artemisinin resistance background markers over two decades of changing anti-malarial drug policy in Kenya. Using the *crt*-76 and *mdr1*-86 and *mdr1*-1246 molecular markers, we found that the withdrawal of CQ and SP as first-line treatment in Kenya shifted the parasite population from CQ resistant to a nearly fixed (99%) CQ sensitive population. On the other hand, *dhps* mutations associated with SP resistance (A437G and K540E) were maintained at a high frequency (greater than 75%). Something that may be attributable to the fact that SP is still readily available in Kenya, with a recent survey reporting a market share of 57% in the private sector. We did not detect any of the artemisinin resistance markers nor the background mutations that precede artemisinin resistance except mdr2 I492V mutation. However, we show a gradual decline in the novel *nfs* (K65 allele) marker, which potentially confers resistance to lumefantrine, from 38% to 20% with a significant allele frequency difference pre- and post-ACT introduction. In summary, the high frequency of CQ sensitive parasites circulating in the population supports the on-going debate on re-introduction of chloroquine for the treatment of malaria and the impact of the decreasing frequency of *nfs* (K65 allele) on the efficacy of Artemether-Lumefantrine combination warrants close monitoring.

256

INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON *PLASMODIUM FALCIPARUM IN VITRO* RESISTANCE TO ARTEMISININ

Sandra Duffy, Vicky M. Avery

Griffith University, Brisbane, Australia

With the goal of malaria eradication firmly on the table, several aspects are undermining this, including the resurgence of malaria cases in some malaria endemic countries and artemisinin combination therapy (ACT) failure within the greater Mekong subregion of Asia. *Plasmodium falciparum* (*Pf*) ACT resistance presents as a slow parasite clearance time (> 5 hours) *in vivo* and *in vitro* greater than 1% survival of early ring stage parasites, 0-3 hours post red blood cell invasion, after a 6-hour dose of 700nM of artemisinin derivative (ART). Both these phenotypes are correlated to mutations within the *Pf*K13 propeller domain, but not exclusively as some genetic predisposition is proposed to pre-empt the K13 mutation acquisition. The *in vitro* acquisition of K13 mutations through escalating ART challenge has only resulted from one study where O_2 levels

throughout incubation were 21% in comparison to 1-5% in other studies. Routinely the *in vitro* culture of *Pf* utilizes a single gaseous environment that is constant and maintained at, for example, 5% O_2 and 5% CO_2. However, *in vivo* the gaseous microenvironment has a diverse range of concentrations and ratios of O_2 and CO_2 depending on the location within the circulatory system and the presence of certain pathologies. To study the impact of alterations in the gaseous environment on parasite susceptibility to ART, the ring stage survival assay (RSA) was performed on *Pf* parasites, designated resistant or sensitive (K13 mutant or WT), cultured under different O_2 and CO_2 concentrations. To advance knowledge on the effect changing gaseous environments has on ART sensitivity and resistance at different stages of *Pf* asexual lifecycle, the RSA was also performed at a range of O_2 and CO_2 concentrations at selected times post red blood cell invasion. The effects of gaseous environments on parasite *in vitro* resistance and susceptibility profiles, relationship to K13 mutations, and biological evaluation of the underlaying mechanisms behind the altered responses will be discussed.

257

PREVALENCE OF ANTIMALARIAL DRUG RESISTANT MARKERS IN KWARA, NORTH-CENTRAL, NIGERIA: A DECADE AFTER REPLACEMENT OF CHLOROQUINE AND ANTIFOLATES AS FIRST-LINE REGIMEN

Olugbenga Akinola[1], Daniel E. Egboro[2], Ese Iyenmoana[2], Bukola F. Abdulkadir[2], Yusuf O. Yakub[2], Abimbola A. Adeosun[2], Hidayah A. Olumo-Abdul[3], Chairat Utaipibull[1], Olusola G. Gbotosho[4]

[1]*National Center for Genetic Engineering and Biotechnology (BIOTEC), Khlong Luang, Pathum thani, Thailand,* [2]*Department of Pharmacology and Therapeutics, University of Ilorin, Ilorin, Kwara State, Nigeria,* [3]*Department of Pharmacology and Toxicology, University of Ilorin, Ilorin, Kwara State, Nigeria,* [4]*Department of Pharmacology and Toxicology, University of Ibadan, Ibadan, Oyo State, Nigeria*

The emergence of "super malaria" parasites, resistant to artemisinins based combinations (ACT), portend danger to malaria control efforts. While measures to curtail the spread must be addressed, preparedness, also becomes necessary. One premonition is to evaluate background status of parasites resistant to previously used drugs, especially as artemisinin resistance selects for sensitive alleles of chloroquine resistant markers. This study was designed to establish the current profile of *Plasmodium falciparum* resistant markers of chloroquine (*Pfcrt*), pyrimethamine (*Pfdhfr*) and *Pfmdr1* in field isolates. In a cross sectional survey, 305 participants were enrolled from four communities in Kwara state, Nigeria. Participants were randomly tested for malaria infections, using rapid diagnostic detection. Parasite densities were determined from thick blood smears by microscopy. Dried blood spots samples were collected on Whatman FTA cards, from which parasite genomic DNA was extracted. Polymerase chain reaction (PCR) and restriction fragment length polymorphism were conducted on genomic samples. The prevalence of *P. falciparum* infections by microscopy was 22.30% (68/305), with a PCR corrected value of 23.28% (71/305). *P. falciparum* chloroquine resistant transporter (*Pfcrt*) mutation (K76T) was predominantly significant ($p<0.05$) in all positive samples (100%), compared to 56% and 38%, for dihydrofolate reductase (*Pfdhfr*) primary point mutation (S108N) and the *P. falciparum* multidrug resistant gene (*Pfmdr1*) mutation (86Y), respectively. Background detection of *Pfcrt* wild-type K76 was recorded in 41% (29/71) of the positive samples. More than ten years after withdrawal of chloroquine (CQ) and PYR from first-line treatment of malaria in the region, there is still a strong presence of resistant markers to these drugs in *P. falciparum* isolates. However, the presence of CQ sensitive strains in mixed population with resistant cousins may suggest development of reversal processes by parasites probably due to fitness cost in the absence of chloroquine and/ or evolving mechanisms towards artemisinin resistance due to ACT pressure.

258

IDENTIFYING MOLECULAR MARKERS OF *PLASMODIUM FALCIPARUM* ARTEMISININ RESISTANCE USING THE CRISPR-CAS9 GENOME EDITING SYSTEM

Oheneba Charles Hagan

University of Ghana, Accra, Ghana

The emergence and spread of *P. falciparum* to artemisinin and the artemisinin combination therapy partner drugs threatens to upend the gains made by the control program in recent times especially in Africa. With hindsight from the devastation chloroquine and sulphadoxine-pyrimethamine resistance wreaked in Africa due to an unprepared public health system, real time monitoring of resistance has therefore been recommended in other to forestall any similar occurrence. Monitoring of the molecular markers of resistance is less logistically and financially constraining compared with *in vivo* and *in vitro* monitoring especially in resource limited setting. However, the kelch 13 molecular markers for monitoring artemisinin resistance have arisen independently, with multiple mutations either conferring resistance or otherwise. Gene-editing system has previously been utilised to validate some of these mutations, we therefore undertook to use the CRISPR-Cas9 genome editing system validate kelch 13 mutations detected from recrudescing parasites sampled from the monitoring sentinel sites in Ghana. We have successfully edited kelch 13 V568G and C580R previously detected in Ghana in addition to C580Y and R539T commonly found in Southeast Asia and C580C (silent mutation) into Dd2 *P. falciparum* strain. We utilised a single plasmid system carrying a chimeric short-guide RNA, a codon-optimised Cas9 sequence and a donor DNA.Cloning by limiting dilution method has been used to clone out isogenic parasites, which would be utilised to perform ring stage survival assay to validate these mutations. In addition, conventional SYBR green drug assays of commonly used antimalarial, fitness and growth assays.

259

THERAPEUTIC RESPONSE OF SINGLE *PLASMODIUM FALCIPARUM* SPECIES VERSUS MIXED SPECIES INFECTIONS TO ARTEMETHER-LUMEFANTRINE AS COMPARED TO OTHER ARTEMISININ COMBINATION THERAPIES IN KISUMU COUNTY, WESTERN KENYA

Gladys C. Chemwor[1], Hoseah M. Akala[1], Benjamin H. Opot[1], Raphael O. Okoth[1], Redemptah M. Yedah[1], Agnes C. Cheruiyot[1], Irene Onyango[1], Dennis W. Juma[1], Ben Andagalu[1], Jim R. Managbanag[2]

[1]*Department of Emerging Infectious Diseases (DEID), United States Army Medical Research Directorate-Kenya (USAMRD-K), Kenya Medical Research Institute (KEMRI), Kisumu, Kenya,* [2]*United States Army Medical Research Directorate-Kenya, Kisumu, Kenya*

Information on treatment outcomes of infections comprising non-*falciparum* species is scanty despite it being reported to be responsible for around 25% of imported malaria from Africa. The recommended first line treatment in Africa is artemether-lumefantrine (AL) with dihyroartemisinin-piperaquine (DHAPPQ) as second line whereas in Asia, artesunate-mefloquine (ASMQ) is the preferred choice of treatment. The aim of this study was to compare the treatment outcomes of mixed *Plasmodium* species versus pure *falciparum* species infections during dosing with AL, DHAPPQ & ASMQ. Samples collected at hours 0, 4, 8, 24 and 30 from individuals enrolled in an ACT efficacy study in Kisumu County, Western Kenya, between 2013 and 2015 were analyzed for species composition using real time polymerase chain reaction (rt-PCR). The assay targeted detection of *Plasmodium falciparum* (*Pf*), *Plasmodium malariae* (*Pm*), *Plasmodium ovale curtisi* (*Poc*) and *Plasmodium ovale wallikeri* (*Pow*). Therapeutic response was evaluated using parasite clearance parameters as well using the World Health Organization criteria and compared with species composition of each infection. Recurrent parasitemia for the subsequent time points specifically hours 24 and 30 was also characterized to rule out re-infection. There was a steady decline in parasites for

infections comprising *Pf* species alone following administration of AL and ASMQ as opposed to those comprising *Pf* and other species. *Pow* frequency persisted in the subjects treated with AL in all the time points with an increase at hour 8 whereas for ASMQ, it appeared at hour four and eight then cleared completely. *Pm* seems to have been cleared early at hours 4 and 8 for AL and ASMQ respectively. Data analysis for DHAPPQ is in progress. Obtaining more information and continued monitoring of non-*falciparum* malaria during implementation of ACTs is therefore warranted to advise clinical intervention and align them to solving challenges of increasing traveler malaria.

260

SELECTIVE IMPACT OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER-LUMEFANTRINE THERAPIES ON THE POLYMORPHISM OF *PFCRT* K76T, *K13 PROPELLER* AND *PFMDR1* (CODONS 86 AND 184) GENES IN THREE MALARIA SENTINEL SITES IN CÔTE D'IVOIRE

Abibatou Konate, Paterne Akpa Gnagne, Valerie A. Bedia, Herve Menan, William Yavo

Felix Houphouet boigny University, Abidjan, Côte D'Ivoire

Plasmodium falciparum resistance to most of antimalarial drugs including the more recent is a concern to malaria elimination. Despite their adoption for the treatment of uncomplicated malaria in Côte d'Ivoire, there is few information regarding the selective impact of *artemisinin-based combination therapies* (ACTs) on *Plasmodium falciparum* strains circulating in the country. The present study aimed to assess *pfcrt* K76T, *K13 propeller* and *Pfmdr1* (codons 86 and 184) mutations before and after treatment with artesunate-amodiaquine (ASAQ) or artemether-lumefantrine (AL) in three sentinel site for malaria monitoring in Côte d'Ivoire. *P. falciparum* infected samples were collected in 2013,2014 in Abidjan and 2016 in Abengourou and San Pedro through routine monitoring of antimalarial drug's efficacy. Only patients with treatment response were included in this study. Samples collection concerned Day 0 before treatment and the day of recurrent parasitaemia during the 42-day follow-up. The single nucleotide polymorphism in the *pfcrt* (codon 76) gene was analyzed PCR-RFLP after *Plasmodium* DNA extraction while *K13 propeller* and *Pfmdr1* genes were analyzed by sequencing. Overall 340 samples were genotyped i.e. 322 from D0 and 18 at follow-up day. No recrudescence was observed in ASAQ arm. For *Pfcrt* K76T, a selection of mutant strains by both therapies was found. The analysis of *Pfmdr1* gene showed a selection of mutant strains *Pfmdr1* 86Y (11.1%) and *Pfmdr1* 184F (66.7%) in case of new infestation after ASAQ treatment. After treatment with AL, in case of recrudescence, the wild-type strains of *Pfmdr1* N86 (100%) and Y184 (42.9%) were selected. This trend was the same in case of new infestation. As regards the *K13 propeller* gene, no mutation was found after treatment by ASAQ as well as AL. This study shows an antagonism in the selection of strains by ASAQ and AL associations. This demonstrates the benefit of the use of several antimalarial drugs as first-line treatments which could lead to the delay the apparition of chemoresistance.

261

EVALUATING THE ANTIMALARIAL ACTIVITY OF GOSSYPOL, A NATURAL PRODUCT AGAINST *PLASMODIUM FALCIPARUM*

Jersley Didewurah Chirawurah, Yaw Aniweh, Gordon A. Awandare

West African Center for Cell Biology of Infectious Pathogens and Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon, Accra, Ghana

Gossypol is a natural product from cotton seeds that has been shown to have good antimalarial activities against both chloroquine resistant and susceptible *Plasmodium falciparum* parasite strains. However, the target and mechanisms of action of this compound have not been clearly demonstrated. This study first evaluated the potency of gossypol using laboratory strains and clinical isolates of *Plasmodium falciparum* parasites. This was followed by the selection of resistant parasites against gossypol for studying the mechanisms of resistance to this compound. The gossypol resistant parasites (*P. falciparum* Dd2 background) were subsequently screened against Chloroquine, Dihydroartemisinin (DHA) and three Malaria Box compounds (MMV006087, MMV085203 and MMV008956). A total of six (6) laboratory strains and eight (8) clinical isolates of *P. falciparum* were screened against gossypol using optimized growth inhibitory assays. Additionally, gossypol resistant Dd2 strains were selected (using media containing 3.5 μM gossypol) and screened against Chloroquine, DHA and the three Malaria Box compounds.The results from this study suggest gossypol was more efficacious against the clinical isolates (IC_{50} value of 5.108 μM) compared to the laboratory strains (IC_{50} value of 6.1135 μM). Interestingly, the gossypol resistant Dd2 parasites were observed to be more sensitive after three months (IC_{50} value changed from 5.049 μM to 2.599 μM) and then resistant after six months (IC_{50} value changed from 2.599 μM to 14.94 μM). The gossypol resistant Dd2 parasites were also observed to be more sensitive to chloroquine, DHA and the three Malaria Box compounds compared to their parental strains.The results from this study suggest gossypol might possess an interesting mechanism of action and potentially new targets. Further experiments are currently underway to determine the mechanisms modulating the sensitivity in the gossypol resistant Dd2 strains. This work will be instrumental in identifying novel targets in *P. falciparum* parasites, which is critical for the discovery of novel antimalarial compounds against drug resistant malaria parasites.

262

GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* PARASITES IN PREGNANT AND NON-PREGNANT WOMEN AND POTENTIAL RESISTANCE TO ANTIMALARIAL DRUGS IN KENYA

Brenda Makena Mugambi

US Army Medical Research Directorate Africa-Kenya, Kisumu, Kenya

Malaria infection during pregnancy has detrimental effects owing to decreased immunity to malaria. In high transmission regions most individuals are presumed malaria-immune; non-pregnant women have been shown to have faster parasite clearance compared to pregnant ones. This suggests non-immune environment in pregnant women may select for parasite populations associated with resistance to artemisinin. This study will determine genetic variations in *Plasmodium falciparum* parasites in pregnant versus non-pregnant women. Blood samples were collected at hours 0, 8, 24 and days 7 and 28 from 75 malaria positive women; 25 pregnant in second trimester (arm1), 25 pregnant in third trimester (arm2) and 25 non-pregnant (arm3); enrolled in an artemisinin combination therapy efficacy study in Ahero, Kenya. All samples were diagnosed for malaria using 18s rRNA rtPCR. Genotyping for both merozoite surface proteins 1 and 2 was done to determine recrudescence and reinfection. Single nucleotide polymorphisms (SNP) genotyping for K13, *Pfmdr1, Pfdhfr, Pfdhps* and *Pfcrt* genes was done to determine mutations in parasites with varying clearance using Sanger sequencing and MassARRAY. Additionally, microsatellite genotyping using 12 markers was performed to determine genetic variations in parasites between the two populations. Of the 75 women; 45 consisting n=15 arm 1, n=12 arm 2 and n=18 arm 3 had samples at all study time points merited to be included in the analysis. The 45 individuals comprised: Hr 0=100.0%; Hr 8=84.4%; Hr 24=26.7%; Day 7=24.4% and Day 28=4.4% samples positive for *Plasmodium*. *P. falciparum* species were observed in: Hr 0=44.4%; Hr 8=44.7%; Hr 24=8.3%; Day 7=0% and Day 28=0% samples. Samples positive for *Plasmodium* on days 7 and 28 were confirmed as reinfections based on MSP genotyping. A total of 225 samples including all subsequent positives have proceeded to MassARRAY, Sanger sequencing and microsatellites genotyping. Findings of this study will present genotypic properties of malaria parasites in pregnant and non-pregnant women and advise on expression of genes associated with resistance to artemisinin among the three arms.

263

DRUG USE IN THE MANAGEMENT OF SEVERE MALARIA IN PUBLIC HEALTH FACILITIES IN DEMOCRATIC REPUBLIC OF CONGO

Aline Biongo Engo, Nsengi Ntamabyaliro, Didier Bomene Nzolo, Yves Ntamba Lula, Gaston Lutete Tona

Clinical Pharmacology Unit, Kinshasa, Democratic Republic of the Congo

Democratic Republic of the Congo (DRC) is the second most affected country by malaria deaths. National Policy recommends the usage of Injectable artesunate or quinine for the management of severe malaria. These drugs, especially injectable artesunate have shown to be effective and safe. The increased number of deaths in DRC may be due to many factors, including irrational use of drugs. The study was conducted in all previous eleven provinces of DRC in 2014. One Referral hospital one Urban Health Centre and one Rural Health Centers were selected in each of the Provinces. In each of the Health Facilities, 100 randomly selected medical files containing the prescription of an antimalarial drug from January to December 2013 were reviewed. For each file, the following variables were recorded: age of patient, diagnosis of malaria (clinical and parasitological), treatment and issues of treatment. Descriptive analysis were performed. Out of the 3254 files collected, 531(16, 2%) concerned complicated malaria. Children under five year of age represented 52.7% of patients affected by severe malaria. Parasitological confirmation of diagnosis have been made by thick smear in 228 patients (43%) and Rapid diagnostic tests (RDTs) in 126(24 %). Treatment of positive parasitology in 51, 4%, treatment despite negative parasitology in 12, 2%. Quinine was used in 465 patients (87, 6 %) but was never associated with Doxycycline or Clindamycin as recommended by National Malaria Program. Apart from antimalarials, patients received an average of 3.9 other drugs among which antibiotics (73.2%), antalgics (48.6%), anemia drugs (22.8%) and corticosteroids (16.8%). Seventy-four patient (14%) patients received blood transfusion. After treatment, 168(3, 6%) patient were cured and 3, 4% died; the issue was not known for 63, 8%. Deaths rates seems to be higher in patient concomitantly treated with corticosteroids (9, 4%) (p=0, 000815) Treatment of malaria is not complete in almost all cases. Numerous concomitant medications are added. There are significantly more deaths in patients treated by corticosteroids. Determinants of this irrational use need to be assessed.

264

PATIENTS' ADHERENCE TO MALARIA TREATMENT IN DEMOCRATIC REPUBLIC OF THE CONGO

Pierre-Michel Nsengi Ntamabyaliro[1], Didier B. Nzolo[1], Aline B. Engo[1], Yves N. Lula[1], Samuel M. Mampunza[1], Eric S. Mukomena[2], Gaston Lutete Tona[1]

[1]*Clinical Pharmacology Unit, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [2]*National Malaria Program, Kinshasa, Democratic Republic of the Congo*

Irrational use of antimalarials in Health facilities is probably hampering the fight against malaria in the Democratic Republic of the Congo (DRC) as shown by a survey conducted in 2014. However, the use of these drugs by the community the adherence of patients to treatment need to be assessed as well in order to have a better picture of the use of antimalarials in DRC. A survey was conducted in December 2017 in all the 11 previous provinces of DRC. In each of them the catchment area of one Rural and one Urban health center were selected. In the selected area, all the household which had had a case of malaria in the 15 days before the survey were selected and the patient or the caring person were interviewed. For all antimalarial that had been prescribed the adherence (considered as non-interruption of treatment before its end) and reasons for treatment interruption were recorded. A total of 1987 households were interviewed. Patients received an average of 3.1 drugs among which the drugs recommended by the National Malaria Control program, Artesunate/amodiaquine (ASAQ), artemether/Lumefantrine (AL) and Quinine were the most cited. Treatment with Quinine was interrupted in 33% of cases (259/790), ASAQ in 16.1% (106/658) and AL in 5.9% (15/255). Reasons for interruption of quinine and ASAQ were most of the time adverse events (51.9% and 60.0% respectively). Adherence to Quinine and ASAQ are low. Adverse events are the major cause of interruption of treatment for the drugs used in the management of malaria especially Quinine and ASAQ. The impact of this in this need to be monitored. Management of adverse events need to be considered at the initiation of antimalarial treatment.

265

INCREASED GLUTATHIONE PRODUCTION CONTRIBUTES TO THE MECHANISM OF ARTEMISININ RESISTANCE IN *KELCH13*-MUTANT *PLASMODIUM FALCIPARUM*

Darren J. Creek, Amanda De Paoli, Ghizal Siddiqui

Monash University, Parkville, Melbourne, Australia

Artemisinin and its derivatives (ARTs) underpin the most effective treatments for uncomplicated *Plasmodium falciparum* malaria. However, resistance to ARTs is becoming increasingly prevalent and the cellular mechanism of resistance requires further elucidation. Untargeted metabolomics and peptidomics analysis of *PfKelch13*-mutant *P. falciparum* revealed a down-regulation of haemoglobin digestion, and an increased abundance of key antioxidant molecules such as glutathione (GSH) and its precursor gamma-glutamyl cysteine in ART-resistant parasites. We propose that these differences enhance the ability of ART-resistant parasites to ameliorate ART-induced free radical damage, as glutathione conjugation has been shown to function as a detoxification mechanism for many drugs that act via the generation of reactive intermediates. This study further investigated the role of glutathione in *PfKelch13*-mediated ART resistance in *P. falciparum* parasites using metabolomics, and tested whether inhibition of this detoxification system can be a strategy for modulation of resistance to ART. ART-resistant isolates were incubated with buthionine sulphoximine (a gamma-glutamylcysteine synthetase inhibitor) and sulfasalazine (a cysteine transport inhibitor) in order to inhibit glutathione production. Pre-incubation with these compounds increased ART ring-stage activity in the ART-resistant parasites (40% survival decreased to 18%, $p < 0.05$) to a level of activity comparable to sensitive isolates. On the contrary, pre-incubation of ART-sensitive parasites with the glutathione precursor, N-acetylcysteine, was sufficient to reduce ART ring-stage activity to a level comparable to the resistant parasites. This confirmed that anti-oxidant capacity plays a key role in the cellular mechanism of ART resistance. In conclusion, we have identified altered glutathione metabolism as a major contributing factor to the mechanism of *PfKelch13*-mediated ART resistance. Furthermore, the *in vitro* ART resistance phenotype can be reversed by inhibiting this detoxification system.

266

EVALUATION OF THERAPEUTIC EFFICACIES OF ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* AND CHLOROQUINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR UNCOMPLICATED *PLASMODIUM VIVAX* IN FECTION IN ETHIOPIA

Ashenafi Assefa[1], Hussein Mohammed[1], Anjoli Anand[2], Heven Sime[1], Mekonnen Tadesse[3], Yehualashet Tadesse[3], Samuel Girma[4], Worku Bekele[5], Kebede Etana[6], Bereket Hailegiorgis Alemayehu[3], Hiwot Teka[4], Matthew Murphy[4], Hiwot Solomon[6], Adugna Woyessa[1], Jimee Wang[7]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*ICAP at Columbia University, Addis Ababa, Ethiopia,* [4]*US President's Malaria Initiative, Addis Ababa, Ethiopia,* [5]*World Health Organization, Addis*

Ababa, Ethiopia, [6]Ethiopian Federal Ministry of Health, Addis Ababa, Ethiopia, [7]Malaria Branch, US Centers for Disease Control and Prevention, Atlanta, GA, United States

Artemisinin-based combination therapy has been used for the treatment of uncomplicated malaria in Ethiopia since 2004. The national malaria diagnosis and treatment guidelines employ a species-specific approach for malaria treatment. Artemether-lumefantrine (AL) and Chloroquine (CQ) are the first line treatments for *Plasmodium falciparum* (Pf) and *P. vivax* (Pv), respectively. In this study, we report the clinical and parasitological efficacy of AL and dihydroartemisinin-piperaquine (DP) against uncomplicated Pf, and of CQ and DP against uncomplicated Pv infections. The WHO guideline for monitoring antimalarial drug efficacy was followed for 42 days. The study was conducted from October 2017-February 2018 in two sentinel sites in Ethiopia. A total of 379 patients were enrolled in four arms (n = 106, AL/Pf; n = 75, DP/Pf; n = 142, CQ/Pv; n = 56, DP/Pv). Only patients above 18 years were enrolled for the DP arms. High cure rate was observed at 28 days (PCR uncorrected): 98% in AL/Pf (95% CI: 93-100); 98% in CQ/Pv (95% CI: 94-100). Efficacy at 42 days was 94% in AL/Pf (95% CI: 87-97), and 82% in CQ/Pv (95% CI: 75-88). There was 100% adequate clinical and parasitological efficacy in the DP/Pf and DP/Pv arms up to 42 days. For secondary endpoints, 98% (95% CI: 93-100) of AL/Pf patients cleared parasites and 99% were afebrile by day three. For all other arms, 100% of patients cleared parasites and were afebrile by day three. Early treatment failure was not detected in any arm. No severe adverse event was observed with a slight hematological recovery in all arms. Despite pending PCR corrected failure rates which will be available shortly, the results support the efficacy of the current anti-malaria drugs used by the malaria control program in Ethiopia. In addition, DP appears to be a good alternative for the treatment of both species. The high recurrence rate observed for Pv with CQ after 28 days is concerning, and highlights the importance of providing anti-relapse therapy.

267

EFFICACY AND TOLERABILITY OF ARTEMISININ- AND QUININE-BASED TREATMENTS FOR UNCOMPLICATED FALCIPARUM MALARIA DURING PREGNANCY: A WWARN INDIVIDUAL PATIENT DATA META-ANALYSIS

Makoto Saito[1], Rashid Mansoor[1], Kalynn Kennon[1], Anupkumar R. Anvikar[2], Elizabeth A. Ashley[3], Daniel Chandramohan[4], Lauren Cohee[5], Umberto D'Alessandro[6], Blaise Genton[7], Elizabeth Juma[8], Linda Kalilani-Phiri[9], Irene Kuepfer[4], Miriam K. Laufer[5], Khin Maung Lwin[10], Steven R. Meshnick[11], Dominic Mosha[12], Victor Mwapasa[13], Norah Mwebaza[14], Michael Nambozi[15], Jean-Louis A. Ndiaye[16], François H. Nosten[10], Myaing Nyunt[17], Bernhards Ogutu[8], Sunil Parikh[18], Moo Kho Paw[10], Aung Pyae Phyo[3], Mupawjay Pimanpanarak[10], Patrice Piola[19], Marcus J. Rijken[20], Kanlaya Sriprawat[10], Harry K. Tagbor[21], Joel Tarning[22], Halidou Tinto[23], Innocent Valéa[23], Neena Valecha[2], Nicholas White[24], Jacher Wiladphaingern[10], Kasia Stepniewska[1], Rose McGready[10], Philippe J. Guérin[1]

[1]*WorldWide Antimalarial Resistance Network, Oxford, United Kingdom,* [2]*ICMR-National Institute of Malaria Research, New Delhi, India,* [3]*Myanmar–Oxford Clinical Research Unit, Yangon, Myanmar,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [6]*Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [7]*Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland,* [8]*Kenya Medical Research Institute, Nairobi, Kenya,* [9]*Department of Medicine, University of Malawi College of Medicine, Blantyre, Malawi,* [10]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand,* [11]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, United States,* [12]*Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania,* [13]*College of Medicine, University of Malawi, Blantyre, Malawi,* [14]*Infectious Disease Research Collaboration, Makerere University, Kampala, Uganda,* [15]*Department of Clinical Sciences, Tropical Diseases Research Centre, Ndola, Zambia,* [16]*Department of Parasitology, University Cheikh Anta Diop, Dakar, Senegal,* [17]*Duke Global Health Institute, Duke University, Durham, NC, United States,* [18]*Yale School of Public Health, New Haven, CT, United States,* [19]*Institut Pasteur du Cambodge, Phnom Penh, Cambodia,* [20]*Department of Obstetrics and Gynecology, Division of Woman and Baby, University Medical Center Utrecht, Utrecht, Netherlands,* [21]*School of Medicine, University of Health and Allied Sciences, Ho, Ghana,* [22]*Mahidol–Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [23]*Clinical Research Unit of Nanoro, Institut de Recherche en Sciences de la Santé, Nanoro, Burkina Faso,* [24]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom*

Highly efficacious treatment is required to reduce adverse effects for the mother and fetus from *Plasmodium falciparum* infection in pregnancy. The current WHO guidelines recommend quinine- and artemisinin-based combination therapy (ACT) for first and second/third trimester women, respectively. There is now a significant body of evidence on the safety of ACTs in pregnancy, including in the first trimester. However, the current guidelines are supported by limited evidence on efficacy because pregnant women have usually been excluded from antimalarial studies. In addition, there is no fixed guideline for antimalarial efficacy studies in pregnant women: most study designs have been variably extrapolated from guidelines for non-pregnant populations; which complicates aggregated data meta-analysis on efficacy. In an effort to assemble the available evidence on antimalarial efficacy in pregnancy in a uniform way with data standardisation for statistical analysis, we conducted a systematic review and an individual patient data (IPD) meta-analysis of antimalarial efficacy studies on uncomplicated falciparum malaria in pregnancy. A one-stage IPD meta-analysis was conducted to identify the factors associated with PCR-corrected treatment efficacy. IPD of 4968 pregnant women (93% of the total targeted IPD) from 19 studies (10 in Asia and 9 in Africa) were analysed. Studied drugs included artemether-lumefantrine (AL, n=1278), artesunate-mefloquine (n=1028), dihydroartemisinin-piperaquine (n=874), artesunate-amodiaquine (n=841), quinine monotherapy (n=244), artesunate monotherapy (n=230), artesunate-sulfadoxine-pyrimethamine (n=173), artesunate-clindamycin (AC, n=142), artesunate-atovaquone-proguanil (n=91) and quinine-clindamycin (n=67). Quinine monotherapy and higher baseline parasitaemia were associated with a higher risk of failure. Adverse abdominal symptoms and tinnitus were more frequently observed with quinine compared to AL. Considering the lower efficacy and lower tolerability, withdrawal of quinine monotherapy from the first-line treatment for pregnant women should be considered.

268

THERAPEUTIC EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN COLOMBIA, 2018-2019

Mario Javier Olivera[1], Angela Patricia Guerra[1], Liliana Jazmin Cortes[1], Roberta Horth[2], Jonathan Novoa[3], Maria de la Paz Ade[4], Naomi Lucchi[2], Dragan Ljolje[2], Wilmer Marquino[3], Martha Renteria[5], Wilman Yurgaky[6], Alexandre Macedo de Oliveira[2]

[1]*Instituto Nacional de Salud, Bogota, Colombia,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*Pan American Health Organization, Bogota, Colombia,* [4]*Pan American Health Organization, Washington, DC, United States,* [5]*Laboratorio de Salud Publica de Choco, Choco, Colombia,* [6]*Hospital Local Ismael Roldan Valencia, Choco, Colombia*

Artemether-lumefantrine (AL) has been the first-line antimalarial treatment for uncomplicated *Plasmodium falciparum* malaria in Colombia since 2010. The World Health Organization (WHO) recommends evaluating antimalarial treatment regimens every three years in low-endemic countries to confirm efficacy. To assess the therapeutic efficacy of AL in uncomplicated *P. falciparum*-infected patients in Quibdo, Colombia, we conducted a 28-day trial following WHO and Pan-American Health Organization guidance for antimalarial efficacy surveillance. From July

2018 to February 2019, febrile patients ≥5 years old with microscopically confirmed *P. falciparum* monoinfection and asexual parasitemia 250-100,000 parasites/μL were enrolled and treated with a supervised 3-day course of AL. The primary endpoint was adequate clinical and parasitological response (ACPR) on Day 28. We used neutral microsatellites to differentiate reinfection and recrudescence during the follow-up. Out of 259 patients offered participation, 86 consented and were ultimately enrolled, two of those were lost to follow-up, and 84 (97.7%) reached a valid endpoint: treatment failure or ACPR. One patient had recurrent infection with low-level parasitemia (12 parasites/μL) on Day 28. The parasite responsible for this infection was not isolated on molecular testing; therefore, this patient was excluded from the microsatellite-corrected analysis. The uncorrected and microsatellite-corrected ACPR rates were 98.8 % (83/84) (95% confidence interval [CI]: 93.5-100%) and 100% (83/83) (95% CI: 95.7-100%), respectively. In addition, no patient remained positive on Day 3, which would signal delayed parasite clearance, commonly associated with artemisinin resistance. AL remains efficacious for falciparum malaria in Quibdo, Colombia. Due to the time and effort, 8 months in our study, required to conduct efficacy trials in low-endemic settings, routine use of molecular markers for antimalarial resistance could greatly expand the coverage of antimalarial drug efficacy monitoring.

269

EFFICACY OF THREE REGIMENS OF CHLOROQUINE AND PRIMAQUINE FOR THE TREATMENT OF *PLASMODIUM VIVAX* MALARIA IN CRUZEIRO DO SUL, ACRE STATE, BRAZIL, 2018-2019

Sarah-Blythe Ballard[1], Suiane Negreiros[2], Samela Farias[2], Giselle Maria R. Viana[3], Stella Chenet[4], Paola Marchesini[5], Cassio Peterka[5], Marinete Marins Povoa[3], Alexandre Macedo de Oliveira[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Acre State Health Secretariat, Cruzeiro do Sul, Brazil,* [3]*Instituto Evandro Chagas, Belem, Brazil,* [4]*Instituto de Salud Publica, Santiago, Chile,* [5]*Brazil Ministry of Health, Brasilia, Brazil*

Treatment of *Plasmodium vivax* malaria infections requires the administration of chloroquine (CQ) and an aminoquinoline, such as primaquine (PQ), to eradicate hypnozoites, dormant liver forms that can lead to relapses. The World Health Organization (WHO) recommends a total PQ dose of either 3.5 mg/kg or 7.0 mg/kg depending on the expected relapse rates for each region of the world. After reports of frequent malaria recurrent infections, likely relapses and not new infections, with the low PQ dose, we evaluated the efficacy of three regimens of CQ and PQ for the treatment of uncomplicated *P. vivax* malaria in Brazil. This was a randomized 3-arm study among patients presenting to malaria diagnostic posts in Cruzeiro do Sul from April 2018 to February 2019. We enrolled patients ≥5 years of age with microscopy-confirmed *P. vivax* monoinfection and no evidence of severe disease. Patients received CQ under direct observation for 3 days (total dose: 25 mg/kg). After confirmation of normal glucose-6-phosphate dehydrogenase activity levels, we randomly assigned patients to receive a total PQ dose of 3.5 mg/kg divided over seven days in group 1 (non-observed therapy) and group 2 (observed), or a total dose of 7.0 mg/kg over 14 days in group 3 (observed). We monitored patients clinically and parasitologically on days 1, 2, 3, 7, 14, 21, 28, and every 4 weeks until day 168. We enrolled 257 patients: 63 in group 1, 98 in group 2, and 96 in group 3. The median asexual parasitemia at admission was 3,988 parasites/μL. By day 28, one patient in group 1, two patients in group 2, and no patient in group 3 presented with *P. vivax* infection; while 21, 33, and 11 presented with *P. vivax* parasitemia by day 168 in groups 1, 2, and 3, respectively. Adequate clinical and parasitological response was estimated at 59.6% (95% confidence interval [CI], 46.3-73.0%), 57.1% (95% CI, 46.1-68.2%), and 85.9% (95% CI, 78.2-93.6%), for groups 1, 2, and 3, respectively. While all regimens were efficacious for treatment of the acute infection, the higher WHO-recommended PQ dose was associated with superior efficacy in preventing recurrent infections within 168 days post treatment.

270

UNRAVELING NEW ANTIMALARIAL TARGETS

Sonia Moliner Cubel[1], Frank Schwach[2], Marcus Lee[2], Julian C. Rayner[2], Esther Fernandez Velando[1], Francisco Javier Gamo[1], Maria de Gracia Gomez Lorenzo[1]

[1]*GlaxoSmithKline, Tres Cantos, Spain,* [2]*Wellcome Sanger Institute, Hinxton, Cambridgeshire, United Kingdom*

Resistance to all known antimalarial drugs has been reported, highlighting the urgent need to discover new chemical entities with novel modes of action. Target based screening is an excellent approach to identify novel chemical diversity to enable development of new antimalarial drugs. However, in malaria this approach is restricted due to the limited number of validated drug targets. One method that has been used extensively in malaria parasites for target discovery and chemical validation is *in vitro* resistance evolution and whole genome sequencing (WGS). In many cases, new mutations are found in genes that are predicted to encode the target. Our aim was to follow this strategy with whole cell antimalarial inhibitors with unknown mode of action to unravel new targets for future target-based screenings. Antimalarial compounds with good parasitological profile from several MedChem programs were selected and progress through a battery of functional and cross-resistance assays to discard those with already known modes of action. After this triage, *in vitro* resistance selection studies under constant drug pressure were performed for 9 compounds. Resistance selection attempts were succssesful with 3 of them. Degree of resistance was confirmed in IC50 assays. Experiments were repeated in triplicate to obtain a wider diversity of genotypes and 3 different clones from the 3 independent flasks were isolated from the resistant pools. Results from WGS of those clones as well as hints toward the mode of action of the compounds will be presented. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

271

EFFICACY OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER-LUMEFANTRINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MADAGASCAR

Antsa Rakotondrandriana[1], Arinomenjanahary Rakotoarisoa[1], Tovonahary Angelo Rakotomanga[1], Marie Ange Rason[1], Catherine M. Dentinger[2], Laura Claire Steinhardt[3], Samaly Souza[3], Naomi Lucchi[3], Venkatachalam Udhayakumar[3], Eric Halsey[4], Arsène Ratsimbasoa[1]

[1]*National Malaria Control Program, Antananarivo, Madagascar,* [2]*Malaria Branch, Centers for Disease Control and Prevention, US President's Malaria Initiative, Antananarivo, Madagascar,* [3]*Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Malaria Branch, Centers for Disease Control and Prevention, US President's Malaria Initiative, Atlanta, GA, United States*

Since 2006, artemisinin-based combination therapy has been recommended to treat uncomplicated malaria in Madagascar. Artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) are the first- and second-line treatments, respectively. To understand whether ASAQ and AL remain efficacious, a therapeutic efficacy study was conducted. Patients between 6 months and 15 years with uncomplicated *Plasmodium falciparum* malaria (1,000-100,000 parasites/μl) were assessed from May—September 2018 in a 28-day *in vivo* efficacy trial in two sites: Ankazomborona (tropical, north) and Matanga (equatorial, southeast). The 2009 World Health Organization (WHO) protocol for monitoring antimalarial efficacy was followed. Polymerase chain reaction (PCR) was used to distinguish recrudescence from reinfection, which allows determination of the PCR-corrected day 28 efficacy. Assays for molecular markers of resistance, including *K13*, *Pfcrt,* and *Pfmdr1*, were carried out. A total of 344 patients were enrolled; 163/170 (96%) in the ASAQ arm and 166/174 (95%) in the AL arm reached day 28. The day 28 uncorrected efficacy was 98.2% (95% CI 94.5-100%) for ASAQ and 93.4% (95%

CI 89.7-97.1%) for AL. PCR-corrected analyses are being finalized. Sequencing was attempted in 85 pre-treatment samples for the *K13, Pfcrt,* and *Pfmdr1* genes. Of the 83 successfully sequenced samples for the *K13* gene, no mutations associated with artemisinin resistance were observed. Of the 82 successfully sequenced samples for the *Pfcrt* gene, all were wild type for codons 72-76. Of the 74 successfully sequenced samples for the *Pfmdr1* gene, a majority carried the NFD (30 single and 7 mixed infections) and NYD (23 single and 7 mixed infections) haplotypes corresponding to codons 86, 184, and 1246. Prior to PCR correction, which will eliminate reinfections from the analysis and reveal an even higher efficacy, this study indicates that both ASAQ and AL have therapeutic efficacies above the 90% WHO acceptable cut-off. Periodic monitoring of therapeutic efficacy should continue routinely to ensure these treatments remain efficacious.

272

MUTATIONS IN *PLASMODIUM FALCIPARUM* PRO-DRUG ACTIVATION AND RESISTANCE ESTERASE MEDIATES RESISTANCE TO A SUB-CLASS OF SESQUITERPENE DIMER ANTIMALARIAL NATURAL PRODUCTS

Joshua H. Butler[1], Emilio F. Merino[1], Rodrigo P. Baptista[1], Judith I. Okoro[2], Ryan M. Scales[3], Philip J. Rosenthal[4], Roland A. Cooper[5], Jessica C. Kissinger[1], Jian-Min Yue[6], Bin Zhou[6], Maria B. Cassera[1]

[1]*University of Georgia, Athens, GA, United States,* [2]*University of California Berkeley, Berkeley, CA, United States,* [3]*University of North Carolina, Charlotte, Charlotte, NC, United States,* [4]*University of California San Francisco, San Francisco, CA, United States,* [5]*Dominican University of California, San Rafael, CA, United States,* [6]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, and University of Chinese Academy of Sciences, Beijing, China*

Plasmodium falciparum is the deadliest of the *Plasmodium* species that cause human malaria, and the emergence of drug resistant parasites is a constant threat. Many parasitic mechanisms of drug resistance to antimalarials have been both observed in nature as well as demonstrated *in vitro*. Recently, *P. falciparum* Prodrug Activation and Resistance Esterase (PARE) was identified and its deactivation implicated in conferring *P. falciparum in vitro* resistance to antimicrobial pepstatin esters as well as the benzodiazepine, MMV011438. Our work, suggests *Pf*PARE deactivation is also a resistance mechanism to the potent antimalarial ester-containing sesquiterpene dimers: Fortunilide A, Fortunilide E and Chlorajaponilide C. In addition, we screened the open source compound libraries called the "Malaria box" and "Pathogen box" using our mutant resistant cell line. Among these libraries, MMV011438 and MMV011576 presented reduced efficacy in parasites resistant to the sesquiterpene dimers. Using a combination of *in vitro* cell-based assays with parasites harboring mutations in *Pf*PARE gene, recombinant *Pf*PARE enzymatic assays and whole genome sequencing and analysis, we provide evidence that mutations in *Pf*PARE confer resistance to the sesquiterpene dimers. *Ex-vivo* efficacy studies performed suggest this parasitic mechanism of resistance to several different classes of small molecules is not currently found in field isolates. Analysis of structure-resistance relationships of 11 lindenane type sesquiterpene dimers provides insights for possible future development of these compounds as an antimalarial therapy aimed at preserving low nanomolar efficacy, high selectivity and circumvent possible *Pf*PARE mediated resistance.

273

LONG-TERM EFFECT OF SEASONAL MALARIA CHEMOPREVENTION WITH AMODIAQUINE PLUS SULFADOXINE-PYRIMETHAMINE ON MOLECULAR MARKERS OF RESISTANCE IN OUELESSEBOUGOU, MALI

Almahamoudou Mahamar[1], Kelsey Sumner[2], Brandt Levitt[2], Betsy Freedman[2], Aliou Traore[1], Amadou Barry[1], Djibrilla Issaka[1], Adame B. Dembele[1], Moussa B. Kanoute[1], Oumar Attaher[1], Issaka Sagara[1], Abdoulaye Djimde[1], Patrick Duffy[3], Michal Fried[3], Steve Taylor[2], Alassane Dicko[1]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Division of Infectious Diseases, Duke University Medical Center, Durham, NC, United States,* [3]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Seasonal malaria chemoprevention (SMC) was recommended as policy for malaria control by the World Health Organization (WHO) in Sahel and sub-Sahel in 2012 along with monitoring of drug resistance. To assess the long term impact of SMC on *Plasmodium falciparum* resistance to Sulfadoxine-Pyrimethamine (SP) and Amodiaquine (AQ), blood smears and blood spots on filter papers were obtained from children aged 0-5 years, randomly selected each year during two cross-sectional surveys before and after SMC rounds in areas of Ouelessebougou where SMC had been implemented for 0, 1, 2 and 3 years. Frequencies of molecular markers of resistance to SP and AQ were assessed by PCR and genotyping. None of the molecular markers of resistance significantly increased in frequency over the period of study (2014-2016). At the beginning of the transmission season, the frequency of the *pfdhfr*59R was 93.9% at baseline and 98.2% after two years of SMC implementation (p = 0.12). The frequencies of *Pfdhps*540E, *Pfmdr1*-86Y and *Pfcrt*K76T mutation were also similar at baseline and after two years of SMC implementation 4.0% vs. 3.5%, p = 0.87; 5.6% vs. 9.8, p = 0.35 and 71.3% vs 80.7%, p = 0.20 respectively. At the end of transmission season after SMC drug administration, the frequencies of *Pfdhfr*59R, *Pfdhps*540E, *Pfmdr1*-86Y and *PfcrtK*76T mutation were also similar in areas where SMC was implemented for one or three years 97.0% vs. 100%, p = 0.21; 0.8% vs. 1.4%, p = 0.64, 10.8 % vs. 18.6%, p = 0.14 and 85.1% vs. 67.4% p = 0.01, respectively. The results of genotyping are consistent with those by PCR for the mutations tested by PCR, but also indicate for the first time the presence of the *dhps*581G mutation at low frequency, varying between 0-6.2% between areas and years, and only associated with the additional *dhps*431V mutation. In conclusion, two and three years of SMC implementation were not associated with a significant increase in the frequencies of the molecular markers of SP and AQ resistance. The detection of the *dhps* haplotype bearing the I431V and A581G mutations for the first time in Mali even at low frequency warrants further long-term surveillance.

274

EVALUATION OF AUTOMATED MALARIA DIAGNOSIS USING HEMATOLOGY ANALYZER WITH FINGER PRICK BLOOD FOR DETECTION OF *PLASMODIUM FALCIPARUM* PARASITEMIA IN RURAL AFRICA

Daisuke Usuda[1], Mamadou Ousmane Ndiath[2], Nuredin Ibrahim Mohammed[2], Haddy Nyang[2], Jane Achan[2], Grant Mackenzie[2], Yasuhiro Kawai[3], Yoshitsugu Iinuma[3], Kento Takeshima[1], Kinya Uchihashi[4], Abdoulie Jammeh[5], Ignatius Baldeh[6], Davis Nwakanma[2], Koya Ariyoshi[7], Umberto D'Alessandro[2]

[1]*Kanazawa Medical University Himi Municipal Hospital, Himi, Japan,* [2]*Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Fajara, Gambia,* [3]*Kanazawa Medical University, Uchinada, Japan,* [4]*Sysmex Corporation, Kobe, Japan,* [5]*Basse*

District Hospital, Basse, Gambia, [6]National Public Health Laboratories, Ministry of Health and Social Welfare, Banjul, Gambia, [7]Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

Light microscopy (LM) remains the gold standard for malaria diagnosis. Sysmex Corporation has recently developed an automated hematology analyzer, XN-30 equipped with a new violet laser that was able to count both malaria-infected red blood cells and white blood cells. The good performance of XN-30 in clinical setting has been demonstrated with venous blood (Pillay et al., Malar J 2019), but it has not been evaluated using finger-prick blood in a rural African setting. The study was done in The Gambia during the 2018 malaria transmission season (August-December). Malaria suspected patients were recruited at Basse district hospital. In addition, a malariometric survey was carried out in 6 villages near Basse, Upper River Region. A finger prick blood sample (250 μL) was collected for XN-30 measurement, high sensitive rapid diagnostic test (RDT; the Alere™ Malaria Ag P.f test), thick blood film, and molecular analysis (dry blood spots on filter paper). One hundred forty two suspected malaria patients and 404 randomly selected healthy individuals were recruited. Malaria prevalence was 31% (44/142) by molecular methods, 15% (22/142) by XN-30, 23% (33/142) by RDT, and 16% (23/142) by LM in suspected malaria patients and 15% (60/404) by molecular methods, 6% (24/403) by XN-30, 17% (67/404) by RDT, 5% (20/404) by LM in 404 randomly selected individuals. A scattergram pattern was judged suboptimal for the XN-30 analysis in 43% (61/142) malaria suspected patients and 73% (293/404) healthy individuals. When those suboptimal results were excluded, the sensitivity and the specificity of XN-30 in the community to compare with molecular methods were 70% and 94% respectively. To compare with LM or RDT, the sensitivity/ the specificity of XN-30 in the hospital were 90.9%/96.6%, 84%/98.2%, respectively. XN-30 has a potential role in malaria elimination program as it demonstrated similar performance to current standard diagnostic methods in rural Africa. However it requires substantial improvement to reduce the proportion of suboptimal results when a finger prick blood sample was applied.

275

EXTERNAL ASSESSMENT OF PROFICIENCY OF MALARIA MICROSCOPISTS AND LABORATORY CAPACITY IN DISTRICTS STRATIFIED FOR MALARIA ELIMINATION IN ETHIOPIA

Desalegn Nega Wada, Abnet Abebe, Adugna Abera, Bekuretsion Gidey, Ababa Gebretsadik, Sindew Mekasha, Eyasu Tigabu, Geremew Tasew, Adugna Woyessa

Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia

Successful Malaria Elimination Relies on Accurate Case Detection and Prompt Treatment by Qualified Professionals and Well Equipped Laboratories. Light Microscopy is an Easily Accessible and Routine Diagnostic Tool for Malaria in Ethiopia. Hence, This Study Assessed the Competency of Malaria Microscopists and Laboratory Capacity in Terms of Reagents and Equipments in the 6 Regional States Targeted for Malaria Elimination in Ethiopia, February to March 2018. A Total of 106 Facilities: 17 District Hospitals, 71 Health Centers (hcs) and 18 Private Clinics (pcs) Were Assessed. Of the Total Facilities, 91.5%(97) Was Using Light Microscopy While the Others Were Using Microscopy and Rapid Diagnostic Tests to Diagnose Malaria. Availability and Appropriate Storage of Giemsa Was Reported by 58.8%(10/17) Hospitals, 81.7%(58/71) hcs and 72.2%(13/18) pcs. In 84.6%(11) Hospitals, 35.7%(25) hcs and 26.7%(4) pcs, Malaria Laboratories Comply with the National Quality Assurance Guidelines. Technical Manuals and Laboratory Bench Aids Were Observed in 56.2%(9) Hospitals, 57.1%(40) hcs and 35.3%(6) pcs. Only 55.6%(10) pcs Had License of Registration. Of the 1896 Malaria Positive and 474 Negative PCR-Corrected Slides Administered to 237 Study Participants, 318 Slides Were Reported Falsely Negative (Rate: 42.7%) and 47 Slides Were Reported Falsely Positive (Rate: 2.9%). The Participants Skill Was Graded Good (Agreement: 84.6%, Kappa: 0.6) on Parasite Detection and Poor (A: 43.8%; K: 0.11) on Species Identification. Agreement Was Even Very Slight for Differentiation of *P. Falciparum* from Other Species (A: 28.41%; Kappa:0.29). Above 95% of Participants Didn't Count Parasitemia or Reported Plus System of Estimation That is Unacceptable Per Who Guideline. Therefore, the Low Competency of Malaria Microscopists Together with Poor Laboratories in the Current Study Implies the Further Need of Comprehensive Laboratory Supervisions and *In-Service* Trainings of Professionals by the Ministry of Health and Relevant Partners, Not to Miss-Diagnose the Low Number but Highly Significant Malaria Cases in Elimination Settings.

276

PREVALENCE OF MALARIA PARASITEMIA AMONG UNDER-FIVE CHILDREN LIVING WITH HIV ATTENDING SELECTED HEALTH FACILITIES IN JOS, NORTH CENTRAL NIGERIA 2017

Kayode Abraham Olawuyi[1], Adebola Olayinka[2], Saad Ahmed[3], Patrick Nguku[2]

[1]*National Veterinary Research Institute, Vom Nigeria/Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria, [2]Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria, [3]Ahmadu Bello University Teaching Hospital, Zaria, Nigeria*

Malaria and HIV contributes to the burden of diseases among children less than five years globally. In Africa an estimated 86% of death due to malaria occurs among children under 5 years of age. The Nigeria National Malaria Elimination Program reported that 30% Under 5 (U5) mortality is due to malaria infection. Children living with HIV are more vulnerable to malaria infection because of their immune status. It is diagnosed by the presence of parasitaemia in the peripheral blood. We conducted this study to determine the prevalence and factors associated with malaria parasitaemia in children 6-59 months living with HIV in Jos, North Central Nigeria. A cross sectional study was conducted among children aged 6-59 months living with HIV. Blood specimen was collected from 264 U5 children and tested for the presence of parasitaemia by microscopy. Malarial parasitaemia was defined as the presence of at least one asexual parasite in the blood film. Haemoglobin estimation was carried out and CD4% determined. Structured, interviewer administered questionnaire was used to obtain demographic information and potential risk factors from caregivers. The mean age in months (± Standard deviation) was 40.6 (± 13.7) among children 6-59 months living with HIV. The Prevalence of parasitaemia was 16%. Mild anaemia was significantly associated with malaria parasitaemia among children living with HIV (aOR, 2; 95% Confidence interval (CI), 1.7-3.3). No association was found between CD4% and presence of malaria parasitaemia among children living with HIV. However, the presence of stagnant water and grasses surrounding the vicinity where these children resides were significant associated with parasitaemia (aOR 3; 95% CI, 1.2-11). Therefore we conclude that the prevalence of parasitaemia was relatively high among children 6-59 months living with HIV. Parents/caregivers of these children were educated on the need to intensify efforts in ensuring clean environment devoid of stagnant water and grasses as this will reduce the breeding site for mosquitoes.

277

ANGIOGENIC AND ANGIOSTATIC FACTORS IN THE SALIVA OF MALARIA PATIENTS

Cecilia Elorm Lekpor[1], Kwadwo Asamoah Kusi[2], Andrew Anthony Adjei[1], Nana Otu Wilson[3], Jonathan Stiles[3]

[1]*University of Ghana/korleBu Teaching Hospital, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]Morehouse School of Medicine, Atlanta, GA, United States*

Malaria death is associated with deregulation of host immune responses to inflammatory factors such as C-X-C motif chemokine 10 (CXCL10) and host angiogenic factors such as angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2). Current diagnosis of malaria relies on microscopic detection of the parasites in the blood film. This approach is invasive, increases accidental infections and uncomfortable for some patients. The aim of this study was to investigate biomarkers -CXCL10, Ang-1 and Ang-2 levels in the saliva of malaria patients and compare with plasma levels with regard

to their potential use as biomarkers in malaria. This may be useful for further development of highly efficient non-invasive malaria detection methods using saliva. This was a case control study involving a total of 213 (119 malaria subjects and 94 non malaria subjects) aged 1 -16 years. Plasma and saliva levels of CXCL10, Angiopoietin (Ang)-1 and Ang-2 were measured among the study participants using Quantikine Elisa kit. The data was analyzed at 450 nm wavelength using a Spectra Max 190 fluorescence micro plate reader. Data was presented as mean ± standard error or median and interquartile range (IQR). Pearson's rank test was used to determine if there was any association between the biomarkers and malaria infection. A p-value < 0.001 was considered statistically significant. There was decreased plasma levels of Ang-1 ($p<0.009$) and increased plasma levels of CXCL10 ($p<0.001$) and Ang-2 ($p<0.001$) in individuals with malaria compared to those without malaria. Similar trends were observed in the saliva samples from study participants. Saliva biomarkers CXCL10, Ang-1 and Ang-2 levels correlated significantly with plasma levels of malaria patients. Finally, Ang-2 was informative when combined with CXCL10 to predict the risk of malaria and could be useful in clinical decision-making. These results provide insight into the use of saliva as a non-invasive diagnostic method and demonstrate that Ang-2 combined with CXCL10 is a promising predictive biomarker in malaria.

278

VALIDATION OF *PFHRP2/3* GENE DELETION ASSAYS USING CULTURED MALARIA PARASITES

Josphat N. Nyataya, George Awinda, John Waitumbi

Kenya Medical Research Institute/Medical Research Unit, Kisumu, Kenya

To encourage rational treatment of malaria based on confirmed diagnosis, WHO has spearheaded the development of antigen-based rapid diagnostic tests (RDT). These efforts could be eroded by the reported increase in *Plasmodium falciparum* that have deleted *pfhrp2/3* genes and therefore do not produce Histidine Rich Protein 2 and 3. To facilitate evaluation of these deletions in complex samples, we first validated the assay performance in *P. falciparum* strains grown in culture. Cultures of 3D7 and Dd2 strains were grown in RPMI 1640 medium at 5% hematocrit of 0+ human red blood cells supplemented with 10% heat inactivated human serum. At 3% parasitemia, the parasites were synchronization using Sorbitol to obtain 95% of rings stage parasites. The parasites were then diluted to a parasitemia of 7,500 parasites/μL allowed to grow for a 48-hr complete cycle to reach a parasitaemia of 15,000 parasites/μL. A 200 μL aliquot was removed and used for DNA extraction and subsequent amplification of exon 2 of *pfhrp2/3* genes and their respective flanking regions. Because detection of the deleted gene is based on absence of amplification, a quality control step included amplification of *msp1* and *msp2* genes. Another 200 μL aliquot of each culture was diluted serially in RPMI and 5 μL of each dilution read on RDTs. *msp1* (K1 in 3D7 and MAD20 in Dd2) and *msp2* (FC27 and IC3D7) genes were amplified in both strains. In 3D7, *pfhrp2* and *pfhrp3* genes and flanking regions were amplifiable, while for Dd2, only the *pfhpr3* gene and its flanking region were amplifiable. In agreements with these results, the limit of detection of HRP2 antigen in 3D7 was 300 parasites/μL and while for Dd2, the limit of detection was 2 log higher (30,000 parasites /μL). Our data validates the use of 3D7 and Dd2 parasites as appropriate quality control specimens for inclusion of *pfhrp2/3 gene* deletions assays. Future studies will need to include HB3 strain of *P. falciparum* that lacks the *pfhrp3* gene and the flanking regions.

279

A NOVEL HIGHLY SENSITIVE FLUORESCENCE BASED CARTRIDGE READER FOR RAPID DETECTION OF MALARIA PARASITES

Allan P. Lemtudo[1], Katie Todorof[2], Mike Lochhead[2], John N. Waitumbi[3]

[1]*United States Army Medical Research Directorate , Kenya, Kisumu, Kenya,* [2]*MBio Diagnostics, Inc., Boulder, CO, United States,* [3]*United states Army Medical Research Directorate , Kenya, Kisumu, Kenya*

The currently approved rapid diagnostic tests for malaria (mRDT) lack sufficient sensitivity, may not differentiate current and historical infections of *Plasmodium falciparum* and have false negatives due to deletions of histidine rich protein (HRP) genes. We describe the performance of a novel fluorescence based cartridge device that uses waveguide planar technology for detection of the pfHRP2 and pan-malaria pLDH. For this study, 326 frozen blood samples that had prior microscopy results were retested on the cartridge reader and a commonly used mRDT. Discordant samples between these two tests were resolved by qPCR. Of the 326 blood samples, 293 (89.6%) were HRP2 positive on the cartridge reader device and 289 (88.6%) on mRDT. The calculated HRP2 agreement was 98.2% (320/326). By qPCR, and in agreement with the cartridge reader, five of the discordant samples were positive by qPCR and one was negative. Significant discrepancy was observed on pLDH. By the cartridge reader, 302/326 (92.6%) were positive and only 220 (67.5%) by mRDT. The calculated pLDH agreement was 74.2% (242/326). For the 84 discordances, qPCR validated the cartridge reader's pLDH score in 83/84 samples. On speciation by qPCR, the 84 discordant samples comprised 35.7% (30/84) monoinfections of Pf with parasitemia of 46-1,376 Pf/μL, 64.3.2% (54/84) mixed infections of Pf/Pm and Pf/Po with parasitemia ranging between 46-9120 parasites/ μL. Taken together, our results indicate that, by HRP2, the performance of the novel cartridge reader is comparable to mRDT. The greatest improvement is in the performance of pLDH. The new pLDH assay will solve common false positives attributable to HRP2 persistence in blood long after parasite clearance and false negative results due to HRP2 gene deletions. Importantly, the test will improve detection of non-Pf infections and thereby allow better management of G6PD deficient patients who may have non-Pf infections.

280

THE ROLE OF MRDTS IN SCREENING OF ASYMPTOMATIC INDIVIDUALS TO BE ENROLLED IN CLINICAL TRIALS

Catherine Sumbi[1], Agneta Ogolo[1], Cornel Arima[1], Cephas Aguko[1], Michael Ayaya[1], Everlyne Omondi[1], Victor Otieno[1], Vincent Akolo[1], Rose Adeny[1], Hosea M. Akala[1], Bernhards Ogutu[2], Jim R. Managbanag[1]

[1]*US Medical Research Directorate-Africa/Kenya, Kisumu, Kenya,* [2]*US Medical Research Directorate-Africa/Kenya, Kenya Medical Research Institute, Kisumu, Kenya*

Malaria is one of the world's most prevalent parasitic diseases with the highest prevalence reported in Sub- Saharan Africa. The parasites that cause malaria are from the plasmodium genus. Clinical trials for effective treatments against malaria are reliant on microscopy and malaria rapid diagnostic kits (mRDTs) for determining treatment response. The antigen specific mRDTs are preferred for their ease of usability where microscopy may not be available. Recent studies showing genotype-based variability in response to mRDT marker proteins warrant assessment of performance of this method in clinical efficacy studies. 1451 participants were enrolled in Blood Collection Protocol 1 , a clinical efficacy study between 2007 and 2011 in Kombewa HDSS .They were tested for malaria using microscopy and Parascreen mRDTs as part of assessing eligibility for enrollment into the study. While mRDT indicated the presence or absence of malaria parasites in whole blood samples, microscopy was done to determine the presence or absence of malaria parasites, species and parasites enumeration. Data generated readouts from microscopy were compared with those from mRDT using descriptive statistics. Of the 1451, 1175

(81%) were positive while 272 (19%) were negative by mRDT. 940 (64%) were positive while 503 (35%) were negative by microscopy. this finding showing higher positive predictive rates for mRDTs than microscopy suggests the mRDTs usability alongside microscopy in efficacy study.

281

ASSESSING THE IMPACT OF MALARIA DIAGNOSTICS CENTRE COMPREHENSIVE MALARIA MICROSCOPY TRAINING COVERAGE IN KENYA AND BEYOND

Rose A. Adeny[1], Agneta Ogolo[1], Cornel Arima[1], Cephas Aguko[1], Catherine Sumbi[1], Michael Ayaya[1], Everlyne Omondi[1], Victor Otieno[1], Vincent Akolo[1], Hosea M. Akala[1], Bernhards Ogutu[2], Jim R. Managbanag[1]

[1]*U.S. Army Medical Research Directorate - Africa/Kenya, Kisumu, Kenya,* [2]*U.S. Army Medical Research Directorate - Africa/Kenya Medical Research Institute, Nairobi, Kenya, Kisumu, Kenya*

A survey of research institutions and hospital facilities globally revealed limited expertise in performance of microscopy for malaria diagnosis. Microscopy as a gold standard for malaria diagnosis is dependent on microscopists's expertise, periodic re-training and certification. The malaria Diagnostics Training Centre (MDC) was established in 2004 to enhance qualitative and quantative malaria diagnostic skills in laboratories globally. The aim of this study was to estimate training impact since its inception. Between 2004 and 2008, MDC implemented a 14 day training syllabus for individuals with no malaria microscopy training and a 5-day refresher for previous trainees. Prior to training commencement, a pre-test was conducted to gauge the knowledge of participants and a post test to gauge the skill sets acquired. Microscopy was alloted 50% of overall training time. Training comprised of theoretical and practical sessions. A total of 1843 laboratory trainees from 28 countries were trained between 2005 and 2018. Countries reached by regions were; East Africa 10, West 8, North 2, South4, Central 2 and Asia 2. The total number of countries trained in Africa 26(93%), outside Africa 2(27%). Number of Microscopy classes held was 126, within Kenya 109(87%), outside Kenya 17(13%). 1843 laboratory trainees comprised those from Kenya 1472(80%), outside Kenya 371(20%). MDC then established sister malaria diagnostic centers in three countries after 10 day mentorship program for facilitators. These results show that East and West Africa had the highest number of trained personnel compared to other regions with Africa at 93% compared to Asia at 7%. This findings show the need for establishment of malaria diagnostic centres across regions. This argues for dedicated funding for this framework as it would warrant an increase in coverage of trained malaria microscopists globally as a prerequisite for adopting pre-elimination phase.

282

MALARIA DIAGNOSTIC PRACTICES AND ACCURACY OF BLOOD SMEAR MICROSCOPY IN PRIVATE HEALTH FACILITIES OF ENTEBBE MUNICIPALITY, UGANDA

Tobius Mutabazi[1], Emmanuel Arinaitwe[2], Alex Ndyabakira[2], Simon P. Katongole[3], Pauline B. Kibwika[1], Moses R. Kamya[1], Joaniter I. Nankabirwa[1]

[1]*Makerere University College of Health Sciences, Kampala, Uganda,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*Faculty of Health Sciences, Uganda Martyrs University, Kampala, Uganda*

Malaria treatment policy in Uganda recommends that all suspected cases have a laboratory diagnosis prior to treatment. Studies have shown that almost half of the patients visiting public facilities are still presumptively treated despite availability of the diagnostics and personnel to do the testing. The practices in private facilities is still unknown despite most patients seeking care at these facilities.We evaluated the malaria diagnostic practices and the accuracy of the malaria blood smear test in private health facilities in Uganda. Between April and May 2018, we enrolled 700 patients suspected to have malaria at 26 private health facilities in Entebbe municipality. Patients records were reviewed following the clinical evaluation and all patients sent to the laboratory for a blood smear had a study smear collected in addition to clinical smear to evaluate for the accuracy of blood smear microscopy. Of the 700 participants enrolled, 695 (99.3%) had a malaria diagnostic test done before treatment. The sensitivity and specificity of the facility microscopy was 95.8% and 90.1 respectively. Factors associated with accurate facility microscopy included having five or more years experience of reading smears (OR=0.134, P<0.001), conducting 5 or more smear readings a day (OR= 0.040, P<0.001) and good laboratory illumination (OR= 5.685, P=0.015). Adherence to the malaria diagnostic guidelines in the private facilities located in this setting is higher than what is observed in the public facilities and accuracy of smear reading is high. The main factor associated with accuracy microscopy is the experience of the laboratory personnel either through conducting many smear readings or duration of reading smears. Private facilities in this setting should act as model facilities to improve the malaria diagnostic practices at public facilities in Uganda.

283

EVALUATING THE PREVALENCE OF *PLASMODIUM* PARASITES AMONG ASYMPTOMATIC INDIVIDUALS USING MICROSCOPY AND MALARIA RAPID DIAGNOSTIC TEST IN KOMBEWA HDSS AREA, WESTERN KENYA

Agneta Ogolo, Cornel Arima, Cephas Aguko, Catherine Sumbi, Michael Ayaya, Everlyne Omondi, Victor Otieno, Vincent Akolo, Rose Adeny, Hoseah M. Akala, Bernhards Ogutu, Jim Ray Managbanag

USAMRD-A Kenya, Kisumu, Kenya

Plasmodium parasite is the main cause of malaria infection in man. In *malaria* endemic areas, *people* tend to develop partial immunity, allowing occurrence of *asymptomatic* infections. The asymptomatic infections are an important reservoir for sustaining vector infections, and anchor the disease in eco-epidemiological settings. Though asymptomatic individuals significantly impacts transmission dynamics, they are often obscure to the health systems since they do not seek treatment. The aim of this study is to determine frequency of plasmodium infections in asymptomatic persons using Microscopy and malaria rapid diagnostic test (mRDT) diagnostic methods. 1451 individuals between 5 months to 65 yrs, from randomly selected households were enrolled between 2007 and 2011 within Kombewa HDSS. 250µl finger prick EDTA blood was collected from each individual. 2 thick and thin smears were made, complete blood count and RDT done using Parascreen®. Giemsa stained slides were read by expert microscopists to determine plasmodium species and parasitemia. Data was analyzed using descriptive statistics for frequency of positive infections per test methods used. Inferential statistics was used to describe variations in between diagnosis methods. Of the 1451, 940 (65%) samples were positive by microscopy and 1175 (81%) samples were positive by RDT. Of the 213 (23%) asymptomatic individuals by microscopy, the distribution of *Plasmodium spp* was; *Plasmodium falciparum* 197 (21%), *Plasmodium falciparum* with *Plasmodium malariae* 7(0.7%), *Plasmodium malariae* 3(0.3%), *Plasmodium ovale* 2(0.2%), *Plasmodium falciparum* with *Plasmodium ovale* 4 (0.4%). This findings shows high burden of asymptomatic infections using the most frequently used microscopy and RDT diagnostic method. Asymptomatic individual presents the risk of transmitting infections since they would not seek treatment. Strategy should be developed for diagnosis and treatment of asymptomatic individuals.

284

EVALUATING MICROSCOPY PERFORMANCE AMONG TRAINEES TRAINED IN MALARIA DIAGNOSTICS CENTER IN KISUMU.

Everlyne Omondi, Rose Adeny, Agneta Ogolo, Cornel Arima, Cephas Aguko, Catherine Sumbi, Michael Ayaya, Victor Otieno, Vincent Akolo, Hoseah M. Akala, Bernhards Ogutu, Jim Ray Managbanag

USAMRD-K/A, Kisumu, Kenya

Lack of metrics to uniformly ascertain effects of intervention such as drugs, vaccines impact and control hinders efforts to transition to pre-elimination phase. Malaria Diagnostics Center (MDC) was established in 2004 by United States Medical Research Directorate - Africa/Kenya, Kenya Medical Research Institute to standardize malaria diagnostics in the region. MDC designed a curriculum which is able to assess the competence of candidates at various proficiency levels. As part of entrenching this habit among malaria diagnosis professionals, it is essential to rate impact of the training on trainees. The aim of this study was to determine the uptake of diagnostic skill sets acquired before and after training among trainees who attended MDC's training in the year 2013. Ten-day malaria microscopy course was conducted for microscopists drawn from both private and public institutions. The course contained theoretical and practical sessions. The impact of training was evaluated by practical and theoretical pre- and post-training assessments on parasite detection (sensitivity and specificity), species identification and parasite quantification. A total of 61 participants completed the training which comprised of Ministry of health personnel at 48(79%) and other research organizations 13(21%). The knowledge of basic malariology (theory) at pre- and post-tests were 64% (95% CI 61-67%) and 93% (95% CI 91-94%), respectively (P<0.001). The mean parasite detection (sensitivity) were 57% (95% CI 50-64%), during the pre-test and 90% (95% CI 87-93%) for the post-test (P<0.001), improvement of 32% (95% CI 26-39%). However for specificity, there was negative reduction from a mean of 57% (95% CI 51-64%) in pre-test to 51% (95% CI 42-60%) post-test (P= 0.254), improvement -6%, (95% CI -12 to -0.7%). Conclusion: Overall improvement was observed in all areas tested except for specificity which had negative (-6) improvement. Findings show improvement in performance, if maintained would improve the quality of microscopic diagnosis of malaria.

285

COMPARING COINFECTION OF *PLASMODIUM OVALE* AND *P. MALARIAE* IN ASYMPTOMATIC INDIVIDUALS IN KOMBEWA HDSS AREA, WESTERN KENYA

Cephas Aguko Oyieke[1], Cornel Arima[1], Agneta Ogolo[1], Catherine Sumbi[1], Michael Ayaya[1], Everlyne Omondi[1], Victor Otieno[1], Vincent Akolo[1], Rose Adeny[1], Hoseah M. Akala[1], Bernhards Ogutu[2], Jim Ray Managbang[1]

[1]*MDC, Kisumu, Kenya,* [2]*KEMRI, Kisumu, Kenya*

The prevalence of malaria infection in Kombewa,Western Kenya still remains high despiteheightened interventions. Three *Plasmodium* species; *P. falciparum* (Pf), *P. ovale* (Po) and P. malariae (Pm), *P. falciparum* plus *P. malariae* (pfm), *P. falciparum, P. malariae* and *P. ovale* (pfmo) and *P. falciparum* and *P. ovale* (pfo) exist sympatrically. Bites from mosquitoes carrying different species of parasites often leads to co-infection in humans. Co-infection withdifferent species has been shown to modulate disease progression to either asymptomatic or symptomatic. As studies continue to highlight mortality among children less than 5 years, it isessential to understand variability in species carriage among the different age groups. The aim ofthis study was to estimate the role of species composition in malaria mortality in Kombewa,Western Kenya. 1451 samples were collected under the Blood Collection protocol between 2007and 2011. These samples from Kombewa HDSS randomly screened for malaria included both asymptomatic and symptomatic individuals. Each blood sample was tested for presence orabsence of *Plasmodium* parasites, species present determined by malaria rapid diagnostic test kit(mRDT) Parasreen® and confirmed by microscopy. Counts of positive Pf, Po, Pm, Pfm, Pfo,Pfmo were obtained and recorded. From asymptomatic individuals screened, a total of 41(3%)had pfm, 18(1%) pfo, 2(0.1%) pfmo, 322(22%) pf and 204(14%) were negative. As for childrenbetween 6-17 years, 37 (3%) had pfm, 5(0.3%) pfo, 1(0.1%) pfmo, 331(23%) pf and 121(8%)were negative. Adults above 18 years had 2(0.1%) pfm, 2(0.1%) pfo, 149(10%) pf 164(11%)were negative, no pfmo species were found in adult samples. The findings above show thatchildren <17 years were more vulnerable to *Plasmodium* infections compared to adults. Mortality could be as a result of poor health seeking behavior especially in the absence of fever and lack ofmechanisms to trace them within house holds

286

IMPLICATIONS OF *PLASMODIUM* PARASITES WITH HRP2 GENE DELETION ON THE PERFORMANCE OF HRP2 BASED RAPID DIAGNOSTIC TESTS IN MIDDLE GHANA OF AFRICA

Dennis Adu-Gyasi[1], Linda E. Amoah[2], Abagna Hamza Bukari[2], Prince Agyapong Darko[1], Felix B. Oppong[1], David Dosoo[1], Oscar Agyei[1], Seeba Amenga-Etego[1], Kwaku Poku Asante[1]

[1]*Kintampo Health Research Centre, Kintampo North, Ghana,* [2]*Noguchi Memorial Institute for Medical Research, Accra, Ghana*

From a previous study that evaluated HRP2 based RDTs to probe other causes aside low parasitaemia that might give false negative results, we sought to explore further using molecular techniques to assess the presence of *Plasmodium* parasites that lack *hrp2 genes* infecting human in the middle-belt of Ghana. The current study pooled together data of two cross sectional studies that evaluated malaria RDTs in the middle-belt of Ghana from 2014 to 2016. Blood samples were collected from participants and used to screen for malaria using microscopy and RDT. With the RDT results, false negative, false positive and samples with parasite densities below detectable limits were selected. DNA was extracted and used for speciation, multiplicity of infection identifying the presence of MSP2. Samples that were positive for MSP2 were further screened for HRP2 and HRP3 deletants. Performance of the malaria RDT was evaluated using the ROC and sensitivity with the specificity. The studies were approved by the Kintampo Health Research Centre Institutional Ethics Committee (FWA_ No.:00011103). The prevalence of *Plasmodium falciparum* infection based on microscopy in the studies pooled were 39.1% (281/754) and 28.1% (441/1569) respectively. The performance of the RDTs in the two studies had sensitivity (SE), specificity (SP) and ROC of 98.2% (95.9%, 99.4%), 66.3% (58.7%, 73.3%) and 0.82 (0.79, 0.86), and SE, SP and ROC of 92.1% (88%, 95.2%), 81.6% (79.2%, 83.8%) and 0.87 (0.85, 0.89) respectively. Of the selected samples screened with PCR, 53.2%(41/77) were speciation positive. Of these, 31.7% (13/41) were MSP2 positive. Screening among these 13 samples for HRP2 and HRP3 gene deletions, 30.8% (4/13) had pfhrp2 gene deletion in exon 1-2 segment. About 50.0% (2/4) of the pfhrp2 deleted parasites had pfhrp3 which did not compensate for pfhrp2 deletions. This study describes the prevalence of *pfhrp2/3 gene* deletions in the middle-belt of Ghana based on genotyping of *Plasmodium falciparum* positive samples from health facility and community levels, with 95% CIs for all point estimates. It is necessary to use standard guided protocol to assess the spread of HRP2 gene deletions.

287

MALARIA RAPID DIAGNOSTIC TESTING IS COMPROMISED BY *PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2* AND *3* DELETIONS IN ETHIOPIA: A MULTI-SITE, CROSS-SECTIONAL SURVEY

SindewMekasha Feleke[1], Ozkan Aydemir[2], Hussein Mohammed[1], BokretsionGidey Brhane[1], Hassen Mamo[3], Beyene Petros[3], Madeline Denton[4], Steven R. Meshnick[4], Jonathan J. Juliano[4], Jeffrey Bailey[5], Jane Cunningham[6], Jonathan B. Parr[7]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*Brown University, Providince, RI, United States,* [3]*Addis Ababa University, Addis*

Ababa, Ethiopia, [4]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [5]Brown University, Providence, RI, United States, [6]World Health Organization, Geneva, Switzerland, [7]University of North Carolina, Chapel Hill, NC, United States

Plasmodium falciparum parasites with deletions of the *histidine-rich protein* 2 and 3 (*pfhrp2/3)* genes are not detected by commonly deployed malaria rapid diagnostic tests (RDTs) in Africa. Because RDTs account for the majority of diagnostic testing for suspected malaria in Ethiopia, increasing reports of these parasites has raised concerns that existing diagnostic strategies are threatened. High prevalence of *pfhrp2/3* deletions in neighboring Eritrea recently forced a shift away from PfHRP2-based RDTs to less sensitive, less heat stable alternatives. To determine the impact of these parasites in Ethiopia, we conducted the first study using the World Health Organization's (WHO's) protocol for *pfhrp2/3*-deleted parasite surveillance. 3,095 subjects with suspected malaria who presented to government health facilities in the West Armachiho district of the Amhara Region and the K/Humera and Atsede Tsimbila districts of the Tigray Region were enrolled and underwent testing with two distinct, WHO pre-qualified RDTs. Molecular and serological testing for parasites with *pfhrp2* and/or *pfhrp3* gene deletions is underway. Pilot testing of samples collected from 61 subjects with PfHRP2-negative but PfLDH-positive RDT results identified *pfhrp2/3*-deleted *P. falciparum* parasites in all three districts. Initial Luminex antigen testing confirmed the absence of PfHRP2 in 90% (55/61) of *P. falciparum* PCR-positive samples tested. Nested PCR with prolonged cycling failed to amplify *pfhrp2* in 34% (21/61) and *pfhrp3* in 74% (45/61). Deep sequencing of the *pfhrp2/3* genes and 29 flanking *loci* provided high resolution mapping of deletion regions. This work is being complemented by additional antigen profiling, PCR assays, and whole-genome sequencing. In summary, largescale surveillance near Ethiopia's northern border with Eritrea identified *pfhrp2/3*-deleted parasites in all surveyed districts. We are employing a multi-omics approach to characterize regionally specific deletion breakpoints, identify mutations associated with impaired PfHRP2 expression, and explore the evolutionary history of these parasites.

288

MALARIA IN SEMI-ISOLATED AMAZONIAN INDIGENOUS COMMUNITY: HETEROGENEITY OF TRANSMISSION AND PREDOMINANCE OF SUBMICROSCOPIC INFECTION

Daniela Rocha Robortella

Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

In Latin America, inequitable access to healthcare has a profound impact on the indigenous populations of the Amazon rain forest that typically suffer from an excess burden of infectious disease. Malaria official data are underestimated due to the difficulty of access to ethnic groups, such as the Yanomami, a traditionally semi-nomadic group living in widely dispersed communities in the Amazon border of Brazil and Venezuela. Here, we investigate malaria infection among 981 individuals (median age 14 years) from four Ianomâmis villages from Marari indigenous community (North of the Amazonas State). The methodological approach involved cross sectional surveys carried out during dry season (September/ November, 2014) and at the beginning of the rainy season (January, 2015). Different PCR protocols (ribosomal and non-ribossomal targets) were used to increase sensitivity in detection of submicroscopic malarial infection. Taken together, the results demonstrated that: (i) at the enrollment, malaria prevalence was 1.6% by conventional microscopy and 6% for PCR-based protocols, confirming the high frequency of submicroscopic malaria infections. Although the number of cases decreased during the second cross sectional, the submicroscopic infections remained relatively high (4% *vs.* 0.7%); (ii) *Plasmodium vivax* and *P. malariae* were responsible for the majority of malaria infections (35% and 32%, respectively); (iii) in the Yanomani area, malaria transmission was not homogeneous transmitted, with prevalence ranged from 1.5% to 12% between villages; (iv) while the frequency of malaria infection was similar between genders, there was a clear tendency in decreased malaria-positivity according to the increase in age, suggesting acquired immunity against malaria.

289

RETINOPATHY IN CEREBRAL MALARIA IN CHILDREN IN KINSHASA

Mireille Ngale Amba, Joseph M. Bodi, Joseph T. Kelekele, Dieudonné N. Mumba, Nsengi Ntamabyaliro, Celestin N. Nsibu

University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Democratic Republic of the Congo (DRC) is the second most affected country by malaria worldwide, and cerebral malaria is the deadliest complication. However, its diagnosis difficult by the non-specificity of its signs and the lack of appropriate equipment in the context of DRC. Fundus is an accurate and early non-invasive means of observing pathognomonic ocular abnormalities and is very important for understanding pathophysiology, diagnosis and prognosis of cerebral malaria. This is an observational study conducted in the 4 districts of Kinshasa. One Health Facility was randomly selected in each of the districts, and all children admitted for cerebral malaria from 2012 to 2014 were included in the study after informed consent by parent or legal guardian. A fundus was performed with direct ophthalmoscopy within 24 hours of admission. Analyzes were performed on SPSS 20.0 and EXCELL. Pearsonian Chi- squared test was used to compare averages. Seventy-nine patients were included in our study. Median age of 5.8 ± 2.6 years. Children aged 3 to 5year accounted for 60.8%. More than three quarters of patients had retinal changes (77.2%). These changes consisted of hemorrhages (100%), bleaching (34.2 %), vascular discoloration (3.8 %) and papilledema (11.4 %). Patients also had seizures (60.7%), anemia (78.7%), thrombocytopenia (45.9%), acidosis (54.1%) and hypoglycemia (10%). A total of 22.8% did not have retinal changes. This may be due to genetic factors leading to cerebral malaria without retinal expression or incidental parasitemia in patient with other cause of coma. Fundus should be taken in account for rapid diagnosis and prompt treatment of severe malaria in Kinshasa. A training on the use of the ophthalmoscope to emergency department practitioners would be needed.

290

THE INFLUENCE OF TRAINING OUTCOME AND COMPETENCY ON EFFECTIVE UTILIZATION OF MALARIA MICROSCOPY RESULT BY HEALTH PROFESSIONALS IN SOUTHEASTERN NIGERIA

Chinyere Ihuarulam Okoro[1], Jide Bamiro[2], Kingsley E. Dunga[3], Oluchi I. Okoro[4], Francis Ihenetu[5], Ikechukwu V. Ejiogu[6]

[1]*Federal Medical Center Owerri, Owerri, Nigeria,* [2]*College of Medicine, University of Lagos, Lagos, Nigeria,* [3]*Department of Medical Laboratory Science, Madonna University Elele, Owerri, Nigeria,* [4]*Beulah Medical Diagnostic Laboratory and Research, Owerri, Nigeria,* [5]*Department of Microbiology, Federal University of Technology, Owerri, Nigeria,* [6]*Haematology Department, Federal Medical Center, Owerri, Nigeria*

Malaria diagnosis in Nigeria was largely done based on clinical presentations until recently when the policy on parasitological confirmation of all suspected malaria cases before treatment was released by the government in 2011. One of the possible causes of over-diagnosis and over treatment of malaria in Nigeria is poor utilization of Malaria test results in health care delivery. This study demonstrates the influence of in-service training on malaria microscopy amongst medical laboratory scientists on the utilization of malaria microscopy results in a selected Government Health Facility in Nigeria. The base line study was conducted in 2014 while a follow up study where pre tested questionnaire on perception of Health care providers on malaria diagnostic results were administered to end users of laboratory results in selected secondary health facilities, Basic malaria microscopy training conducted for medical laboratory scientists working in these selected facilities in accordance with the World Health Organization (WHO) basic microscopy training manual. Level of improvement and confidence in malaria result utilization by health workers was assessed. To assess the performance after the training intervention, paired-test was used to determine if there was any significant difference between the performance scores before and after

the training and between the basic and refresher training. The study demonstrates a significant improvement in the mean written pre-and post-tests scores from 28.4% (95% CI 30.4-30.4%) to 75.2% (95% CI 73.6-78.2%) ($P < 0.001$). . Comparing the baseline and follow up study, there was significant difference when comparing the rate of utilization of malaria result from the Trained Med Lab Scientists. ($p < 0.001$)The increase in utilization of malaria microscopy result for effective case management of malaria in the study area was influenced by training outcome and competency of medical laboratory scientists.

291

THE LACK OF A STANDARD DEFINITION FOR SUSPECTED MALARIA CASE—HOW CAN WE DIAGNOSE WHAT WE DON'T FIRST SUSPECT? DIAGNOSE WHAT WE DON'T FIRST SUSPECT?

Julie I. Thwing[1], Jordan Burns[2], Erin Eckert[1], Erin Eckert[3], Kevin Griffith[2], Mateusz Plucinski[1], Peter Thomas[1], Eric Halsey[1], Meera Venkatesan[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*United States Agency for International Development, Washington, DC, United States,* [3]*United States Agency for International Development, Atlanta, GA, United States*

Since 2010, the World Health Organization has recommended parasitological confirmation by microscopy or rapid diagnostic test (RDT) for all patients with suspected malaria. Most sub-Saharan African countries have now widely scaled up RDTs for diagnosis; however, less than half of children seeking care for fever receive a diagnostic test. While there are multiple reasons for this poor coverage, malaria case management guidelines developed by national malaria control programs set the standard for provider behavior and are used to guide training and supervision of malaria case management. We reviewed 25 case management guidelines from malaria-endemic sub-Saharan African countries and assessed whether a suspected malaria case was defined, or if not defined, what guidance was given in regard to malaria diagnosis. Of the guidelines reviewed, 14 gave a definition of suspected malaria or guidance as to the population to test. Of these 14, all included fever and 12 included history of fever, though temperature cut-offs for fever and duration for history of fever varied. However, four of the 14 did not recommend testing if there were signs of another fever source, and two required at least one additional symptom of malaria. Of the remaining 11 guidelines, seven defined malaria as a febrile syndrome plus parasitologic confirmation without giving guidance as to whom to test and four gave only a general discussion of malaria symptoms. While all 25 guidelines discussed fever, seven did not include history of fever. If providers are not clearly directed to test all cases of febrile illness for malaria, they must use clinical judgment, which is notoriously inaccurate for malaria diagnosis, to decide if a febrile illness is suspected malaria and requires testing. Patients with malaria infection seeking care for fever who are not tested and therefore left untreated are missed opportunities to reduce morbidity and mortality as well as transmission in the community. Malaria case management guidelines for endemic zones should clearly define a suspected case of malaria as anyone with a fever or history of fever and require testing of all suspected malaria cases.

292

SENSITIVITY AND SPECIFICITY OF A NOVEL HIGHLY SENSITIVE RAPID DIAGNOSTIC TEST FOR DETECTING LOW DENSITY *PLASMODIUM FALCIPARUM* INFECTION DURING A CONTROLLED HUMAN MALARIA INFECTION STUDY IN EQUATORIAL GUINEA

Maxmillian G. Mpina[1], Jose Raso[2], Anna Deal[1], Ludmila A. Pupu[2], Elizabeth L. Nyakarungu[3], Maria del Carmen Ovono Davis[2], Tobias Schindler[1], Vicente Urbano[2], Ali Mtoro[3], Ally Hamad[3], Maria Silvia A. Lopez[2], Beltran Ntutumu Pasialo[2], Marta Alene Eyang[2], Matilde Riloha Rivas[4], Carlos Cortes Falla[2], Guillermo Garcia[5], Juan Carlos Momo[2], Raul Chuquiyaur[6], Elizabeth Saverino[6], Peter F. Billingsley[6], Preston Church[6], B. Kim Lee Sim[6], Thomas Richie[6], Bonifacio Manguire[7], Marcel Tanner[1], Salim Abdulla[3], Carl Maas[7], Stephen L. Hoffman[6], Said Abdallah Jongo[8], Claudia A. Daubenberger[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Medical Care Development International, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [4]*Ministry of Health and Social Welfare of Equatorial Guinea, Malabo, Equatorial Guinea,* [5]*Medical Care Development International, Silver Spring, MD, United States,* [6]*Sanaria Inc., Rockville, MD, United States,* [7]*Marathon Oil Corporation, Malabo, Equatorial Guinea,* [8]*Ifakara Health Institute, Basel, Switzerland*

Plasmodium falciparum (*Pf*) malaria continues to pose a threat despite the range of extensive interventions currently implemented. The inability of conventional malaria rapid diagnostic tests (cRDTs) to routinely detect low-densityparasitemia that contributes to ongoing transmission is perhaps one reason why the 2018 World Malaria Report failed to show continued declines in the global burden of malaria. The relatively high limit of detection (>50 pf/μl) makes cRDTs insufficient to support malaria elimination efforts. The recently developed highly sensitive RDTs (hsRDTs) present an attractive improvement to cRDTs. However, information regarding which proportion of malaria infections are missed by hsRDTs during asymptomatic subpatent infections in the field are currently scarce. Additionally, data surrounding the point at which hsRDTs can be expected to give a positive result during an individual malaria infection are limited. Controlled human malaria infections (CHMI) provide a valuable platform to evaluate novel diagnostic tools and hence could be used to answer such questions. During our EGSPZV3 study (ClinicalTrials.gov ID: NCT03590340) a total of 98 healthy Equatoguinean adults, aged 18-35 years, underwent CHMI with non-attenuated 3.2 $x10^3$ live, cryopreserved sporozoites of *Pf* NF54 given intravenously (Sanaria® PfSPZ Challenge). Pf parasitemia was monitored by qPCR and thick blood smear (TBS) starting from day 8 post-CHMI until onset of TBS positivity which resulted in treatment of volunteers. Whole blood samples collected from all subjects during and after CHMI will be analysed in parallel by cRDT (Carestart Combo, Access Bio Inc) and hsRDT (Alere Malaria Ag P.f, Standard Diagnostic Inc). We will present data comparing results from cRDT, hsRDT, TBS and qPCR from identical samples collected during a CHMI study performed in malaria-pre-exposed population. This information will provide insight on which proportion of parasite infections may be missed by hsRDTs during asymptomatic, sub-patent *Pf* infections and help to establish when hsRDT start detecting parasites after infection and until when after malaria treatment.

293

SOLUBILITY OPTIMIZATION AND DOCKING STUDIES OF ANTIMALARIAL AMINOPYRAZINES

Peter Mubanga Cheuka[1], Kelly Chibale[2]

[1]*The University of Zambia, Lusaka, Zambia,* [2]*The University of Cape Town, Cape Town, South Africa*

Malaria was responsible for 219 million cases and 435,000 deaths in 2017. Once effective drugs like chloroquine have been rendered ineffective by wide-spread resistance. Eradication efforts are further jeopardized by rising resistance to current first line treatment options, the artemisinin-

combination therapies (ACTs). Thus, new antimalarial drugs are urgently needed. Recently, aminopyrazines, as a novel antimalarial chemotype, have been shown to demonstrate impressive *in vitro* activity and *in vivo* efficacy in animal models of malaria infection. Amongst these, one analogue has since progressed to an optimized late lead stage. Unfortunately, this compound has been beset by poor solubility, which can present challenges in clinical development if the compound were to progress to that stage. Thus, it became important to address such developability issues by improving solubility in follow-on compounds. In this study, we sought to generate other second-generation candidates with improved solubility by replacing the aryl rings in the parent molecules with saturated motifs. We hypothesized that such chemical modifications would potentially improve solubility by disrupting solubility-suppressing ϖ-ϖ stacking. The new analogues were tested for *in vitro* antiplasmodium activity and aqueous solubility. In order to help rationalize the *in vitro* antiplasmodium data, we retrospectively studied the binding of the new analogues in a homology model of *Pf*PI4K (*Plasmodium falciparum* phosphatidylinositol 4kinase), a known molecular target of this chemotype. Although the new analogues showed compromised antiplasmodium potency, with only one retaining sub-micromolar activity (IC_{50} = 0.51 μM), aqueous solubility was improved by 4 - > 20-fold compared to the initial lead. Our docking studies showed that the introduced molecular features resulted in the disruption of key binding interactions to the ATP binding pocket which corroborated the parasite-based SAR (structure-activity relationship).

294

DISCOVERY OF A NOVEL, FAST-ACTING ANTIMALARIAL COMPOUND THAT RETAINS POTENCY AGAINST A PANEL OF RESISTANT PARASITES

Stacie Canan

Celgene Global Health, San Diego, CA, United States

With examples of alternative data visualizations and decisions based on sufficient (minimum) data, this presentation will highlight how biopharma medicinal chemistry research efforts can contribute to our goal of discovering new, safe treatments for malaria. In collaboration with global partners, Celgene Global Health (CGH) has created a novel class of anti-malarial compounds. CGH identified this proprietary series from a phenotypic screen against *Plasmodium falciparum*, and simultaneously optimized potency, properties, and selectivity of the series. Lead compounds successfully achieved proof-of-concept *in vivo* efficacy and retained potency against resistant lab and field strains of *P. falciparum*, an early indication of a possible novel mechanism of action. Additional non-clinical studies toward progressing the late lead compound will be discussed.

295

EXPLOITING *IN VITRO* SYNERGISTIC INTERACTIONS BETWEEN THE CONSTITUENT PLANTS OF SOME ANTIMALARIAL POLYHERBALS TO IMPROVE THERAPEUTIC SELECTIVITY

Protus Arrey Tarkang[1], Regina Appiah-Opong[2], Michael F. Ofori[2], Lawrence S. Ayong[3], Alexander K. Nyarko[4]

[1]*Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaounde, Cameroon,* [2]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [3]*Centre Pasteur du Cameroun, Yaounde, Cameroon,* [4]*School of Pharmacy, University of Ghana, Accra, Ghana*

The low structural diversity of currently available anti-malarial drugs and the increasing ability of malaria parasites to quickly develop resistance to them underscores the need to explore new therapeutic strategies. Combination therapy improves efficacy by synergistic effects and slows down parasite resistance, as reported previously. Hence, the potential of combining plant extracts is a tool to be systematically explored. This study was ubdertaken to exploit *in vitro* synergistic interactions between the constituent plant extracts of selected polyherbals, to explore strategies of improving their therapeutic selectivity. A plant extract library was generated by accelerated solvent extraction (ASE). *In vitro* antiplasmodial activities of polyherbal constituents were previously evaluated on multidrug resistant *Plasmodium falciparum* strain, followed by cytotoxicity screening. Extract interactions were analyzed using an equipotency ratio drug combination approach. The 50% fractional inhibitory concentration (FIC_{50}) and combination indices (CI) were calculated from determined EC_{50} values. ASE yields: non-polar (1-5%); polar solvents (20-25%). 18/96 extracts exhibited good antiplasmodial activities (SI > 250). Exhibited fold increases in activity of polyherbal extracts (aqueous; ethanol): *Nefang* (5; 3), *PFC* (8; 0.8), *PFH* (6; 39), *PFA* (4; 0.3), *PFT* (1.3; 12.6), *PFS* (5.7; 0.2), indicating improved therapeutic selectivity, potential efficacy and safety for fold increases ≥ 4. Out of 120 paired extracts, 21 aqueous and 16 ethanol exhibited synergism (CI < 0.8). Observed synergism informed conditions for improving therapeutic selectivity. The outcomes are likely to substantially advance malaria phytotherapy.

296

METHYLENE BLUE ACTIVITY AGAINST HEPATIC STAGES OF *PLASMODIUM*

Henriette Bosson Vanga[1], Jean François Franeitich[2], Valérie Soulard[2], Steffen Borrmann[3], Olaf Müller[4], Olivier Sylvie[2], Dominique Mazier[2]

[1]*Félix Houphouët Boigny, Abidjan, Côte D'Ivoire,* [2]*Sorbonne Universités, UPMC Univ Paris* [06]*, INSERM, CNRS, Centre d'Immunologie et des Maladies Infectieuses, U*[1135]*, ERL*[8255]*, Paris, France,* [3]*German Center for Infection Research (DZIF), Tübingen, Germany,* [4]*Institute of Public Health, Medical School, Ruprecht-Karls-University, Heidelberg, Germany*

In the context of malaria elimination/eradication, drugs effective against the different developmental stages of the parasite are highly desirable. The oldest synthetic antimalarial drug, the thiazine dye Methylene blue (MB), is known for its activity against *Plasmodium* blood stages, including gametocytes. The aim of the present study was to investigate a possible effect of MB against malaria parasite liver stages. MB activity was investigated using both *in vitro* and *in vivo* models. *In vitro* assays consisted of testing MB activity on *P. falciparum*, *P. cynomolgi* and *P. yoelii* parasites in human, simian or murine primary hepatocytes, respectively. MB *in vivo* activity was evaluated using intravital imaging in BALB/c mice infected with a transgenic bioluminescent *P. yoelii* parasite line. MB shows no activity on *Plasmodium* liver stages, including hypnozoites, *in vitro* in primary hepatocytes. In BALB/c mice, MB has moderate effect on *P. yoelii* hepatic development but is highly effective against blood stage growth. In conclusion, while confirming activity of MB against both sexual and asexual blood stages, our results indicate that MB has only little activity on the development of the hepatic stages of malaria parasites.

297

AN EXTENSIVE COMPUTATIONAL APPROACH TO INHIBIT MEROZOITE SURFACE PROTEIN-1 OF *PLASMODIUM VIVAX* ELUCIDATES FURTHER HORIZON IN THE ESTABLISHMENT NEXT GENERATION THERAPEUTICS AGAINST MALARIA

Parag Palit[1], Md. Ohedul Islam[1], Jakaria Shawon[1], Md. Kamrul Hasan[2], Mustafa Mahfuz[1], Tahmeed Ahmed[1], Dinesh Mondal[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh*

Malaria represents a life-threatening disease caused by the obligate intra-erythrocytic protozoa of the *Plasmodium* genus. It exerts a sinister global health burden and accounting for approximately 660,000 deaths annually. Additionally, 219 million new cases are reported each year, most of which result from the growing issue of artemisinin resistance shown by the *Plasmodium* parasite. Much of the research done for the purpose of development of therapeutics against malaria has traditionally been focused on *Plasmodium falciparum*, which is responsible for majority of the cases of mortality due to malaria. However, *Plasmodium vivax* is also known to contribute greatly towards the malaria relate morbidities

particularly in vivax endemic areas. In this study, we have used two different computational approaches aimed at establishing newer concepts towards the development of advanced therapeutics against vivax malaria by targeting the surface antigen, merozoite surface protein-1 (MSP-1). In-silico approach involving computational siRNA designing against MSP-1 resulted in a total of four candidate siRNAs being rationally validated following corroboration with a plethora of algorithms. Additionally, molecular docking analysis unraveled a total of three anti-parasitic peptides. These peptides namely: AP02283, AP02285 and AP00101 were found to exhibit considerable binding affinity with MSP-1 of *P. vivax,* thus providing an apparent indication of their anti-malarial property and affirming their potency to be used as novel molecules for development of next generation anti-malarials. However, irrespective of the prospective magnitude of these in-silico findings, the results require extensive validation by further rigorous laboratory experiments involving both in-vitro and in-vivo approaches.

298

INVESTIGATION OF *PLASMODIUM FALCIPARUM* CYTOADHERENCE AND EXPORTED PROTEINS INTERACTIONS WITH SULFATED POLYSACCHARIDES CONTAINING ANTI-MALARIAL PROPERTIES

Jennifer Mumbe Mutisya[1], Victor Mobegi[2], Johnson Kinyua[2], Hoseah Akala[1], Ben Andagalu[1], Dennis Juma[1], Marcel Nyabute[1], Martha Kivecu[1], Edwin Mwakio[1], Raphael Okoth[1], Benjamin Opot[1], Charles Okello[1], Brenda Makena[1], Gladys Chemwor[1], Redemptah Yeda[1], Agnes Cheruiyot[1], Jim Managbanag[1]

[1]*KEMRI-USAMRDIA, Kisumu, Kenya,* [2]*Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Drugs are key interventions for malaria control. There are many diverse molecules in anti-malarial drugs and the parasite has developed resistance to most of the known available molecules. Diversity in merozoite proteins mediating pathogenesis is a challenge in development of drugs targeting intracellular parasites. Merozoite surface protein 2 (MSP2) with Duffy Binding-Like Domain (DBLMSP2) is a cytoadherence protein found in complex with MSP1 which mediates initial attachment of merozoite to erythrocytes. Poly-Helical Interspersed Subtelomeric domain b with RESA (Ring-infected Erythrocyte Antigen) Like Protein (PHISTb/RLP1) is an exported protein across the parasite parasitophorous vacuole to the erythrocytes and mediates remodelling and cytoadherence to microvascular of infected red blood cells. We explored inhibitors of these proteins in the sulfated polysaccharides compounds which contain anti-malarial properties and have pre-determined efficacy. *Plasmodium falciparum* 3D7 strain protein sequences were obtained from PlasmoDB and their structures predicted using I-TASSER. The chemical compounds were screened from PubChem. Interaction prediction was achieved through docking using autodock vina. Ten sulfated polysaccharides whose chemical properties fulfilled rules of a drug compound were obtained. The autodock vina results were visualized in pymol. The ligands bound closely to *Pf*DBLMSP2 binding sites inferring protein-ligand interaction. None of the ligands interacted with *Pf*PHISTb/RLP1. Further analysis on genetic variations within these proteins and how they affect interaction with the drug compounds is underway. These results support use of sulfated polysaccharides as lead compounds in development of antimalarials targeting merozoite-egress. In *Plasmodium falciparum*, the compounds block sialic independent pathway, which is used by the parasite to invade erythrocytes. Research on chemical inhibitors of exported proteins in *Plasmodium* parasites is further recommended given the sulfated polysaccharides failed to interact with PHISTb/RLP1 protein.

299

ANTIMALARIAL ACTIVITY OF TRADITIONAL MEDICINE COPTIS RHIZOME AND ITS MAJOR ACTIVE COMPOUNDS

Awet A. Teklemichael

Nagasaki University Institute of Tropical Medicine, Nagasaki City, Japan

Malaria has gained significant interest with various cases reporting high mortality and morbidity due to the emergence of resistance to first-line artemisinin-based combination therapy (ACT). To circumvent the threat posed by these, a new drug is needed. Kampo is originated from traditional Chinese medicine, mainly obtained from herbal plants which have been used for centuries to treat various aliment. Therefore, here we design a comprehensive screening to identify the antimalarial activity of Kampo extracts & their main active components using *in vitro* & *in vivo* assay. We designed a comprehensive screening to identify novel antimalarial drugs from a library of Kampo crude drug extracts (n = 120). The antimalarial activity was initially evaluated *in vitro* using chloroquine/mefloquine-sensitive (3D7) & -resistant (Dd2) strains of *Plasmodium falciparum*. The cytotoxicity was also evaluated using primary Adult Mouse Brain cells. Subsequently, major active components of Kampo crude drug extract showing high antimalarial activities and low cytotoxicity was further evaluated. Finally, the *in vivo* antimalarial activities of promising Kampo crude drug extract was investigated using a *P. yoelii* infected mouse model. Out of 120 extracts, Coptis Rhizome showed the highest antimalarial activity (IC_{50} 1.9 μg/mL of 3D7 & 4.85 μg/mL of Dd2) with a high selectivity index (SI) > 263 (3D7) & > 103 (Dd2). Three major components in Coptis Rhizome also showed antimalarial activities with IC_{50} ranging from 1.1 to 6.0 μM (against 3D7) & from 3.1 to 11.8 μM (against Dd2). Among them, coptisine chloride exhibited the highest antimalarial activity (IC_{50} 1.1 μM against 3D7 & 3.1 μM against Dd2) with SI of 37.8 & 13.2, respectively. Furthermore, Coptis Rhizome exhibited significant antimalarial activity in mice infected with *P. yoelii* 17X strain with respect to its activity on parasite suppression consistently throughout the entire test period ($P < 0.05$). In conclusion, Coptis Rhizome is a potential natural resource for antimalarials & it›s component coptisine chloride is a promising antimalarial lead compound.

300

A SINGLE ORAL DOSE OF DECOQUINATE FOR PREVENTING MALARIA INFECTION AND RELAPSING MALARIA

Hongxing Wang[1], Shuanghong Liang[1], Yinzhou Fan[1], Yucheng Liu[2], Xiaohui Ning[1], Sumei Zeng[1], Siting Zhao[3], Li Qin[2], Xiaoping Chen[3]

[1]*Bluelight Pharmatech. Co. LTD, Guangzhou, China,* [2]*CAS Lamvac Biotech Co., Guangzhou, China,* [3]*Guangzhou GIBH CAS, Guangzhou, China*

A liver stage antimalarial in man with no known drug resistance is in need of development for widespread use in resource-poor, disease-endemic areas. Lately, we have successfully created decoquinate (DQ) solid solutions (HME DSS) as an oral dosage form by using hot melt extrusion (HME) technology. In the optimized HME formulations after standing for 24 hours without mixing or vortex before taking samples for analysis, the amount of DQ in the aqueous phase determined by HPLC analysis is greater than 98%. No precipitation, no floating and no agglomeration of the drug particles appear. The consistency from batch to batch preparations was demonstrated by dissolution rate experiments. HME DSS suspended in aqueous solution (saline) had no change in both mean particle size (207 nm) and bioactivity for >12 months. The formation of the true solid solution of DQ was also evidenced by the analyses of thermogravimetry, differential scanning calorimetry and x-ray diffraction, indicating that DQ is thermodynamically stable during HME process and completely miscible with excipients over the whole composition range after HME. In vitro assays show that HME DSS has excellent antimalarial activity against both the liver stage (*Plasmodium berghei* in Hep G2 cells) compared to atovaquone and primaquine and the blood stage infection (*Plasmodium falciparum 3D7, Dd2* and *803* in human RBC) compared to chloroquine and artemisinin. In the efficacy study of causal prophylaxis

in mice, 1 mg/kg of DQ in HME DSS (a single dose of 3 consecutive days given by intragastric method) provided complete prophylaxis for most animals in the group whereas 3 mg/kg was totally effective in preventing *Plasmodium* infection (*P.b* 50,000 sporozoites (SP), IV) for all animals in the group. Intragastric dose of up to 2000 mg/kg of DQ of HME DSS to mice did not result in any signs of toxicity. The blood DQ concentration in Sprague Dawley rats was greater than 1000 ng/ml after intragastric dose of HME DSS (DQ 20mg/kg). We will further test in monkey model using inoculation of *P. cynomolgus* SP the effectiveness of our innovative HME DSS on preventing new malaria infection and malaria relapse.

301

INCORPORATION OF AN INTRAMOLECULAR HYDROGEN BONDING MOTIF IN THE SIDE CHAIN OF ANTIMALARIAL BENZIMIDAZOLES

Henrietta Dede Attram[1], Sergio Wittlin[2], Kelly Chibale[3]

[1]*University of Cape Town, Cape Town, South Africa,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*South African Medical Research Council Drug Discovery and Development Research Unit, Cape Town, South Africa*

Malaria continues to cause significant morbidity and mortality globally, especially in sub-Saharan Africa where the disease is endemic. Recent reports of emerging resistance to Artemisinin-based combination therapies (ACTs), the current first-line antimalarial drugs, present an even grimmer picture regarding future control and eradication of malaria. Moreover, antimalarial medications in current clinical use are fraught with challenges of high cost, low availability and undesirable adverse effects associated with their use. These factors call for accelerated research efforts to identify novel, safe and efficacious agents for treatment of malaria. Benzimidazole is a heterocyclic aromatic organic compound. The benzimidazole motif is a recognized privileged scaffold in medicinal chemistry due to its capacity to interact with numerous biological systems, leading to a wide variety of biological activities, including antimalarial activity. Likewise, the incorporation of an intramolecular hydrogen bond (IMHB) into a molecule is gaining a great deal of interest in drug design due to its ability to significantly alter molecular properties because of the formation of various conformers that in turn influence solubility, permeability, pharmacokinetic and pharmacodynamic processes, as well as protein binding affinity. As part of the malaria eradication campaign, analogues of a novel class of benzimidazoles with an intramolecular hydrogen bonding motif were synthesized and evaluated *in vitro* for their antiplasmodium activity against chloroquine-sensitive (NF54) and multi-drug resistant (K1) strains of the human malaria parasite *Plasmodium falciparum*. Compounds were also screened for their cytotoxicity towards a mammalian Chinese Hamster Ovarian (CHO) cell line. Most of the compounds exhibited good antiplasmodium activity (*Pf*NF54 IC_{50} <1 μM) and were relatively noncytotoxic. Moreover, towards establishing the possible mode of action of these molecules, potential effect on the host haemoglobin degradation pathway was investigated. Single crystal X-ray data confirmed the existence of an intramolecular hydrogen bond.

302

DISCOVERY OF NEW INHIBITORS OF PLASMEPSINS TARGETING MULTIPLE LIFE STAGES OF THE MALARIA PARASITE

Neha Sharma[1], Prem P. Sharma[1], Poonam FNU[2], Prakasha Kempaiah[3], Agam P. Singh[4], Brijesh Rathi[1]

[1]*Laboratory for Translational Chemistry and Drug Discovery, Department of Chemistry, Hansraj College University Enclave, University of Delhi, Delhi, India,* [2]*Department of Chemistry, Miranda House, University of Delhi North Campus, Delhi, India,* [3]*Department of Medicine, Loyola University Stritch School of Medicine, Chicago, IL, United States,* [4]*Infectious Diseases Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, Delhi, India*

Malaria remains accountable for abundant lethal cases worldwide. New therapeutics with novel modes of actions are urgently needed to enlarge the extent of treatment and to conquer developing drug resistance. Hydroxyethylamines are high-valued chemical frameworks with various biological activities, particularly antimalarial. Encouraged with our recent observations, we developed a library of new chemical scaffolds based on hydroxyethylamine and heterocycles. The toxicity assays against HepG2 cells supported all the compounds as nontoxic up to 1300 μM concentrations. Firstly, all the compounds were tested for their inhibitory actions against malarial aspartyl proteases (Plasmepsins X) that indicated two hits with inhibitory concentrations of ~900 nM. Further, compounds were examined against *P. falciparum* (3D7) culture that afforded several hits with inhibitory concentrations of <1 μM. The compounds were additionally examined for their potency chloroquine-resistant strain, Dd2 and showed remarkable efficacy. The hit molecules showed significant inhibition in *P. berghei* infected mouse models. Besides, liver stage malarial infection of *P. berghei* in culture was examined for hits, which were further tested in mouse model. All the hits were evaluated against the gametocyte culture of *P. falciparum*. It was noticed that both heterocyclic scaffolds and hydroxyethylamine were essentially important for the activity. The interesting observations will be presented in the form of a poster.

303

SMALL MOLECULE ASPERACULANE B INHIBITS MALARIA INFECTION AND TRANSMISSION

Guodong Niu, Jun Li

Florida International University, Miami, FL, United States

Mosquito-transmitted *Plasmodium* parasites infect millions of people worldwide. Drug-resistant *Plasmodium* parasites and insecticide-resistant mosquitoes make this disease hard to control. A drug that inhibits malaria infection and transmission is desperately needed. We screened our Global Fungal Extract Library (GFEL) and obtained a candidate extract that completely inhibits *P. falciparum* infection in *Anopheles gambiae*. The candidate fungal isolate was determined as *Aspergillus aculeatus*. The bioactive compound was purified with a flash column and high-performance liquid chromatography (HPLC) and was identified to be Asperaculane B. The concentration of 50% inhibition on *P. falciparum* transmission (IC_{50}) is 7.89 μM. Moreover, Asperaculane B also inhibits the development of asexual *P. falciparum* with IC_{50} of 3 μM. Therefore, Asperaculane B can be used to develop a novel antimalarial drug to treat malaria as well as to block malaria transmission.

304

MULTISTAGE ANTIMALARIAL ACTIVITY OF NEW CHEMICAL LIBRARIES TARGETING MULTIPLE PROTEASES

Brijesh Rathi[1], Jiang He[2], Poonam FNU[1], Kailash C. Pandey[3], Daniel E. Goldberg[4], Agam P. Singh[5], Prakasha Kempaiah[6]

[1]*University of Delhi, Delhi, India,* [2]*Massachusetts Institute of Technology, Cambridge, MA, United States,* [3]*National Institute of Malaria Research, New Delhi, India,* [4]*Washington University, Washington, WA, United States,* [5]*National Institute of Immunology, New Delhi, India,* [6]*Loyola University Stritch School of Medicine Chicago, Chicago, IL, United States*

Despite the significant advances, malaria remains a major threat to human population in many parts of the tropical world including India. Complex life cycle of *Plasmodium* and the increasing drug resistance to the front-line treatments are the hard-hitting obstacles to achieve the eradication of malaria. The discovery of new chemical scaffolds with multistage antimalarial activity are urgently required to combat this lethal disease. As a part of our ongoing interest in this direction, we synthesized a library of hydroxyethylamine analogs and studied their antimalarial activity in *Plasmodium falciparum (Pf)* culture and in mouse models of the malaria.

Several of these compounds displayed a significant growth inhibition of blood stage drug-resistant parasites (*Pf*D6 and *Pf*Dd2) at sub-micromolar concentrations, with the best showing 50% inhibitory concentrations (IC_{50}) of 300 nM. The cytotoxicity of these compounds was tested in peripheral blood mononuclear cells, leukemic monocytic cell lines (U937), and HepG2 cells. I*n vitro* drug-drug interaction of potent compounds with dihydroartemisinin indicated synergistic effect against *Pf*Dd2. A significant decrease in blood parasite load was noted for the hits in chloroquine-resistant *P. berghei* and *P. berghei* ANKA infected mouse models. Of these, a notable activity was exhibited by few analogs against the gametocyte stage of *P. falciparum* and liver stage infection of *P. berghei* in culture with IC_{50} values of ~1 micromolar. Few potent compounds exerted strong and comparable efficacy against *P. berghei* liver-stage infection in mouse model. Further, few analogs showed significant activity against malarial aspartyl proteases and cysteine proteases with inhibitory concentrations at sub-micromolar range. We have also identified that these compounds act as a potent lead for optimization as an antimalarial drug as revealed good membrane permeability and long endurance in the bloodstream as supported by the preliminary pharmacokinetic experiments.

305

COMPLIANCE AMONG PATIENTS/CAREGIVERS AND SERVICE PROVIDERS IN LIBERIA USING FIRST LINE ANTIMALARIALS DRUG (ARTESUNATE+AMODIQUINE-ASAQ AND ARTEMETHER + LUMEFATHRINE-AL)

Victor S. Koko[1], Oliver J. Pratt[1], Paye K. Nyansaiye[1], Levi D. Hinneh[1], Joseph O. Alade[1], Moses Badio[2], Eric Diboulo[3]

[1]*Ministry of Health/National Malaria Control Program, Monrovia, Liberia,* [2]*Local consultant, Monrovia, Liberia,* [3]*Measure Evaluation, Monrovia, Liberia*

Liberia adapted the use of ACT as the first line treatment for uncomplicated malaria 2008. Efficacy studies have shown high parasite clearance of this drug. Its effectiveness depends on adherence to treatment by both service providers and patients but todate, NMCP has no current empirical data on adherence. In wake of the lack of current evidence on adherence to treatment by both patients/caregivers and service providers, NMCP conducted an adherence study to determine adherence/compliance among patients/caregivers and service providers. The study used cross sectional survey design in randomly selected 10 health facilities throughout the country. (Two from each of the five health regions). Study participants were followed-up to determine their compliance status. The study consisted of two major components; observations made at facilities on dispensers interaction with patients diagnosed and confirmed positive with uncomplicated malaria and given ACT and exit interview furthered by follow-up at patients' homes on day 4. Descriptive and analytical analysis conducted in finding association. On the overall, 50% patients/caregivers complied/adhered with treatment regardless of drug type. By drug type, ASAQ had the highest compliance/adherence 61% than AL 39%. Children under five years had the highest (33%) compared with 12-17 year reporting the lowest 7%. Regarding dispensers' interaction with patients/caregivers, over 94% of the patients/caregivers were told how to take the drugs, with 12% told the importance of completing the doses and 15% asked if they understood the instruction. The result poses threat to the alternative first line antimalarial (AL) as the drug is considered new in Liberia and irrational use may lead to early treatment failure. Service providers&patients/caregivers need to be advised on the importance of explaining and completing treatment as there is an increased chance of complying with treatment the more explanations a patient/caregiver receives.

306

MALARIA IN VENEZUELA: TRACKING THE WORST MALARIA EPIDEMIC IN THE AMERICAS REGION AND THE ROLE OF CIVIL SOCIETY IN THE MALARIA RESPONSE

Leonor Pocaterra[1], Luis F. Chavez[2], Maria M. Villegas[3], Jorge Moreno[4], Angela Martinez[5], Elsy Rojas[1], Maria E. Guevara[3], Jose Oletta[6], Gustavo Bretas[3], Aurora Hernan[1], Mary A. Torres[7], Michelle Gonnet[8], **Leopoldo Villegas**[9]

[1]*Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela,* [2]*Instituto Costarricense de investigación y Enseñanza en Nutrición y Salud (INCIENSA), San Juan, Costa Rica,* [3]*Global Development One, Silver Spring, MD, United States,* [4]*Centro de Investigacion de Campo Dr. Francesco Vitanza", Tumeremo, Bolivarian Republic of Venezuela,* [5]*Instituto de Salud Publica, Ciudad Bolivar, Bolivarian Republic of Venezuela,* [6]*Sociedad Venezolana de Salud Publica, Caracas, Bolivarian Republic of Venezuela,* [7]*International Council Of Aids Service Organizations (ICASO), Toronto, ON, Canada,* [8]*Fundación Educando en Salud, Caracas, Bolivarian Republic of Venezuela,* [9]*Asociacion Civil Impacto Social (ASOCIS), Tumeremo, Bolivarian Republic of Venezuela*

Venezuela is facing a complex humanitarian crisis, including the worst malaria epidemic in the continent. Since 1955, the Malaria Program consolidates weekly cases from all states with reporting rates ≈85%. Historically, the malaria information system (MIS) includes only "new cases"(NC), excluding relapses (R) and recrudescences (Rc) cases. In Bolivar State (where 75% of malaria occurs), weekly reporting of R/Rc was incorporated into the MIS from 2008 onwards. Although malaria information is being routinely collected, the Venezuelan government stopped publishing data since 2015. In response to this malaria situation, as part of the civil society, we aim.to describe and update the number of malaria cases in Venezuela (1995-2018), including data on relapses, recrudescences, self-medication and underreporting. Estimations of 2019-2020 cases are also presented. Nationwide, malaria NC rose 2.200% from 22501 cases in 1995 to 530414 cases in 2018. Similarly, annual incidence (per 1000 pop) increased from 1.27 in 1995 to 32 (2.4K%) in 2018. Over the study period, imported malaria increased 6.8 times and the median percentage of *Plasmodium vivax* infections (range) were 80.6% (64.8-91.2), and 18.15% (8.6-28.9) for *P. falciparum*. The percentage of mixed infections (Pf+Pv) increased from 0.01% in 1997 to 6.4% in 2018. The annual percentage of R and Rc shifted in 2013, coinciding with major contextual shifts in the country. Estimated malaria cases (range) for 2019 and 2020 were 2 M (1.1M-3.8M) and 3.9 M (1.9M-7.9M), respectively. All observations in this study are consistent with increased transmission over space and time. and we estimate that at least 1.4 M cases were not accounted for between 2008-2018. Venezuela has lost previous gains in malaria control and local transmission is occurring in areas that have been malaria-free since the 1960s. There is an urgent need for effective targeted interventions to reduce the burden of malaria in Venezuela and its regional spread. The organized civil society can play a key role in promoting accountability and transparency about the current malaria situation, and the implementation of targeted interventions.

307

BASELINE MALARIA PREVALENCE IN DISTRICTS TARGETED FOR MALARIA ELIMINATION IN ETHIOPIA

Adugna A. Hirpa[1], Desalegn Nega Wada[1], Sindew Mekasha Feleke[1], Bokretsion Gidey[1], Geremew Tasew Guma[1], Abnet Abebe[1], Honelegn Nahusenay[2], Semira Abdulmenen[2], Ayele Zewude[2], Dereje Dillu[3], Degu Mehari[3], Gudisa Aseffa[3], Gezahegn Tesfaye[3], Hiwot Teka[4], Mattew Murphy[4], Adugna Woyessa Gemeda[1]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*Addis Continental Institute of Public Health, Addis Ababa, Ethiopia,* [3]*Federal Ministry of Health Ethiopia, Addis Ababa, Ethiopia,* [4]*President Malaria Initiative-USAID Ethiopia, Addis Ababa, Ethiopia*

Baseline malaria prevalence in elimination targeted areas enables us to measure the impact of the intervention in malaria control program. The objective of this survey was to assess the baseline malaria prevalence at community level in elimination targeted districts of Ethiopia. Community based cross sectional survey was conducted from October to December 2017. Samples of malarious villages were randomly selected from 20 districts, among the 239 elimination targeted districts in Ethiopia. Open Data Kit programmed household questionnaires and laboratory formats were used to collect the data. Blood samples were examined with Care Start™ Malaria HRP-2/pLDH. Data analysis was done using STATA 14. The overall prevalence of malaria detected by RDTs in this survey was 1.17% (339/28983). High proportion of malaria infection was reported in some districts: Harari 4.7% (46/979), followed by 3.7% (87/2358) in Kersa, 2.7% (81/2999) in Misrak Badawacho, 1.7% (27/1597) in Kolla Tembien, 1.4% (32/2281) in Habru and 1.07% (22/2055) in Raya Kobo. Whereas, in half (50%) of the surveyed districts, the prevalence was less than 1%. Interestingly, four districts namely Berehet, Sire, Gemechis and Damboya reported zero prevalence. The total prevalence of fever was 9.2% (2766/29993). Symptomatic malaria was 75.2% (255/339) and symptomatic 24.8% (84/339) among malaria positives. In conclusion, the total malaria prevalence of 1.17% by RDT in the elimination setting is yet high which calls for further strong intervention measures. The presence of the considerable asymptomatic malaria prevalence indicated the presence of asymptomatic reservoirs for continuity of malaria transmission in the elimination settings. This residual malaria in low transmission sustains uninterrupted malaria transmission in the areas. Active case detection is highly suggested to break the transmission sources. The conventional diagnostic methods such as microscopy and RDT may not provide conclusive determination; therefore inclusion of sensitive methods such as PCR is highly necessary.

308

HOTSPOT BASED INTERVENTIONS: A SUCCESS IN REDUCTION OF MALARIA TRANSMISSION FROM HIGH ENDEMIC CHATTOGRAM HILL TRACT (CHT) DISTRICTS IN BANGLADESH

Shamsun Naher, Akramul Islam, Mohammad Moktadir Kabir, Abu Saeid

BRAC, Dhaka, Bangladesh

Malaria is endemic in 13 eastern and north-eastern border belt districts of Bangladesh with variable transmission potentials (high, moderate and low). A total of 13.25 million people are at risk of malaria inhabited in those areas. Three Chattogram Hill Tract (CHT) districts have the highest endemicity and mortality due to their socio-economic and geographical factors which cover about 11.78% (2.13 million) of the endemic population but contribute more than 90% malaria burden over the years nationally. BRAC has been performing malaria program since 1998 in close collaboration with the government of Bangladesh. BRAC applied its community-based approach in providing malaria diagnostic and preventive services to door-steps of the community. *Para* (the smallest administrative unit) wise micro-stratification data were collected since 2015 and hot-spot based intensified interventions were taking, such as distribution of community health workers, mobile campaign, Long Lasting Insecticidal Nets (LLINs) distribution, awareness activities etc. Here we analysed past two years (2017 and 2018) data in CHT in order to categorize the endemicity of the sub-districts and villages on basis of Annual Parasite Incidence (API). About 64 % reduction of malaria incidences in 2018 compare to the year 2017 in Bangladesh. In CHT which contributed 90.6 % in 2018 showed reduction of malaria cases to 65% percent in 2018 compare to the year 2017. Only 2 out of 7 deaths due to malaria were reported in CHT in 2018. Eight sub-districts out of 25 where population covered 28% of CHT reported 77.4% malaria cases in 2018. Among the community health workers 37.5% were deployed in those areas. Among 2180 villages, 318 (14.6%) villages reported malaria where API >50 per 1,000 population in the last year contributed 50% of malaria cases. In 2017, 582 villages showed API >50 per 1,000 which in 2018 reduced to 45.4 %. Our data suggest that intensive interventions and continuous surveillance targeting hot-spot area and high risk group, and sustaining political commitment and finance are essential to achieve the target of malaria free Bangladesh by 2030.

309

PREVALENCE AND DRIVERS OF *PLASMODIUM* INFECTION ACROSS VILLAGES IN SOUTHEASTERN TANZANIA

Elihaika G. Minja, Johnson Kyeba Swai, Emmanuel Mrimi, Halfan Ngowo, Fredros Okumu

Ifakara Health Institute, Ifakara, United Republic of Tanzania

Malaria prevalence has significantly declined over the past decade in Tanzania, due to scale-up of key interventions such as the long-lasting insecticidal nets (LLINS), indoor residual sprays (IRS) and improved diagnosis and treatment. In the south-eastern districts of Ulanga and Kilombero, detailed entomological surveys have been done to track the declining malaria transmission and its drivers, but there have been no survey of actual malaria prevalence in recent times. We therefore conducted a cross-sectional active-case detection to assess prevalence *Plasmodium* infections and associated factors in 12 wards of Ulanga and Kilombero districts, south-eastern Tanzania. Malaria infection was tested using rapid malaria diagnostic test (mRDT), and positive cases confirmed using microscopy and PCR. Structured interviews were done to identify households at risk. A total of 2,912 individuals were tested across all villages. Multivariate analyses showed no difference in malaria infections between males and females (OR: 1.25 [0.99-1.57], P=0.064), but living in urban or peri-urban areas was associated with 99.7% less malaria (OR: 0.02 [0.01-0.07], P<0.001). Village by village prevalence varied from as high as 52% (Tulizamoyo) and 43% (Igota) in the north, to 2% (Minepa) or 1% (Lipangalala) in the southernmost part of the study area. Three wards including Ifakara town and two of its surrounding wards had no positive malaria cases detected. Malaria prevalence was higher among under-fives than other age groups (OR: 1.25 [0.99-1.57], P<0.001). These findings highlight high levels of spatial variability in malaria transmission, and a major decline in prevalence in some of the villages previously experiencing hyper to holoendemic transmission. Specific underlying drivers are not yet known until final analysis is completed.

310

RISK FACTORS FOR PATENT AND SUB-PATENT GAMETOCYTE CARRIAGE OF *PLASMODIUM FALCIPARUM* (*PFS25*) AND *PLASMODIUM VIVAX* (*PVS25*) IN HYPO- TO MESO-ENDEMIC WEST TIMOR IN EASTERN INDONESIA

Ayleen A. Kosasih[1], Cristian Koepfli[2], Rintis Noviyanti[3], Dwi A. Pujianto[4], Decy Subekti[1], William A. Hawley[5], Frank H. Collins[2], J. Kevin Baird[6], Ivo Mueller[7], Neil F. Lobo[2], Inge Sutanto[8]

[1]*Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia,* [2]*Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States,* [3]*Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [4]*Department of Biology, Medical Faculty, Universitas Indonesia, Jakarta, Indonesia,* [5]*UNICEF Jakarta, Jakarta, Indonesia,* [6]*Center for Tropical*

Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, [7]Infection and Immunity Division, Walter & Eliza Hall Institute, Melbourne, Australia, [8]Department of Parasitology, Medical Faculty, Universitas Indonesia, Jakarta, Indonesia

Asymptomatic gametocyte carriage at low densities may often drive malaria transmission in low to moderate endemic settings. We examined risk factors for gametocyte carriage employing RT-qPCR diagnostics among residents of hypo- to meso-endemic West Timor in eastern Indonesia. Two mass blood surveys were conducted in June and September 2013. 1,551 blood samples were collected from 875 residents. Light microscopy identified 47 *Plasmodium falciparum*- and 74 *P. vivax*-positives. Later, qPCR was utilised to examine for malaria positivity, and a total of 91 *P. falciparum* and 315 *P. vivax* positives were identified. RNA for gametocyte assay was available from 85 and 227 *P. falciparum*- and *P.vivax* -positive subjects, respectively. Assay for gametocytemia employed *pfs25* and *pvs25* RNA markers. Participant age, sex, microscopic malaria status, plasmodial parasite density, fever, and fever history were examined as potential risk factors for gametocyte carriage using bivariate and multivariate analyses. Among microscopically patent subjects, 23/47 *P. falciparum* positives also had patent gametocytemia, whereas 16/74 *P. vivax* positives did so. Gametocyte carriage was detected in 52% (44/85) and 37% (86/227) *P. falciparum* and *P. vivax* infections. Bivariate analyses suggested younger age (<=17 yrs), microscopic positivity, estimated parasite density, and history of fever were positively associated with gametocyte carriage in both *P. falciparum* and *P. vivax* ($p<0.05$). Gametocytemia for either species did not appear to be impacted by sex or fever. Multivariate analyses suggested age and history of fever were associated with *P. falciparum* ($p=0.015$ and $p=0.006$) gametocytemia, whereas parasite density was the sole predictor for gametocyte carriage in *P. vivax* ($p<0.001$). Most parasitemias in this community occurred below the limit of detection by expert microscopy, as did almost all gametocyte carriage. This study demonstrates sub-patent gametocyte carriage and its apparently species-specific risk factors in a low transmission setting. Gametocytemia in *P. vivax* indeed appears to emerge independently of febrile illness.

311

ASSESSING RELATIONSHIP BETWEEN HUMAN SETTLEMENT PATTERNS AND MALARIA RISK IN A RESIDUAL TRANSMISSION SETTING IN SOUTHEASTERN TANZANIA

Emmanuel W. Kaindoa[1], Arnold S. Mmbando[1], Gustav Mkandawile[1], Maureen Coetzee[2], Sherif Amer[3], Fredros O. Okumu[1]

[1]*Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2]Wits Research Institute for Malaria and Wits/MRC Collaborating Centre for Multidisciplinary Research on Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, [3]Faculty of Geo-information Science and Earth Observation, University of Twente, Netherlands, Netherlands*

Spatial targeting of interventions is increasingly recognized as essential for malaria control, particularly in areas aiming for elimination. The associations between house characteristics and malaria transmission are known, but gaps remain on whether transmission is also influenced by factors such as distances between households or the degree to which houses are clustered. This study examined households densities and their distances influence malaria transmission in a set of rural Tanzanian communities. We performed indoor and outdoor mosquito collection from fixed as well as randomly selected households over 12 months period and assessed effects of spatial clustering of households on malaria transmission risks. The study found that high house densities increased *Anopheles* biting risk but mosquito density declined as distances between houses increased beyond 50m. Despite the recent scale-up of effective vector control interventions, such as LLINs, there are still major household and environmental characteristics contributing to persistent malaria transmission. Additional efforts such as house improvement programs targeted at village-level, could address the challenges by prioritizing high-risk household clusters. Such new efforts could be further improved by involving communities in the control strategies, and removing financial and access barriers associated with poor-housing.

312

PARADIGM SHIFT AND SEASONAL VARIATION IN MALARIA PREVALENCE AND ANAEMIA IN IJEDE, IKORODU LOCAL GOVERNMENT AREA, LAGOS STATE, NIGERIA

Oluwagbemiga Olanrewaju Aina[1], Adeola Yetunde Olukosi[1], Chimere Obiora Agomo[2], Bamidele Abiodun Bamidele Iwalokun[1], Olusola Ajibaye[1], Bassey A. Orok[1], Adeniyi K. Adeneye[1], Chinendum T. Oparaugo[1], Samuel K. Akindele[1], Olajumoke M. Akinyele[1], Samson T. Awolola[1]

[1]*Nigerian Institute of Medical Research, Lagos, Nigeria, [2]Department of Medical Laboratory Science, University of Lagos, Lagos, Nigeria*

Seasonal variation is the change that occurs as a result of climate change including rain fall, humidity and temperature. There are two main seasons in Nigeria wet season and dry season. The objective of this study is to compare the prevalence of malaria and anaemia in Ijede community in both the wet and dry seasons and to assess the malaria prevalence of children under-five and above five years old. This is a cross sectional study, the study participants were screened for malaria using mRDTs and microscopy. The anaemia status of the participants were defined using the WHO haematocrit cut-off for mild, moderate and severe anaemia based on age and sex. Participants with confirmed malaria parasite were treated with artemether-lumefantrine. Blood spots were made on filter paper for studies on antimalarial drug resistance. A total of 813 and 833 participants were screened in the dry and wet seasons respectively , majority of them were females 556 (68.4%) and 480 (57.6%) in both seasons, The mean age was 30.07 ± 20.5 years and 26.3 ± 20.6 years in dry and wet seasons respectively. Children under the age of 5 were 134 (16.5%) and 130 (15.6%) in dry and wet seasons respectively, while those above 5 years of age were 679 (83.5%) and 704 (84.4%) in dry and wet seasons respectively. The prevalence of aneamia was 100 (22.4%) and 206 (47.7%) in dry and wet seasons respectively. Malaria prevalence in Ijede community was 5.7% and 9.7% in dry and wet seasons respectively. Malaria prevalence was 122(3.2%) and 590 (6.2%) in Children under 5 years and above 5 years respectively in dry season while in wet season the malaria prevalence was 113 (13.1%) and 610 (13.4%) in Children under 5 years and above 5 years respectively. Malaria prevalence was higher in children above 5 years of age irrespective of methods of assessment ($P<0.05$) in both dry and wet seasons. There is a paradigm shift in high malaria prevalence from children under-fives to above five years in this study. Malaria control strategies should be also be targeted at Children above the age of 5 years mostly during the wet season.

313

A SYSTEMATIC REVIEW OF THE CHANGING MALARIA DISEASE BURDEN IN SUB-SAHARAN AFRICA SINCE 2000: COMPARING MODEL PREDICTIONS ANDEMPIRICAL OBSERVATION

Alice Kamau[1], Polycarp Mogeni[1], Emelda A. Okiro[1], Robert W. Snow[2], Philip Bejon[2]

[1]*KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, [2]Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom*

The changing burden of malaria in Africa has been modelled using sparse data on parasite prevalence, environmental covariates and intervention coverage which are linked to historical estimates of clinical incidence. Uncertainty remains regarding the spatial consistency of these modelled declines. We undertook a systematic review (PROSPERO International Prospective Register of systematic reviews; ID= CRD42019116834) to identify empirical data on the temporal changes in clinical malaria in Africa since 2000, where reports covered at least 5 continuous years. These data were then compared with time-space matched estimates of the predicted

changes in clinical burden using data from the Malaria Atlas Project (MAP). The magnitude of relative change and correlation between changes in empirical clinical malaria and modelled estimates of clinical burden was assessed. Sixty-six articles met our inclusion criteria representing 120 sites surveyed between 5-15 consecutive years since 2000 from 23 African countries. Forty-one (34%) site-specific data showed evidence of an increase in malaria over the surveyed period, 79 (66%) showed a decline. Comparisons between changes in empirical clinical malaria and modelled estimates of clinical burden showed a mixed pattern of concordance, depending of data types, location and period of observation. Models provide a broad picture of changing global disease burden but cannot replace empirical data. The paucity of high quality, temporal clinical data in Africa must be redressed, to avoid a continued dependence on models or to help train future models.

314

HIGHLY DYNAMIC CHANGES IN IRON STATUS DURING PREGNANCY AND POSTPARTUM AND ASSOCIATIONS WITH ADVERSE MATERNAL OUTCOMES IN A MALARIA ENDEMIC REGION OF PAPUA NEW GUINEA: A COHORT STUDY

Eliza Davidson[1], Michelle Scoullar[1], Herbert Opi[1], Elizabeth Peach[1], Chris Morgan[1], Pele Melepia[2], Ruth Fidelis[2], Willie Pomat[3], Philippe Boeuf[1], Ricardo Ataide[1], Julie Simpson[4], James Beeson[1], Freya Fowkes[1]

[1]*Burnet, Melbourne, Australia,* [2]*Burnet, Kokopo, Papua New Guinea,* [3]*Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea,* [4]*Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia*

Iron deficiency is the most common nutrient deficiency worldwide and is the leading risk factor for anaemia; approximately half of all anaemia cases are attributed to iron deficiency. The highest prevalence of iron deficiency globally is observed in pregnant women, who are highly susceptible to both iron deficiency and anaemia; while postpartum susceptibility is less well known. However, iron is also an essential nutrient for a number of pathogens, and so a deficiency in host iron is hypothesized to reduce pathogen fitness and decrease infection susceptibility and/or severity. This interaction is of particular concern in malaria endemic countries. Here we determine temporal changes in iron deficiency during pregnancy and postpartum; and investigate associations with anaemia and *Plasmodium* species infection. This study utilized 700 samples and corresponding epidemiological data from women living in a malaria endemic region of Papua New Guinea, where the prevalence of iron deficiency and poor maternal outcomes are high. Iron deficiency, defined as ferritin<15μg/L, was highly prevalent at enrolment (80.6%) and delivery (84.9%); far less prevalent at 6 months (22.2%) and 12 months postpartum (28%). The prevalence of anaemia, defined as haemoglobin <11g/dL, was also very high at enrolment (82.3%) and delivery (57.6%), but less so at 6 (37.7%) and 12 (49.4%) months postpartum. Ferritin measures within women were highly dynamic over time, with huge fluctuations between enrolment and 12 months postpartum. Haemoglobin levels were more stable, gradually increasing from enrolment through to delivery and 6 months postpartum. Iron deficiency was significantly associated with lower haemoglobin levels at enrolment, delivery and 6 months postpartum ($p<0.05$), compared to iron replete women. Multivariate analyses quantified the relationship between *Plasmodium* species infection, iron status and anaemia. Findings highlight the high burden of deficiencies and disease in resource-poor settings and the complex interactions that exist between morbidities transitioning from pregnancy to postpartum.

315

COMPARISON OF *PLASMODIUM* INFECTION BETWEEN CHILDREN AND ADULTS IN KOMBEWA

Cornel Obonyo Arima, Jim Ray Managbanag, Cephas Aguko, Agneta Ogolo, Catherine Sumbi, Michael Ayaya, Everlyne Omondi, Victor Otieno, Vincent Akolo, Rose Adeny, Hoseah M. Akala, Bernhards Ogutu

[1]United States Medical Research Directorate - Africa/Kenya, [2]Kenya Medical Research Institute

Malaria remains one of the major causes of preventable illnesses and mortality in developing countries. In 2005, WHO estimated 214 million new malaria infected cases with 438,000 malaria associated mortalities. Malaria morbidity and mortality is higher in children under five years than adults. Studies have shown that recent up-scaled integrated vector control interventions have eased disease burden in Africa. Specifically, the world health organisation recommends that in endemic areas with intense malaria transmission, all infants at their first immunization and all pregnant women as early as possible in pregnancy should receive one long-lasting insecticidal net through immunization and antenatal care visits. As this guideline continues to be followed, it is essential to continue tracking the impact of these guidelines on disease burden across ages in the population. The aim of this study was to estimate the frequency of malaria in Kombewa by age group. A total of 1451 potential study subjects were screened in the blood collection protocol survey between 2007 and 2011 in Kombewa sub county, Kisumu. A total of 20μl of whole blood was drawn from each study subject. Each sample was tested by both RDT and confirmed by expert microscopy. Out of 1451, 940(64.8%) samples tested positive for *Plasmodium* species by microscopy. Of the 940 positive cases, children < 5 years were 400(43%), ages of 5-17years 385(26.6%) while adults above 18yrs were 150(10.5%) positive cases. Further, children ages <5 years had highest parasite counts than older children aged 7-17 years and adults based on expert microscopy confirmed read-outs ($P<0.005$). These findings show decreasing frequency of infection with increase in age suggesting sustained burden among children despite heightened intervention targeting this age group.

316

PARASITAEMIC PROFILES AND DISTRIBUTION OF HOST AND MATERNAL FACTORS AMONG INFANTS LIVING IN A HIGH MALARIA TRANSMISSION AREA OF GHANA

Akua Kyerewaa Botwe[1], Seth Owusu-Agyei[2], Ulf Hammar[3], Felix Boakye Oppong[1], Stephaney Gyaase[1], Gabriel Jakpa[1], George Adjei[4], Muhammad Asghar[5], Faith Osier[6], Anna Färnert[7], Kwaku Poku Asante[1]

[1]*Kintampo Health Research Centre, Kintampo, Ghana,* [2]*Institute of Health Research, University of Health and Allied Sciences, Ho, Ghana,* [3]*Unit of Biostatistics. Department of Epidemiology, Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,* [4]*University of Cape Coast, Cape Coast, Ghana,* [5]*Division of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden,* [6]*Kenya Medical Research Institute, Kilifi, Kenya,* [7]*Department of Infectious Diseases. Karolinska University Hospital, Stockholm, Sweden*

Insight into determinants of asymptomatic and symptomatic infections in infants can contribute to our understanding of malaria immunity and guide control interventions. The aim of study was to identify infants immune to malaria based on their temporal profile of episodes in the first year of life and identify pre-disposing factors to susceptibility. A birth cohort in Kintampo, Ghana (N = 1855) was followed with monthly blood sampling and passive surveillance of malaria between 2008 and 2011. Malaria parasites were detected by light microscopy. Host, maternal, parasite and hematological parameters were collected from infants and their mothers. Profiles of infections and distribution of host and maternal factors among infants through their first year of life were examined. Analyses were based on 1264 infants that had at least eight monthly visits in twelve months. Infants were either parasite negative in all samples

(36 %), or when parasites detected were always asymptomatic (7 %), always symptomatic (35 %) or alternated between asymptomatic and symptomatic episodes (22 %). Compared to infants who were parasite negative only, significantly higher proportion with symptomatic malaria only had low socioeconomic status (SES), were born in low transmission season, had mothers receiving one to three doses of intermittent preventive treatment during pregnancy (IPTp) and resided in rural areas. Compared to symptomatic malaria only, significantly lower proportions of infants with asymptomatic infections only had illnesses and fever before first infection, lower age at first infection, placental malaria, IPTp directly observed therapy and low SES. Birth weight, congenital abnormalities, age of mother, sickle cell status of mother or infant, insecticide treated bed-net use by mother and tetanus immunizations during pregnancy were not significantly different between the profiles. Although most infants experienced episodes of symptomatic malaria, some remained asymptomatically infected through the first year of life. Future studies will examine risk of asymptomatic infections and identify antibody correlates of protection for infants.

317

ABO BLOOD GROUP AND MORTALITY IN CHILDREN WITH SEVERE MALARIAL ANEMIA DUE TO *PLASMODIUM FALCIPARUM*

Jean-Bertin Kabuya[1], Luc Kamavu[2], Mike Chaponda[1], James S. Lupiya[1], Manuela Hauser[3], Jessica Schue[4], William J. Moss[4], Philip Thuma[5], Matthew Ippolito[4]

[1]*TDRC, Ndola, Zambia,* [2]*St Paul's Hospital, Nchelenge, Zambia,* [3]*University Children's Hospital, Zurich, Switzerland,* [4]*Johns Hopkins, Baltimore, MD, United States,* [5]*Macha Research Trust, Macha, Zambia*

Previous studies of ABO blood group and malaria describe a protective effect of blood group type O. To assess the possible relationship between blood group and survival in children with severe malarial anemia due to *P. falciparum* infection, we combined data from two observational studies of severe malaria conducted in two hospitals in Zambia. Study participants were children ≤12 y with falciparum malaria and severe anemia, defined as hemoglobin (Hb) concentration ≤5 g/dl. For comparison, we included children with severe anemia due to causes other than malaria. We examined associations between ABO blood group and mortality in unadjusted and adjusted logistic regression models. We identified 384 children with severe malarial anemia and 45 children with severe anemia due to another cause. Other causes included sickle cell, malnutrition, renal disease, and infections other than malaria. Among children with severe malarial anemia, blood group type O was the most prevalent (43%) followed by types A (28%), B (23%) and AB (5%). The median age was 23 m (IQR: 12-36) and 52% were girls. The median hemoglobin concentration was 3.8 g/dl (IQR: 3.1-4.3). Most (86%) received blood transfusion. The case fatality ratio was 13%. Children with other causes of severe anemia were similar across all characteristics. Adjusting for age, sex, Hb, and blood transfusion we found no statistically significant association between ABO blood group and mortality in children with severe malarial anemia, or in children with severe anemia due to other causes. Higher Hb and receipt of blood transfusion were associated with improved survival (OR 1.6, 95% CI: 1.1-2.3, p=0.02 per g/dl increase in Hb; OR 3.3, 95% CI: 1.5-7.4, p<0.01 for blood transfusion). Although previous studies demonstrated a relationship between ABO blood group and malaria-related outcomes, we did not find an association between blood type and mortality in Zambian children with severe malarial anemia, or severe anemia due to another cause. Further studies that assess the association between ABO blood group and malaria may lend insight into the pathophysiology of malaria caused by *P. falciparum*.

318

PREDICTORS OF MALARIA PARASITEMIA AMONG CHILDREN UNDER FIVE YEARS IN GHANA: A SUBNATIONAL COMPARATIVE ANALYSIS OF POPULATION SURVEY DATA, 2019

Samuel Kweku Oppong, Alexander Asamoah, Mildred Kommey

National Malaria Control Programme, Accra, Ghana

Ghana has 3 ecological zones(coastal, forest and savanna) with different malaria transmission patterns. With the current drive for elimination at subnational level, identifying predictors of malaria parasitemia to help inform appropriate area specific intervention mix is needed.Data was analyzed to assess factors contributing to malaria parasitemia across the ecological zones.Secondary data analysis on 2016 Malaria Indicator Survey was done.Variables included were age,sex,place of residence of child,wealthindex,IndoorResidualSpraying(IRS),mother's educational level,age,exposure to malaria messages,source of exposure,use of ITNandhousehold ownership of net.Odds Ratio(OR) was used to determine associations at 95% confidence interval(CI).Of the 2601 children data included in the analysis,39%(1014),38%(988)and23%(599) were from coastal, forest and savanna zones respectively. The odds of parasitaemia OR;CI was significantly associated with place of residence 2.64;1.47-4.76,Wealth index (middle 0.43;0.21-0.87,Richer0.29;0.13-0.63,Richest0.06;0.02-0.21,Mother's education (secondary1.33;0.13-0.84,Age of Child(24-35 months1.95;1.06-3.61,48-59 months1.95;1.06-3.61 in coastal zone; wealth index (middle0.34;0.16-0.71,Richer0.19;0.08-0.46,Richest0.03;0.00-0.17),Mother's education (secondary0.40;0.16-0.98, source of malaria message 0.49;0.25-0.97 in forest zone and wealth index (poorer0.25;0.09-0.70,middle0.24;0.07-0.82,Age of Child(36-47 months 2.63;1.35-5.13, 48-59 months2.67;1.48-4.83,IRS 0.53;0.30-0.93 in savanna zone. The odds of parasitemia reduces with increasing wealth index across all zones. Children 48-59 months are more likely to have malaria parasites in coastal and savannah zones whiles children of mothers with secondary or higher education are less likely to have malaria parasites in coastal and forest zones. IRS reduces the odds of malaria parasitaemia in the savannah zone. More data should be gathered on interventions implemented in specific areas of the country to assess their effect on parasitaemia to better inform program strategies.

319

HUMAN MOBILITY AND MALARIA HISTORY IN A PERIURBAN COMMUNITY OF THE PERUVIAN AMAZON

Andree Valle Campos[1], Jorge L. Maguiña[1], Gabriela Ulloa[1], Katty M. Arista[2], Viviana Pinedo-Cancino[2], Lastenia Ruíz-Mesia[2], Meddly Santolalla[1], Adam Bennett[3], Andres G. Lescano[1]

[1]*Emerge, Emerging Diseases and Climate Change Research Unit, School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Universidad Nacional de la Amazonía Peruana, Iquitos, Peru,* [3]*Malaria Elimination Initiative, Global Health Group, University of California San Francisco (UCSF), San Francisco, CA, United States*

Human mobility is a main driver of *Plasmodium vivax* local transmission in rural communities of the Peruvian Amazon. However, this factor may not apply for a periurban Amazonian setting that develop different behavioral dynamics due to their proximity to roads and the city. Even more in populations that have a broad range of night activities due to their electricity access. We performed a cross-sectional household survey to adults (> 18 years old) in the periurban community of Zungarococha, 5km to the southwest of Iquitos city, during the dry season (July-August 2017). We applied a malaria history survey to assess past events diagnosed by a health worker by microscopy. Movement and travel patterns survey to assess the range of hours out of home and frequency of short (1 night - 1 month) and long (>1 month) travels. To identify associated variables to malaria history in the last year we performed a poisson-log multiple regression and reported adjusted prevalence ratio (PR) with 95% confidence intervals (95%CI). We surveyed 427 individuals within 247 households. Agriculture was the highest reported occupation (11.6%)

over fishing or logging. 26.9% of subjects reported being out of home between 18:00 and 05:59 hours and 20.5% had a malaria episode in the last year. Our model showed five associated factors: the sum of reported hours out of home between 18:00-05:59 h (PR 1.04 95%CI 1.00-1.08) and between 06:00-17:59 h (PR 1.04 95%CI 1.01-1.06), presence of long travels (PR 1.49 95%CI 1.00-2.22), and living in one village to the other three (PR 0.56 95%CI 0.34-0.91). We included outdoor work as confounder and walking transport as mediator. We did not identified difference in the prevalence of malaria history in the last year between hours spent out of home at night or day, although both were detected as associated factors. Additionally, its prevalence in subjects with travels greater than a month showed to be 49% higher than subject without long travel history. Lastly, urban village conditions decrease its prevalence in 44%. In conclusion, longer travels showed a different scenario for periurban settings with respect to malaria history, independent of occupation.

320

PREGNANT WOMEN MAY BE A SENTINEL GROUP FOR MALARIA SURVEILLANCE: PRELIMINARY RESULTS FROM A STUDY IN SOUTHERN MOZAMBIQUE

Alfredo Mayor[1], Gloria Matambisso[2], Gizela Bambo[2], Beatriz Galatas[1], Pau Cisteró[1], Sonia Maculuve[2], Caterina Guinovart[1], Francisco Saúte[2], Clara Menéndez[1], Pedro Aide[2], Eusébio Macete[2]

[1]*Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain,* [2]*Manhiça Health Research Center (CISM), Manhiça, Mozambique*

Antenatal care (ANC) clinics may provide accessible routine information of theoretically healthy women, which might serve as a proxy for several health indicators at the community level. Several studies have suggested that the prevalence of malaria among pregnant women attending the ANC may reliably reflect malaria patterns in their communities. However, evidence on the relationship between malaria parasite prevalence in the community and in pregnant women at first ANC visit is still lacking. To test this approach, pregnant women at first ANC visit were recruited between November 2016 and November 2019 at three ANC clinics located in two districts from southern Mozambique (Manhiça and Magude, in Maputo province). At this first visit, clinical and demographic information was collected, together with a dried blood spot for detection of *P. falciparum* (*Pf*) by real-time quantitative PCR (qPCR). In parallel, cross-sectional surveys conducted in May 2017-2019 estimated *Pf* infection prevalence by rapid diagnostic test (RDT) in an age-stratified simple random sample of the population of Manhiça and Magude districts. *Pf* prevalence by qPCR among pregnant women attending the first ANC clinic (PR_{ANC}) between November 2016 and 2018 was 7% in Manhiça, 33% in Ilha Josina and 5% in Magude. *Pf* infection prevalence by RDT (PfPR) in children 2-10 years old ranged from 30.9% (95% CI 14.7-53.5) in Ilha Josina 2017 to 1.6% (95% CI 1.1-2.3) in Magude 2018. Overall, *Pf* prevalence (determined by qPCR) was higher in pregnant women when compared with 2-10 year-old children (detected by RDTs). There was a good correlation between PR_{ANC} and $PfPR_{2-10}$, with a Pearson r of 0.9994 (p=0.0006). This preliminary analysis suggests a strong correlation between *P. falciparum* prevalence detected in May in children 2-10 years from the community and in pregnant women at their first ANC visit recruited between November and May. Further analysis should consider the impact of parity, HIV infection, gestational age and parasite densities on this correlation.

321

MONITORING MALARIA CASES IN THE STATE OF RORAIMA, BRAZIL

Rispah A. Abdallah[1], Jaime Louzada[2], Venkatachalam Udhayakumar[1], Joseli Oliveira-Ferreira[3], Naomi W. Lucchi[1]

[1]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Federal University of Roraima, Boa Vista, Brazil,* [3]*Institute Oswaldo Cruz- Fiocruz, Rio De Janeiro, Brazil*

In Brazil, Roraima State is considered moderate risk for malaria transmission. However, the state has recently seen an increase in the number of reported *P. falciparum* infections believed to be coming from residents travelling from Guyana and Venezuela. The objective of this study was to determine the prevalence of *Plasmodium* species among patients attending health clinics in Boa Vista (state capital), Pacaraima (a health post located in the Venezuelan border town) and Rorainopolis (211 Km south of the capital) in Roraima State and quantify the infections attributable to imported malaria. Information about residence, occupation and travel history was collected from all enrolled patients. Microscopic diagnosis was made during presentation at the clinic and a blood spot was collected to confirm diagnosis and for species identification using PET-PCR. For PCR, the samples were first screened for the presence of *Plasmodium spp; Plasmodium*-positive samples were then evaluated for the four human infecting species. A total of 751 patients were enrolled between 2016-2017 from the three sites: Boa Vista [340], Rorainopolis [57], and Pacaraima [354]. Field microscopy identified 489 positive samples: 283 (57.9%) *P. vivax* infections, 189 (38.6%) *P. falciparum* infections and 17 (3.5%) mixed *P. falciparum* and *P. vivax* infections. PET-PCR identified 541 positive samples: 257 (47.5%) *P. vivax*, 241 (44.5%) *P. falciparum*, 33 (6.1%) mixed infections (*P. falciparum* and *P. vivax*), 1 (0.2%) mixed infection (*P. falciparum* and *P. ovale*), 9 samples (1.7%) species could not be identified. Based on reported residence and travel history, 90% of the PCR-positive patients were likely to have acquired infection in Venezuela (76.0%) or Guyana (14.0%), which supports the hypothesis that imported malaria contributes to the bulk of malaria cases diagnosed in these health facilities. Given the high mobility of people between this state and the neighboring countries, these findings are not surprising and need to be considered when developing malaria control interventions in this region.

322

MOLECULAR SURVEILLANCE FOR ANTIMALARIAL DRUG RESISTANCE MARKERS IN *PLASMODIUM FALCIPARUM* CASES — RORAIMA, BRAZIL, 2016-2017

Christina M. Carlson[1], Julia N. Kelley[1], Rispah A. Abdallah[1], Dhruviben Patel[1], Jaime Louzada[2], Bryan C. Ezema[1], Venkatachalam Udhayakumar[1], Joseli Oliveira-Ferreira[3], Eldin Talundzic[1], Naomi W. Lucchi[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Federal University of Roraima, Boa Vista, Brazil,* [3]*Institute Oswald Cruz - Fiocruz, Rio De Janeiro, Brazil*

Artemisinin-based combination therapies (ACTs) are standard treatment for *Plasmodium falciparum* (*Pf*) malaria. ACT resistance in Southeast Asia has generated concern over the potential spread of these resistant parasites to other regions, threatening ongoing malaria elimination efforts and case management effectiveness. Molecular markers of resistance are a critical tool to monitor the emergence and spread of antimalarial resistance. Reports of the emergence of *Pf* parasites with the C580Y allele in the *k*13 gene (a marker of artemisinin resistance) in Guyana motivated a molecular surveillance study in the neighboring state of Roraima, Brazil to gather information about the prevalence of resistance alleles in this region and guide local antimalarial treatment policy. In 2016-2017, we collected dried blood spots from patients diagnosed with uncomplicated *Pf* malaria in three sites in Roraima State: Pacaraima, Boa Vista, and Rorainopolis. A next generation sequencing Malaria Resistance Surveillance (MaRS) method was used to generate single nucleotide polymorphisms

data for 6 drug resistance genes (*crt, cytb, dhfr, dhps, k*13*, mdr*1). Data were analyzed with the MaRS Next-generation Sequence-analysis Toolkit (NeST; https://github.com/CDCgov/MaRS). Of 204 samples, 194 were successfully sequenced. No *k*13 artemisinin resistant mutations were found. All samples exhibited known *crt* mutations associated with chloroquine resistance and mutations in *dhfr* and *dhps* genes associated with sulphadoxine-pyrimethamine resistance. Interestingly, the C350R mutation, associated with ACT partner drug piperaquine resistance and a reversal to chloroquine sensitivity, was found at an allele frequency of 18% in a pool of ten samples. We found no molecular markers associated with artemisinin resistance, supporting the use of the current ACT. However, molecular markers associated with chloroquine, sulphadoxine-pyrimethamine, and piperaquine resistance were observed. Given the high mobility of people in this region, continuous molecular surveillance and periodic assessment of the therapeutic efficacy of ACTs are necessary.

323

VERY LOW MALARIA TEST POSITIVITY RATES IN THE HIGHEST MALARIA BURDEN COUNTRY: A CASE FOR TESTING AND TREATING

Tobi Bamigbade[1], Ameh Akoji[1], **Nirmal Ravi**[2]

[1]*EHA Clinics, Kano, Nigeria,* [2]*Ehealth Africa, Kano, Nigeria*

Nigeria, a country of nearly 200 million people in Sub-Saharan Africa has the highest burden of malaria in the world. It accounts for 1 in every 4 cases and 1 in 5 deaths due to malaria globally. A previous population-based study found malaria prevalence as high as 60.6% in Kano, a city in Northern Nigeria. Clinicians and lay people frequently attribute clinical symptoms such as fever and malaise to malaria without any confirmatory laboratory test. This practice has lead to widespread misuse of anti-malarials. This retrospective study was done to determine malaria test positivity in 386 patients who reported clinical symptoms suggestive of malaria at a private primary care clinic in Kano, Nigeria. Clinical symptoms of malaria were defined as fever, malaise, headaches, bitter taste, nausea/vomiting and loss of appetite. The study period was from July 2018 to March 2019. *Plasmodium* spp testing was done using SD Bioline Malaria Ag P.f/Pan Rapid Diagnostic Test (RDT) kits and microscopy. Of the 386 patients tested, 84.5% were adults. Malaria RDT was positive in 34 (8.8%) of the 386 patients tested. Among the 85 patients who had fever with temperature greater than 37.5 Celsius, only 14% had positive malaria RDT. We tested a small subset of 75 samples among the 386 patients with both RDT and microscopy to determine concordance between the two test methods. Concordance between RDT and microscopy results among these 75 samples was 100%, indicating the reliability of the RDT in accurately diagnosing malaria. Malaria test positivity rate of 8.8% among our patient population is significantly lower than previously reported from Nigeria. This could be because most of our patients were from middle to high income households. Our study shows that prevalence of malaria can vary widely even within the same city in a high malaria burden country. Testing each presumptive malaria patient before treating with anti-malarials is imperative to avoid misdiagnosis and overuse of anti-malarials.

324

CHARACTERIZING THE HUMAN INFECTIOUS RESERVOIR FOR MALARIA IN TORORO, UGANDA: AN AREA UNDER EFFECTIVE MALARIA CONTROL

Chiara Andolina[1], John Rek[2], Joseph Okoth[2], Alex Musiime[2], Kjerstin Lanke[1], Melissa Conrad[3], Peter Olwoch[2], Lisette Meerstein-Kessel[1], Jessica Briggs[3], Emmanuel Arinaitwe[2], Joaniter Nankabirwa[2], Bryan Greenhouse[3], Moses Kamya[2], Chris Drakeley[4], Grant Dorsey[3], Sarah Staedke[5], Teun Bousema[1]

[1]*Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, United States,* [4]*Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Department of Clinical Research, London School of Hygiene & Tropical Medicine, London, United Kingdom*

A better understanding of how malaria is transmitted from human hosts to mosquito vectors is needed to support malaria elimination efforts. Especially in low-endemic areas, our understanding of this human infectious reservoir is incomplete. In Tororo, an area in Uganda under intensive malaria control, we followed an all-age cohort (n=492) to characterize factors associated with gametocyte production and infectivity to mosquitoes. *P. falciparum* parasite density was determined by ultrasensitive varATS qPCR. All qPCR positive individuals were eligible for mosquito membrane feeding assays on their next visit, 4 weeks after parasite detection. Between November 2017-December 2018, 299 membrane feeding experiments were successfully conducted, including 10 on clinical malaria cases and 289 during asymptomatic infections; mosquitoes were dissected 9-10 days later for oocyst detection. Mosquito infections were observed in 10% (30/299) of these experiments; overall 1.8% (338/18,397) mosquitoes were infected with the proportion of mosquitoes per donor ranging from 1.1% to 81.4%. Only one clinical case infected mosquitoes (10.0% ; 1/10) whilst asymptomatic children aged 5-15 years comprised the majority of infectious individuals (80%, 24/30). The likelihood of being infectious and the proportion of infected mosquitoes increased with increasing varATS qPCR parasite density ($p<0.001$). Nevertheless, 13.9% (47/338) of all infected mosquitoes were infected by individuals with <100 parasites/μL. During the study period, parasite prevalence and parasite density declined in the cohort. In individuals with repeated membrane feeding assays, this decline in parasite burden was mirrored by a slowly declining likelihood of infecting mosquitoes. The unique longitudinal nature of our study allowed us to assess the human infectious reservoir for malaria during intensive malaria control. In this scenario, we find that asymptomatic infections in school aged children are responsible for the majority of onward transmission events and that the likelihood of infecting mosquitoes is strongly associated with parasite density.

325

ASYMPTOMATIC *PLASMODIUM FALCIPARUM* MALARIA INFECTION AMONG SCHOOL AGE CHILDREN IN KUMASI, GHANA

Natalie Olson[1], Santosh George[1], Sunil Parikh[1], Michael Kusi Addai[2], Lisa M. Harrison[1], Kweku Djan[1], Apongnwu Fopenawoh[3], Tsiri Agbenyega[4], Michael Cappello[1]

[1]*Yale University, New Haven, CT, United States,* [2]*HopeXchange Medical Centre, Kumasi, Ghana,* [3]*University of Maryland, College Park, MD, United States,* [4]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

As the landscape of malaria in sub-Saharan African changes due to improved control efforts, there is increasing interest in elucidating the epidemiology of asymptomatic *P. falciparum* infection and its role in disease transmission, particularly in school age children. We conducted a cross-sectional survey in the summer of 2018 of children attending 4 primary/junior high schools within a 5 km radius of HopeXchange Medical Centre in Kumasi, Ghana, a city of 2.5 million inhabitants with year-round malaria transmission. Informed consent/assent and demographic information was obtained from 634 subjects (ages 5-17 years), after which measurements of height/weight/body temperature were recorded and a blood sample was obtained for evaluation by blood film microscopy, rapid diagnostic test (RDT) and nested polymerase chain reaction (PCR) for *P. falciparum*. Factors associated with malaria parasitemia were analyzed using a qualitative questionnaire covering socioeconomic factors, malaria prevention behaviors, and healthcare access. The overall prevalence of asymptomatic parasitemia varied by diagnostic test (microscopy: 5.5%; RDT: 11.8%; PCR: 23.4%). Agreement between methods was highest for samples with higher levels of parasitemia as measured by microscopy. Bivariate analysis showed that factors associated with a positive malaria test included school, lack of bed net usage and age. After controlling for

relevant factors, school of attendance was the single greatest predictor of a positive malaria test in the study population. Across the 4 schools, PCR (n=555) substantially increased the sensitivity for parasitemia detection when compared to microscopy (1.7 vs 7.6%; 2.8 vs 10.3%; 3.2 vs 28.4%; 12.2 vs 40.1%). These data are in agreement with previously described higher sensitivity of PCR for detection of asymptomatic parasitemia. More importantly, these results capture the significant heterogeneity in asymptomatic malaria risk in Kumasi, underscoring the importance of characterizing the epidemiology of asymptomatic malaria, even within small geographic areas.

326

BLACKWATER FEVER IN UGANDAN CHILDREN WITH SEVERE ANEMIA IS ASSOCIATED WITH POOR POST-DISCHARGE OUTCOMES. A PROSPECTIVE COHORT STUDY

Robert O. Opoka[1], Ali Waisswa[2], Harriet Nambuya[3], Ch C. John[4], James K. Tumwine[1], Charles Karamagi[5], Charles Karamagi[1]

[1]*Makerere University, Kampala, Uganda,* [2]*Global Health Uganda, Kampala, Uganda,* [3]*Jinja Regional Referral Hospital, Jinja, Uganda,* [4]*Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States,* [5]

Blackwater fever (BWF), one of the complications of severe malaria, has recently re-emerged as a cause of severe anemia (SA) in African children. However, post-discharge morbidity in children with BWF has hitherto not been described. This was a descriptive cohort study in which children, aged 0-5 years, admitted to Jinja Regional Referral Hospital with acute episodes of SA (Hb ≤ 5.0 g/dl) were followed up for 6 months after hospitalization. Incidence of readmissions or deaths during the follow-up period was compared between SA children with BWF and those with no BWF. A total of 279 children with SA including those with BWF, n=92, and no BWF, n=187, were followed for the duration of the study. Overall 128 (45.9%) of the study participants were readmitted at least once while 22 (7.9%) died during the follow up period. After adjusting for age, sex, nutritional status and parasitemia SA children with BWF had higher risk of readmissions, HR 1.68 (95% CI, 1.1, 2.5), and a greater risk of death, HR 3.37 (95% CI, 1.3 to 8.5), compared to those with no BWF. Malaria and recurrence of SA were the most common reasons for readmissions. There is a high rate of readmissions and deaths in the immediate 6 months after initial hospitalization amongst SA children in Jinja hospital. SA children with BWF had increased risk of readmissions and deaths in the post discharge period. Post-discharge malaria chemoprophylaxis should be considered for SA children living in malaria endemic areas. Future studies should explore factors that predispose SA children with BWF to high post discharge morbidity.

327

CHARACTERIZING MALARIA BURDEN IN TURKANA REVEALS THE IMPACT OF DEVELOPMENT ON MAINTAINING TRANSMISSION

Hannah R. Meredith[1], Amy Wesolowski[1], Timothy M. Shields[1], James Maragia[2], Daniel Esimit[2], Samuel Lokemer[2], Joseph Kipkoech[3], Diana Menya[3], Andrew Obala[4], Wendy Prudhomme-O'meara[5]

[1]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Department of Health and Sanitation, Turkana County Government, Lodwar, Kenya,* [3]*School of Public Health, Moi University College of Health Sciences, Eldoret, Kenya,* [4]*School of Medicine, Moi University College of Health Sciences, Eldoret, Kenya,* [5]*Department of Medicine and Duke Global Health Institute, Duke University, Durham, NC, United States*

Malaria transmission has not been well characterized in the semi-arid areas of northern Africa, largely due to the assumption that the environment and people's pastoral lifestyles do not support stable malaria transmission. Brief, epidemic transmission could arise after unusually large rainfalls, but those are uncommon and isolated events. Here, we characterize malaria transmission in a prototypical example: Turkana, a vast county in northern Kenya with semi-arid conditions and semi-nomadic people. The discovery of oil in Turkana in 2012 has led to a rise in settlement and an increase in accessible water sources. While some studies suggest urbanization may decrease the risk of malaria, others predict that rapid, unplanned urbanization may increase it through the creation of open water surfaces where mosquitoes breed. Thus, we hypothesized that more settled areas in Turkana were more likely to support stable malaria transmission, despite rainfall patterns. We evaluated the impact of settlement and rainfall patterns on the malaria test positivity rate collected from routine surveillance reports of 36 health facilities from 2016-2018. These health facilities are located across Turkana and captured varied rainfall patterns and settlement levels. Satellite imagery was used to quantify rainfall and settlement in health facility catchment areas. Results revealed a range of malaria test positivity rates in both magnitude and duration that did not necessarily correspond with rainfall patterns. For instance, neighboring health facilities experiencing the same rainfall patterns reported malaria test positivity rates that were low and episodic in one and high and sustained in the other. Additionally, although 2017 lacked the long rain pattern observed in 2016 and 2018, its peak malaria test rates were similar to or greater than those recorded in the other years for many health facilities. These results highlight the role that dynamic populations play in creating environments suitable for malaria transmission. Consequently, malaria should be continuously monitored in areas assumed inapt for transmission.

328

SPATIAL AND TEMPORAL PATTERNS OF *PLASMODIUM FALCIPARUM* INFECTIONS IN BHUTAN, 2006-2014

Kinley Wangdi[1], Zhining Xu[1], Darren J. Gray[1], Kathryn Glass[1], Archie C. Clements[2]

[1]*Australian National University, Canberra, Australia,* [2]*Curtin University, Peth, Australia*

Malaria in Bhutan has dwindled in recent years and is aiming for malaria elimination in Bhutan. This study aims to identify *Plasmodium falciparum* clusters at a high geographical resolution and to determine the association between local environmental characteristics and the distribution and transmission of the disease. Notifications of *P. falciparum* cases from January 2006 to December 2014 were obtained from the Vector-borne Diseases Control Program under the Ministry of Health, Bhutan. The population of each sub-district (second-level administrative sub-division) was obtained from the Population and Housing Census 2007. Spatial autocorrelation in dengue incidence was explored using Moran's I statistic, Local Indicators of Spatial Association (LISA), and the Getis-Ord statistics. A multivariate, Zero-Inflated, Poisson regression model was developed with a conditional autoregressive (CAR) prior structure, and with posterior parameters estimated using Bayesian Markov chain Monte Carlo (MCMC) simulation with Gibbs sampling. There was a total of 2,062 *P. falciparum* cases during the study period. Malaria clusters were observed in the sub-districts in Sarpang district. Only rainfall lagged at one month was significant variable with *P. falciparum* cases increased by 6% (95% credible interval [CrI]: 3%, 9%) for a one mm increase in rainfall. There was no significant residual spatial clustering after accounting for climate and demographic variables. *P. falciparum* incidence had a positive association only with rainfall lagged at one month. This factor explained the observed spatial heterogeneity of infection.

329

FACTORS ASSOCIATED WITH ACCESS AND ADHERENCE TO ARTEMISININ-BASED COMBINATION THERAPY (ACTS) FOR TREATMENT OF FEVER IN CHILDREN UNDER FIVE: A SECONDARY ANALYSIS OF THE 2012 SIERRA LEONE MALARIA KNOWLEDGE, ATTITUDES, AND PRACTICES SURVEY

Kristin Banek[1], Emily L. Webb[1], Emily Bostick Doogue[2], Samuel Juana Smith[3], Daniel Chandramohan[1], Sarah G. Staedke[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Catholic Relief Services, Baltimore, MD, United States,* [3]*National Malaria Control Program, National Malaria Control Programme, Ministry of Health and Sanitation, Freetown, Sierra Leone*

To ensure effective malaria case management, patients must have access to artemisinin-based combination therapies (ACTs) and adhere to treatment guidelines. A secondary analysis of the 2012 Sierra Leone malaria Knowledge, Attitudes and Practices (mKAP) survey was conducted to investigate access and adherence to ACTs for treatment of fever in children under-five. The mKAP was a nationally representative, two-stage cluster-sample survey. The secondary analysis was restricted to children under-five with fever in the past two weeks. Factors associated with access and adherence were assessed using logistic regression. Of 5,169 enrolled households, 1,456 reported a child under-five with fever in the past two weeks, including a total of 1,641 children. Of these, 982 (59.8%) received malaria treatment and were analyzed for access; 467 (28.5%) received an ACT and were analyzed for adherence. Only 220 (13.4%) children with fever received an ACT and completed the recommended 3-day treatment. In a multivariable analysis, factors associated with ACT access included knowledge of ACTs (yes vs no; odds ratio [OR] 2.84, 95% CI: 2.07-3.89; p<0.001), knowledge of insecticide treated nets (ITNs) (yes vs no; OR 1.80, 95% CI: 1.25-2.58; p=0.001), source of care (public health facility vs other; OR 1.85, 95% CI: 1.27-2.71, p=0.002), geographic region (East vs West; OR 2.40, 95% CI: 1.26-4.55; p=0.018), and child age (24-59 vs 0-23 months; OR 1.46, 95% CI: 1.08-1.39; p=0.014). The only factor associated with ACT adherence was time to treatment; children treated within 24 hours were less likely to adhere (OR 0.58, 95% CI: 0.35-0.95; p=0.032). In 2012, access and adherence to ACTs remained low in Sierra Leone. Knowledge of ACTs and ITNs, and seeking care in the public sector, were most strongly associated with ACT access. Future national household surveys could expand the malaria treatment seeking section to capture indicators related to treatment adherence thereby providing critical information needed to realize optimal malaria treatment effectiveness.

330

RISK FACTORS FOR MALARIA, AND SPATIAL CLUSTERING OF CASES FROM A HOUSEHOLD SURVEY IN ARTIBONITE, HAITI

Karen E. Hamre[1], Nishant Kishore[2], Amber M. Dismer[1], Anyess R. Travers[3], Kathleen McGee[4], Baby Pierre[5], Kathleen Holmes[1], Eric Rogier[1], Jean Frantz Lemoine[5], Michelle A. Chang[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Harvard T.H. Chan School of Public Health, Cambridge, MA, United States,* [3]*University of Georgia, Athens, GA, United States,* [4]*Population Services International - Haiti, Peguy-Villy, Haiti,* [5]*Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti*

Malaria Zero Consortium partners are working together to accelerate malaria elimination in Haiti, where malaria distribution is heterogeneous. In such elimination settings, targeting interventions is essential to ensure efficient use of resources. Following a census in the study area, a cross-sectional household survey was conducted in the communes of La Chapelle and Verrettes, Artibonite, Haiti to identify and characterize spatial clustering of malaria cases. Of 8,818 sampled households, 79% participated; all consenting household members (N=21,566) were administered a questionnaire and tested for malaria. A case was defined as a person testing positive for *Plasmodium falciparum* by either conventional rapid diagnostic test (RDT) or a novel highly-sensitive RDT. Clusters were identified using SaTScan™ software, assuming a Poisson distribution. The associations between potential individual, household, and environmental risk factors for individual malaria risk and for risk of living in a spatial case cluster were evaluated using the *svy* estimation commands in Stata to account for the sampling weights and clustering at the household level. There were 161 malaria cases identified; median age was 15 years. Weighted malaria prevalence was low (0.56%, 95% CI: 0.45-0.71%), yet two predominant significant spatial case clusters were identified. Preliminary data from univariate logistic regression analyses suggest individuals who sleep under a bed net have lower risk of malaria as compared to those who do not (OR = 0.20, p &lt 0.01); as do shopkeepers when compared to farmers (OR = 0.41, p = 0.04). Individuals living in higher altitudes were at increased risk for having malaria and for living in a spatial cluster. Additional analysis and adjusted models will be presented. Haiti's National Malaria Control Program conducted a focus investigation, entomologic investigation, and vector control interventions. Identifying malaria clusters in low-transmission settings is an essential step for targeting interventions.

331

RAPID CHARACTERIZATION OF URBAN MALARIA TRANSMISSION — CONAKRY, GUINEA, 2018

Dean M. Sayre[1], Alioune Camara[2], Yaya Barry[2], Toure B. Deen[2], Denka Camara[2], Mohamed Dioubate[2], Ibrahima Camara[2], Kalil Keita[2], Nouman Diakite[2], Youssoufa Lo[2], Ibrahima Bah[3], Hadja F. Camara[4], Mohamed S. Conde[4], Aissata Fofana[4], Abdoulaye Sarr[5], Eugene K. Lama[2], Seth Irish[1], Mateusz Plucinski[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*National Malaria Control Program, Conakry, Guinea,* [3]*Catholic Relief Services, Conakry, Guinea,* [4]*Stop Palu+, Conakry, Guinea,* [5]*Centers for Disease Control and Prevention, Conakry, Guinea*

In 2018 the Guinean malaria control program requested assistance to rapidly estimate the extent of malaria transmission in Conakry to inform the planning for a national bednet distribution campaign. As is typical for urban settings, the reported malarial burden in Conakry was small compared to the remainder of the country, but the true risk of local disease acquisition was unknown because the proportion of cases resulting from importation was undefined. To inform decision-making on the need for bednet distribution in the city, we performed a combined entomological and epidemiological study to determine the risk of local acquisition of malaria in ten sites across Conakry. Mosquito populations were characterized by human landing catches, and cross-sectional surveys were used to measure household access to bednets and malaria infection prevalence (by rapid diagnostic test). Data regarding recent travel-related exposures from outpatients tested for malaria in 25 health centers were used to calculate risk ratios (RR) and population attributable fractions of risk (PAR) related to travel. *Anopheles* mosquitoes were found throughout Conakry, with an average of 7.1 (range: 0 - 21) females captured per night. Only 16.7% (170/1016) of sleeping spaces were protected by a hanging bednet. Prevalence of parasitemia among all ages was 5.2% (57/1102), with 75.4% (43/57) of those testing positive reporting no travel outside the city in the last 4 weeks, and 50.9% (29/57) not traveling in the last year. Among 4,678 outpatients, travel within 4 weeks of clinical presentation was a risk factor for malaria infection (RR: 2.2; 95% confidence interval: 2.0-2.4), though the PAR for travel was 8.7%. Malaria transmission in Conakry appeared heterogeneous, with the highest disease prevalence (10.7%) and most favorable conditions for transmission (21 female *Anopheles* captured per night, 7.5% of sleeping spaces with a bednet hanging) in Kaloum, the most urbanized portion of the city. Many malaria cases reported from Conakry appear to result from local transmission. Results of this assessment should inform decision-making on where to target future bednet distribution.

332

USING AMPLICON DEEP SEQUENCING TO CHARACTERIZE CLONAL EXPANSION OF A KELCH 13 R622I MUTANT IN GONDAR, ETHIOPIA

Daniel R. Castaneda-Mogollon[1], Abebe Genetu Bayih[2], Aberham Abere[2], Ranmalee Amarasekara[1], Habtie Tesfa[2], Dylan R. Pillai[1]

[1]*University of Calgary, Calgary, AB, Canada,* [2]*University of Gondar, Gondar, Ethiopia*

According to the WHO, almost two thirds of the Ethiopian population are at risk of contracting malaria, where infection with *Plasmodium falciparum* accounts for approximately 60% of cases today. The risk of artemisinin resistance spreading from SE Asia to Africa is a major concern. We conducted a 28-day *in vivo* efficacy trial of Artemether-Lumefantrine (Co-Artem) for treatment of uncomplicated malaria (n=97) in Gondar Region, NW Ethiopia in 2017-2018. Our data showed 100% adequate clinical and parasitological response (ACPR). Further analysis of day 0 samples showed the expansion of a kelch13 mutation R622I to 9.5% from 2.4% over a 3 year period. In order to better understand the emergence of this mutation and whether it is a sentinel of emerging resistance, we assessed the haplotypes and complexity of infection (COI) by employing PCR and amplicon deep sequencing of the genetic markers msp1, msp2 and the conserved *Plasmodium* membrane protein (cpmp) at day 0 and day 7. Sequences were subject to a bioinformatics pipelines (SRST2, PoolHap, DADA2) that could assess SNP and haplotype calling across each data point. The complete dataset is currently under analysis and will be presented at the meeting. Understanding the infection dynamics of *P. falciparum* in this region could provide a better insight of the geographic extension of emerging artemisinin-resistant clones and its implications in public health. Furthermore, clone dominancy variation over time may explain why some individuals have different clinical manifestations. Nevertheless, additional genotyping studies are required to assess the surveillance of artemisinin-resistant *Plasmodium* species across the region

333

GLOBAL SURVEILLANCE OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG RESISTANCE AND DIAGNOSTIC TEST EVASION

Christiane Prosser[1], Rogan Lee[1], Wieland Meyer[2], John Ellis[3]

[1]*University of Sydney, Sydney, Australia,* [2]*Westmead Institute for Medical Research, University of Sydney, Sydney, Australia,* [3]*School of Life Sciences, University of Technology Sydney, Sydney, Australia*

Emerging drug resistance in *Plasmodium falciparum* imperils malaria control and elimination. Resistance to the frontline drug artemisinin, and partner drugs, is spreading throughout South East Asia. Deletion of *pfhrp2/3* genes (results in false negatives from HRP-based rapid diagnostic tests) has emerged and is thought to be under section globally. This project aimed to investigate imported malaria cases from travellers and refugees entering Australia, and field isolates from Southern Thailand. Malaria diagnostic samples from the NSW parasitology reference lab (n=205) and samples collected from health centres in 4 provinces of South Thailand (n=91) were screened for known markers of drug resistance including *pfK13* mutations underlying artemisinin resistance. The cohort was also screened for the presence of exons 1 and 2 for both *pfhrp2* and *pfhrp3*. This cohort captured many endemic regions with limited ongoing surveillance or no previous data on these markers - notably South Sudan, Sudan, Sierra Leone, and districts of Thailand bordering Malaysia. Epidemiologically relevant deidentified patient data associated with each case was analysed. Seven microsatellites were typed for the Sudanese and South Sudanese cohort (n=56) to identify phylogenetic clustering of HRP2/3 positive and negative parasites found. Propeller domain *pfK13* mutations were observed, including the C580Y mutation most strongly associated with artemisinin resistance. C580Y was found in an indigenous parasite originating from Papua New Guinea, where resistance *pfK13* mutations were previously unreported. C580Y mutations were additionally observed (n=31/91) in Southern districts of Thailand, including Yala, where resistance mutations were previously unreported. The findings demonstrate the utility of screening travellers as sentinels and the need for more comprehensive molecular surveillance of emerging malaria drug resistance and diagnostic test evasion.

334

FRAGMENTED POPULATION STRUCTURE OF *PLASMODIUM VIVAX* ASSOCIATED WITH THE DECLINE TRANSMISSION FACILITATE THE MALARIA SURVEILLANCE AND TARGET CONTROL IN THE GREATER MEKONG SUBREGION

Li Yuling[1], Cao Yaming[1], Wang Qinghui[1], Cui liwang[2]

[1]*China Medical University, Shenyang, China,* [2]*Department of Internal Medicine, South Florida, SC, United States*

The Asia Pacific Leaders in Malaria Alliance (APLMA) have committed to eliminate malaria from the region by 2030. Since the initiation of the WHO's Mekong Malaria Program a decade ago, malaria situation in the Greater Mekong Subregion (GMS) has greatly improved, but with the dramatically decline in annual malaria incidence and deaths, *Plasmodium vivax* has become the dominant malaria infection in some parts of this region. Therefore, to gain a better understanding of transmission dynamics and population structure of *P. vivax* in the GMS is imperative and beneficial to malaria control programme. We investigate genetic diversity and population structure in four geographically and ecologically distinct regions of The Greater Mekong Subregion. A total of 200 *P. vivax* isolates were from Yunnan, Anhui province of China, Thailand and China-Myanmar border regions (50 for each population) were analysed using 10 microsatellite markers. A wide range of genetic diversity was observed in the four populations reflecting a spectrum of transmission intensities across Greater Mekong Subregion. Fragmented population structure and genetic differentiation between regions was evident, suggest limited gene flow among these sites. a small panel of microsatellite markers was sufficient to assign the parasites to their geographic origins. In conclusion, with enhanced control efforts on malaria elimination, *P. vivax* population in the Greater Mekong Subregion has fragment into a limited number of clustered *foci*, but the presence of large *P. vivax* reservoirs still sustain transmission. The insights into *P. vivax* transmission dynamics and population structure will inform targeted strategies to *P. vivax* malaria surveillance and track parasites in this area.

335

SELECTIVE SWEEPS AND GENETIC LINEAGES OF *P. FALCIPARUM* CHLOROQUINE RESISTANCE GENE, *PLASMODIUM FALCIPARUM* DIHYDROPTEROATE SYNTHASE AND *P. FALCIPARUM* DIHYDROFOLATE REDUCTASE GENES IN KENYA

Marcel Nyabute[1], Dennis Juma[1], Penninah Muiruri[2], Benjamin Opot[1], Raphael Okoth[1], Martha Nginya[1], Jennifer Mutisya[1], Brenda Mugambi[1], Agnes Cheruiyot[1], Gladys Chemwor[1], Redemptah Yeda[1], Charles Okello[1], Hoseah Akala[1], Ben Andagalu[1], Jim Ray Managbanag[1], Edwin Kamau[3]

[1]*US Army Medical Research Directorate, Kenya, Kisumu, Kenya,* [2]*Department of Biochemistry; Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [3]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Washington, MD, United States*

With the continued widespread use of antimalarial agents, molecular tools are valuable for determining evolutionary history and the prevalence of drug-resistant malaria parasites. These tools have helped to predict decreased sensitivity to antimalarials and fixation of multidrug resistance genotypes in some regions. Systematic studies of selective sweeps provides information on origin and evolution and have previously shown that chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) resistance originated from Thailand - Cambodia border before eventually spreading to other Asian countries and sub-Saharan Africa. This trend has not been established for East Africa. This study investigated putative CQ and SP

drug resistance-associated gene mutations and genetic lineages for field isolates collected across Kenya to describe their origin and evolution. The *Pfcrt*, *Pfdhps,* and *Pfdhfr* mutations associated with CQ and SP resistance and the microsatellite *loci* flanking these genes were genotyped in 350 *Plasmodium falciparum* isolates from Kenya to give insights into the origin and spread of drug resistance *loci*. For *Pfcr*t, proportion of isolates carrying wild-type CVMNK was 90.1% while 9.5% had the mutant haplotype CVIET. 94.4% isolates had the *Pfdhfr* triple mutant haplotype CIRNI at positions 51, 59 and 108. For *Pfdhps*, 97.8% of the isolates had double mutant haplotype SGEAA at position 437 and 540. Initial analysis of genetic diversity and differentiation at microsatellite *loci* flanking all 3 genes appears to suggest that they have been under strong selection, because of CQ and SP use. The high SP resistance witnessed across Kenya was likely due to fixation of key mutations in the *Pfdhfr* and *Pfdhps* genes as well as drug pressure from other antifolate drugs being used to treat malaria and other infections. The sustained SP-resistant haplotypes, *Pfdhfr* 51I/59R/108N and *Pfdhps* 437G/540E despite withdrawal of SP as the first-line treatment in Kenya warrants continued evaluation of SP efficacy as long as IPTp-SP is in force. Wild-type *Pfcr*t 76 heralds possibility of re-introduction of CQ in combination with other malaria drugs.

336

EVALUATION OF *PLASMODIUM FALCIPARUM* HISTIDINE RICH PROTEIN 2 AND 3 (*PF*HRP2 *AND PF*HRP3) GENES POLYMORPHISMS IN KENYA

Martha N. Kivecu[1], Victor Mobegi[2], Brenda Makena[1], Raphael Okoth[1], Gladys Chemwor[1], Marcel Juma[1], Edwin Mwakio[1], Jackline Juma[1], Charles Okello[1], Redemptah Yedah[1], Agnes Cheryuiyot[1], Benjamin Opot[1], Dennis Juma[1], Hoseah Akala[1], Ben Andagalu[1], Jim R. Manangbanang[1]

[1]*Us Army Medical Research Directorate- Africa, Kenya (USAMRD-A), Kisumu, Kenya,* [2]*The University of Nairobi, Nairobi, Kenya*

Globally, 74% of malaria diagnoses are by malaria Rapid Diagnostic Test (RDT). The accuracy of *Pf*HRP2-based RDTs can be impaired by either deletion in *Pf*HRP2 gene or cross-reaction with *Pf*HRP3 antibodies. The World Health Organisation (WHO) criteria that require greater than 95% accuracy as the threshold for selection or withdrawal of RDTs argue for active mapping of *Pf*HRP2 deletions. As part of the on-going epedemiology of malaria drug resistance work, this study aims to determine the frequency and trends of *Pf*HRP2 and *Pf*HRP3 gene mutations and deletions in four of five malaria transmission regions in Kenya. 350 samples collected between 1998 and 2017 were diagnosed for malaria by microscopy, RDT and 18S rRNA PCR. All *P. falciparum* PCR positive samples were amplified then sequenced and multiple sequence alignment (MSA) done to detect *Pf*HRP2 and *Pf*HRP3 mutations. Additionally, twelve neutral microsatellites markers were amplified and parasites population structure determined. 255 samples of the 350 were collected between 2013 and 2017. 220 of 255 samples were *P. falciparum* positive by PCR, of these, 37(16.8%) were negative by *Pf*HRP2 based RDT. This appears to suggest possible *Pf*HRP2 and *Pf*HRP3 genes deletion. However, 29 of the 37 RDT negative samples were positive for both *Pf*HRP2 and *Pf*HRP3 amplification, 5 and 3 samples were positive for *Pf*HRP2 and*Pf*HRP3 respectively. 95 of the 350 samples were collected between 2003 and 2005, 89(93.7%) of these samples were positive for *P. falciparum* by PCR. RDT diagnosis was not carried out on these samples as only DNA samples were available. *Pf*HRP2 and *Pf*HRP3 genotyping did not clarify variability in RDT reactivity. Therefore, sequence analyses of these fragments are underway for additional clarification of polymorphisms within the *Pf*HRP2 and *Pf*HRP3 regions. Additionally, 12 microsatellites genotyping is underway to determine the population structure. Findings will guide policy decision on the relevance of continued deployment of *Pf*HRP2 based RDTs across Kenya. Temporal and population structure analysis will verify the role of evolution in the distribution of the *Pf*HRP2 and *Pf*HRP3 aberrant genotypes.

337

TEMPORAL CHANGE OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF *PLASMODIUM VIVAX* IN THREE CONTRASTING SETTLEMENTS IN THE PERUVIAN AMAZON

Paulo C. Manrique Valverde[1], Roberson Ramírez Saavedra[1], Mitchel Guzman Guzman[1], Alejandro Llanos Cuentas[2], Joseph Vinetz[3], Ananias A. Escalante[4], Dionicia Gamboa Vilela[5]

[1]*Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofa, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Yale School of Medicine, Section of Infectious Diseases, Department of Internal Medicine, New Haven, CT, United States,* [4]*Institute for Genomics and Evolutionary Medicine (IGEM), Temple University, Philadelphia, PA, United States,* [5]*Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru*

Despite efforts made to eliminate malaria, *Plasmodium vivax* remains a challenge in Peru. The high heterogeneity of its transmission has limited the intervention measures based on global and generalized epidemiological information at medium and long term. Population genetic tools may provide information on the spatial heterogeneity of transmission by linking parasite genetic patterns with epidemiological information. In this study, the genetic diversity and population structures from three settlements located in the Peruvian Amazon, Cahuide (CAH), Lupuna (LUP) and Santa Emilia (STE) were measured on samples collected from September 2012 to March 2015. A total of 777 *P. vivax* mono-infections were genotyped. LUP showed the lowest proportion of polyclonal infections (0.197 CI95% 0.159 - 0.241) and genetic diversity (*Hexp* 0.544 ± 0.0012) in the whole study period. Moreover, in this study area results showed a significant increment of polyclonal infections and *Hexp* ($p < 0.005$), and the introduction and persistence of a new parasite population from March 2014 onward. STE showed the highest proportion of polyclonal infections (0.401 CI95% 0.323 - 0.485) and *Hexp* (0.596 ± 0.0028) among study areas, the presence of 4 genetic clusters without signals of clonal expansion and infections with lower parasite densities compared to the other two areas. Even though CAH showed the introduction of at least 4 parasite populations in 2012, a clear reduction of cases (from 213 to 61), a subtle reduction of polyclonal infections (from 0.286 to 0.18), and an unpredicted behavior of *Hexp* were seen since June 2014. These results suggest that LUP and mainly STE are two areas that maintain basal levels of transmission and that there are factors that allow the reintroduction of *P. vivax*. In contrast, malaria cases in CAH are circumstantial, thus, receptivity to malaria transmission and vulnerability to the importation of new parasite population vary drastically among the different study areas analyzed, and this information must be considered in the design of current control strategies.

338

PLASMODIUM VIVAX GENETIC POLYMORPHISM IN DIFFERENT BRAZILIAN ENDEMIC AREAS

Natália Ketrin Almeida de Oliveira[1], Rebecca Abreu Santos[1], Anielle Pina Costa[2], Patrícia Brazil[2], Cláudio Tadeu Daniel-Ribeiro[1], Maria de Fátima Ferreira da Cruz[1]

[1]*Oswaldo Cruz Institution, Rio de Janeiro, Brazil,* [2]*National Institute of Infectology/Fiocruz, Rio de Janeiro, Brazil*

Plasmodium vivax is the predominant species in Brazil, representing 87% of malaria cases. The country presents two malaria transmission autochthonous profiles: one in Amazon Basin (AB), where 99.7% of reported cases are occurred, and the other in non-endemic regions of Atlantic Forest (AF). The polymorphisms dynamic of circulating genotypes from different geographical areas provide insights into population structure generating inferences on gene flow patterns. Polymorphic genes encoding antigens, potential candidates to compose a malaria vaccine, have been useful to monitor changes in parasite genetic diversity. There are no data on evaluation of *P. vivax* antigen diversity in isolates

from endemic and non-endemic Brazilian regions. Thus, we investigated polymorphisms in MSP-1 and DBP-II genes in these two Brazilian malaria transmission scenarios. For this end, genomic DNA was isolated from 1 ml-whole blood using QIAamp columns and gene fragments were amplified by PCR using previously published primers. DNA sequencing was carried out after purification using the Wizard SV Gel and PCR Clean-Up System. Analysis of 248 isolates, 180 from AB and 68 from AF, showed that polymorphisms in MSP-1 (57 SNPs; 53 non-synonymous) was higher than those of DBP (27 SNPs; 26 non-) in all studied localities. Among MSP-1 mutations, 7 (12%) were specific of AF and 5 (9%) of AB localities. While in DBP, 5 (18%) mutations were specific of AF and 1 (4%) of AB. We concluded that these genes are continuous evolving under immune selective pressure and polymorphic sites are indicative of balancing positive selection on antigen molecules. In addition, these preliminary data suggest that the presence of 12 SNPs is indicative of *P.vivax* parasites from non-endemic Brazilian areas (AF).

339

POPULATION STRUCTURE OF *PLASMODIUM MALARIAE* USING MICROSATELLITES AND SELECTIVE WHOLE GENOME SEQUENCING

Eniyou C. Oriero[1], Deus S. Ishengoma[2], Lucas Amenga-Etego[3], Soulama Issiaka[4], Tobias Apinjoh[5], Umberto D'Alessandro[1], Abdoulaye Djimde[6], Martin Meremikwu[7], Alfred Amambua-Ngwa[1]

[1]*Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine, Fajara, Gambia,* [2]*National Institute for Medical Research, Tanga, United Republic of Tanzania,* [3]*University of Ghana, WACCBIP, Department of Biochemistry, Cell and Molecular Biology, Legon, Ghana,* [4]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [5]*Faculty of Health Science, University of Buea, Buea, Cameroon,* [6]*Malaria Research and Training Center, University of Science, Techniques and Technology, Bamako, Mali,* [7]*Calabar Institute Of Tropical Disease Research & Prevention, University Of Calabar Teaching Hospital, Calabar, Nigeria*

Plasmodium malariae, one of five species that infect humans, is prevalent across sub-Saharan Africa. Recent completion of its reference genome paves way for new genomic studies aimed at understanding the biology of this parasite species. We characterized *P. malariae* samples from across sub-Saharan Africa using microsatellites and selective whole genome amplification (sWGA) techniques. Samples from Nigeria were obtained from rapid diagnostic test (RDT) positive individuals who attended health facilities in Cross River State, Nigeria, in October - November 2017, following community sensitization and consent. Additional samples were accessed from strategic collaborations (including DELGEME and PDNA) across sub-Saharan Africa, namely Tanzania, Burkina Faso, Mali, Ghana, Guinea and Cameroon. *Plasmodium* species were confirmed by real-time PCR (PrimerDesign®, UK). Five highly divergent microsatellites (Pm_02, Pm_09, Pm_11, Pm_34 and Pm_47) were used to genotype the *P. malariae* samples. Confirmatory real-time PCR revealed a total of 74 *P. malariae* samples (Nigeria - 18, Tanzania - 19, Burkina Faso - 17, Mali - 9, Ghana - 3, Guinea - 4 and Cameroon - 4). No population structure was detected with the five microsatellites. Towards population genome sequencing, dried blood spot (DBS) and leucocyte depleted (CF11 column filtration) *P. malariae* infected blood samples were subjected to sWGA using primers designed in-house. A combination of Illumina and Nanopore sequencing approaches are envisaged. Initial *P. malariae* genome sequence coverage remain patchy as most infections were mixed (*P. falciparum)*. Bioinformatics techniques for deconvolution of mixed genomes will be explored. Knowledge of population structure and diversity of *P. malariae* across endemic regions will help in monitoring local and regional transmission patterns. New sWGA approaches are needed to selectively improve *P. malariae* coverage in mixed species infections and to determine its contribution to the malaria burden.

340

EVALUATION OF A POOLED STRATEGY FOR TARGETED AMPLICON DEEP SEQUENCING OF *PLASMODIUM FALCIPARUM* DRUG RESISTANT ASSOCIATED GENES *DHFR* AND *MDR1*

Camelia Herman[1], Julia Kelley[1], Eldin Talundzic[2]

[1]*CDCF, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Sequencing large numbers of individual samples, which is often needed for countrywide surveillance-based studies, remains expensive despite the decreasing costs of next generation sequencing (NGS). Pooling several individual samples prior to gene enrichment, NGS library prep and sequencing is potentially an alternative cost and time effective method. Using 100 *P. falciparum* samples from a nationwide Haiti drug resistance surveillance study, we compared allele frequency (AF) calls for *dhfr* and *mdr1* drug resistance genes between individual and pooled deep sequenced samples. The individual sequenced samples were used to determine the expected allele frequency of the pooled samples. Samples with similar real-time PCR cycle threshold (CT) values (+/- 1.0 CT value) were combined into ten different pools, with 10 samples per pool. The CT value was used to estimate parasite DNA in each individual sample and ensure that similar parasite DNA volumes (5.0uL) were pooled. In *dhfr* only the S108N and in *mdr1* the Y184F drug resistant mutations were identified. The actual AF calls for the pooled samples were as follows, *dhfr* S108N: group 1 to 6: 45%, 12%, 47%, 51%, 86% and 60%, showing a difference of +/- 10.15% when compared to the expected AFs (e.g., sum of individual AFs /total number of pooled samples = expected pooled sample AF). In comparison, *mdr1* Y184F had an AF of 100% for all six groups and a difference of 1.67% when compared to the expected AFs. The remaining four pooled sample groups with a CT values ≥34.0 (e.g., <100 parasites per uL/blood) showed a difference of greater than 34% or more between the actual and expected AFs. This may have been due to low amounts of parasite DNA (indicated by a CT value ≥ 34.0), which can lead to variable PCR amplification efficiencies of the *dhfr* and *mdr1* genes. This method of grouping samples based on CT values prior to pooling can provide higher confidence that unbiased AFs are obtained in pooled deep sequencing experiments. With further validation, this method may be used as a screening approach to rapidly genotype large number of samples for drug resistant markers in population-based studies.

341

INSECTICIDE RESISTANCE GENE MUTATIONS IN *ANOPHELES STEPHENSI, AN. ARABIENSIS*, AND *CULEX PIPIENS S.L.* IN EASTERN ETHIOPIA

Tamar E. Carter[1], Lambodhar Damodaran[2], Shantoy Hansel[3], Callum Montgomery[3], Victoria Bonnell[4], Karen Lopez[3], Daniel Janies[3], Solomon Yared[5]

[1]*Baylor University, Waco, TX, United States,* [2]*University of Georgia, Athens, GA, United States,* [3]*University of North Carolina at Charlotte, Charlotte, NC, United States,* [4]*Pennsylvania State University, State College, PA, United States,* [5]*Jigjiga University, Jigjiga, Ethiopia*

The recent detection of the primarily South Asian malaria vector species *Anopheles stephensi* in the Horn of Africa emphasizes the importance of continued vector surveillance. Genetic analysis of insecticide resistance mutations across multiple species of Culicidae in the Horn of Africa provides an important ecological perspective on the potential impact of insecticides on *An. stephensi*. In this study, the voltage gated sodium channel (*vgsc*) and acetylcholinesterase (*ace-1*) genes were analyzed in *An. stephensi, An. arabiensis*, and *Culex pipiens s. l.* collected in east Ethiopia between 2016 and 2017. A portion of both genes that contain known insecticide resistance mutations (*vgsc* kdr L1014 and ace-1 G119) were amplified by polymerase chain reaction (PCR) and then sequenced with Sanger technology. Frequencies of resistance mutations were calculated, and nucleotide diversity was analyzed. *Vgsc* sequence analysis revealed no L1014 mutations in any *An. stephensi* (n=129) specimens, while 100% of

Cu. pipiens s.l. (n = 42) collected in the same location were homozygous for the L1014F mutation. Of the *An. arabiensis* (n=67), 71.6% carried the kdr L1014F mutation (heterozygous and homozygous). Analysis of the *vgsc* sequence including a downstream intron in all three species revealed nucleotide diversity only in *An. stephensi* (Pi= 0.001; haplotypes, h = 5; haplotype diversity; H_d = 0.135). The results of neutrality tests varied across species, with no evidence of non-neutral evolutionary processes for *An. stephensi* sequences. The *ace-1* G119 mutation was examined in *An. stephensi* and *An. arabiensis* and was absent in both species. Overall these results indicate that some Culicidae have been impacted by pyrethroid insecticides. The absence of the kdr mutation and lack of evidence for selection on the *vgsc* in the *An. stephensi* may be explained by one of the following: 1) *An. stephensi* is new to the region and not impacted by resistance like sympatric *Cu. pipiens* s. l. or 2) *An. stephensi* has other mechanisms of resistance. Further studies on additional sites will provide insight into the status of *An. stephensi* resistance in Ethiopia and its history in the region.

342

DISTINGUISHING AMONG *PLASMODIUM VIVAX* RELAPSES AND NEW INFECTIONS IN A LOW ENDEMIC AREA: A POPULATION GENETICS APPROACH

Christopher Delgado Ratto[1], Verónica E. Soto-Calle[2], Annette Erhart[3], Peter Van den Eede[4], Eliana Torres[2], Luis Sánchez-Martínez[2], Juan Contreras-Mancilla[5], Anna Rosanas-Urgell[4], Hugo Rodríguez Ferrucci[6], Alejandro Llanos-Cuentas[2], Umberto D'Alessandro[3], Jean-Pierre Van geertruyden[1], Dionicia Gamboa Vilela[2]

[1]*University of Antwerp, Antwerp, Belgium,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Medical Research Council Unit at the London School of Hygiene & Tropical Medicine, Fajara, Gambia,* [4]*Institute of Tropical Medicine, Antwerp, Belgium,* [5]*Universidad Peruana Cayetano Herediaerp, Lima, Peru,* [6]*Ministry of Health of Peru, Iquitos, Peru*

We used the population genetics information of the local parasite population retrieved from a 2-year follow up study carried in the Peruvian Amazon to provide an estimation of the origin of the 1st *P.vivax* recurrence experienced after the radical cure treatment, chloroquine (CQ) plus primaquine (PQ), was administered. A systematic blood sampling was performed weekly up to day 28 and later monthly after a direct observed treatment (3-day CQ+ 7-day PQ) until month 24. Recurrent parasites were detected by microscopy and PCR; and genotyped using 14 microsatellites. Population genetics analysis was performed. We compared D0 and 1st recurrence to classify recurrent parasites as homologous, highly related heterologous or heterologous parasites. Finally, the probability of reinfection with homologous or highly related heterologous parasites (*Pmatch)* was calculated to estimate the origin of the 1st recurrence. After treatment, 197 (65.2%) participants had at least one recurrence within the 2-year follow up and the median time to the 1st recurrence was 242 days. Up to month 6, recurrences carried predominately heterologous parasites (75%) and less homologous parasites (25%). Geographic population sub-structure and no significant temporal changes along the follow-up within areas were found. The likelihood of experiencing a 1st recurrence with relapsing homologous parasites varied according to the origin ($p<0.001$). Moreover, relapses with highly related heterologous parasites were also found. At 6-month post-treatment, the unadjusted relapse cumulative incidence (all homologous considered as relapses) was 10.7% (95%CI:7.4,15.3) whereas after adjustment decreased to 7.5% (*Pmatch*: homologous + highly related) and 5.1% (*Pmatch*: only homologous). The likelihood of experiencing a relapse with homologous or highly related parasites was affected by the genetic population structure of the local parasites. Unraveling of population genetics of local parasites can be used as additional tool to assess the efficacy of radical cure treatments.

343

RBTAP; A NOVEL REFERENCE BASED TOOL TO ASSEMBLE POLYMORPHIC GENES IN MALARIA

Saikou Y. Bah[1], Gordon A. Awandare[1], Thomas D. Otto[2]

[1]*West African Centre for Cellular Biology of Infectious Pathogens, Accra, Ghana,* [2]*Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom*

Thousands of malaria parasites have been sequenced and analysed often ignoring highly polymorphic genes which often hold significant genomic information. The sequencing reads do not map, or the amount of variation is too high to reliably detect polymorphisms by mapping based approaches. Therefore, alternative approaches are needed because de novo assembly requires high level bioinformatics skills and compute. Available reference genomes from long read sequencing, can be used as templates to obtain more information on these variable genes. Our primary objective is to develop a tool that allows laboratories with little computational power to access the sequencing data from large consortia to assess diversity of genes of interest. The tool needs the following inputs 1) The genome assemblies of the samples to be analysed, 2) The genomic reference of the *Plasmodium*, 3) Coordinates of the gene, and 4) Reference genes of interest from long reads. The multiplicity of infection (MOI) is estimated using the estMOI package. Reads mapping to genes of interests and unmapped reads are extracted from genomes and mapped to long reads gene alleles. If the MOI is 1 a consensus fasta is generated from the best mapping allele and gaps closed using iCORN2 and IMAGE. If MOI is greater than 1, consensus sequence is generated for the MOI with best coverage for phylogenetics. We applied the tool to the MSP gene family of 2500 P falciparum genomes in the Pf3k dataset from different regions. As reference set, we used 16 PacBio reference genomes. It took 15-20 minutes on laptop computer to run a sample to generate a consensus sequence with closed gaps for phylogenetic analysis. The tool accurately generates consensus sequences from highly polymorphic genes for phylogenetics and can be used in laboratories with limited computational and bioinformatic capacity for genomics comparisons. The tool will be made freely available on GitHub.

344

GENOMIC ANALYSIS OF *PLASMODIUM VIVAX* CLINICAL ISOLATES FROM THE CHINA-MYANMAR BORDER AND NEIGHBORING COUNTRIES IN SOUTHEAST ASIA PROVIDES INSIGHTS INTO GENETIC DIVERSITY AND PARASITE POPULATION STRUCTURE

Sonia Agrawal[1], Fang Huang[2], Biraj Shrestha[1], Matthew Adams[3], Sandra Ott[4], Lisa D. Sadzewicz[4], Hui Lui[5], David Serre[4], Myaing M. Nyunt[6], Joana C. Silva[4], Christopher V. Plowe[6]

[1]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*National Institute of Parasitic Diseases, Chinese Centre for Disease Control and Prevention, Shanghai, China,* [3]*University of Maryland School of Medicine, Baltimore, MD, United States,* [4]*Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States,* [5]*Yunnan Institute of Parasitic Diseases, Pu'er, China,* [6]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Plasmodium vivax malaria has been refractory to malaria elimination efforts in Southeast Asia, especially in the border areas shared between the countries. Better understanding of the genomic epidemiology of *P. vivax* could inform these efforts by improving our understanding of parasite population structure, potentially leading to new tools for distinguishing between local and imported infections, and for identifying locations that serve as transmission sources and sinks. The objective of this study was to characterize and compare genetic diversity and population structure of circulating isolates of *P. vivax* in areas of low, intermediate and high *P. vivax* prevalence in South and Southeast Asia, using parasite whole genome sequences from clinical infections. Whole genome sequence data were generated from 73 leukocyte-depleted, RDT-positive, field samples with

low *P. vivax* genomic DNA collected from a refugee camp located adjacent to the border between Laiza Township in Myanmar's Kachin State and the town of Nabang in China's Yunnan Province. These new data were compared with publicly available sequences from 361 *P. vivax* clinical isolates from India, Sri Lanka, Myanmar, China, Thailand, Lao, Cambodia and Vietnam. Samples from the China-Myanmar border showed extensive population structure with 79% monoclonal infections and 46% genetically similar isolates. Furthermore, measures of population differentiation revealed relatively low between-country genome-wide differentiation ($0.006 < FST < 0.058$) but high differentiation between all Asian countries and this border population ($0.15 < FST < 0.26$). Taken together these results suggest the presence of an isolated *P. vivax* population in the China-Myanmar border. A modest decline in genetic diversity and proportion of polyclonal infections with decreasing transmission across Southeast Asia was also observed. The analyses showed that genomic surveillance could be used as a powerful tool for monitoring and tracking parasite populations using field-collected samples in these pre-elimination settings.

345

ROLE OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR AND HUMAN LEUKOCYTE ANTIGEN-C GENETIC VARIANTS IN TRANSMISSION AND SEVERITY OF MALARIA IN UGANDA

Stephen Tukwasibwe[1], James Traherne[2], Olympe Chazara[2], Arthur Mpimbaza[1], Jyothi Jayaraman[2], John Trowsdale[2], Ashley Moffett[2], Francesco Colucci[2], Moses R Kamya[1], Joaniter I Nankabirwa[1], Grant Dorsey[3], Philip J Rosenthal[3], Stephen Cose[4], Annettee Nakimuli[1]

[1]*Makerere University, Kampala, Uganda,* [2]*University of Cambridge, Cambridge, United Kingdom,* [3]*University of California, San Francisco, CA, United States,* [4]*London School of Hygiene & Tropical Medicine/MRC, London, United Kingdom*

The ability of NK cells to produce inflammatory cytokines early in malaria infections and their role in direct cytotoxic killing of malaria infected red blood cells suggest a beneficial impact of NK cells in malaria. NK cell activity is modulated by interactions between Killer Cell Immunoglobulin-like Receptors (KIR) and Human Leucocyte Antigen (HLA). Several studies have shown certain KIR and HLA genes to be associated with decreased risk of severe malaria and to have varied prevalence in different populations. However, these studies have been limited by low resolution genotyping. Structural and copy number variation (CNV) in KIR and HLA genes have not been considered in malaria studies. We hypothesized that specific KIR and HLA-C genetic variants are associated with severe malaria among Ugandan children and that the prevalence of KIR and HLA-C variants differ in Ugandan populations with varied malaria transmission. We used high resolution multiplex qPCR to genotype for KIR and HLA-C structural and CNV in samples from two clinical studies in Uganda. First, we evaluated samples from 900 children, aged 6 months to 14 years enrolled in a case control study of children presenting with severe or uncomplicated malaria. Second, we evaluated samples from 1500 individuals residing in regions of Uganda with historically different malaria transmission. Preliminary results showed that the prevalence of KIR2DL1 was significantly higher in children with severe malaria compared to those with uncomplicated malaria (93% vs 76%; $p<0.0001$). There was no association seen between HLA-C variants and malaria severity. The prevalence of KIR2DL1 was significantly lower in Tororo, a historically high malaria transmission region compared to Jinja and Kanungu (84% vs 93%; $p=0.0014$ and, 84% vs 95%; $p<0.0001$). The prevalence of HLA-C2C2 was significantly higher in Kanungu compared to Tororo and Jinja (34% vs 27% vs 21%; $p=0.0039$). Malaria evolutionary pressure may have selected for KIR and HLA genetic variants that reduce transmission and susceptibility to severe malaria, but potentially increase risks of other diseases.

346

A SUICIDE-RESCUE-BASED CRISPR/CAS9 SYSTEM COMPETENT FOR LARGE DNA FRAGMENT KNOCK-IN AND SEQUENTIAL GENE EDITING IN *PLASMODIUM FALCIPARUM*

Ying Tong[1], Junnan Lu[2], Minghong Zhang[1], Rui Dong[1], Li Qin[1], Xiaoping Chen[3]

[1]*CAS-lamvac Biotech Company, Guangzhou, China,* [2]*Center for Synthetic Genomics, Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China,* [3]*Laboratory of Pathogen Biology, State Key Laboratory of Respiratory Disease, Center for Infection and Immunity, Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou Institutes of Biomedicine and Health (GIBH), Guangzhou, China*

CRISPR/Cas9 technology applied to *Plasmodium falciparum* offers the potential to greatly improve gene editing, but such expectations including large DNA fragment knock-ins and sequential gene editing, have remained unfulfilled. Here, we achieved an advance in addressing this challenge, especially for creating large DNA fragment knock-ins and sequential editing, by modifying our suicide-rescue-based system that has already been demonstrated to be highly efficient for conventional gene editing. This improved approach was confirmed to mediate efficient knock-ins of DNA fragments up to 6.3 kb, to produce "marker-free" genetically engineered parasites and to edit genes sequentially using the same select marker. This represents an important advancement in establishing platforms for large scale genome editing, which might gain a better understanding of gene function for the most lethal cause of malaria and contribute to adjusting synthetic biology strategies to live parasite malaria vaccine development.

347

MULTIPLICITY OF *PLASMODIUM FALCIPARUM* INFECTIONS IN ASYMPTOMATICALLY INFECTED CHILDREN IN UGANDA

Adnan Gopinadhan[1], Dibyadyuti Datta[1], Bob Opoka[2], Chandy John[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Makerere University, Kampala, Uganda*

Prior studies have shown that Ugandan children with asymptomatic parasitemia are less likely to have subsequent uncomplicated or severe malaria episodes than children with severe malaria. It is unclear whether multiplicity or persistence of infection contributes to development of clinical immunity. To investigate this question, we assessed persistence of parasitemia over time and genetic diversity of parasitemia in 79 Ugandan children with asymptomatic parasitemia enrolled between 2008 and 2013. The presence of asymptomatic parasitemia was assessed by testing DNA from filter paper dried blood spots for *P. falciparum* by nested PCR at enrollment at 6 and 12 months after enrollment. Parasite genotype was assessed by PCR detection of variants in polymorphic region of merozoite surface protein-2 (*msp2*) with capillary electrophoresis. Prevalence of AP at baseline, 6 and 12 month collections was 73/73 (100%), 38/73, (52%), and 23/73 (31.5%), respectively. Children had similar number of parasite genotypes present (mean [SD]) at enrollment (2.98 [1.7]), 6 months (3.5 [1.6]) and at 12 months (3.2 [1.6]). Sixteen children tested positive for parasites at all 3 time points. MSP2 variants amplified from 13/16 enrollment samples all 6- and 15/16 12-month samples. 35, 46, and 39 unique *msp2* alleles were identified in enrollment, 6 month and 12 month samples, respectively. The number and frequency of infections with at least one identical genotype at two time points was: enrollment and 6 months, 4/13 (30.8%); 6 and 12 months, 2/16 (12.5%); enrollment and 12 months, 4/13 (30.8%). Only 1 of 16 children had one or more identical parasite genotypes at all three time-points. Ugandan children with asymptomatic *P. falciparum* parasitemia are often infected with multiple *P. falciparum* genotypes, and parasitemia at subsequent time points is usually due to new infections. Future studies will assess if infection with multiple or specific genotypes is associated with protection from subsequent episodes of clinical malaria

348

ANTIBODIES TO PFRH5-LIKE DOMAIN OF PFRH2B PROTEIN IS ASSOCIATED WITH INCREASE IN PARASITEMIA

Jonathan Suurbaar[1], Collins M. Morang'a[1], Prince B. Nyarko[1], Katherine Wright[2], Kwadwo A. Kusi[3], Felix Ansah[1], Eric Kyei-Baafour[3], Evelyn B. Quansah[1], Jessica Asante[3], Laty G. Thiam[1], Matthew Higgins[2], Gordon A. Awandare[1], Yaw Aniweh[1]

[1]*West African Centre for Cell Biology of Infectious Pathogens, Accra, Ghana,* [2]*Department of Biochemistry, University of Oxford, Oxford, United Kingdom,* [3]*Immunology Department, Noguchi Memorial Institute for Medical Research, Accra, Ghana*

Plasmodium falciparum virulence is characterized by the use of surface proteins as well as organelles such as the micronemes and the rhoptries to facilitate erythrocyte invasion aimed at proliferation. The multigene *P. falciparum* reticulocyte homolog (PfRh) family play a pivotal role in merozoites invasion. Structural polymorphism within the PfRh2b gene has been implicated in mechanisms to evade immune attack. More specifically, a 0.58 Kb deletion, at the C-terminus has been reported in high frequencies in Senegalese and Southeast Asian parasite populations. However, the outlook of this deletion mutation in parasite population across all malaria endemic locations has not been established. We therefore hypothesized that, the observed deletion in Rh2b is skewed towards hyper-endemic areas where humoral acquired immunity is normally predominant. Here, by analysing 1,674 *P. falciparum* isolates, we have successfully shown that this deletion is present within the parasite populations from Ghana (37.3%) and observed mainly in the Kintampo (holoendemic, 56.7%). Interestingly, some parasite isolates possessed mixed PfRh2b deletion status (7.4%), indicative of multiple clonal isolates. Globally, 4,032 parasites were analyzed, which showed varying deletion frequency with the west African sub-region as hotspot. The copy number evaluation indicated that the full-length gene could be duplicated in high endemic setting. although PfRh2b harbours a PfRh5-like domain, antibodies to the PfRh2b domain associated with increase parasitemia where as PfRh5 showed the inverse. In all, we have successfully characterized the Rh2b gene deletion polymorphism within parasite isolates globally. We have also indicated that, the deletion could have an important role to evade antibodies that will have effect on parasite survival whilst the duplication enhances the usage of the ligand. Antibodies to the PfRh5-like domain of PfRh2b is of diagnostic importance as antibodies persist in patients from one season to the other, though generally low.

349

EFFECT OF MALARIA PATHOLOGY ON CD4 AND IMMUNE CELLS

Emmanuel C. Amadi[1], Emmanuel Eze[2], Vincent Chigor[2]

[1]*Enugu State University of Science and Technology, GRA, Enugu, Nigeria,* [2]*University of Nigeria, Nsukka, Nigeria*

Knowledge of immunity in malariology is very essential to understanding the pathology, treatment and vaccine production. The effects of malaria disorder on CD4 and immune cells counts was carried out at a Teaching Hospital in Enugu, Eastern Nigeria between October-December 2018. Patients on doctor's provisional diagnosis of malaria were examined for *Plasmodium* infections and the degree of parasitaemia (0, +, ++, and +++). Positive samples and negative ones (0) were thereafter examined for their CD4 (Flow cytometry) and immune cells (Automated blood cell counter) counts. 45 patients were studied. All the *Plasmodium*-negative specimens were within reference ranges of CD4 cell count (464-1308); with mean value of 835. The (+) parasitaemia showed lower ranges of CD4 count (502-1282); mean = 678; with immune falls in one (427). The 12 (++) parasitaemia showed crash in 9 CD4 cells counts; range: 301-415; mean =399. All the 7 (+++) parasitaemia showed crashes in CD4 cell counts, range: 160-357; mean =225. The CD4 cells falls and crashes were detected only in *Plasmodium falciparum* parasitaemia infections. Depending on the CD4 cells count, also a reflex of parasitaemia, variances occur in the various immune cells' percent and numeric. Total WBC count fell in only 3 patients (1.99, 3.42 & 3.64) x 10^9cells/L, corresponding to (+++), (++) & (+) parasitaemia, respectively. Low CD4 counts do not always produce low lymphocyte numeric, probably because other CDs or killer cells compensate it. In conclusion, *Plasmodium falciparum* infection causes immuno-suppression in patients. Corollary, it means that malaria infection in the immunodeficients and AIDS patients will accelerate the complications as well as death, unlike prevailing reports.

350

VARIATION AND HOST GENETIC CONTROL OF MIRNAS IN RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION

Aissatou Diawara[1], Mame M. Dieng[1], Vinu Manikandan[1], Hala Tamin El Jarkass[1], Alfred B. Tiono[2], Sodiomon B. Sirima[2], Issiaka Soulama[2], Youssef Idaghdour[1]

[1]*NYU Abu Dhabi, Abu Dhabi, United Arab Emirates,* [2]*Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso*

Malaria is one of the greatest global public health problems and the host mechanisms responsible for protection against it remain poorly understood. Here, we report the landscape of human whole blood miRNA response to natural *Plasmodium falciparum* infection and its genetic control through genome-wide differential miRNA analysis, total RNASeq and whole genome sequencing of malarial children profiled before infection, during asymptomatic parasitemia, during symptomatic parasitemia and after treatment. The signature of miRNA expression differentiation associated with infection (127 out of 320 miRNAs, FDR 5%) and parasite load (72 miRNAs, FDR 5%) is striking and implicate core immune processes. In particular, we highlight several infection-responsive miRNAs affecting adaptive immune processes or erythrocyte function. Using miRNA *cis* eQTL analysis (111,367 cis SNPs and 274 miRNAs), we have identified 486 genetic variants associated with expression of 34 miRNAs (at permuted level 5%). We also show that host miRNA response to infection is predictable from allelic variation in the identified SNPs suggesting that the transcriptional mechanisms coping with controlling malaria infection are under host genetic control. Together, our findings highlight the role of miRNA and host genotype in modulating response to infection and advocate the use of integrative genomic approaches as companion to GWA mapping for linking molecular genetics to epidemiology in settings where profiling large cohorts is challenging.

351

INVESTIGATING THE IMMUNE PREDICTORS OF NATURALLY ACQUIRED IMMUNITY IN MALIAN CHILDREN USING SYSTEMS BIOLOGY APPROACHES

Jyoti Bhardwaj

Indiana University, Indianapolis, IN, United States

Parasite-specific IgG antibodies play a central role in naturally acquired immunity (NAI) to *Plasmodium falciparum (Pf)* malaria. However, the precise antigenic targets of these antibodies and the specific cellular correlates of immunity to malaria remain elusive. We sought to identify the immunological predictors of clinical or sterile immunity to *Pf* infection in a prospective cohort study of Malian children using systems biology approaches. We hypothesized that clinical outcomes can be predicted by global analyses of host immune factors at baseline which can influence the immune response during subsequent *Pf* exposures. Using whole blood, plasma, and peripheral blood mononuclear cells collected from children who were prospectively defined as clinically protected from febrile malaria or apparently protected from *Pf* infection, we assessed differences in gene expression, antibody responses, cytokines, and cellular profiles between classes. Whole-blood transcriptomics by RNA-seq revealed differential expression of blood transcription modules between protected and susceptible children for both clinical and apparently sterile immunity. We detected enhanced interferon (IFN) and dendritic cell (DC) module responses with increasing age in clinically immune children relative to clinically susceptible children. We observed upregulated enrichment of DC, monocyte, immune activation, toll-like receptor and inflammation and cell

cycle modules but decreased response of B-cell signatures with increasing age in infection-protected relative to infection-susceptible children. Taken together, our expression data suggests the possible role of DCs and monocyte subsets along with pro-inflammatory and interferon related cytokines in NAI to *Pf* infection. We will integrate the transcriptomic profiles with data obtained from *ex vivo* plasma cytokine analysis, cellular immunophenotyping, and IgM/IgG antibody profiles generated against a panel of 250 *Pf* liver and blood-stage antigens to identify features that are predictive of the clinical classes. Overall, this study may provide important insights into NAI to *Pf* infection.

352

CHARACTERIZATION OF MONOCYTES IN MALAWIAN CHILDREN PRESENTING WITH EITHER UNCOMPLICATED OR CEREBRAL MALARIA

Visopo Chizaso Harawa[1], Madi Njie[2], Iset Vera[3], Anne Kessler[4], Anthony Jaworowski[5], Terrie Taylor[1], Wilson Mandala[6], Karl Seydel[1], Kami Kim[3], Stephen Rogerson[2]

[1]*Blantyre Malaria Project, Blantyre, Malawi,* [2]*University of Melbourne, Melbourne, Australia,* [3]*University of South Florida, Tampa, FL, United States,* [4]*Albert Einstein College of Medicine, Bronx, NY, United States,* [5]*RMIT University, Melbourne, Australia,* [6]*Malawi University of Science and Technology, Thyolo, Malawi*

Monocytosis has been reported in malaria however their roles remain unclear. Intravascular influx of monocytes has been associated with placental malaria and fatal cerebral malaria (CM). Depletion of inflammatory monocytes abrogated experimental CM in mice. We hypothesised that monocytes could be driving local tissue inflammation through secretion and shedding of activation markers. We aimed to characterize monocyte numbers and their activation levels in children with uncomplicated malaria (UM) and those with CM. Children aged between 6 months and 12 years with malaria were recruited in Blantyre, Malawi. We quantified numbers and plasma monocyte activation markers soluble CD14 (sCD14) and soluble CD163 (sCD163) in 163 children [UM =87 and retinopathy positive (ret+) CM =76] using haematology analyser, ELISA and Luminex. *Mann Whitney* test was used to compare groups medians and a p value of ≤ 0.05 was considered statistically significant. Total monocyte counts were higher in ret+ CM than UM: ret+ CM 1000 median cells/μL IQR [615-1800] vs UM 630 cells/μL [430-900], p value <0.0001. Soluble CD14 was higher in ret+ CM than UM (ret+ CM median 2.56 x 10^6 pg/mL IQR [2.3 x 10^6 -3.15 x 10^6] vs UM 2.14 x 10^6 median pg/mL IQR [1.77 x 10^6 -2.42 x 10^6], p value <0.0001). Soluble CD163 concentration was similar between the two groups. Monocytes numbers and concentrations of sCD14 were higher in children with retinopathy-confirmed CM than matched children with UM. The expansion of monocytes in ret+ CM and elevated sCD14 could suggest a direct or indirect role in CM pathogenesis. . Further studies are needed to elucidate a role for monocytes in severe malaria.

353

FLOW CYTOMETRIC-BASED MULTIPLEX ASSAY TO ASSESS STRAIN TRANSCENDING ANTIBODIES TO *PLASMODIUM VIVAX* DBPII REVEAL AN EFFICIENT TOOL TO IDENTIFY BINDING-INHIBITORY ANTIBODIES (BIABS) RESPONDERS

Jéssica Rafaela Alves

René Rachou Institute, Belo Horizonte, Brazil

Malaria remains a major public health problem across the globe. In particular, *Plasmodium vivax* is the most prevalent species and its ability to cause relapses and subpatente infections challenge control and elimination strategies. Currently, leading *P.vivax* malaria vaccine target parasite ligands that are critical to reticulocyte invasion such as the Duffy binding protein II (DBPII). Naturally acquired Binding-Inhibitory antibodies (BIAbs) to DBPII is associated with reduced risk of clinical malaria. Due to the methodological issues to evaluate the functional proprieties of DBPII antibodies, so far, few studies have investigated DBPII BIAb responses in *P. vivax*-exposed population. Here, we hypothesized that the detection of high levels of DBPII strain-transcending antibodies is indicative of the presence of BIAbs response. For that, we standardized a sensitive multiplex flow cytometry-based serological assay to detect IgG antibodies against multiple DBPII variants, including an engineered DBPII, DEKnull-2, with conserved functional epitope targets of strain-transcending immune responses. The study design included long-term malaria exposed individuals (n=85) living in a well-characterized rural community of the Brazilian Amazon. Three cross-sectional surveys (baseline, 6 and 12 months) allowed us to characterize individuals as those with or without DBPII BIAbs response, as detected by standard COS7 cell assay. Taken together, the results demonstrate that the multiplex assay developed here increases the chance of identifying responders with BIAbs by at least 12 times. Of interest, around 80% of samples from BIAbs responders present high titers to DEKnull2 as detected by the multiplex assay. In conclusion the standardized multiplex assay confirms our hypothesis that DBPII-BIAbs activity is associated with high titers of strain-transcending antibodies. Futures studies involving different populations are required to confirm the multiplex assay as an alternative to screening individuals harboring DBPII inhibitory antibodies.

354

INFLUENCE OF EPSTEIN-BARR VIRUS INFECTION ON HUMAN MALARIA CAUSED BY *PLASMODIUM VIVAX*

Michelle Hallais Dias

René Rachou Institute, Belo Horizonte, Brazil

In highly endemic malaria areas of sub-Saharan Africa, it has been long recognized an association between *Plasmodium falciparum* malaria and the most common childhood lymphoma, the endemic Burkitt›s lymphoma (eBL) caused by the Epstein-Barr virus (EBV). Although eBL seem to be not associated with *Plasmodium vivax* malaria, it is not clear whether EBV may influence the morbidity and/or the development of a specific immune response against that parasite.Therefore we sought to investigate whether EBV infection may influence *P. vivax* morbidity. The study design include two groups of *P. vivax*-exposed individuals: (i) acute *P. vivax* infection (n=154); individuals sporadically exposed to malaria transmission who sought care at malaria reference centers in the Amazon area; and (ii) long-term malaria-exposed Amazonian individuals who present (n=38) or not (n=503) acute *P. vivax* infection at the time of enrollment; these individuals living in an agricultural community of the Amazon rain forest (Rio Pardo, Amazonas, Brazil) and their age basically correspond to their time of malaria exposure. Methodological approach included a real-time PCR amplification of BALF-5 gene from EBV (EBV-DNA) from peripheral blood from all volunteers. Taken together the results showed that (1) the frequency of EBV-DNA varied between the two groups studied, with virus been detected in 6% (9/154) of sporadically exposed individuals and in 23% (123/541) of long-term Amazonian residents, suggesting that continuous exposure to malaria transmission may have contributed for the difference (2) co-detection of *P.vivax* infection and EBV-DNA was associated with altered hematological parameters, including low levels of hemoglobin, hematocrit, and platelets; (3) a positive association between EBV-DNA and malaria-related clinical symptoms. In a prove-of-concept study, we demonstrated for the first time that EBV infection may influences on *P. vivax* morbidity and immunity. Future studies should include population of different endemic areas and individuals harboring different levels of *P. vivax* morbidity including severe disease.Financial support: CNPq, FAPEMIG, CAPES.

355

A PEPTIDE-BASED CHECKPOINT INHIBITOR THERAPEUTICALLY RESCUES MICE FROM LETHAL MALARIA AND ENHANCES PRIMING OF T CELLS FOLLOWING VACCINATION

Timothy W. Phares[1], Vinayaka Kotraiah[1], Deshapriya Karunarathne[2], Michelle Wykes[2], Jing Huang[3], Moriya Tsuji[3], Jim A. Pannucci[1], Gabe M. Gutierrez[1]

[1]*Explorations in Global Health / Leidos, Frederick, MD, United States,* [2]*QIMR Berghofer, Brisbane, Australia,* [3]*Aaron Diamond AIDS Research Center, New York, NY, United States*

Checkpoint receptors are highly expressed on T cells from chronically-infected individuals, arguing T cell exhaustion contributes to pathogenesis and lack of immunity. Targeting checkpoint receptors has shown to improve outcomes in models of chronic infections. In malaria, increasing evidence suggests that inhibitory receptors (e.g., PD1, LAG3), mediate immune suppression. Studies have shown that PD1-deficient mice rapidly clear chronic *Plasmodium chabaudi* and develop sterile immunity. Moreover, PD1 mediates loss of long-term protection against *P. chabaudi*. These data argue that blocking PD1 may rescue T cell exhaustion in malaria-infected individuals. While mAb-based checkpoint therapeutics are efficacious in oncology, they are not ideal for infectious diseases. We created a platform for discovering short peptides and evolving these peptide scaffolds by *in silico* design. Our lead checkpoint inhibitor, LD01, inhibits both the PD1 and CTLA4 receptors and has shown efficacy in oncology models. We evaluated the LD01 checkpoint inhibitor in therapeutic and prophylactic malaria vaccine mouse models. In the lethal *P. yoelii* model 100% mortality occurs 10 days post-infection. Treatment with LD01, as late as 3 days post-infection, decreased clinical severity and induced 50% protection. Further, all of the LD01 treated surviving mice were protected after re-challenge, indicating long-lived memory. Studies in the *P. yoelii* model to elucidate immunological mechanism of action are ongoing. In a recombinant replication defective AdPyCS vaccine model, a significant increase in splenic PyCS-specific, IFN-γ^+ CD8 T cells was detected in mice treated with LD01 compared to AdPyCS alone. Overall, LD01 appears to mediate release of immune suppression and acts as a T cell focused adjuvant when co-administered with a vaccine antigen, supporting further studies to understand its potential as a therapeutic peptide-based checkpoint inhibitor or as part of a prophylactic vaccine regimen.

356

IMMUNOGLOBULIN REPERTOIRES IN MEMORY B CELLS AND PLASMABLASTS FROM CHILDREN AND ADULTS WITH DIFFERENT LEVELS OF PRE-EXISTING EXPOSURE TO *PLASMODIUM FALCIPARUM*

Jacqueline Mutai[1], Velislava N. Petrova[2], Philip Bejon[1], Julian C. Rayner[2], Francis M. Ndungu[1]

[1]*KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya,* [2]*Wellcome Trust Sanger Institute, Cambridgeshire, United Kingdom*

An effective preventive malaria vaccine would add significantly to current malaria control methods, and would add significantly to the drive to malaria elimination. The goal of many vaccines is to generate long lasting and high affinity antibodies, which have been shown to be important mediators of naturally acquired anti-malaria immunity, but it is currently unclear how these responses are generated and maintained. We are analysing the immunoglobulin (Ig) repertoire, including key features such as clonal structure, rates of somatic hypermutation and Ig class-switching, in adults who present with different outcomes in controlled human malaria infection (CHMI) trials, and in children recovering from acute *Plasmodium falciparum* malaria. We determined the kinetics of memory B cells and plasmablasts in both populations using B cell ELISPot and flow cytometry assays. The proportions of plasmablasts increased during acute malaria infection in children, but decreased after treatment. By contrast, there was no synchronized peak in the proportions of plasmablasts in adults following CHMI, which could be indicative of suboptimal antigenic stimulation. Based on these results, we sorted plasmablasts (by flow cytometry) from children of different ages at various timepoints before, during and after acute malaria. These samples are currently being processed for B-cell receptor sequencing (BCRseq) to characterize the evolution of responding plasmablasts, and provide insights into whether there is a qualitative improvement of the response with time from diagnosis, and with increasing age and/or exposure. We have also sorted memory B cells from the "most highly immune" and the "least immune" adults, as determined by their ability to control parasite growth during CHMI, and will use BCRseq for the identification and comprehensive characterization of protective antibodies within these cohorts. This project will provide insight into characteristics of protective antibody responses, as well as the processes leading to them.

357

MALIAN CHILDREN WITH BOTH CEREBRAL MALARIA AND SEVERE MALARIAL ANEMIA HAVE A SEROLOGIC AND CYTOKINE PROFILE DISTINCT FROM THOSE WITH OTHER SEVERE MALARIA SUBTYPES

Abby R. Goron[1], Andrea A. Berry[1], Jason A. Bailey[1], Drissa Coulibaly[2], Matthew Adams[1], Abdoulaye K. Kone[2], Bourema Kouriba[2], J. Alexandra Rowe[3], Ogobara K. Doumbo[2], Marcelo B. Sztein[1], Philip Felgner[4], Christopher V. Plowe[1], Mahamadou A. Thera[2], Kirsten E. Lyke[1], Mark A. Travassos[1]

[1]*Malaria Research Program, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Malaria Research and Training Center, University Science, Techniques and Techologies, Bamako, Mali,* [3]*Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom,* [4]*Division of Infectious Diseases, Department of Medicine, University of California Irvine, Irvine, CA, United States*

Children aged less than five years accounted for 66% of malaria deaths in sub-Saharan Africa in 2018. Pediatric severe malaria subtypes with the highest mortality include cerebral malaria (CM) and severe malarial anemia (SMA). We recently showed that Malian children with these severe malaria subtypes differed in serologic responses to particular *Plasmodium falciparum* variant surface antigens. A third group of children with both cerebral malaria and severe malarial anemia (CM+SMA) had serologic responses that differed from both of these groups. Unlike children with either severe malaria subtype, Malian children with CM+SMA had less parasite burden and higher overall mortality. Here, we aimed to further determine how children with CM+SMA differed from other severe malaria subtypes, by examining serologic responses to Apical membrane antigen-1 (AMA1), a merozoite surface antigen and surrogate for *P. falciparum* exposure, and cytokine profiles using cytometric bead array technology and flow cytometry. Using a protein microarray populated with 268 AMA1 field variants, we measured AMA1 reactivity of sera for 78 Malian children aged 4.7 to 79.4 months with severe malaria compared to that for age-, region- and ethnicity-matched controls with uncomplicated malaria. Serorecognition was defined as a fluorescence intensity at least two standard deviations above the mean for sera from malaria-naïve North American controls. Overall, sera from children with CM, SMA, or CM+SMA recognized similar numbers of AMA1 variants (87.8%, 92.1%, and 97.5%, respectively; NS). Unlike sera from children with CM or SMA, sera from children with CM+SMA responded more intensely to 50 AMA1 variants than matched controls. Serum cytokine level measurements showed that children with CM+SMA had lower levels of IL-1β and IL-12 than those with CM alone and higher levels of IL-6 and IL-10 than children with SMA alone. Children with both cerebral malaria and severe malarial anemia represent a distinct severe malaria subtype suggesting a unique parasite exposure pattern compared to other severe malaria subtypes and a distinct cytokine profile that merits further exploration.

358

MULTI-SITE COMPARISON OF CHANGES IN CYTOKINE AND CHEMOKINE EXPRESSION PROFILES OVER 1-YEAR IN CHILDREN <5 YEARS WITH ASYMPTOMATIC MALARIA

Katrina E. Co[1], Elizabeth C. Okafor[2], Dibyadyuti Datta[1], Elizabeth Fernander[1], Estela Shabani[3], Eliud O. Onyango[1], George Ayodo[4], Robert O. Opoka[5], Chandy C. John[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*University of Minnesota School of Medicine, Minneapolis, MN, United States,* [3]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [4]*Kenya Medical Research Institute, Kisumu, Kenya,* [5]*Makerere University, Kampala, Uganda*

Understanding the cytokine and chemokine profiles over time in children with asymptomatic parasitemia (AP) may help explain the underlying immunity that keeps these children from developing symptomatic febrile malaria. To explore this question further, we studied children <5 years of age with AP in a region of low to moderate malaria transmission in Kampala, Uganda, and a region of high transmission in Ajigo, Kenya. Samples were collected at baseline and 6 and 12 months later (Uganda) and baseline and 12 months later (Kenya). *Plasmodium falciparum* was detected by nested PCR and cytokines, chemokines, angiogenic growth factors, and endothelial activation markers by cytometric bead assay. In the Ugandan cohort, AP was present at 34% (baseline), 22% (6 months), and 18% (12 months). At baseline, children with AP had lower levels of IL-1β, IL-8, IL12p70, IFN-γ, MIP-1α, RANTES, and IL-4 and higher levels of TNF-α, MIP-1β, IP-10, and IL-10 compared to children with no parasitemia (NP), showing a mixed anti- and pro-inflammatory picture. Levels of the angiogenic growth factors FGF-basic and G-CSF were lower and endothelial markers vWF, VCAM-1, and ICAM-1 higher in AP compared to NP. At 6-months, children with AP had a clear pro-inflammatory profile compared to NP, with higher levels of IL-6, IL-8, IL-17a, TNF-α, IFN-γ, MIP-1α, and MCP-1 than NP. FGF-basic and VCAM-1 levels were also higher in AP than NP (all $p<0.05$). In the Kenyan cohort, AP was present at 19% (baseline) and 36% (12 months). AP in this area had higher levels of pro-inflammatory cytokines/chemokines (TNF-α, MIP-1β, IP-10, MCP-1, and IL-1ra) and one anti-inflammatory cytokine (IL-10) compared to NP (all $p<0.05$). Testing of 12-month time point samples is ongoing. Children with AP in both sites generally showed increased levels of pro-inflammatory cytokines and endothelial activation markers compared to children with NP. However, the specific factors upregulated varied by time and area, possibly due to co-existing infectious or inflammatory stimuli.

359

DOES IT MATTER IF *PLASMODIUM* PREFERENTIALLY INVADES RETICULOCYTES OR NORMOCYTES?

Palmer Masumbe Netongo[1], Palmer Masumbe Netongo[2], Spencer Seely[2], Patrice Mimche[3], Aubree Earl[3], Nathan T. Jacobs[4], Tracey J. Lamb[3]

[1]*University of Yaounde I, Yaounde, Cameroon,* [2]*Department of Pathology, University of Utah, Salt Lake City, UT, United States,* [3]*Department of Pathology, University of Utah, Salt Lake City, UT, United States,* [4]*Population Biology, Ecology and Evolution Graduate Program, Laney Graduate School, Emory University, Atlanta, GA, United States*

Malaria is caused by infection with *Plasmodium* parasites that infect red blood cells to propagate infection. It is known that there is preference in some *Plasmodium* species for a particular age of red blood cells with *P. vivax* preferring to invade young reticulocytes (immature red blood cells). Infection of B cell deficient υMT mice turns a normally non-lethal *P. yoelii XNL* infection into a lethal one whereas υMT mice infected with *P. chabaudi-AS* all survive. One major difference between these two infections is that *P. yoelii XNL* infects primarily reticulocytes whereas *P. chabaudi-AS* infects normocytes. This implies that there could be a difference in the requirement for antibodies in the control of *Plasmodium*-infected red blood cells that are reticulocytes vs normocytes. Control of the primary peak of parasitemia in mice infected with the non-lethal reticulocyte-invading *P. yoelii XNL* requires antibody while mice infected with the more promiscuous-invading non-lethal *P. chabaudi-AS* does not. We investigated whether there were differential requirements for phagocytosis of *Plasmodium*-infected reticulocytes as opposed to more mature infected normocytes infected with *Plasmodium*. We also discuss results of the impact of altering the timing of infection on differences in requirement for parasitemia control by antibody-mediated vs non-antibody-mediated phagocytosis *during P. yoelii XNL* and *P. chabaudi-AS* infections.

360

PLASMA CYTOKINE AND CHEMOKINE SIGNATURES DURING ACUTE INFECTION BUT NOT CONVALESCENCE DISTINGUISH BETWEEN CHILDREN WITH DIFFERENT LEVELS OF PRIOR EXPOSURE TO MALARIA

Nancy K. Nyakoe[1], Jean Langhorne[2], Gordon A. Awandare[1], Kwaku P. Asante[3], Yaw Bediako[1]

[1]*West African Centre for Cell Biology of Infectious Pathogens, Accra, Ghana,* [2]*The Francis Crick Institute, London, United Kingdom,* [3]*Kintampo Health Research Centre, Kintampo, Ghana*

Regulation of cytokines plays an important role in malaria anti-disease immunity in naturally exposed individuals. Acquisition of natural immunity is influenced by variations in exposure due to differences in transmission intensity. This study aimed to investigate cytokine responses during acute and convalescent phases of *Plasmodium falciparum* infection in children exposed to different malaria transmission intensities. Children of ages 5-14 years were recruited from two regions in Ghana with distinct malaria transmission intensities: Accra (low transmission) and Kintampo (high transmission). Luminex magnetic 25-plex bead array was used to determine plasma levels of 25 cytokines and chemokines, including interleukins (IL), interferons (IFN), Tumor necrosis factor (TNF), IFN-γ-inducing protein 10 (IP-10), monocyte chemotactic protein-1 (MCP-1) and Monokine induced by (IFN-γ) (MIG), during infection (D0) and convalescent phases (D7 and D21). Cytokine levels were also compared between the 2 sites. *P. falciparum* infection induced a proinflammatory response driven by IL6, IFNγ, IFNα, MIG, MCP1 and IP10 cytokines, associated with an immunomodulatory profile mediated by IL10, IL-2R, and IL-1RA. These were significantly elevated during acute infection compared to convalescence with significantly higher levels in children from low transmission sites compared to those from high transmission sites. Only IFN-α, IL-6, IL-1RA and MCP-1 were significantly associated with parasitemia levels. Correlation network analysis identified a signature that distinguished individuals from the low transmission area. Interestingly, we identified a cytokine signature dominated by IL-10 and IL-1RA associated with asymptomatic parasitemia in a subset of children from the high transmission area with detectable parasitemia at D21. The study reveals significant differences in cytokine responses during active malaria infection in individuals with different levels of prior exposure. These differences are however transient and are not maintained during convalescence.

361

MALIAN ADULTS MAINTAIN SEROLOGIC RESPONSES TO NON-CD36-BINDING PFEMP1S AMID SEASONAL PATTERNS OF FLUCTUATION

Noah Thomas Ventimiglia[1], Emily M. Stucke[1], Andrea A. Berry[1], Drissa Coulibaly[2], Kirsten E. Lyke[1], Matthew B. Laurens[1], Jason A. Bailey[1], Matthew Adams[1], Amadou Niangaly[2], Abdoulaye K. Kone[2], Shannon Takala-Harrison[1], Bourema Kouriba[2], Ogobara K. Doumbo[2], Mahamadou A. Thera[2], Phillip L. Felgner[3], Christopher V. Plowe[4], Mark A. Travassos[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Sciences, Techniques and Technologies, Bamako, Mali,* [3]*University of California, Irvine, CA, United States,* [4]*Duke University, Durham, NC, United States*

Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) antigens, a family of malaria parasite proteins expressed on the infected erythrocyte surface, play an important role in malaria pathogenesis, mediating infected erythrocyte adhesion to host vascular endothelium while evading immune recognition through genetic diversity. Antibody responses to PfEMP1s are likely essential to immunity to clinical malaria, with antibodies to non-CD36-binding PfEMP1s associated with protection against severe disease. Using a protein microarray, we aimed to determine how serologic responses to 166 3D7 strain PfEMP1 fragments changed during a dry season and subsequent malaria transmission season in 18 Malian adults. We defined antigen seroreco gnition using malaria-naive N. American adults as controls. We hypothesized that adults, given lifelong exposure to the *P. falciparum*, would recognize most PfEMP1 antigens and possess high levels of seroreactivity during the year of follow-up. We also expected that serologic responses would decrease during the dry season and increase during the malaria transmission season due to the intense, seasonal nature of parasite exposure in Mali. Considered as a group and by individual, Malian adult sera had significant serologic responses to PfEMP1s throughout the study year. While the group rarely gained or lost seroreco gnition, it had decreased seroreactivity to a subset of PfEMP1s during the dry season (22 fragments, $p < 0.05$) and increased seroreactivity to another subset during peak malaria transmission (18 fragments, $p < 0.05$) [Wilcoxon Signed-Rank test (WSR)]. Individual Malian adults generally followed these seasonal trends [McNemar's test (MT); WSR] and were more likely to experience a significant change in seroreco gnition of CD36-binding PfEMP1s than non-CD36-binding PfEMP1s (MT; Binomial test), which remained seroreco gnized throughout the year. If sustained seroreco gnition of non-CD36-binding fragments into adulthood amid seasonal patterns of fluctuation reflects underlying protective clinical immunity, future studies for potential vaccine development may be warranted.

362

MECHANISMS DRIVING ALTERED VΔ2+ ΓΔ T CELL FUNCTION DURING RECURRENT MALARIA INFECTION

Kathleen W. Dantzler[1], Sandy Klemm[1], Rafael Polidoro[2], Aditya Rao[1], Caroline Junquiera[3], Mai Dvorak[1], John Rek[4], Moses Kamya[5], Peggie Cheung[1], Alex Kuo[1], Grant Dorsey[6], Margaret Feeney[6], Judy Lieberman[2], Purvesh Khatri[1], William Greenleaf[1], Prasanna Jagannathan[1]

[1]*Stanford University, Palo Alto, CA, United States,* [2]*Harvard University, Boston, MA, United States,* [3]*Fiocruz Minas, Belo Horizonte, Brazil,* [4]*Infectious Disease Research Collaboration, Kampala, Uganda,* [5]*Makerere University, Kampala, Uganda,* [6]*University of California San Francisco, San Francisco, CA, United States*

Naturally acquired immunity to the most deadly human malaria parasite, *Plasmodium falciparum (Pf)*, provides some protection against symptomatic disease in older children and adults but is unable to eliminate parasite replication. Immune mediators, such as pro-inflammatory cytokines produced from innate-like γδ T cells, can reduce parasitemia but can also lead to chronic inflammation. Repeated malaria exposure among Ugandan children leads to attenuation of the pro-inflammatory response from the Vδ2+ γδ T cell subset, which associates with a reduced likelihood of symptoms upon subsequent *Pf* infection. Building on evidence that altered Vδ2+ T cell function is important in the development of antimalarial immunity, we are using epigenetic and transcriptional approaches to identify mechanisms driving this process. We used 2 parallel methods to examine epigenetic reprogramming: 1) EpiTOF, which uses CYTOF to interrogate specific histone modifications at the single cell level, and 2) Omni-ATAC, which reveals sites of open chromatin across a population of cells. Results indicate significant differences in chromatin accessibility and histone methylation between Vδ2+ T cells from children with varying malaria exposure (low *vs*. high transmission) or infection status (uninfected, asymptomatic *vs.* symptomatic infection), suggesting that epigenetic regulation may play a role in altering functional responses of these cells after repeated infection. We are further establishing *in vitro* systems both to quantify changes in various Vδ2+ T cell functions (cytotoxicity, growth inhibition, antibody-dependent cytotoxicity) and to replicate the phenotype of altered function following repeated *in vivo* infection. By deepening our understanding of the molecular mechanisms driving inefficient acquisition of antimalarial immunity in children, this work could enable novel therapeutic approaches that enhance parasite clearance and/or reduce disease severity.

363

STATISTICAL MODELLING OF SURVEILLANCE DATA TO IDENTIFY CORRELATES OF URBAN MALARIA RISK: A POPULATION-BASED STUDY IN THE AMAZON BASIN

Rodrigo Corder, Gilberto Paula, Anaclara Pincelli, Marcelo Ferreira

University of Sao Paulo, Sao Paulo, Brazil

Despite the dramatic decrease during the last decades in the malaria burden in the Americas, focal transmission persists across the Amazon Basin. Characterizing high-risk individuals and households is crucial for better targeting control interventions.Here we analyzed 5,480 laboratory-confirmed malaria episodes and combined with demographic and socioeconomic information to identify malaria risk factors in the main urban transmission hotspot of Brazil, themunicipality of Mâncio Lima, situated intheJuruá Valley, westernmost Brazil, close to the border with Peru. After determining the number of malaria episodes for each urban resident during 33 months of follow-up, overdispersed count data clustered into households were fitted with random-effects zero-inflated negative binomial (ZINB) regression models. Random-effect predictors and alpha-shapes were used to characterize the spatial heterogeneity in malaria risk. We found evidence for local and imported transmission. Poor housing and residence in the periphery of the town were key predictors of malaria risk, consistent with a substantial local transmission. On the other hand, adult males were identified as the population at greatest risk, likely due to increased occupational exposure away of the town. Around two thirds of the 8,878 urban residents remained uninfected after 23,975 person-years of follow-up and nearly 14% of them, mostly children and older adults living in the center of the town, were estimated as the excess of zeros and, therefore, free of malaria risk (unexposed, naturally unsusceptible, or immune to infection). Random-effects ZINB model of routinely collected malaria surveillance data can be explored to characterize drivers of transmission heterogeneity at the community level, thus providing evidence for the rational deployment of control interventions.

364

DEVELOPMENT OF A COMMUNITY-DELIVERED MALARIA ELIMINATION MODEL FOR MYANMAR

Win Han Oo[1], Lisa Gold[2], Elizabeth Hoban[2], Kyu Kyu Than[1], Aung Thi[3], Paul A. Agius[4], Freya J. Fowkes[4]

[1]*Burnet Institute, Yangon, Myanmar,* [2]*School of Health and Social Development, Faculty of Health, Deakin University, Melbourne, Australia,* [3]*Department of Public Health, Myanmar Ministry of Health and Sports, Nay Pyi Taw, Myanmar,* [4]*Burnet Institute, Melbourne, Australia*

The malaria program in Myanmar is currently transitioning from control to elimination phase in order to achieve its 2030 malaria elimination goal. As a consequence, malaria volunteers in Myanmar have been transforming into Integrated Community Malaria Volunteers (ICMVs) since 2017. This ICMV model was designed with no evidence base to include integrated services for malaria, lymphatic filariasis, dengue, tuberculosis (TB), HIV/AIDS and leprosy. To inform the evidence base, a series of studies aiming to develop an optimal and scientifically proven integrated community-delivered malaria elimination model for Myanmar was conducted. Firstly, a systematic review and meta-analysis of 28 studies investigating the impact of community-delivered models was undertaken to estimate the effectiveness of these community-delivered models. Secondly, inductive thematic analyses of qualitative data collected from focus group discussions (n=8), participatory workshops (n=2) and semi-structured interviews (n=19) were used to consolidate the perspectives of community leaders and members, and malaria stakeholders from Myanmar. In qualitative consultations, community leaders and members identified common health concerns in addition to malaria and included respiratory illness, childhood diarrhoea, skin infections and TB (in this order) and recommended incorporating preventive, and whenever possible curative, services for those diseases into the malaria volunteer model. Although stakeholders perceived that disease combination in ICMV model was optimal, community members perceived that more illnesses should be included. Stakeholders also suggested ways that volunteer recruitment, training, supervision and reporting in the current ICMV model could be optimized. Based on international evidence and recommendations from community consultations, a community-delivered malaria elimination model should consist of malaria, TB, dengue, respiratory illness and childhood diarrhoea. Providing a range of services together with malaria will ensure a positive advancement towards the malaria elimination goal.

365

TREATMENT ALGORITHMS WITH RADICAL CURE OF VIVAX MALARIA BASED ON SEX: A COST-EFFECTIVENESS ANALYSIS TO SUPPORT ROLL OUT STRATEGIES

Angela Devine[1], Sandra Incardona[2], Ric N. Price[1], Sabine Dittrich[2], Xavier Ding[2]

[1]*Menzies School of Health Research, Darwin, Australia,* [2]*FIND, Geneva, Switzerland*

The recent license of single-dose tafenoquine for radical cure of *Plasmodium vivax* has changed the landscape for malaria elimination. Tafenoquine will overcome the issue of poor adherence to a prolonged course of primaquine; however, its clinical use requires prior screening for glucose-6-phosphate-dehydrogenase (G6PD) deficiency to exclude patients at risk of drug-induced haemolysis. G6PD deficiency is a sex-linked disorder: whilst diagnosis at a 30% cut-off excludes hemizygous deficient males at risk of haemolysis, it will not accurately exclude heterozygous females with intermediate deficiency (30-70% activity). The diagnosis of intermediate deficiency requires a quantitative test, associated with increased logistical and infrastructure complexity as well as cost. To support a phased implementation and to bridge potential infrastructure gaps, we explored a treatment algorithm for tafenoquine stratified by sex. In this algorithm, after testing with the less costly qualitative test (which can diagnose G6PD deficiency at the 30% threshold), males who test normal will be prescribed tafenoquine. Females would be offered the choice of either primaquine or referral to a higher facility where quantitative testing (which can diagnose reliably at the 70% threshold) is available, enabling the use of tafenoquine after appropriate assessment of G6PD activity. The cost-effectiveness of this strategy was explored using a decision model for Afghanistan, Ethiopia, Indonesia and Vietnam. The model was parameterized for these countries using data on local costs, G6PD deficiency and hemoslysis from a large randomized controlled trial. In settings where quantitative testing is not readily available, this strategy would offer a cost-effective and easy to implement approach for enabling the safe and effective radical cure of vivax malaria, an essential step to achieving elimination.

366

REFINING GLOBAL MAPS OF G6PD DEFICIENCY: HARNESSING NEW DATA AND ANALYTICAL METHODS

Daniel A. Pfeffer[1], Timothy C. Lucas[2], Colin Johnston[2], Jia Wei[3], Jonas Sandbrink[2], Benedikt Ley[1], Archie Clements[4], Peter W. Gething[2], Ric N. Price[1], Rosalind E. Howes[2]

[1]*Menzies School of Health Research, Darwin, Australia,* [2]*University of Oxford, Oxford, United Kingdom,* [3]*Peking University, Beijing, China,* [4]*Curtin University, Perth, Australia*

Up to 2.5 billion people are at risk of *Plasmodium vivax* malaria. Clearance of dormant parasites in the liver ('radical cure') requires treatment with an 8-aminoquinoline, however this is associated with haemolysis in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd). G6PDd is common, especially in malaria endemic regions. Understanding the spatial distribution of G6PDd, its prevalence, and local variants is essential to informing safe treatment policies. In order to refine global maps of G6PDd prevalence, we undertook a systematic literature review to update an existing global database of G6PDd prevalence surveys, identifying new studies published between 2010 and 2018. Surveys from the general population and among malaria patients were analysed separately. Surveys were georeferenced using published coordinates, information from authors or online mapping software (e.g. Google Maps). Global genomic datasets were explored to create a spatial covariate surface of broad-scale population relatedness. Final maps of G6PDd prevalence were produced via a Bayesian geostatistical model implemented using Integrated Nested Laplace Approximation (INLA). Of 7,447 papers screened, 813 were included. Population representative point prevalence data was extracted and added to the existing database of 1,734 spatially unique sites. Spatially continuous maps of G6PDd prevalence were produced at a resolution of approx. 5x5km, along with national estimates of the prevalence of G6PDd and number of affected individuals. Preliminary analysis demonstrated improved model performance and reduced uncertainty compared with previous research, particularly in areas where data coverage was previously poor. Improved input data, informative covariate data and computationally efficient methods have enhanced our ability to map G6PDd. The up-to-date maps of G6PDd synthesize our current knowledge on the local burden of G6PDd, while enabling informed inference where data are not yet available. These data will be critical for risk-benefit analysis of *P. vivax* radical cure and highlight priority areas for G6PD screening programs.

367

IN SILICO PREDICTION OF THE STRUCTURE OF *PLASMODIUM FALCIPARUM* GAMETOCYTE DEVELOPMENT PROTEIN 1 AND ITS EVALUATION AS A DRUG TARGET

Josephat K. Bungei[1], **Victor A. Mobegi**[2], Steven G. Nyanjom[1]

[1]*Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya,* [2]*University of Nairobi, Nairobi, Kenya*

Malaria transmission is a critical point in the disease cycle but the current drugs and vaccines target pre-erythrocytic and erythrocytic stages of malaria parasites. Therefore, transmission blocking strategies should be designed to target the sexual stage of the parasite. The exploration of transmission mechanism of the malaria parasite, *Plasmodium falciparum,* reveals that gametocytogenesis is critical in human host to mosquito vector transmission. Molecular studies demonstrate that gametocyte

development protein 1 (*Pfgdv1*) is an upstream epigenetic activator of gametocytogenesis. Genetic characterization of *Pfgdv1* gene and prediction of its structure forms the basis of drug design and development to prevent the transmission of malaria. Prediction of protein structure, ligand-binding sites, and the nature of ligands provide critical information regarding functional sites and mechanism of action in gametocytogenesis as a molecular target. *Pfgdv1* amino acid sequence of *P. falciparum* 3D7 strain was retrieved from PlasmodDB and used in the prediction of protein structure and its ligand-binding sites. Sequence data obtained from clinical isolates was also used to ascertain the effect of Non-synonymous substitutions. Protparam tool at ExPASy, was used to predict physical and chemical properties of *Pfgdv1*. InterPro 71 was used to predict domains, family, and functional sites of *Pfgdv1*. Iterative threading assembly refinement (I-TASSER) was utilized to model the protein structure and visualized using Jmol. Protein-ligand docking was performed using both I-TASSER and COACH. Secondary structure prediction reveals that *Pfgdv1* constitutes 33% of α-helix, 16% of beta-sheet, and 49% of coil structures. The predicted 3D structure of *Pfgdv1* has three apparent domains, which cover 1-277, 278-437, and 438-599 segments of the amino acid sequences. Protein-ligand docking has identified some ligands of *Pfgdv1* protein which can be used as a basis for evaluation of this protein as a drug target.

368

VALIDATION OF PIPERAQUINE CONCENTRATIONS PROTECTIVE AGAINST *PLASMODIUM FALCIPARUM* INFECTION WHEN DIHYDROARTEMISININ-PIPERAQUINE IS GIVEN ASINTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY

Emma Hughes[1], Richard Kajubi[2], Erika Wallender[1], Liusheng Huang[1], Teddy Ochieng[2], Abel Kakuru[2], Prasanna Jagannathan[3], Miriam Nakalembe[4], Bishop Opira[2], John Ategeka[2], Patience Nayebare[2], Tamara D. Clark[1], Moses Kamya[5], Philip Rosenthal[1], Grant Dorsey[1], Francesca Aweeka[1], Rada Savic[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Infectious Disease Research Collaboration, Kampala, Uganda,* [3]*Stanford University, Palo Alto, CA, United States,* [4]*Department of Obstetric and Gynecology, Makerere University College of Health Sciences, Kampala, Uganda,* [5]*School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda*

Dihydroartemisinin-piperaquine (DHA-PQ) is currently being tested for intermittent preventive treatment of malaria during pregnancy (IPTp). Accurate definition of the preventive PQ concentration is imperative to define dosing and regimen recommendations. Previously, using a prevention study in pregnant women residing in Tororo, Uganda, our group defined that a PQ concentration of 10.3 ng/mL or higher would protect 95% of pregnant women against *P. falciparum* parasitemia by loop mediated isothermal amplification, and reported that at least monthly dosing of standard 3-day DHA-PQ is required to achieve this concentration. The current study aims to validate established preventive targets and if monthly DHA-PQ dosing achieves those targets. The analysis was conducted using a pharmacokinetic/pharmacodynamic (PK/PD) model which described the relationship between PQ concentrations and risk of parasitemia. The validation trial was conducted in Busia, Uganda, a region with high transmission intensity. In total, 373 women received DHA-PQ every month during pregnancy with pre-dose trough blood samples collected for PQ quantitation and assessment of parasitemia using quantitative PCR (qPCR). In total, 1230 PQ samples and 1757 qPCR results were available for analysis. The observed PQ concentrations were higher than expected: the median PQ trough concentration in this trial was 12.8 ng/mL (2.8-41 ng/mL; 2.5-97.5% percentile) compared to 6.9 ng/mL (1.2-23 ng/mL; 2.5-97.5% percentile) from the previous study in Tororo. In the current study 63% of PQ concentrations were above 10.3 ng/mL compared to 27.6% in the previous study. Three women experienced symptomatic malaria, all with PQ concentrations <6 ng/mL at the time of diagnosis Poorer adherence to DHA-PQ in the Tororo study compared to the Busia study has been identified as one potential explanation for the observed PK differences. Further work will refine the estimate of preventive concentrations using quantitative qPCR as the PD marker. These findings confirm that monthly DHA-PQ dosing achieves PQ plasma concentrations which are sufficiently protective against symptomatic malaria.

369

INCREASED PATENT MALARIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC): GEOGRAPHIC AND EPIDEMIOLOGICAL CHANGES FROM 2007 TO 2013

Molly Deutsch-Feldman[1], Jonathan Parr[2], Nicholas F. Brazeau[2], Kyaw Thwai[2], Melchior Kashamuka[3], Antoinette Tshefu[3], Jonathan J. Juliano[2], Robert Verity[4], Steven R. Meshnick[2]

[1]*University of North Carolina - Chapel Hill, Carrboro, NC, United States,* [2]*University of North Carolina - Chapel Hill, Chapel Hill, NC, United States,* [3]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo,* [4]*Imperial College, London, United Kingdom*

Malaria remains a significant public health problem in the Democratic Republic of the Congo (DRC), with 17 million confirmed cases in 2016, accounting for 11% of cases worldwide. To combat the spread of malaria, the DRC implemented several intervention programs over the past decade, including long lasting insecticide treated bed-nets (LLIN) distributions and increased intermittent preventive therapy for pregnant women (IPTp). However, the heterogeneous spatial epidemiology of malaria across the country complicates prevention efforts. This study explores changes in the spatial distribution of malaria infections in the DRC over the past decade and the impact of intervention programs on these changes. We performed quantitative PCR (qPCR) on 17,968 samples collected during the 2013-2014 DRC Demographic and Health Survey and leveraged previously generated qPCR data from the 2007 survey. Using data from these two national, cross-sectional surveys, we compared the prevalence and spatial distribution of patent *Plasmodium falciparum* infections (at least 100 parasites/uL of blood) amongst adults over time. We created risk maps to identify changes in province-level prevalence between 2007 and 2013. Additionally, we used geospatial Bayesian modeling to assess the causal effect of LLINs and IPTp on malaria prevalence. Among 26,965 subjects enrolled in the two studies, the overall prevalence of patent infections increased from 2.4% in 2007 to 7.5% in 2013. Province-level prevalence changes ranged from -1.0% to +12.8%, with a median change of +6.0%. Modeling results indicate heterogeneous effects for both interventions, from highly protective in certain provinces to no effect in others. The results of this study will aid the DRC in its efforts to reduce malaria transmission. Our findings demonstrate important differences in malaria transmission across the DRC and highlight variation of the effects of two interventions across the country. Understanding where malaria transmission increased and where recent interventions were least effective over the past decade is essential for the development of effective, targeted interventions.

370

THE ROLE OF LONG-LASTING SYSTEMIC INSECTICIDES IN ACCELERATING MALARIA CONTROL AND ELIMINATION: A MODELING STUDY OF THREE DIVERSE TRANSMISSION SETTINGS

Prashanth Selvaraj[1], Joshua Suresh[1], Edward Wenger[1], Caitlin Bever[1], **Jaline Gerardin**[2]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Northwestern University, Chicago, IL, United States*

While bednets and insecticide spraying have had significant impact on malaria burden in many endemic regions, outdoor vector feeding and insecticide resistance may ultimately limit their contribution to elimination and control campaigns. Complementary vector control methods are therefore generating much interest. Here we explore the conditions under which systemic insecticides would have a substantial impact on transmission and burden. Hypothetical long-lasting systemic insecticides with effective durations ranging from 14 days to 90 days are simulated

using an individual-based mathematical model of malaria transmission. The impact of systemic insecticides when used to complement existing vector control and drug campaigns is evaluated in three settings - a highly seasonal high-transmission setting, a near-elimination setting with seasonal travel to a high-risk area, and a near-elimination setting in southern Africa. At 60% coverage, a single round of long-lasting systemic insecticide with effective duration of at least 60 days, distributed at the start of the season alongside a seasonal malaria chemoprevention campaign in a high-transmission setting, results in further burden reduction of 30-90% depending on the sub-populations targeted. In a near-elimination setting where transmission is sustained by seasonal travel to a high-risk area, targeting high-risk travelers with systemic insecticide with effective duration of at least 30 days can result in likely elimination even if intervention coverage is as low as 50%. In near-elimination settings with robust vector control, the addition of a 14-day systemic insecticide alongside an antimalarial in mass drug administration (MDA) campaigns can decrease the necessary MDA coverage from about 85% to the more easily achievable 65%. While further research into the safety profile of systemic insecticides is necessary before deployment, we find that long-lasting systemic insecticides can play a critical role in reducing burden or eliminating malaria in a range of contexts with different target populations, existing malaria control methods, and transmission intensities.

371

EVIDENCE-BASED TOOLS TO SUPPORT DECISION MAKING FOR MALARIA ELIMINATION

Sheetal Prakash Silal[1], Lisa White[2]

[1]*Modelling and Simulation Hub, Africa, Cape Town, South Africa,* [2]*Mathemical and Economic Modelling (MAEMOD), Mahidol Oxford Research Unit, Bangkok, Thailand*

With malaria elimination being high on the political and funding agenda globally, many countries are strengthening efforts towards this ambitious goal. There is no "one size fits all" intervention for malaria elimination due to the spectrum of available sub-optimal interventions acting at different stages of the parasite life-cycle and the heterogeneous transmission landscape. Every district of every country has its own unique challenges, conditions and solutions. Mathematical modelling is a leading approach for combining the many interacting factors that must be considered. A study has been conducted to create evidence-based tools to support decision making for malaria elimination. It involves the development of mathematical disease transmission models and costing tools to provide a platform to turn national surveillance data into strategic information to support the policy-makers in programme and funding decisions. User-friendly computer applications have be designed to allow policy-makers to run simple mathematical models and navigate the output of millions of simulations of more complex models with the aid of interactive graphs. These tools focus on implementation and translation of the modelling results allowing policy-makers to design an elimination/control strategy incrementally by combining candidate interventions to predict the desired impact and cost. Applications of the tool in several countries are highlighted.

372

AN INTEGRATED SOFTWARE PACKAGE FOR MODELLING CLINICAL TRIALS OF VECTOR CONTROL INTERVENTIONS FOR *PLASMODIUM FALCIPARUM* MALARIA REDUCTION

Keith J. Fraser, Lazaro Mwandigha, Azra Ghani

Imperial College London, London, United Kingdom

The public health benefit of vector control interventions including interior residual spraying (IRS), long-lasting insecticide-treated nets (LLINs) and attractive targeted sugar baits (ATSBs) can only be assessed in cluster randomized trials (cRCTs). Typically, such trials involve randomisation of the intervention at village level, with outcomes measured in a cohort formed of subsets of those residing in the villages. Standard statistical methods are available to estimate power for cRCTs given a single outcome metric. However, because typical outcomes including malaria prevalence and clinical incidence vary non-linearly by age and transmission intensity, mathematical models are often needed to estimate the expected effect size for a given location. To aid future trial design, we developed an integrated software package using an existing mathematical model of *Plasmodium Falciparum*. The software includes the effect of three vector-control interventions (LLINs, IRS and ATSBs, alone or in combination) on the vector population and on malaria transmission in the human population using a deterministic model for a range of transmission intensities (defined by EIR) and seasonality profiles. A linked stochastic individual-based model is incorporated that simulates a range of malaria outcomes (incidence of disease, incidence of infection, parasite prevalence by microscopy/RDT or PCR) in the trial cohort. The cohort model also captures censoring of observations due to treatment. Outputs include expected outcomes in the intervention and control villages by calendar time, and calculations of statistical power for a given set of design parameters (EIR, seasonality, number of clusters, number of individuals per cluster, coverage of interventions, outcome measure).

373

HAPLOTYPE FREQUENCY *VS.* PREVALENCE IN THE CONTEXT OF SEASONAL MALARIA

Kristan A. Schneider

University of Applied Sciences Mittweida, Mittweida, Germany

A plethora of empirical studies monitor prevalence of parasites, such as drug-resistance associated haplotypes or variants with deletions at the HRP2/3 *loci*. While prevalence of such haplotypes provides a proxy for the occurrence of treatment failure or false-negative RDT results, evolutionary processes drive the spread of such haplotypes. To correctly infer the evolutionary dynamics, haplotype frequencies need to be considered. Although the terms 'frequency' and 'prevalence' are often used synonymously, they actually refer to different concepts. A haplotypes' prevalence is the probability to observe it in an infection, and hence can be regarded a feature of infected patients. On the opposite, a haplotype's frequency is its relative frequency in the population of sporozoites within the mosquitoes' salivary glands ready to infect human hosts. Here, it is shown by a formal model that frequency and prevalence are linked by the process of infection. In particular, a haplotype's prevalence is a function of its frequency and multiplicity of infection (MOI). Importantly, higher MOI yields higher prevalence. This is especially relevant in the context of seasonal malaria, where MOI varies between seasons, leading to zigzag-like oscillations in prevalence, even if frequencies stay constant. Such oscillations also occur when estimating frequencies as relative haplotype prevalence. Notably, these estimates have an unpredictable bias depending on the overall frequency distribution and MOI and might severely underestimate the true frequency and prevalence of a common haplotype. Methods to correctly and accurately estimate frequency and prevalence alongside with MOI are presented and exemplified by various data applications.

374

WITS RESEARCH INSTITUTE FOR MALARIA (WRIM)

Miles B. Markus, Lizette L. Koekemoer, Robyn L. van Zyl

University of the Witwatersrand, Johannesburg, South Africa

The Wits Research Institute for Malaria (WRIM) in Johannesburg, South Africa, undertakes studies (especially work relevant to Africa) in collaboration with many national and international partners. There are opportunities for graduate and postdoctoral research by appropriately qualified individuals. Subject areas that are covered are diverse. Members of WRIM have expertise in regard to mosquitoes, and investigations are undertaken on insecticide resistance, systematics, population genetics, control of malaria-transmitting mosquitoes, and mosquito-parasite interactions. Novel drug discovery is also a focus of WRIM. This includes the design and evaluation of antimalarial and insecticidal activities of natural and synthetic compounds on various stages of the malaria parasite

within both hosts. Also, elucidation of the mechanisms of action and interaction of novel compounds with standard antimalarial agents, taking toxicity into account. All of this research falls within the broad fields of Molecular Parasitology, Pharmacology and Entomology. Clinical Medicine staff collaborate with Pharmacology and other experts to investigate the clinical incidence of malaria and treatment outcomes in tertiary hospitals, along with constant monitoring for drug resistance. A member of the Institute will, of course, be in attendance at the poster to answer any questions about WRIM.

375

MALARIA MANAGEMENT IN BÉNIN: FROM ETHNOBOTANICAL APPROACH TO AN EFFICIENT PHYTOTHERAPY

Gérard Hodévè Tiko[1], Rock Djehoue[1], Rafiou Adamou[1], Adandé Assogba Medjigbodo[2], Abdou Madjid O. Amoussa[1], Luc Salako Djogbenou[2], Latifou Lagnika[1]

[1]*Laboratoire de Biochimie et Substances Naturelles Bioactives/University of Abomey Calavi, Cotonou, Benin,* [2]*Institut Régional de Santé Publique (IRSP)/Université d'Abomey Calavi, Ouidah, Benin*

Benin is a malaria endemic country where more than 70% of the population uses a large number of medicinal plants for prophylaxis and malaria healing based on their cultural practices or ancestral knowledge. Unfortunately, there are no data that guarantee the efficiency, quality, and safety of these practices. Therefore, these plants need to be investigated assessing their properties. The goal of this study is to identify follows ethnobotanical approach efficient antimalarial plants for development of phytomedicine. A malaria-specific ethnobotanical survey was conducted by computer search and other supports to carry out antimalarial plants reported in Benin from 1989 to 2016. Comparative analysis of these survey followed by the bibliographical study have made it possible to identify the commonly used traditionally. Ethanolic and aqueous extracts of aerial part prepared was tested *in vitro* against CQ-sensible and field isolate of *Plasmodium falciparum* based on plasmodial lactate dehydrogenase detection. Hemolytic effect of extracts was also evaluated on human red blood measuring the absorbance of hemoglobin released by spectrophotometer and acute toxicity was performed on Wistar rats. 195 medicinal plants are listed in which 42 are commonly used. Both extracts of *Ampelocissus bombycina* and *Hibiscus surratensis* (IP> 60%) showed an interesting percentage of growth inhibition (IP) at 100 μg/ml whereas the same extracts of *Cola milenii* and *Costus afer* showed low activity (<40%). All extracts have hemolytic power less than 1% and no recorded mortality or difference between hematological and biochemical parameters at 5000 mg/kg of body weight. The results obtained in this study strengthen the use of *A. bombycina* and *H. surattensis* as antimalarial plants. They could be considered as a source of new anti-malarial drugs. A complementary study is ongoing on the capacity of these plants to block malaria transmission in order to develop an efficient phytomedicine

376

PROTECTING THE PERI-DOMESTIC ENVIRONMENT: THE CHALLENGE FOR ELIMINATING RESIDUAL MALARIA

Edgar J. Pollard, David MacLaren, Tanya L. Russell, Thomas R. Burkot

Australian Institute of Tropical Health & Medicine, Cairns, Australia

Malaria transmission that occurs after universal access and use of malaria preventive services is known as residual malaria transmission. The concurrent spatial-temporal distributions of people and biting mosquitoes in malaria endemic villages determines where and when residual malaria transmission occurs. This study tracked people movements in Solomon Island villages using structured questionnaires and daily movement diaries to identify where humans during peak biting by malaria vectors s. Although 84% of people sleep under an LLIN, only 7% of people are sleeping under an LLIN during the 18.00 to 21.00 hr peak biting period of the dominant vector, An. farauti when 76% of bites are delivered. During this critical exposure time to malaria vector bites, people are near but outside their houses, either in their kitchens or on the verandas of their primary residence. Novel vector control tools that protect individuals from mosquito bites in these peri-domestic areas have the potential to contribute significantly to ending residual malaria transmission.

377

PLASMODIUM OVALE AND *PLASMODIUM FALCIPARUM* CO-INFECTION AMONG SYMPTOMATIC AND ASYMPTOMATIC MALARIA ASSOCIATED WITH SYMPTOMS IN ENDEMIC REGIONS OF WESTERN KENYA

Jackline Juma, Hosea Akala, Dennis Juma, Benjamin Opot, Agnes Cheruiyot, Redemptah Yeda, Gladys Chemwor, Edwin Mwakio, Charles Okudo

Kenya Medical Research Institute-Walter Reed Project, Kisumu, Kenya

Plasmodium falciparum (*Pf*) infections occur sympatrically with *Plasmodium ovale curtisi (Poc), Plasmodium ovale wallikeri (Pow)* and *Plasmodium malariae (Pm).* Detection of these species has been conducted by semi-convenient sampling layouts that screen symptomatic rather than asymptomatic cases. Estimating the burden of species among symptomatic and asymptomatic cases is significant. This study assesses the composition of *Plasmodium* species among symptomatic and asymptomatic cases in Kisumu. Between 2013 and 2016, 435 symptomatic malaria individuals presenting at Kombewa Sub-County hospital were recruited for a malaria drug resistance surveillance study. Concurrently, 454 asymptomatic individuals within the same area were enrolled in a transmission dynamics study. About 2mL of blood drawn from participants, tested for presence of malaria parasites by 18s rRNA real-time PCR, typed for *Plasmodium* species composition using the genus specific small subunit ribosomal RNA gene (ssrRNA). Of 435 symptomatic cases, *Pf* had the highest prevalence at 96%, followed by *Pow*, Poc and *Pm* at 28%, 9% and 7% respectively. Co-infections between *Pf*/*Pow* were highest 21%, *Pf*/*Pm*, *Pf*/*Poc, Pf*/*Poc*/*Pow, Pow*,*Pf*/*Pm*/*Poc and Poc*/*Pow* at 6%, 5% 3%,0.5% and 0.2% respectively. In asymptomatic cases *Pf, Pm, Pow* and *Poc* prevalent at 90%, 15%, 11% and 10% respectively. *Pf*/*Poc* and Pf/*Pow* co-infections were observed at 5%, 2% while *Pf*/*Pm, Pf*/*Pm*/*Pow, Pm*/*Pow*,*Pf*/*Pm*/*Poc*,*Pm*/*Poc , Pf*/*Pm*/*Pow*/*Poc and Pm*/*Poc* were prevalent at 6%, 4%, 2%, 4%, 0.6% and 0.4% respectively. Comparison of overall species frequency between two infections showed stronger association between *Pow*/*Pf* co- infections with symptomatic malaria (OR of 10.4, 95% CI range [5.6 - 19.4 and *P< 0.0001*). Higher frequency of non-*falciparum* species especially *Pow*/*Pf* co-infections among symptomatic than asymptomatic malaria cases was observed. However higher *Pm* frequency was observed among asymptomatic than symptomatic cases suggesting low virulence. This argues for consideration of asymptomatic malaria matrices and simulation estimating malaria burden based on analyses of treatment seeking habits.

378

MOLECULAR DETECTION OF PARASITIC CO-INFECTION AND DETERMINATION OF INFECTION PREVALENCE IN PREGNANT WOMEN OF GHANA

Sarah Alhakimi[1], Abraham Anang[2], Nilanjan Lodh[1]

[1]*Marquette University, Milwaukee, WI, United States,* [2]*University of Ghana, Legon, Accra, Ghana*

In sub-Saharan Africa, a significant proportion of the population is exposed to malaria, schistosomiasis and soil transmitted helminths (STHs). Most importantly, about [40] million pregnant women are infected with STHs and Schistosome spp. along with malaria. The consequences include intrauterine growth retardation, low birth weight, pre-term delivery and neonatal mortality. When parasitic diseases overlap in distribution, high rates of co-infection occur and there is a shortage of data about co-infection prevalence in pregnant women in Ghana. We have detected single, dual and multiple infections (malaria, schistosomiasis, and Strongyloides) among pregnant women by amplifying cell-free repeat DNA (CFRD) fragments via polymerase chain reaction (PCR) from single

filtered urine samples collected from two districts (Adidome and Battor) of Ghana. In addition, sensitivity and specificity of PCR was evaluated against parasitological tests based on stool, urine and blood. Out of 163 samples, Schistosoma haematobium had the highest prevalence (47%) by PCR and then 37% for S. mansoni. The prevalence of malaria infection for Adidome district was 18% by rapid diagnostic test (RDT), whereas no infection was detected in Battor by RDT. Also, 10 positive infections for Strongyloides stercoralis were detected by PCR. We found malaria co-infection with schistosomes and with Strongyloides, when RDT and PCR were compared. Detection of CFRD by PCR from single urine sample is more cost-effective than individual parasitological tests and more sensitive. The study addressed the weaknesses in the current diagnostic techniques available for malaria, schistosomes and Strongyloides. Our approach and method can be optimized to the use in the clinical set up in endemic countries to determine multiple infection prevalence and for surveillance for this vulnerable group of population.

379

RISK FACTOR FOR CRITICAL CARE ADMISSION AMONG CHILDREN WITH MALARIA IN PUBLIC HOSPITALS OF THE PERUVIAN AMAZON

Nataly Atarama[1], Karen Arica[1], Amy C. Morrison[2], Raul Seminario[3], Stalin Vilcarromero[4]

[1]*Universidad Nacional de la Amazonia Peruana, Iquitos, Peru,* [2]*University of California Davis, Davis, CA, United States,* [3]*Hospital Regional de Loreto, Iquitos, Peru,* [4]*Stony Brook University, New York, NY, United States*

In Peru, malaria is a major public health problem affecting children and adults in endemic areas concentrated in the Amazon Region of the contry. Despite the high disease burden, there is a lack of clinical data on severe malaria especially in the pediatric population. To identify risk factors associated with admission to critical care units for severe malaria we conducted a retrospective clinical chart review of pediatric inpatients from two public hospitals located in Iquitos City where most of the severe malaria cases from this region are treated. Charts of pediatric inpatients (≤15 years old) with malaria (thick smear), and with at least one of the WHO severe malaria criteria during 2013-2017 were included. In total, 144 met the criteria. Of these, 58% (84/144) were male, 30.6% (44/144) were age 2-5 years, 73.6% (16/144) lived in rural or peri-urban settings, and 91% (131/144) reported travel to malaria endemic locations. Interestingly, 75.7%(109/144) of the cases were caused by *Plasmodium vivax.* Clinical characteristics included an average of 6.22 days between symptom onset and hospitalization, 59% (85/144) had parasitemia < 3+, 6.3% (9/144) presented complications at admission, 34.7% (50/144) had an acute comorbidity, only 43.3% (62/144) received intravenous antimalarial treatment, 33.3% (62/144) received an additional antibiotic, and 13.9% (20/144) had blood transfusions. The principal signs and symptoms were: fever (92.4%), vomiting (54.9%), headache (41.7%) and chills (40.3%). Only 21/144 (14.6%) were admitted to CCU. The most reported severity criteria were: coagulation disorders (44.4%), hyperparasitemia (36.8%), severe acute anemia (34%), hyperpyrexia (20.1%) and consciousness compromise (15.3%). Multiple logistic regression to identified severe anemia, hemoglobinuria, use of vasoactives drugs, need for blood transfusion, complications at admission, and parasitemia less than 3 crosses were significantly associated with admission to the CCU. Because anemia is common in children from this malaria endemic areas, programs to address anemia in the pediatric population should improve outcomes do to malaria infection.

380

EVOLUTION OF AVAILABILITY IN ACT, RDT AND SULFADOXIN/PYRIMETHAMIN AT THE LEVEL OF DIRECT CUSTOMERS OF COTE D'IVOIRE NEW PHARMACY OF THE PUBLIC HEALTH FROM JANUARY TO FEBRUARY 2019

Yepie Armande Eve Yapi

NMCP, Abidjan, Côte D'Ivoire

RCI has received two external funding in the fight against malaria since 2017 from Global fund and PMI. Its performance improves in the interventions against this scourge. We have analyzed specific reports relating to the availability of health products used against malaria and from eLMIS (electronic application) from January to February 2019. This analysis concerned the direct customers of the New Pharmacy of Public Health (Nouvelle PSP CI) regroups in: - health districts (DS) - and other Nouvelle PSP direct customers (CHU, CHR, general hospitals and FSU, CSU...) At the DS level, the results did not differ significantly, the percentage of DS with stock out in Artemether/Lumefantrin move from [1%-6%] to [2%-5%]. For DS in Overstock [22%-34%] to [14%-34%]. Finally, the well-stored DS percentage is going from [18%-51%] to [20%-45%]. Other direct customers show less satisfactory results. Thus, the percentage of these facilities with stock out in Artemether/Lumefantrin increase from [9%-17%] to [5%-16%]. For DS in Overstock, the percentage is going from [20%-46%] to [22%-51%]. Finally, the percentage of well-stored DS change from [22%-44%] to [20%-33%]. The DS, over the same period, have a decrease in RDT availability from 36% to 27% of DS well-stored, while other direct clients show a satisfactory evolution of 10% to 13%. However, the availability of RDT remains better at the DS level. DS and other direct clients have improved their availability of Sulfadoxin/Pyrimethamin (SP) over the period of [37%-41%] and [31%-43%] for well-stored sites and for sites with stock out [1%-1%] and [5%-2%]. An improvement in the availability of these facilities is observed in terms of the reduction of cases of SP and RDT stock out and the increase of well-stored facilities in SP. We note, a slight improvement in the availability of ACT, RDT and SP at the level of all Nouvelle PSP customers. However, some efforts are to be made to obtain at the end of each month no cases of stock out for all health product of malaria control in all facilities.

381

HEME SCAVENGER HEMOPEXIN, HAPTOGLOBIN AND HEMEOXEGENASE-1 LEVELS IN PREGNANT WOMEN WITH ASYMPTOMATIC MALARIA CORRELATE WITH ADVERSE BIRTH OUTCOMES

Annette Nti[1], Hassana Salifu[1], Felix Botchway[2], Nelly Yatich[3], Mingli Liu[1], Andrew Adjei[2], Pauline Jolly[3], Jonathan K. Stiles[1]

[1]*Morehouse School of Medicine, Atlanta, GA, United States,* [2]*Korle Bu Teaching Hospital, Accra, Ghana,* [3]*University of Alabama, Birmingham, AL, United States*

Malaria caused 435,000 deaths globally in 2017, with pregnant women and children at the highest risk. Malaria in pregnancy involves severe symptoms and outcomes including anemia, higher rates of miscarriage, premature delivery, low birth weight neonates, intrauterine, neonatal and maternal death. Current effective treatment for malaria targets parasite burden. Therefore, there is an urgent need to understand the causes of poor birth outcomes and to identify novel interventions as well as predictive biomarkers to determine at-risk individuals. However recent studies have shown that malaria pathogenesis is mediated by parasite -derived factors as well as host factors such as heme, a by-product of parasite-infected erythrocyte destruction. Our lab has previously determined that free serum heme levels and cytotoxicity in pregnant women were dependent on the robustness of their heme scavenging systems. We hypothesize that individuals with effective heme scavenging mediators will result in improved birth outcomes and that alternatively, those individuals with poor heme regulation will have poor birth outcomes. We assessed pregnant women in Ghana and identified *Plasmodium* infection by rapid diagnostic testing (RDT). Plasma samples

obtained from these women were assessed for heme, Heme Oxygenase-1, Haptoglobin, and Hemopexin levels to correlate results with birth outcomes. The results indicate that RDT *Plasmodium*-positive women had significantly different low HO-1: Hemopexin ratios that correlated with pre-term delivery.

382

EVALUATION OF A HOMEMADE SALIVA KIT FOR THE STABILIZATION OF *PLASMODIUM* DNA AT ROOM TEMPERATURE

Eric Berenger E. Tchoupe Kamoua

University of Yaounde [1], Yaounde, Cameroon

Malaria diagnostic require blood collection. Saliva from Malaria-infected individuals contains trace amounts of *Plasmodium* DNA, PCR showed great benefit using saliva; conserving parasite DNA in saliva is still problematic.. We designed this study to evaluate the effectiveness of a homemade kit to stabilize plasmodium DNA in saliva at room temperature. Blood and saliva samples were collected from 33 patients. Saliva samples were collected in two separate kits: OMNIGENE•DISCOVER kit as the standard kit and a newly formulated kit as the test kit. Samples were stored at room temperature for 12 months. Blood samples were analyzed using microscopy to detect Parasites. *Plasmodium* DNA was extracted from saliva by Chelex method and molecular detection of the parasite DNA was based on nested PCR amplification of the multicopy 18s rRNA gene. Products were separated on 2.0 % agarose gel and visualized under UV light. The frequency of malaria in this study was 78 % using microscopy. Saliva PCR-f1 detected 21 positive samples, saliva PCR-so detected 18 positive sample. When microscopy was used as gold standard, the sensitivities of PCR-so and PCR-f1 were all recorded at 100%; however the specificities were at 80%, and 85%, respectively. PCR-f1 had a "very good" agreement (kappa 0.81) compared to PCR-so (kappa 0.64).

383

TRACKING SPENDING ON MALARIA BY SOURCE IN 106 COUNTRIES, 2000-2016: AN ECONOMIC MODELLING STUDY

Annie Haakenstad[1], Anton C. Harle[2], Golsum Tsakalos[2], Angela E. Micah[2], Tianchan Tao[2], Joseph L. Dieleman[2]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Institute for Health Metrics and Evaluation, Seattle, WA, United States*

The Global Technical Strategy for Malaria, 2016-2030, articulates an ambitious agenda to make progress toward malaria elimination, including reducing case incidence and mortality rates by 40% by 2020. An estimated $6.6 billion in investments annually is required to meet these targets. We estimated malaria expenditure by source over 2000-2016 in 106 countries. We collected 36,038 data points reporting government, out-of-pocket (OOP), and prepaid private malaria spending, as well as malaria treatment-seeking, costs of patient care, and drug prices. We estimated government spending on patient care for malaria, which was added to government spending by National Malaria Control Programs. For OOP malaria spending, we used data reported in National Health Accounts and estimated OOP spending on treatment. Spatiotemporal Gaussian process regression was used to ensure estimates were complete and comparable across time and to generate uncertainty. In 2016, $4·3 billion (95% uncertainty interval 4·2–4·4) was spent on malaria worldwide, with 8·5% (8·1-8·9) in annual increases occurring since 2000, but still more than $2 billion short of the global goal. OOP spending amounted to $556 million (487-634) or 13·0% (11·6 -14·5) of all malaria spending in 2016. Governments spent $1·2 billion (1·1 -1·3) or 28·2% (27·1 -29·3) of all malaria spending in 2016. The source of malaria spending varied depending on whether countries were in the malaria control or elimination stage. Because most countries with a high burden of malaria are low- or lower-middle income, mobilizing additional government resources to meet the $6.6 billion target may be challenging. Furthermore, the major share of malaria expenditure sourced from DAH and OOP makes the fight against malaria vulnerable. The high dependence on DAH makes malaria endemic countries vulnerable to drops in contributions from development assistance partners. The high reliance on OOP spending may deter treatment-seeking and prevent countries from realizing malaria reduction aims.

384

NEURODEVELOPMENTAL PERFORMANCE IN PRESCHOOLERS AFTER SEVERE ANEMIA AT LIRA REGIONAL REFERRAL HOSPITAL, UGANDA

Andrew Sentoogo Ssemata[1], Robert O. Opoka[1], Noeline Nakasujja[1], Chandy C. John[2], Paul Bangirana[3]

[1]*Makerere University, Kampala, Uganda,* [2]*Indiana University, Indianapolis, IN, United States,* [3]*Makerere University, Kampala, Uganda*

Severe anemia (SA) is a global public health challenge commonly associated with morbidity and mortality among children <5 years of age in Sub-Saharan Africa. In this study, we investigated neurodevelopmental performance in preschool children (<5 years) diagnosed with severe anemia (SA) in Northern Uganda. We conducted a hospital based cross-sectional study among children < 5 years of age with SA (Hb≤5.0 g/dl; n=171) presenting to Lira Regional Referral Hospital, Uganda. Neurodevelopmental outcomes (cognitive, language [receptive communication and expressive communication] and motor [fine motor and gross motor]) were assessed 14 days post discharge using the Bayley Scales of Infant and Toddler Development, 3rd edition. Age-adjusted z-scores for each domain were calculated using scores from controls of healthy community children (n=88) from the same environment for each age category. Multiple linear regression was used to compare z-scores in the cognitive, language and motor scales between the two groups after adjusting for weight-for-age z score, social economic status, mother's education, father's education and father's employment on all the scales. Significant differences were observed between the severe anemia group and control group for cognitive adjusted mean score (SE), [-0.20, (0.64) vs. 0.01, (0.06), P=0.02]; receptive communication [-0.16, (0.05) vs. 0.01, (0.06), P=0.05]; expressive communication [-0.32, (0.05) vs. 0.01, (0.07), P<0.01]; overall language [-0.25, (0.04) vs. 0.00, (0.06), P<0.01]; Gross motor [-0.25, (0.06) vs. 0.00, (0.09), P<0.01]; Overall motor [-0.18, (0.05) vs. 0.00, (0.07), P=0.05]; but not for the fine motor scale [0.08, (0.07) vs. 0.00, (0.09), P=0.46]. In children <5 years of age, severe anemia was associated with worse cognitive ability, language and motor skills scores in the immediate period post clinical recovery. Neurodevelopmental assessment, especially in areas with high SA prevalence, could identify at risk children for early interventions.

385

ANALYSIS OF UNITED STATES PRESIDENT'S MALARIA INITIATIVE-SUPPORTED OPERATIONAL RESEARCH STUDIES, 2006-2018

Robin Miller[1], Michael Elhardt[1], Meera Venkatesan[1], Carrie Nielsen[2], Richard Steketee[3], Martin Alilio[1], **Achuyt Bhattarai**[2]

[1]*United States President's Malaria Initiative, United States Agency for International Development, Washington, DC, United States,* [2]*United States President's Malaria Initiative, Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*United States President's Malaria Initiative, Washington, DC, United States*

The U.S. President's Malaria Initiative (PMI) has supported operational research (OR) studies to help overcome malaria control implementation challenges, test promising new tools, identify methods to scale-up malaria control interventions, find local solutions to optimize intervention effectiveness, and identify the most cost-effective mix of proven interventions under varying malaria transmission settings in PMI focus countries. We analyzed PMI-supported OR studies conducted during 2006-2018 with the aims to 1) examine completed and ongoing studies by technical areas, countries, and other descriptive metrics and 2) perform a pilot assessment to ascertain the dissemination, use, and influence of findings from a sample of PMI-supported OR studies. From 2006 to

2018, one hundred PMI-funded studies were implemented in twenty countries across eight technical areas. Vector control (n=41) and malaria case management (n=31) and were the most common technical areas funded. These studies mainly aimed to answer country-specific operational concerns (n=70) while other studies (n=30) addressed larger initiative-wide questions. The pilot assessment of a purposively selected sample (n=15) of studies completed during 2012-2016 demonstrated that the study results were often published in peer-reviewed journals (n=11), presented at scientific conferences (n=11), and communicated to broader audiences (n=9) through national or regional meetings and various media sources. The assessment also showed that PMI-supported OR studies have included training of research investigators (n=12) and involved National Malaria Control Programs staff (n=14) and in-country institutions (n=14) in the design, execution, analysis, and/or dissemination of study results. The findings of the pilot assessment indicate that it may useful to systematically document the impact and influence of PMI-supported OR studies, and prospectively collect data related to dissemination, use, and influence of ongoing and future OR studies.

386

SEVERE MALARIA WITH REPEATED MALARIA ATTACKS AND ACADEMIC ACHIEVEMENT IN UGANDAN CHILDREN

Ann J. Nakitende[1], John Ssenkusu[1], Richard Idro[1], Noeline Nakasujja[1], Chandy C. John[2], Margaret Semrud-Clikeman[3], Paul Bangirana[1]

[1]*Makerere University, Kampala, Uganda,* [2]*Indiana University, Indianapolis, IN, United States,* [3]*University of Minnesota, Minneapolis, MN, United States*

Children with a history of severe malaria are at higher risk of hospitalization for subsequent episodes of malaria. While severe malaria(SM) has been associated with poorer neurocognitive functioning, the additive effect of subsequent malaria attacks (RMA) has not been studied. This study compared academic achievement in children with a history of SM and RMA to those without RMA and to healthy community children. This study was nested in a prospective study of cognitive functioning after SM conducted at Mulago Hospital, Kampala, Uganda from January 2015 to August 2017. Children aged 5 to 12 years with SM and community children (CC) were assessed for academic achievement in word reading, sentence comprehension, spelling and arithmetic calculations using the Wide Range Achievement Test Fourth Edition. Age adjusted z- scores for academic achievement outcomes were calculated using scores of the CC children. Multiple regression was used to compare scores between the groups with caregiver employment and income entered into the regression model. 232 participants (CM=75, SMA =58 and CC=99) were enrolled in the study with a mean age of 9.6 (SD=2.6) years. Children with SM and RMA had statistically significant lower z- scores than SM without RMA for math (95% CI,-0.50- -0.05, p=0.016), reading (95% CI,-0.66 to -0.17, p=0.001) and spelling (95% CI -0.61 to -0.10, p=0.007). There was no difference in z-scores for sentence comprehension between the two groups. Children with a history of SM and RMA have poor academic performance compared to those with a history of SM without RMA two years after the illness. This study highlights the need for malaria chemoprevention for children in malaria endemic areas. It also provides evidence for the need to develop appropriate interventions for these children at the earliest possible time.

387

CARE SEEKING FOR AND MANAGEMENT OF FEBRILE ILLNESS IN SENEGAL IN THE CONTEXT OF MALARIA CONTROL AND ELIMINATION. WHAT DO SURVEY DATA TELL US?

Katharine M. Sturm-Ramirez[1], Yaya Ly[2], Leah Moriarty[1], Mame Birame Diouf[3], Alioune Badara Gueye[4], Moustapha Cisse[4], Samba Ndiaye[2], Doudou Sene[4]

[1]*Centers for Disease Control and Prevention and the President's Malaria Initiative, Atlanta, GA, United States,* [2]*Agence Nationale de la Statisique et de la Démographie, Dakar, Senegal,* [3]*United States Agency for International Development, Dakar, Senegal and U.S. President's Malaria Initiative, Dakar, Senegal,* [4]*Programme National de la Lutte contre le Paludisme (PNLP), Dakar, Senegal*

Senegal has made great strides against malaria with aggressive scale-up of control interventions, such as improved access to diagnosis and treatment in health facilities and communities and phasing in of universal testing of fevers for malaria. Malaria transmission remains heterogeneous but as Senegal shifts to an elimination phase, universal access to diagnosis and treatment is critical. We analyzed survey data from 2005-17 to characterize spatio-temporal trends and identify gaps in care seeking and case management. Since 2012, Senegal implements a nationally representative, continuous Demographic and Health Survey, composed of annual household surveys and health facilities' service provision assessments (SPAs). The proportion of children < 5 years (CU5) who presented with fever in the 2 weeks prior to a household survey was 29.8% in 2005, 12.7% in 2016 and 20% in 2017; medical care was sought for only half of febrile CU5 (range: 49-57%). The most frequent sources of care or advice were public health posts (57-63%) and private pharmacies (10-12%) while informal sources including traditional practitioners were sought less over time (7% in 2015 and 2.3% in 2017). Data from the SPAs (2012-17) indicate that most of the observed medical consultations in both private and public facilities included fever evaluation (range: 80-100%) yet the proportion of CU5 that were diagnosed as having fever or malaria ranged from 4-32%, with marked regional differences. The malaria service readiness index (SRI) captures availability of diagnostic capacity, treatment, trained personnel, and guidelines at health facilities. The national SRI increased from 38% in 2012 to 64% in 2017, with clear regional differences. Nationwide, primary and secondary health structures were better equipped than tertiary structures to provide malaria care and the SRI was higher for the public than the private sector (71% vs 21% in 2017). Despite improved access to and quality of malaria services in the public health sector, the rate of care seeking for febrile illness in CU5 remains low in Senegal. There is an urgent need to strengthen the malaria service readiness of the private sector.

388

MALARIA DATA QUALITY AND USE IN SELECTED CENTERS OF EXCELLENCE IN MADAGASCAR: RESULTS FROM A CROSS-SECTIONAL BASELINE SURVEY

Maurice Ye[1], Jean Marie N'Gbichi[2], Thierry Franchard[3], Solo Harimalala[3], Brune Ramiranirina[3], Mauricette N. Andriamananjara[3], Laurent Kapesa[4], Jocelyn Razafindrakoto[4], Yazoume Ye[5]

[1]*MEASURE Evaluation, ICF Macro Madagascar, Antananarivo, Madagascar,* [2]*MEASURE Evaluation, ICF USA, Chapel Hill, NC, United States,* [3]*Ministry of Public Health, National Malaria Control Program, Antananarivo, Madagascar,* [4]*U.S.President's Malaria Initiative, Madagascar, Antananarivo, Madagascar,* [5]*MEASURE Evaluation, ICF USA, Rockville, MD, United States*

In Madagascar, the U.S. President's Malaria Initiative through MEASURE Evaluation conducted a health information system performance assessment in 2015 that reported low data quality, subpar reporting completeness (65.3%) and timeliness (45.5%), and weak evidence of data analysis and use that could lead to inappropriate decision making and impaired program management. To improve data quality, the National Malaria Control Program will implement Centers of Excellence (COE). COEs will be health centers selected to receive training, equipment, data management tools, and direct coaching. In April 2018, a baseline assessment was done in 12 health centers, eight which will become COEs (intervention) and four controls. We explored the availability of data collection tools, data accuracy, completeness and timeliness of reporting, data analysis, and data use for decision making. Baseline data quality indicators were compared across intervention and control centers. Data were analyzed using Stata14. A Kruskal-Wallis test estimated the difference between the two groups and *p-values* were calculated. Completeness of reporting was similar in both groups: 95.3% and 95.0%, respectively (*p=0.240).* There was no significant difference in timeliness

of reporting: 31.8% and 44.9%, respectively *(p=0.364)*. Both groups reported similar use of rapid diagnostic tests (RDT) for malaria diagnosis. Availability of RDTs and artemisinin-based combination therapy was equally high: above 90% in both groups. Data accuracy (data in monthly reports versus data in patient registries) showed similar ranges of discrepancy in both groups: around 25%. Availability of data collection tools was 79.7% in the intervention group and 81.0% in the control group ($p>0.05$). Only 25.0% of centers in the intervention group and 28.0% in the control group were performing data analysis *(p=0.926).* Similarly, data use was low—25.5% and 27.0%, respectively—in intervention and control groups. The assessment provided baseline information on comparable groups of health facilities to measure improvement in data quality because of the implementation of COEs in Madagascar.

389

HEALTH MESSAGE ANNOUNCEMENTS THROUGH LOUDSPEAKERS BOUT MALARIA CARE - PREVENTION AND PRACTICE AMONG PEOPLE LIVING IN A MALARIA ENDEMIC AREA OF BANMAUK TOWNSHIP, SAGAING REGION, MYANMAR

Pyae Linn Aung[1], Tepanata Pumpaibool[2], Than Naing Soe[3], Myat Phone Kyaw[4]

[1]*Southeast Asia International Centers of Excellence for Malaria Research, Yangon, Myanmar,* [2]*College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand,* [3]*Department of Public Health, Ministry of Health and Sports, Naypyidaw, Myanmar,* [4]*Department of Medical Research, Yangon, Myanmar*

Various approaches towards community awareness-raising interventions have been delivered through a variety of channels, but evidence for the effect of these practices has been minimal. This study aimed to determine the effectiveness of announcements made through loudspeakers regarding malaria care and prevention practices among people living in the malaria endemic villages of Banmauk Township, Sagaing Region, Myanmar. Four villages among the most malaria-burdened areas were randomly chosen: two villages were assigned as the intervention group and another two as a control. Before the peak season of malaria (June 2018), a baseline study was conducted. The announcement was regularly repeated at 7:00pm every other day using local messages that were conveyed through loudspeakers. A six-month follow-up survey was carried out in both groups using the same questionnaire to compare them against the baseline results. Descriptive statistics, chi-square, and the *t*-test for addressing statistical differences were determined. Among a total of 270 respondents with similar socio-economic characteristics, the baseline knowledge, attitude and practice mean scores were not found to be significantly different between intervention and control groups. After six months' post intervention, improvements in scores were observed at p-value<0.001 in both groups, but the increase in score was much greater in the intervention group. The declining trend of malaria was also noticed as an impact. In addition, more than 90% of people showed positive opinions of the intervention. The intervention was found to be effective, as shown by the significant improvement in scores relating to prevention and care-seeking practices for malaria in the targeted community. An expansion of study areas and long-term follow-up measures will be encouraged in addition to the maintenance of sustainability.

390

EFFECT OF SEASONAL MALARIA CHEMOPREVENTION ON MALARIA IN CHILDREN UNDER 5 YEARS: A COHORT STUDY IN DANGASSA, MALI

Drissa Konaté[1], Sory I. Diawara[1], Mahamoud Touré[1], Seidina AS Diakité[2], Agnes Guindo[2], Ayouba Diarra[3], Bourama Keita[1], Sibe Thiam[3], Moussa Keita[3], Ibrahim Sissoko[3], Nafomon Sogoba[3], Sekou F Traoré[1], Donald J Krogstad[4], Seydou Doumbia[1], Mahamadou Diakité[5]

[1]*MRTC/FMOS/USTTB, Bamako, Mali,* [2]*MRTC/FAPH/USTTB, Bamako, Mali,* [3]*MRTC/USTTB, Bamako, Mali,* [4]*Tulane Universtity, New Orleans, WA, United States,* [5]*MRTC/FMOS/FAPH/USTTB, Bamako, MaliSeasonal*

Malaria Chemoprevention (SMC) is a new control strategy recommended by the world health organization since ^{2012} to prevent malaria during the high transmission season in children under five. In this study, we assessed change in malaria indicators after two consecutive years of routine implementation of SMC providing Sulfadoxine-pyrimethamine + Amodiaquine (SP+AQ) to children less than ^{5} years in Dangassa. We conducted a cohort study in the village of Dangassa from ^{2013} to ^{2016}. In the course of our study, a routine SMC was implemented by the National Malaria Control Program in the village from ^{2015} to ^{2016} at a monthly basis from August to October each year (corresponding to the transmission season). We tracked all clinical malaria cases in children less than five years in the health center from ^{2013} to ^{2016}. We used Cox regression model to assess the changes in malaria risk from ^{2013} (before SMC introduction) to ^{2016} (two years after SMC introduction. Results of the Cox regression model shows a significant reduction in the incidence of malaria in ^{2015}-^{2016} after SMC introduction compared with ^{2013} (HR=$^{0.23}$ [$^{0.15}$-$^{0.35}$] in October (the pick of transmission season). The gametocyte prevalence decreased from $^{15.6}$% to $^{2.1}$%, hemoglobin level increased from $^{10.32}\pm^{0.09}$ to $^{10.81}\pm^{0.05}$ and parasite density. However, a slight increase in malaria incidence was observed in December after the SMC provision ends in October. In conclusion, SMC has significantly reduced malaria incidence, gametocyte prevalence and improve hemoglobin level in children less than five years after two years of SMC implementation.

391

AN ASSESSMENT OF QUALITY OF DELIVERY OF SEASONAL MALARIA CHEMOPREVENTION USING LOW LITERATE COMMUNITY HEALTH WORKERS IN NIGERIA

Olusola B. Oresanya[1], Abraham Ahmadu[1], Olatunde Adesoro[1], Louise Maranda[1], Diego Morosso[2], Kolawole Maxwell[1]

[1]*Malaria Consortium, Abuja, Nigeria,* [2]*Malaria Consortium, Kampala, Uganda*

The effectiveness of seasonal malaria chemoprevention (SMC) depends not only on the provision of quality-assured medicines, but also on the quality of planning and implementation. Community health workers (CHWs) delivering SMC follow key steps including directly observing treatment of eligible children (3-59 months) with sulfadoxine-pyrimethamine and administering the first dose of amodiaquine (AQ). They also advise caregivers on how to administer and record subsequent doses of AQ and what to do in case of drug reaction. To ascertain the quality and effectiveness of CHWs' performance and SMC messaging, we assessed those trained to administer SMC to about 3.2 million eligible children in four states of Nigeria through interviews of caregivers from 4,090 randomly selected households in which SMC had been administered. The interviews took place immediately after the last cycle of SMC in 2018, and covered caregivers' knowledge of SMC and adherence to SMC doses. Overall, 92.9% of all eligible children seen by the CHWs had been treated at least once. However, CHWs also appear to have treated a large proportion of ineligible children (46% of those aged 5-10 years). Only 44.4% of caregivers said CHWs had administered the first dose and, although 93.6% said that CHWs had left subsequent doses with them, only around a third (35.7% and 35.4%) reported having administered the second and third doses of AQ respectively. Despite this, most caregivers

(86.8%) reported knowing how to administer these correctly. Caregivers' retention of SMC record cards was also found to be low (37.3%) and only few (13.0%) were aware of the need to record their at-home administration of AQ dose. 61.0% reported knowing that SMC was for malaria prevention; however, 28.3% believed it was to treat malaria. These findings show that although CHWs' coverage of SMC eligible children in Nigeria is high, the quality of their delivery could be better. Thus, closer monitoring and supervision of CHWs is required to ensure they correctly adhere to protocols, and provide effective messaging and support to caregivers around the importance of administering subsequent AQ doses and retaining SMC cards.

392

MALARIA PREVENTIVE PRACTICES AMONG OUTDOOR WORKERS IN BILIN TOWNSHIP, MON STATE, MYANMAR

Pyae Sone Tint

ARC/Defeat Malaria, Yangon, Myanmar

Bilin Township, one of the malaria endemic areas in Myanmar, is a township where most workers went to the forest for bamboo cutting, rubber plantation and coal making. A cross-sectional study was conducted in this township with the purpose of identifying malaria preventive practices and their related factors among outdoor workers. A two stage probability proportional to size (PPS) cluster sampling was used to select 240 outdoor workers for face-to-face interviewing with structured questionnaires. Descriptive statistics and Chi-square test of association were used for data analysis. The age of the outdoor workers ranged from 18 to 73 years, with mean of 38.4 years. More than half of them (69.2%) attended primary school and their main occupation (45.8%) were forest activities (bamboo cutting/rubber plantation/coal workers). Majority (87.9%) of them had their own bed nets. The results revealed that only 37.5% of them had good level of malaria preventive practices, while the others (62.6%) were at needed to be improved level. The knowledge on malaria were 59.6% at good level. The perception on susceptibility, severity, benefits and barriers were 95.4%, 92.9%, 77.5% and 45.4% respectively at good level. The factors significantly associated with malaria preventive practices were ownership of bed nets (p-value<0.001), owners of insecticide treated bed nets (p-value<0.001), overall knowledge on malaria (p-value <0.001), knowledge on its transmission (p-value=0.001), knowledge on malaria signs and symptoms (p-value=0.032), knowledge on malaria prevention (p-value=0.003) and perception of malaria severity (p-value=0.004). Based on the studied results, it is recommended that the improvement on malaria knowledge especially on transmission, signs and symptoms and prevention should be focused to these outdoor workers as well as their perception on severity of malaria. The ownership of the bed nets is also an important factor for better malaria preventive practice among this group.

393

REVIEW OF ACCESS AND USE OF LONG LASTING INSECTICIDAL BED NETS (LLINS) IN MALARIA ENDEMIC AREAS OF BANGLADESH

Akramul Islam, Shamsun Naher, Moktadir Kabir

BRAC, Dhaka, Bangladesh

BRAC has successfully implemented community health service model in close collaboration with National Malaria Elimination Programme particularly in 13 districts in the north-east and south-east areas with the specific goal of malaria free Bangladesh within the year of 2030. Started in 2007, the current thrust is on diagnosing and treating malaria cases from plain land to hard reach area by community healthcare service providers and prevention through provision of long-lasting insecticidal nets for free among the people living in malaria endemic area. To obtain the views of the access and use long lasting insecticidal bed nets (LLINs), several cross-sectional qualitative and quantitative studies have been conducted since 2008. In this present article, we used review of the published and unpublished literatures available from these studies done by BRAC. The result shows, in 2008 (one year after start of the programme), only 41% household members were sleeping under LLIN whereas in 2018 it was increased to 91.4%. In case of high risk groups, 36% under-five children and 30% pregnant women were sleeping under LLIN in 2008; while in 2018 it was increased to 96.2% and 95.4% respectively. Adequate supply of LLINs, door-to-door mobilisation of the community and regular monitoring have played major role in achieving this result. In addition, a network of mobile health services uses outreach heath worker in hard to reach pockets of remote places lacking of usual transport route since 2014. That generates a good progress to access and uses LLINs to hard reach area. These increase in LLIN coverage and use, along with other interventions, has contributed to reduction of malaria patients by 87.6% and deaths by 95.5% during the same time period. This declining trend has encouraged the country to move towards elimination strategy. Challenges remain targeting highly mobile populations who are exclusively engaged in forest work for achieving and ensure of 100% uses of LLINs.

394

MOSQUITO EXPOSURE TO ANTIMALARIALS PREVENTS TRANSMISSION OF *PLASMODIUM FALCIPARUM*

Douglas G. Paton[1], Lauren M. Childs[2], Maurice A. Itoe[1], Inga E. Holmdahl[1], Serge Yerbanga[3], Thierry Lefevre[3], Caroline O. Buckee[1], Flaminia Catteruccia[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Virginia Tech, Blacksburg, VA, United States,* [3]*Institut de Recherche en Sciences de la Sante, Bobo Dioulasso, Burkina Faso*

Since the turn of this century, efforts to prevent the transmission of malaria through the mass distribution of insecticide-treated bed nets have been extremely successful and have contributed to an unprecedented reduction in deaths from malaria. However, resistance to insecticides has become widespread in *Anopheles* populations, which has led to the threat of a global resurgence of malaria and makes the generation of effective tools for controlling this disease an urgent public health priority. We recently demonstrated that the transmission of *Plasmodium falciparum* to *Anopheles gambiae* can be rapidly and completely blocked when female mosquitoes take up low concentrations of the antimalarial atovaquone from treated surfaces. Mosquito exposure to atovaquone up to 24 hours before, or 12 hours after, *P. falciparum* infection causes full parasite arrest in the midgut preventing midgut escape and transmission of infection. Although at an early stage, targeting the parasite during human-mosquito transmission, or sporogonic development, could be a powerful and complimentary future tool for malaria elimination. Towards this goal, here, we will present data investigating the interaction of mosquito-targeted anti-parasitic compounds and vector insecticide resistance mechanisms in field-derived *An. coluzzii*, and our further work to fully characterize the effects of atovaquone on *P. falciparum* throughout sporogony and mosquito-human transmission.

395

POLYESTER OR POLYETHYLENE? DIFFERENCES IN LLIN USE DRIVEN BY FABRIC TYPES IN CAMBODIA AND MYANMAR

May Me Thet[1], Ye Kyaw Aung[1], Sochea Phok[2], Si Thu Thein[1]

[1]*Population Services International Myanmar, Yangon, Myanmar,* [2]*Population Services International Cambodia, Phnom Penh, Cambodia*

Use of Long lasting insecticide nets (LLIN) for malaria prevention is influenced by several factors. Past literature suggested that preferences existed for particular fabric type of LLINs. However, there is still no evidence that the LLIN usage per se is associated with fabric types.With the objective of examining the relationship between LLIN use and fabric type, a cross-sectional household survey was conducted in Cambodia and Myanmar in 2018. Single-stage cluster sampling approach was applied. Households with ownership of at least one polyester or polyethylene LLIN were recruited from recent distribution campaign areas. A total of 1362 households and 1414 households were recruited in rural areas of Cambodia and Myanmar.We assessed the usage of polyester and

polyethylene LLIN in two different scenarios: in households with two fabric types, and households with only one type. In Cambodia, there were 620 households with only polyester LLINs and 691 households with only polyethylene LLINs. Only 51 households owned both fabric types. In households with both fabric types, 41.8% of polyester LLINs and 29.4% of polyethylene ones were used at last night. In households with only polyester LLINs, 26.9% of those nets were used. In households with only polyethylene LLINs, only 13.1% were used. Use of polyester LLIN was higher than that of polyethylene in both scenarios. In Myanmar, 636 households had both types of LLINs, 686 had only polyester LLINs, and 92 had only polyethylene LLINs. In households with both fabric types, use of polyester LLINs was 62% and that of polyethylene was 45.1%. In households with a single fabric type, usage of either one was similar (66.5% in households with only polyester LLINs and 67.8% in households with only polyethylene ones).The preliminary findings revealed that the use of LLIN could be determined by fabric type. Given a choice, polyester LLINs enjoyed higher usage than polyethylene ones in both countries. Cambodia findings suggested strong preference of polyester LLINs regardless of availability, whereas availability appeared to determine the usage in Myanmar.

396

QUANTIFYING SEASONAL VARIATION IN INSECTICIDE-TREATED NET USE BEHAVIOR

Hannah Koenker[1], Cameron Taylor[2], Julie Thwing[3], Clara Burgert-Brucker[4], Tom Fish[2], Albert Kilian[5]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*ICF, Rockville, MD, United States,* [3]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*RTI International, Washington, DC, United States,* [5]*Tropical Health, Montagut, Spain*

Seasonal variation in the proportion of the population using an insecticide-treated net (ITN) is well-documented and is widely believed to be dependent on mosquito abundance and heat, driven by rainfall and temperature. However, seasonal variation in ITN use has not yet been quantified controlling for ITN access. Demographic and Health Survey and Malaria Indicator Survey datasets, their georeferenced data, and public rainfall and climate layers were downloaded and pooled for 21 countries. Nine rainfall typologies were developed from rainfall patterns in Köppen climate zones. For each typology, the odds of ITN use among individuals with access to an ITN within their households ("ITN use given access") were estimated for each month of the year, controlling for region, wealth quintile, residence, year, and malaria parasitemia level. Seasonality of ITN use given access was observed over all nine rainfall typologies and was most pronounced in arid climates and less pronounced in typologies where rainfall was constant or nearly constant throughout the year. Peak ITN use occurred 1-3 months after peak rainfall and corresponded with peak malaria incidence and average malaria transmission season. Temperature was positively associated with ITN use given access in southern and equatorial zones where hot season extends through the rainy season, and negatively associated elsewhere. The observed lags between peak rainfall and peak ITN use given access suggest that net use is triggered by mosquito density. In equatorial areas, ITN use given access is likely to be high year-round given the presence of mosquitoes and an associated year-round perceived malaria risk. These results can be used to inform social and behavior change interventions to improve ITN use in specific times of the year, and to inform geospatial models of the impact of ITNs on transmission.

397

EVALUATION OF SIERRA LEONE'S INTERMITTENT PREVENTATIVE TREATMENT FOR INFANTS (IPTI) PILOT IN KAMBIA DISTRICT

Laura C. Steinhardt[1], Roberta Sutton[2], Oliver Eleeza[3], Adewale Akinjeji[3], Anthony Mansaray[3], Michael John[3], Brigette Gleason[4], Michael Friedman[5], Samuel J. Smith[6], Miriam Rabkin[2], Maria Lahuerta[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*ICAP-Columbia, New York, NY, United States,* [3]*ICAP-Columbia, Freetown, Sierra Leone,* [4]*Centers for Disease Control and Prevention, Freetown, Sierra Leone,* [5]*Centers for Disease Control and Prevention, Dhaka, Bangladesh,* [6]*Ministry of Public Health, Freetown, Sierra Leone*

Intermittent preventive treatment in infants (IPTi) with sulfadoxine-pyrimethamine (SP) is a proven strategy to reduce malaria morbidity in infants and is given at 3 routine immunization visits. Sierra Leone is the only country to implement IPTi, beginning in 4 pilot districts in 2017 and scaling up nationwide in 2018. We evaluated IPTi in the initial pilot district of Kambia to assess quality, coverage, and acceptability of IPTi, and explored potential impact on malaria morbidity in this setting. This mixed-methods evaluation included two data collection phases at 3 and 16 months after IPTi implementation. It included health facility (HF) assessments and register data abstraction at a 25% sample of HFs in Kambia (phases 1 and 2) and a household (HH) survey of children aged 3-15 months in 44 clusters using multi-stage sampling (phase 2). Vaccination coverage from the HH survey was calculated from review of child health cards and maternal recall and weighted for the sampling design. HF register data were analyzed using segmented regression comparing 12 months pre- vs. 15 months post-IPTi. Nearly all HFs (17/18 in phase 1 and 18/18 in phase 2) had SP for IPTi in stock and most had supervisory visits in the last six months that addressed IPTi provision (15/18 in phase 1 and 18/18 in phase 2). Improved drinking water for IPTi administration was available at 7/18 and 11/18 HFs in phases 1 and 2, respectively. According to the HH survey, 67.4% of 459 children at least 10 weeks old had received the first dose of IPTi, and 80.4% received Penta 2, given at the same time. Among 444 children ≥14 weeks old, 62.5% received the second dose of IPTi, and 65.4% received Penta 3. Among 217 children ≥ 9 months old, 36.4% received the third dose of IPTi and 52.2% the first measles vaccination. HF register data for patients <12 months old indicated little change in outpatient visits or confirmed malaria after IPTi, but a 23% decrease in malaria test positivity rate was found (p=0.027). Kambia district was able to scale up IPTi swiftly and provide health systems support required for introduction. IPTi coverage could be further improved, as evidenced by gaps between IPTi and childhood vaccines.

398

THE IMPACT OF INDOOR RESIDUAL SPRAYING FOR MALARIA PREVALENCE IN HOMA BAY COUNTY, KENYA: AN OBSERVATIONAL STUDY

Wataru Kagaya[1], Aya Konishi[2], Kyoko Kurihara[2], Chim Chan[1], Jesse Gitaka[3], James Kongere[4], Kinya Uchihashi[2], Gordon Okomo[5], Akira Kaneko[1]

[1]*Osaka City University, Osaka, Japan,* [2]*Sysmex Corporation, Kobe, Japan,* [3]*Mount Kenya University, Thika, Kenya,* [4]*NUITM/KEMRI, Nairobi, Kenya,* [5]*Homa Bay County Ministry of Health, Homa Bay, Kenya*

The estimated global malaria incidence and death decreased by 16% and 48% respectively between 2000 and 2017, as a consequence of increasing global financial aids to support the scale-up of novel tools such as artemisinin-based combination therapy (ACT), insecticide treated bed nets (ITNs), and rapid diagnostic test (RDT). Homa Bay County in Kenya experienced a dramatic reduction of malaria prevalence after the implementation of a large scale indoor residual spraying (IRS) campaign in February 2018. Our cross-sectional malariometric surveys in school children from 2012 to 2017 revealed high and stable malaria prevalence in the inland Ungoye area. However the prevalence significantly

decreased between January and September 2018 from 40.6% to 15.0% by microscopy. On the contrary, Ngodhe Island in Lake Victoria, where IRS was not implemented, had an increase of prevalence from 4.5% to 15.1% by microscopy over the same period. We also investigated suspected clinical malaria cases at Homa Bay County Teaching and Referral Hospital in February 2019 by testing their malaria infection status together with complete blood count (CBC). Although the number of suspected malaria cases did not significantly decrease compared with previous year, slide positivity rate significantly decreased from 11.7% to 6.5%. These results suggest that the IRS program had significant impacts on malaria prevalence and incidence in the target area. The transition of febrile cases from malarial to non-malarial causes necessitates a more precise differential diagnosis of febrile cases in health facilities. We discuss the applicability of a novel hematology analyzer, which can rapidly and simultaneously measure both malaria-infected red blood cell counts and CBC, in the context of malarial vs. non-malarial febrile case management.

399

EFFICACY OF CONTINUOUS BED NET DISTRIBUTION TO MAINTAIN HIGH COVERAGE AFTER A MASS DISTRIBUTION CAMPAIGN IN MADAGASCAR, 2017

Jacky Aubin Raharinjatovo[1], Mauricette Nambinisoa Andriamananjara[2], Fanjanirina Randrianarivony[1], Gilbert Randrianandrasana[1], Laurent Kapesa[3], Jocelyn Razafindrakoto[3], Jemima Andriamihamina[3], Sarah Baum[4], Mickael Randriamanjaka[1], Hasina Harinjaka Ramiandrisoa[2], Morgan Brown[4]

[1]*PSI Madagascar, Antananarivo, Madagascar,* [2]*NMCP, Antananarivo, Madagascar,* [3]*USAID/PMI Madagascar, Antananarivo, Madagascar,* [4]*Population Services International, Washington, DC, United States*

In 2015, Madagascar conducted a mass campaign to distribute (MCD) 10.7 million insecticide treated bed nets (ITN) in 92 malaria-endemic districts of the country. To maintain universal coverage (one ITN per two people), replace damaged ITNs and cover new sleeping spaces, the National Malaria Control Program (NMCP) and partners piloted a community-based distribution scheme called continuous distribution (CD) one year after the MCD. The pilot was conducted in 11 districts and lasted 10 months. To assess the efficacy of CD, we compared ITN use in two CD districts, Vavatenina on the east coast and Vohipeno in the south, to two non-CD districts that were near or bordering the CD districts, Soanierana Ivongo and Vondrozo districts, respectively. We used multi-stage sampling to select 300 households per district: 10 enumeration areas (EA) per district were selected with probability proportional to size; in each EA, 30 MCD households were systematically sampled. Households that refused or were unable to participate were replaced. In each study household, we identified all ITNs and recorded whether they were from CD. A questionnaire was administered to the head of household regarding ITN use and ownership. Proportion comparisons were conducted using chi-squared tests in STATA 13.0. Information was available from 1,200 households. The proportion of sleeping spaces covered with ITNs was 85.9% and 66.9% in CD and non-CD districts, respectively ($p < 0.001$). In CD districts, the proportion of households with at least one ITN was 95.7% and in non-CD districts it was 78.3% ($p < 0.001$). The proportion of households owning at least two ITNs in CD districts was 63.3% whereas in non-CD districts it was 25.7% ($p < 0.001$). The proportion of people who reported having slept under an ITN the previous night was 80.7% in CD districts compared with 62.5% in control districts ($p < 0.001$). ITN coverage and use were higher in CD districts suggesting that CD after an MCD may increase ITN access and use. Due to the success of this pilot, the NMCP will scale-up CD in 39 districts one year after the 2018 MCD and evaluate the impact of this strategy over a longer period.

400

INDIVIDUAL, HOUSEHOLD AND COMMUNITY FACTORS ASSOCIATED WITH THE UPTAKE OF THREE DOSES OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP3) IN COTE D'IVOIRE: A MULTILEVEL ANALYSIS

Stella Babalola[1], Abdul Dosso[2], Monne Therese Bleu[3], Antoine Kouame[4], Olamide Oyenubi[1], Grace Awantang[1], Michael Toso[1], Gabrielle Hunter[1], Mieko McKay[2], Colette Yah Kokrasset[3], Blaise Kouadio[5], Antoine Mea Tanoh[3], Diarra Kamara[2]

[1]*Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [2]*Johns Hopkins Center for Communication Programs, Abidjan, Côte D'Ivoire,* [3]*Ministry of Health and Hygiene, Cote d'Ivoire National Malaria Prevention and Control Program, Abidjan, Côte D'Ivoire,* [4]*Save the Children Cote d'Ivoire, Abidjan, Côte D'Ivoire,* [5]*United States Agency for International Development-USAID, Abidjan, Côte D'Ivoire*

The World Health Organization has recommended intermittent preventive treatment of malaria in pregnancy (IPTp) for women, achieved by taking multiple doses of Sulfadoxine/pyrimethamine (SP) during pregnancy. In Cote d'Ivoire, the policy recommends that pregnant women take at least three doses of SP. Results of the 2016 Multiple Indicator Cluster Survey reveal that only 22.6% of women that gave birth during the previous two years obtained at least three doses of SP during their pregnancy. Applying multilevel regression to data from a 2018 national malaria behavioral determinants survey, this study examines background factors that are associated with the uptake of three or more doses of SP. The sample for the study included 2225 women who had a child during the two years before the survey. The data showed that the majority (91.4%) of these women obtained at least one antenatal care (ANC) visit and a little over three quarters (78.1%) attended at least four visits. Concerning the uptake of IPTp, the majority (86.5%) received at least one dose whereas only about half (53.0%) received at least three doses. The health facility was the source of IPTp doses for most women: 86.2% received at least one SP doses from this source. It is notable that 26.7% of the women obtained one or more doses of SP from a private pharmacy. Though women who obtained at least four ANC visits were more likely than others to obtain the third dose of SP (IPTp3), less than 60% of these women obtained IPTp3. Results of the multilevel regression show that the variables most strongly and positively associated with obtaining IPTp3 were number of ANC visits, husband's engagement in antenatal care, prevalence of post-primary education in cluster of residence, and the woman's age. The analyses further suggest the existence of unmeasured variables operating at the cluster level that are associated with the uptake of IPTp3. Programs designed to promote the uptake of IPTp3 should encourage increased frequency of ANC attendance among pregnant women by targeting male partners, using community mobilization approaches, and addressing relevant health system-related factors

401

TIMELINESS OF INITIATION OF INTERMITTENT PREVENTATIVE TREATMENT OF MALARIA WITH SULFADOXINE-PYRIMETHAMINE (IPTP-SP) IN PREGNANT WOMEN: EVIDENCE FROM A CROSS SECTIONAL SURVEY IN MALAWI

Jobiba Chinkhumba[1], Ashley Malpass[2], Katherine Wright[3], Xiomara Brown[2], Dziko Chatata[4], John Munthali[4], Michael Kayange[5], Fannie Kachale[5], Don Mathanga[1], Julie Gutman[6]

[1]*University of Malawi, College of Medicine, Malaria Alert Center, Blantyre, Malawi,* [2]*U.S. President's Malaria Initiative, Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Lilongwe, Malawi,* [3]*Management Sciences for Health (MSH), Medford, MA, United States,* [4]*Management Sciences for Health (MSH), Lilongwe, Malawi,* [5]*Ministry of Health, Lilongwe, Malawi,*

[6]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States*

Intermittent Preventive Treatment of pregnant women with sulfadoxine-pyrimethamine (IPTp-SP) is a key strategy to prevent malaria related adverse health outcomes among pregnant women and their infants in malaria endemic areas. Current Malawian National guidelines recommend that pregnant women receive the first dose at 13 weeks gestational age, with subsequent doses four weeks apart, for a minimum of three doses. However, coverage remains low. We aimed to assess timeliness of the first dose of IPTp-SP (IPTp-SP1; receipt between 13 and 16 weeks) among pregnant Malawian women during the initial antenatal care (ANC) visit to identify missed opportunities and highlight key points for programmatic intervention. A cross-sectional survey in two districts in Malawi recruited women who had a live birth in the previous 12 months. IPTp-SP uptake data were collected using a structured questionnaire and ANC card review. A total of 370 recently pregnant women with mean age 25 years (SD 6.2) and average parity 2.5 (SD 1.5) participated in the survey. Few (26.2%, 95%CI: 21.9-30.9) had attained secondary education. A total of 270 women had ANC cards available. At the initial ANC visit, the women's mean gestation age (GA) was 20 weeks (SD 5.1), and 86.6% (95% CI: 77.2-92.5) were ≥13 weeks; 19.0% (95% CI: 12.2-28.4) presented between 13-16 weeks. Overall, considering the 1st ANC visit only, 67.3% (95% CI: 59.9-73.9) of women who were at least 13 weeks GA received IPTp-SP during their initial ANC visit. IPTp-SP was provided to 49.6% (95% CI: 31.2-68.2), 67.6% (95% CI 51.6-86.6), 72.3% (95% CI 62.4-80.4), and 86.2% (95% CI 67.4-91.6) of women attending at 13-16, 17-20, 21-24, and 25-28 weeks, respectively. Few women attend ANC early enough to receive the first dose of IPTp in a timely fashion; even among those who do, half do not receive IPTp-SP. To increase early IPTp-SP uptake and coverage, it is critical to find effective ways to facilitate early ANC attendance, such as improved community engagement and mobilization, and to improve the quality of ANC to ensure women receive SP at all visits, per national guidelines

402

LARVIVOROUS FISH BASED MALARIA ELIMINATION IN INDIA

Susanta Kumar Ghosh

ICMR-National Institute of Malaria Research, Bangalore, India

Larval source management with larvivorous fish is an effective strategy for vector management. This method of larval control was necessitated in areas where routine indoor insecticidal spraying was not possible in most of malaria-endemic sericulture areas in Southern Karnataka State, India. Larval source management was the only option left in this area to contain malaria transmission. It was noted that villages surrounded by ponds and wells had more malaria than villages on streams. Two reported malaria vectors *Anopheles culicifacies* and *An. fluviatilis* were present. Again, sibling species complex analyses of these two vectors revealed that species A of *An. culicifacies* were more numerous in villages with ponds and wells, while species B in villages situated nearer to streams. On the other hand, all *An. fluviatilis* belonged species T. Species B of *An. culicifacies* and species T of *An. fluviatilis* are not efficient vectors. It was also observed that in most of nearby non-malarious areas larvivorous fish were present. These observations led to the release of larvivorous fish for management of malaria vectors. Following this, a detailed geographical reconnaissance was conducted to enumerate the water bodies in each village. Two Poecillid fish *Gambusia affinis* and *Poecilia reticulata* were used for the intervention. It was observed that *Gambusia* is best suited in ponds and large water bodies, while *Poecilia* in wells. A block-level task force model is in place involving the local health and block development officials. The main challenge is to implement this programme at village level where dedicated staff are needed, local government to expand the programme. In the initial stage some window period must be given to establish the fish in the local environment. Monitoring of fish once in 6-month period is good for better results. Now, village-level data through remote sensing are available, and this can be utilized in an effective manner.

403

COSTING MASS DRUG ADMINISTRATION WITH DIFFERENT TARGETING STRATEGIES

Shwe Sin Kyaw[1], Tom L. Drake[2], Wirichada Pan-ngum[1], Sasithon Pukrittayakamee[1], Yoel Lubell[1], Lisa J. White[1], Frank M. Smithuis[3]

[1]*Mahidol Oxford Research Unit, Bangkok, Thailand,* [2]*London School of Tropical Hygiene and Medicine, London, United Kingdom,* [3]*Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom*

Mass drug administration (MDA) is receiving increasing attention as an essential component in the toolkit for malaria elimination. In the Greater Mekong Sub-region (GMS), efforts to eliminate *P. falciparum* malaria must be accelerated to address the global health emergency of anti-malarial drug resistance. There are many options for the implementation of MDA including targeted approaches, each of which has different economic implications. A key piece of information missing so far has been a realistic estimate of the programmatic cost of MDA for various targeting strategies. This paper uses an evidence-based method to extrapolate programmatic costs from the financial data of a trial. We estimate the costs of implementing a range of mass interventions against *P. falciparum* malaria with a range of choices for the extent and method of targeting high-risk sub-populations. The results from this are being used to guide decision-making on malaria elimination in the GMS. An online tool is available to further enhance the utility of this research.

404

COLLECTIVE INTELLIGENCE AND MALARIA: A PRECISION PUBLIC HEALTH-MALARIA ELIMINATION MODEL

Leopoldo Villegas[1], Gustavo Bretas[1], Stephen Vreden[2]

[1]*Global Development One, Silver Spring, MD, United States,* [2]*Academisch Ziekenhuis Paramaribo, Paramaribo, Suriname*

Collective intelligence is a key feature of mankind that is being expanded with the use of computers and digital data. There are several examples of applications of CI in many fields but there is limited information on its use on malaria elimination. Here we describe a public health model for malaria elimination that uses CI. In 2004, Suriname's National Malaria Control Program (NMCP) decided to accelerate its malaria control activities with the support of the Global Fund. A precision public health model was built with all the available information about humans, vectors, and parasites involved in the transmission. An epidemiological stratification was conducted in order to target interventions aiming to eliminate malaria transmission. Three different, well-defined strata were identified, and a combination of interventions was adapted to each of the stratum. The model used included a rigorous M&E plan to track all activities and the epidemiological impact it had. Different inputs were discussed and incorporated in a forum, the National Malaria Board; researchers, malariologists, politicians, clinicians, and representatives of the communities affected. Once the general strategy was decided, a strategic partnership plan was designed to engage all key actors in the malaria elimination campaign. The result was that Suriname experienced a rapid and significant impact on malaria morbidity and mortality rates. Between 2004-2017, *P. falciparum* and *P. vivax* infectious decreased 98.5% and 67.8%, respectively. Using collective intelligence, Suriname experienced a 180-degree turn, from having the highest annual parasite incidence (API) in the Americas (207 per 1000 people) in 2003 to become one of the E2020 malaria elimination countries (API=6 per 1000 people). This is an example illustrating that CI could be used to develop precision public health models that leave an imprint on society. This model could be adapted to other malaria scenarios, benefiting from the synergies between data-driven approaches, information use, expertise, software/hardware, partners, and in-depth participation of a broad range of affected communities.

405

PERSPECTIVES OF KEY STAKEHOLDERS IN TANZANIA ON ALTERNATIVE TECHNOLOGIES FOR MALARIA ELIMINATION

Marceline F. Finda[1], Nicola Christofides[2], Javier Lezaun[3], Ann H. Kelly[4], Fredros O. Okumu[1]

[1]*Ifakara Health Institute, Ifakara, United Republic of Tanzania,* [2]*University of the Witwatersrand, Johannesburg, South Africa,* [3]*University of Oxford, Oxford, United Kingdom,* [4]*King's College London, London, United Kingdom*

Global burden of malaria has significantly reduced since 2000 due to both public health efforts and improvements in socioeconomic conditions. As we approach elimination however, it is crucial to understand and address socio-cultural factors associated with persisting transmission. Novel technologies such as mass releases of genetically-modified mosquitoes, mass drug administrations of Ivermectin and larviciding require strong collaborations between scientists and communities to ensure the technologies are effective and meet local needs. This study aims to use innovative anthropological techniques to bridge current gaps between researchers and key stakeholders on various issues relative to alternative malaria control technologies. Focus group discussions (FGDs) were held with stakeholders including scientists, policy makers, officials from regulatory bodies and community members in Tanzania. The discussions focused on exploring opinions of the stakeholders on the need for alternative technologies for malaria elimination, and their views on novel technologies that are aiming towards elimination. This is an on-going study. All participants of the FGDs agreed that while malaria prevalence and incidence have declined over the past decade, currently available strategies and their levels of utilization are not sufficient for malaria elimination in Tanzania by 2030. However there were varying opinions among the stakeholders on which alternative technologies should be put in place to achieve elimination by 2030. Popular technologies across the stakeholder groups included larviciding, genetically-modified mosquitoes, and spatial repellents. Less preferred options included space-spraying of mosquitoes and mass drug administration of Ivermectin. Community members and scientists also preferred improving housing for the lowest income people for malaria control, but policy makers challenged the sustainability of this strategy given its high costs. More FGDs are in progress and more comprehensive findings will be shared during the conference.

406

MALARIA CONTROL AND ELIMINATION IN AFRICA: WHY ARE SOME COUNTRIES DOING BETTER THAN OTHERS?

Xiaoming Wang, Guofa Zhou, Guiyun Yan

University of California Irvine, Irvine, CA, United States

This study was undertaken to determine social, economic, biological and environmental factors critical to malaria control effectiveness in African countries that are with contrasting responses to the malaria control and elimination efforts, and use this information to inform the development of improved malaria control strategies in Africa. A systematic analysis was conducted using longitudinally collected data from publically available data sources for the period of 2000 and 2016, for 8 African countries with contrasting malaria incidence dynamics. Single- and multivariable linear regression analyses were performed to determine the impact of various risk factors on malaria incidence. The risk factors included autocorrelation with a one-year time lag, study country, and economic status, internal and external malaria control budgets, bednet coverage, indoor residual spray coverage, and insecticide resistance status. Malaria incidence exhibited highly significant autocorrelation. Per capita government funding for malaria control and insecticide resistance were the two significant factors correlating with malaria incidence. In conclusion, this study indicates the significance of malaria control investment from African countries themselves and insecticide resistance management in malaria control and elimination in Africa.

407

LISTENING TO THE VOICES OF THE VULNERABLE: A MIXED METHODS STUDY ON HEALTH-SEEKING BEHAVIORS IN A MALARIA ENDEMIC DISTRICT IN LAO PEOPLE'S DEMOCRATIC REPUBLIC

Ken Ing Cherng Ong[1], Phonepadith Khattignavong[2], Sengdeuane Keomalaphet[2], Moritoshi Iwagami[3], Bouasy Hongvanthong[4], Paul T. Brey[2], Shigeyuki Kano[3], Masamine Jimba[1]

[1]*Department of Community and Global Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan,* [2]*Institut Pasteur du Laos, Ministry of Health, Vientiane, Lao People's Democratic Republic,* [3]*Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan,* [4]*Center of Malariology, Parasitology and Entomology, Ministry of Health, Vientiane, Lao People's Democratic Republic*

Lao People's Democratic Republic (PDR) aims to eliminate malaria by 2030. Past efforts were successful in reducing the number of malaria cases and 80% reduction was observed from 2012 to 2017. To eliminate malaria, previous efforts focused on vector control through the distribution of long-lasting insecticide bed nets, and biomedical approach through the distribution of antimalarials, but the social aspects of malaria always came second. The population approach where a one-size-fits-all strategy is employed through antimalarials and bed nets distribution might not be enough for vulnerable populations such as the ethnic minorities and low-income families in the rural areas. We investigated the health-seeking behaviors of the villagers in two villages in the malaria endemic area of Thapangthong district, Savannakhet Province, in October, 2018. We conducted a mixed methods study using a pre-tested questionnaire in the quantitative part and in-depth interviews and focus group discussions in the qualitative part. We targeted the villagers and the health care providers in the area. We found that although 256 out of 313 villagers (81.8%) responded that they would first go to the village health center or the district hospital to seek treatment, qualitative data from focus group discussions revealed problems faced by the villagers such as lack of medicines and medical equipment. "*Overall everything is good but only one thing is that medicine is always not enough*" (*Male, 40, Teacher)*. Although bed nets coverage was high according to the in-depth interviews with the health care providers, and 276 (88.2%) villagers responded they had bed nets at home, their understanding on the transmission of malaria varied. "*Because your house is not clean. If you drink water with mosquito eggs, you will get fever*" (*Female, 45, Government servant).* In conclusion, the vector control and parasite control strategies are undoubtedly crucial, but the social approach is equally important for malaria elimination. By understanding the local beliefs and perceptions of the vulnerable population, better strategies can be formulated to eliminate malaria.

408

IMPLEMENTATION IMPACT OF INNOVATIVE 1,7-MRCT APPROACH ON MALARIA BURDEN REDUCTION IN TANZANIA

Wang Duoquan

National Institute of Parasitic Diseases, China CDC, Shanghai, China

In 2017, China recorded its first year of zero indigenous cases of malaria transmission. New efforts are in translation of Chinese experiences and lessons to support global malaria elimination efforts in developing countries and particularly in Africa. The China-UK-Tanzania pilot project on malaria control was the first project for China to pilot Chinese experiences on malaria control in Africa. The China-UK-Tanzania pilot project on malaria control was an intervention study with two representative intervention communities receiving proposed interventions and two comparable communities serving as control sites. The project intervention included Test, Treat and Track (T3) Initiative of World Health Organization integrated with Chinese experiences on malaria control and elimination. A joint efforts of Chinese and Tanzanian teams, worked together at the ground level, and customized innovatively China's "1-3-7" model for fast-tracking malaria elimination into local approach for areas

of high and moderate transmission. This new approach, named malaria Reactive Community-based Testing and Response approach (1,7-mRCT), has been implemented through intensive use of existing health facility data to decide on the priority clusters to conduct community testing and treatment on weekly basis between 2015 and 2018. This innovative 1,7 -mRCT response approach significantly reduced malaria parasite prevalence by >70% in the intervention communities, beyond and above the benefit of long-lasting insecticide-treated nets (LLINs). The burden of malaria cases reported at health facilities was reduced by 20% from the week of 1,7-mRCT response at the respective villages and the prophylactic effect was observed to continue for five weeks since treatment. This innovative 1,7 -mRCT approach demonstrated effectiveness in significantly reducing prevalence and incidence of malaria in areas of moderate and high transmission. This could be potentially scaled-up to other areas of African countries including Tanzania, to accelerate the malaria control and elimination progress in Africa.

409

MALARIA SURVEILLANCE IN TANZANIA ELECTRONIC INTEGRATED DISEASE SURVEILLANCE AND RESPONSE (EIDSR) SYSTEM COMPLETENESS AND TIMELINES IN AREAS OF VERY LOW TRANSMISSION

Joseph Joachim Joseph[1], Ssanyu Nyinondi[1], Shabbir Lalji[1], Abdul-wahid Al-mafazy[1], Humphrey Mkali[1], Ally Mohamed[2], Renata Mandike[2], Frank Chacky[2], Anna Mahendeka[2], Chonge Kitojo[3], Naomi Kaspar[3], George Greer[3], Erik Reaves[4], Jeremiah Ngondi[5], Claud John[6], Richard Reithinger[5]

[1]*RTI International, Dar es salaam, United Republic of Tanzania,* [2]*National Malaria Control Program, Dodoma, United Republic of Tanzania,* [3]*US President's Malaria Initiative, United States Agency for International Development, Dar es salaam, United Republic of Tanzania,* [4]*US President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es salaam, United Republic of Tanzania,* [5]*RTI International, Washington, DC, United States,* [6]*Ministry of Health Community Development Gender Elderly and Children, Dodoma, United Republic of Tanzania*

Complete and timely reporting of malaria cases is crucial to detect and mitigate malaria outbreaks. Tanzania uses an electronic Integrated Disease Surveillance and Response (eIDSR) system to report weekly on aggregate malaria cases. eIDSR was piloted in 67 health facilities in 2013 and scaled-up to 7,138 facilities in 25 of the country's 26 regions by November 2018. This study aimed to describe the trends of completeness and timeliness of reports submitted through eIDSR stratified by malaria parasite prevalence, (i.e., very low <1%, low 1≤5 %, moderate 5≤30%, and high >30%), to ascertain its usefulness for malaria outbreak detection and case-based surveillance. Completeness was defined as the proportion of reports containing all malaria indicators submitted over the expected number of reports within a week; timeliness was defined as the proportion of reports submitted by the first day of the following week over the expected number of reports. Overall, the completeness of reporting increased from 51.1% (20,452/40,040) in 2014 to 66.6% (239,715/359,736) in 2018, and timeliness increased from 25.4% (10,187/40,040) in 2014 to 41.4% (149,022/359,736) in 2018. Reporting completeness and timeliness in 2018 varied by malaria prevalence, with completeness significantly higher in areas of very low prevalence compared to areas with high prevalence (90.1% vs 60.9%; p<0.0001) and timeliness significantly higher in areas of very low prevalence (60.5% vs 38.5%; p< 0.001). Both completeness and timeliness of eIDSR reports on aggregate malaria cases were greater in areas with very low malaria prevalence, which might make eIDSR a useful tool for both early outbreak detection and case-based surveillance in these settings. Opportunities remain to strengthen completeness and timeliness of eIDSR reporting across all prevalence strata.

410

A MULTIMEDIA IEC-BCC CAMPAIGN TO MODIFY MALARIA TREATMENT-SEEKING BEHAVIOR IN ESCUINTLA, GUATEMALA: DESIGN, IMPLEMENTATION AND EVALUATION IN ENDEMIC LOCALITIES

José Miguel Echeverría[1], Juan Valentín Santos Salazar[1], Beci López Ojeda[1], Rodrigo Antonio Flores[1], Javier López Sevilla[2], Melissa S. Wiles[2], Madeline Baird[3], Graziella Scudu[2], Darlene Bhavnani[3]

[1]*Ministry of Public Health and Social Assistance, Guatemala City, Guatemala,* [2]*Clinton Health Access Initiative, Guatemala City, Guatemala,* [3]*Clinton Health Access Initiative, Panama City, Panama*

Guatemala has committed to eliminate malaria by 2020. In the endemic region of Escuintla where 50% of Guatemala's 3,084 total cases occurred in 2018, people with malaria symptoms often self-medicate or go to pharmacies/informal shops to purchase low-dose chloroquine instead of visiting official points of care (POCs) (e.g. community health workers [CHWs] and public sector health facilities). These practices prolong the time between symptom onset and diagnosis, increasing risk of transmission. The Ministry of Public Health and Social Assistance (MOH) designed a multimedia campaign to reduce self-medication practices and increase uptake of appropriate POCs for malaria diagnosis and treatment and prevention methods. The campaign was piloted in 11 high-risk localities via radio and television, household visits and educational events at churches, schools and other public spaces, selected as culturally-appropriate dissemination methods based on community interviews conducted by local level MOH staff. Exposure to the campaign and behavior change among care seekers is being evaluated via pre/post-intervention program monitoring information collected by CHWs and health facility staff from all who present with fever between February and April 2019 in target localities. The impact of the various dissemination channels included in the campaign will also be assessed to effectively direct future campaign resources. Baseline results show that self-medication is common: 72% self-medicated and 20% went somewhere else to receive treatment before an appropriate POC. Results also show a difference between localities: prevalence of self-medication varied from 53% in some localities up to 100% in others. If shown to be effective for generating behavior change, this campaign could further advance country efforts for malaria elimination, reducing time between symptom onset and initiation of treatment, and ensuring that all cases are detected, diagnosed, promptly treated and investigated to further understand risk factors for persistent transmission such as treatment non-adherence and *P. vivax* relapse infections.

411

USING MODELS TO INFORM IMPLEMENTATION POLICIES OF SEASONAL MALARIA CHEMOPREVENTION

Kamaldeen O. Okuneye, Jaline Gerardin

Institute for Public Health and Medicine, Northwestern University, Chicago, IL, United States

Malaria transmission is highly seasonal in the Sahel and sub-Sahel regions of Africa, where over 60% of clinical malaria cases occur during the 3 - 4 month rainy season and about 90% of the mortality and morbidity occurs among children under the age of 5 years. In addition to the use of insecticide-treated nets and indoor residual spraying, the WHO recommends seasonal malaria chemoprevention (SMC) for children 3 - 59 months in these regions, and some countries are considering increasing the age of eligibility to 10 years. SMC is an intermittent administration of full treatment courses of antimalarial drugs (usually sulfadoxine-pyrimethamine plus amodiaquine, SP-AQ), during the malaria season to prevent illness by maintaining therapeutic antimalarial drug concentrations in the blood throughout this period. Using data from literature, we constructed pharmacokinetic models of SP and AQ in EMOD, an agent-based stochastic model, then calibrated pharmacodynamics of SP and AQ to clinical trials of SMC and of artesunate-AQ, respectively, to capture the

curative and prophylactic properties of SP-AQ across different age groups. Using this model and data collected from Sahelian regions, we describe the impact of imperfect adherence on the success of SMC and the increase of potential exposure of parasites to subcurative drug concentrations that may select for resistant phenotypes. We explore the role of a novel single-dose SMC drug could play in increasing SMC impact. We quantify the potential burden reduction achievable by expanding the SMC age range, how the gains depend on transmission intensity and seasonality, and which geographical areas stand to benefit most from expanding or introducing SMC, including East African regions. Using SMC surveillance data from multiple countries, we identified which areas have a smaller impact from SMC than expected and should be investigated to determine whether these differences are due to insufficient coverage, participation, and/or drug resistance. Results from this work can be used to improve the implementation of SMC by providing standard strategies and individual approaches to existing and new SMC regions.

412

EXPERIENCE FROM THE FIELD: HOW TO IMPLEMENT A COMPLEX INTERVENTION STUDY TO CONTROL MALARIA IN REMOTE AREAS OF THE AMAZON FOREST, WITHIN A CROSS-BORDER CONTEXT?

Muriel Suzanne Galindo[1], Yann Lambert[1], Martha Cecilia Suarez Mutis[2], Louise Mutricy[1], Helene Hiwat[3], Laure Garancher[4], Lise Musset[5], Cassio Peterka[6], Alice Sanna[7], Antoine Adenis[1], Mathieu Nacher[1], Stephen Vreden[8], Maylis Douine[1]

[1]*Centre d'Investigation Clinique Antilles-Guyane (Inserm 1424), Cayenne Hospital, Cayenne, French Guiana,* [2]*Laboratory of Parasitology, Institute Oswaldo Cruz, Rio de Janeiro, Brazil,* [3]*National Malaria Programme, Ministry of Health, Paramaribo, Suriname,* [4]*Pan American Health Organization, Barbados Office, Bridgetown, Barbados,* [5]*Laboratoire de Parasitologie, Centre National de Référence du Paludisme, Institut Pasteur de la Guyane, Cayenne, French Guiana,* [6]*National Malaria Programme, Ministry of Health, Brasilia, Brazil,* [7]*Health Regional Agency of French Guiana, Cayenne, French Guiana,* [8]*Scientific Research Center Suriname, Paramaribo, Suriname*

In complex intervention reporting, a clear definition of the intervention, including the assumptions behind it, and the description of what and how it is implemented, are often lacking. We present here our experience in implementing a pilot strategy to control malaria, titled *Malakit*, targeting illegal gold miners working in French Guiana. This population is identified as a key malaria reservoir in the Region. The principle is to provide participants with a self-diagnosis and self-treatment kit along with adequate resources to handle rapidly and adequately an episode of malaria symptoms by themselves, when isolated in the Amazon forest. The choice not to develop a usual community-based "test and treat" approach is mainly due to regulatory reasons. Developing this intervention involves facing several issues: a mobile population living in remote areas and poorly educated, and a cross-border context with three countries: France, Brazil and Surinam. Several tools to communicate remotely between stakeholders have been experimented but face-to-face meetings remain the most efficient alternative. Debating and decision-making previously undertaken in four languages, including English, were greatly improved by the presence of interpreters during international meetings. Important rear bases on the two border rivers which are transit and rest areas were previously identified as key places to recruit participants. Malakit facilitators were hired for their belonging or proximity to the *garimpeiros* community to train participants how to use a kit. The information, education and communication tools were designed by taking into account the living conditions, education level and habits of the study population *i.e.* animated and tutorial videos, illustrated instructions printed on the plastified kits and an interactive smartphone app. The training-enrollment procedure is detailed in an illustrated step by step guide but tailoring the intervention to individual participants is partially allowed. Adaptations observed are related to participants individual characteristics such as time availability, reading capacity or smartphone literacy.

413

MALARIA CASE MANAGEMENT PRACTICE AND ELIMINATION READINESS IN FIVE ELIMINATION DISTRICTS OF MADAGASCAR, 2018

Anjoli Anand[1], Favero Rachel[2], **Catherine Dentinger**[3], A. Ralaivaomisa[4], S. Ramamonjisoa[5], Elaine Razafimandimby[5], Jocelyn Razafindrakoto[6], Katherine Wolf[2], Laura C. Steinhardt[7], Julie Thwing[7], Bryan K. Kapella[7], M. Rabary[8], Sedera Mioramalala[9], Jean Pierre Rakotovao[5]

[1]*Epidemic Intelligence Service, Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Maternal Child Survival Program, Washington, DC, United States,* [3]*US President's Malaria Initiative, US Centers for Disease Control and Prevention, Antananarivo, Madagascar,* [4]*Maternal Child Survival Program, Madagascar, Antananarivo, Madagascar,* [5]*Maternal Child Survival Program, Antananarivo, Madagascar,* [6]*US President's Malaria Initiative, Antananarivo, Madagascar,* [7]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [8]*Independent consultant, Antananarivo, Madagascar,* [9]*National Malaria Control Program, Antananarivo, Madagascar*

Madagascar's Malaria National Strategic Plan 2018-2022 calls for progressive malaria elimination beginning in low-incidence districts (< 1 case/1000). Although an elimination plan has not yet been developed, optimizing access to prompt diagnosis and quality treatment will be its foundation, along with improving outbreak detection and response. To assess readiness and inform planning, we surveyed health facilities (HFs) and communities to evaluate diagnosis and treatment (case management [CM]) health worker (HW) knowledge and practices, use of data to inform decision-making, and the availability of commodities, training, and supervision. In September 2018, we randomly selected 35 HFs in 5 of the 8 districts identified for elimination, surveyed 41 HWs and 34 community health volunteers (CHVs), and observed 300 clinical encounters between HWs and patients of all ages. To evaluate elimination readiness, a composite score will be assigned to each HF catchment area that incorporates all survey responses based on commodity availability, malaria CM practices, data management, and supervision practices. In preliminary results, 8 of 34 (24%) CHVs reported that they do not manage children under 5 years (CU5) with fever at the community level. Of 26 CHVs who care for CU5, 18 (69%) identified history of fever as a criterion for suspected malaria, 20 (77%) reported using a malaria rapid diagnostic test (RDT) when evaluating patients reporting fever, and 15 (58%) reported giving antimalarials for a positive RDT. Among treating CHVs, 13 (30%) reported having RDTs, and 11 (42%) reported having antimalarials currently available. Among facility-based HWs, 83% identified history of fever as a criterion for a suspected case. Of 120 patients with reported or recorded fever, 56 (47%) were tested with an RDT. Five RDTs were positive; a first-line antimalarial was prescribed to 4 of those patients. This evaluation is a baseline for CM performance as Madagascar establishes elimination targets. In the evaluated districts, CM could be improved by strategies to increase testing at CHV and HF levels and address availability of commodity stocks in the community.

414

INTEGRATED DRUG EFFICACY SURVEILLANCE OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA IN THAILAND, 2013-2018

Prayuth Sudathip[1], Julien Zwang[2], Rungrawee Tipmontree[1], Suravadee Kitcharkarn[1], Thannikar Thongrad[1], Felicity Young[3], Richard Reithinger[3], Jui A. Shah[2], Pascal Ringwald[4], David Sintasath[5], Augkana Saejeng[1], Preecha Prempree[1], Darin Areechokchai[1], Jersuda Kajanasuwan[1], Cheewanan Lertpiriyasuwat[1]

[1]*Bureau of Vector Borne Disease, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand,* [2]*Inform Asia: USAID's Health*

Research Program, RTI International, Bangkok, Thailand, [3]RTI International, Washington, DC, United States, [4]WHO Global Malaria Programme (GMP), Geneva, Switzerland, [5]US President's Malaria Initiative, Regional Development Mission for Asia, United States Agency for International Development, Bangkok, Thailand

Thailand's Bureau of Vector Borne Disease (BVBD), with support from the US President's Malaria Initiative (PMI) and Inform Asia: USAID's Health Research Program, has launched an integrated drug efficacy surveillance (iDES) program to routinely monitor treatment efficacy in low transmission zones. First-line treatment for uncomplicated *Plasmodium falciparum* malaria is dihydroartemisinin-piperaquine fixed-dose combination plus primaquine (DP+P). In 2017, this replaced the artesunate and mefloquine plus primaquine combination treatment (AM+P) that was showing failure. For *P. vivax,* chloroquine plus primaquine (CQ+P) is used. We used Kaplan-Meier survival analysis with modified intent-to-treat analysis to measure crude drug efficacy, with an endpoint at Day 42 for *P. falciparum* and Day 90 for *P. vivax*. Cox multivariate models assessed risk of recurrence. All microscopy confirmed malaria cases treated according to national guidelines between 2013 and 2018 and followed up at least once after treatment were included in the analysis. Among the 29,508 cases included, 13,940 were treated with AM+P, 382 with DP+P, and 15,186 with CQ+P. The mean age was 29 years and 70% were male. Overall crude efficacy for treating *P. falciparum* was 85.4% by Day 42 (95% CI 82.7-87.7), ranging from 78.3% in 2013 to 94.8% in 2018. For *P. vivax,* the crude efficacy was 95.0% by Day 90 (95% CI 94.3-95.6), ranging from 91.0% in 2013 to 96.7% in 2018. Multivariate analysis showed the risk of recurrent infection significantly decreased for both species over time (related to declining transmission in the country), but cases from border provinces such as Sisaket and Tak were at greater risk of recurrent infection. Our analyses showed that iDES can be used as a tool for monitoring antimalarial drug efficacy. By identifying provinces with lower treatment efficacy, iDES provides strategic information for BVBD to better target resources. In the Thai context of malaria elimination, increasing routine next generation sequencing testing on Day 0 for all or a sample of cases is recommended to expand monitoring efforts to include prevalence of common molecular resistance markers.

415

THE EFFECT OF OPTIMIZED SUPPORTIVE SUPERVISION AND DATA USE ON IMPROVED QUALITY OF MALARIA SERVICES IN LIBERIA

Lauretta Nagbe Se[1], George Toe[2], Anne Fiedler[2], Thomas T. Hallie[2], Mantue Reeves[1], Birhanu Getahun[1], Gladys Tetteh[3], Lolade Oseni[1]

[1]*USAID PMI- MCSP Expansion of Malaria Services, Liberia, Sinkor, Monrovia, Liberia,* [2]*USAID PMI- MCSP Expansion of Malaria Services, Sinkor, Monrovia, Liberia,* [3]*Jhpiego Baltimore, Baltimore, MD, United States*

Malaria is the leading cause of health care seeking and of death in Liberia. Malaria prevalence among children under five years in Liberia, based on rapid diagnostic tests (RDTs) was 45% in 2016. The Ministry of Health (MOH) implemented joint supportive supervision and mentoring for health providers to improve the quality of care including malaria performance standards, particularly in rural settings with high malaria burden and limited continuous learning opportunities for providers. In April 2018, the Maternal and Child Survival (MCSP) project started to support the joint supportive supervision and mentoring visits to promote compliance to overall national quality standards at 399 health facilities (HF) in eleven Counties. Mentors and supervisors used on-the-job training/mentoring to improve areas where gaps were identified, reinforced data review and use for program improvement and ensured implementation of recommended remedial actions through follow up in subsequent visits. Information on supportive supervision and mentoring visits was obtained through direct observation and simulation using Ministry of Health revised joint integrated supportive supervision (JISS) tool. At baseline, 85% (100/117) of supported HF had cumulative JISS scores of less than 49% in all the standards, and 60% for malaria performance standards. By September 2018, after five consecutive supportive supervisions and mentoring visits at 95% (111/117) of the HF, the overall JISS scores increased to intermediate level of 70-79%, and by December 2018, all the 117 facilities reached an acceptable level with JISS scores of 80% for overall standards and above, and 85% in malaria performance standards. The ongoing supportive supervision and mentoring visits contributed to measurable improvements in meeting malaria standards and compliance to overall national quality standards. MCSP will share lessons learned in fostering quality improvement through targeted supportive supervision to health care workers to scale up and improve the quality of malaria service delivery by health care workers in rural Liberia.

416

EVALUATION OF THE CONTRIBUTION OF COMMUNITY HEALTH WORKERS (CHWS) IN IMPROVING HEALTH FACILITY ATTENDANCE, PARTICULARLY FOR TIMELY ANC ATTENDANCE AND IPTP SERVICES, IN SIX DISTRICTS IN THE PROVINCIAL HEALTH DELEGATION OF THE EASTERN LOGONE IN CHAD

Naibei Mbaibardoum[1], Ali Baggar[2], Djimodoum Moyreou[1], Noella Umulisa[1], Elana Fiekowsky[1], Kodjo Morgah[1]

[1]*Jhpiego, N'Djaména, Chad,* [2]*Provincial Health Delegation of the Eastern Logone, N'Djaména, Chad*

Malaria infection during pregnancy is a significant public health problem causing substantial risk to pregnant woman, their fetuses, and newborn children. Malaria is a leading cause of morbidity and mortality in Chad, where an estimated 2.2 million cases of malaria occur every year. Children under five and pregnant women are at increased risk. In 2017, Chad national data revealed that malaria represented 36% of outpatient consultations and 30.8% of hospitalization cases. This study evaluated potential client benefits to Community Health Worker (CHW) interaction and referral, including improvements in health facility attendance, timeliness of scheduled antenatal care (ANC) visits and adherence to intermittent preventive treatment in pregnancy (IPTp) services. From February through March 2019, a register review was conducted which included: reporting forms, referral forms and counter-referral forms for the period of January to December 2018 for all 72 CHWs trained by Improving the Quality of Malaria Control Services project funded by ExxonMobil in six districts in the provincial health delegation of Eastern Logone province in Chad. Data collectors recorded monthly total numbers of referred patients collected by CHWs, and compared them with health facility records and counter-referral documents. The data collectors then assessed whether community-level referred malaria cases and ANC visits for pregnant women resulted in treatment seeking behavior among clients receiving these services. In total, 89% of CHW referred patients accessed health centers. Among pregnant women referred for ANC and IPTp services, 77% reached health centers. Among children under five referred, 62% received malaria testing and treatment services at health centers. Of referred patients, 69% reached health centers within 48 hours after CHW referral. Findings of this evaluation show that CHWs could play a significant role in improving health facility attendance, increasing ANC/IPTp compliance and in promoting malaria curative services at health centers in six districts in the provincial health delegation of Eastern Logone, Chad.

417

DN MINI: A MINIATURIZED DOUBLE-NET TRAP FOR EXPOSURE-FREE ASSESSMENT OF INDOOR AND OUTDOOR MOSQUITO BITING RISK

Alex J. Limwagu, Emmanuel W. Kaindoa, Halfan S. Ngowo, Emmanuel E. Hape, Marceline F. Finda, Rukiya M. Njalambaha, Fredros O. Okumu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Effective malaria surveillance in residual transmission settings require accurate assessment of exposures occurring outdoors relative to indoors. Unfortunately, the best approach currently available requires human

volunteers exposing their legs to catch mosquitoes attempting to bite them (i.e. human landing catches, (HLC)), a procedure that carries significant safety concerns and is cumbersome in large scale operations. We developed and tested a miniaturized double net trap (DN-Mini) to enable exposure free monitoring of indoor and outdoor host-seeking mosquitoes without affecting natural behaviors of those mosquitoes. DN-Mini is easy-to use, scalable, efficient and small enough to be installed both inside and in the peri-domestic spaces. It is made with UV-resistant fiber glass net over four wooden / melted poles and canvas base. Collection of mosquitoes is done by a human volunteer who sits in the middle chamber and collects mosquitoes from the outer chamber without any direct contact with the mosquitoes. Using the DN-Mini, we conducted a series of experiments to sample indoor and outdoor host seeking mosquitoes, test sampling efficacies of different designs of the DN-M-trap and to assess mosquito species diversities, biting pattern, parity and insemination rate. We also compared sampling efficacies of DN-M-trap and HLC. More than twice of the *An. arabiensis* were caught outdoors than indoors (p<0.001) while for *An. funestus,* there was no significance difference in the number of mosquitoes caught between outdoors and indoors (p=0.201). These patterns match previous indoor/outdoor estimates obtained using HLC from the same villages. Catches were higher outdoors than indoors for *Mansonia* (p<0.001) and *Culex* species (p=0.593). Parity was higher outdoors than indoors for *An. arabiensis* but higher outdoors than indoors for *An. funestus.* We conclude that DN-Mini has potential to transform unsafe into a safe and scalable platform mosquito surveillance platform in malaria endemic countries, and could be effectively used for measuring indoor versus outdoor exposures

418

COMPARISON OF *IMMEDIATE EX VIVO* VERSUS *IN VITRO* CULTURE ADAPTATION GROWTH INHIBITION ASSAYS FOR TESTING ANTIMALARIAL DRUG SUSCEPTIBILITY IN FIELD ISOLATES

Agnes Cheruiyot, Hosea Akala, Redempta Yeda, Charles Okudo, Edwin Mwakio, Jackline Juma, Raphael Okoth, Benjamin Opot, Dennis Juma, Ben Andagalu, Jim Ray Managbanag

U.S. Army Medical Research Directorate-Kenya, Kisumu, Kenya

Antimalarial resistance is a major obstacle in elimination of malaria, thus necessitates need for continued surveillance. Growth inhibition testing of fresh isolates as *"immediate ex-vivo"* is increasingly being embraced as it guides treatment management of malaria infections whereas "*in vitro"* culture is further adaptation of isolates to *in vitro* conditions. As part of continued validation of this assay relative to *in vitro* assay, it is important to compare performance of antimalarial drugs in field isolates. 24 samples were collected under an ongoing epidemiology of malaria and sensitivity patterns in Kenya. Isolates were dispensed onto pre-dosed drug plates and incubated for 72 hours. Aliquot of sample was maintained in culture prior to testing against 16 antimalarials: chloroquine (CQ), quinine (QN), atovaquone (AV), primaquine (PQ), doxycycline (DX), artemisinin (AR), halofantrine (HAL), tafenoquine (TQ), dihydroartemisinin (DHA), piperaquine (PPQ), artemether (AT), lumefantrine (LU), amodiaquine (AQ), Artesunic acid (AS), Ether (EA) and mefloquine (MQ). Inhibition curves for immediate ex vivo, parallel to *in vitro* assays, were obtained from the relative fluorescence units using Graph Pad Prism. Antimalarial drugs (13) which had higher median 50% inhibition concentration in immediate *ex vivo* had their median values drop when tested after culture. (AV) had a median value of 0.7121 ng/ml (95% CI 0.4580 to 1.048) in immediate *ex vivo* decreased to 0.1946 ng/ml (95% CI, 0.1111 to 0.3200) in cultured isolates P<0.0001. (DHA) had median value of 2.797 ng/ml (95% CI 0.9972 to 4.082) in immediate *ex vivo* decreased to 0.4491 ng/ml (95% CI, 0.3801 to 0.8553) in cultured isolates P<0.0001. (PPQ) had median value of 9.689 ng/ml (95% CI 4.437 to 15.87) in immediate ex vivo decreased to 3.714 ng/ml (95% CI, 3.292 to 4.996) in cultured isolates P<0.0001. AT, AQ, EA and MQ had P<0.0001. Results showed that there could be a selection in the initial population of *Plasmodium* strains during culture resulting in higher susceptibility. Finding necessitates a strategy to be developed to harmonize both *ex vivo* and *in vitro* methods to yield comparable results.

419

EUPATHDB.ORG: FREE, ONLINE RESOURCES BRINGING OMICS TO EVERY PARASITOLOGIST

Jessica C. Kissinger[1], Brian Brunk[2], Omar S. Harb[2], Susanne Warrenfeltz[1], David Roos[2], For The EuPathDB Team[1]

[1]*University of Georgia, Athens, GA, United States,* [2]*University of Pennsylvania, Philadelphia, PA, United States*

The Eukaryotic Pathogen Database Resources (EuPathDB, http://eupathdb.org) are a family of 12 taxon-specific, free, online genome and other Omics data mining resources that support over 190 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. These resources facilitate the discovery of meaningful biological relationships (hypothesis testing) from large volumes of pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. The intuitive graphic interface allows users to take full advantage of the data without the need for programming. Data types range from genome sequence and annotation to transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using standard bioinformatics workflows and in-house analyses generate data including domain predictions and orthology profiles across all genomes, which permit inferences from data-rich organisms to organisms with limited or missing data. EuPathDB offers several perspectives for data mining - record pages compile all data for genes, pathways, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system that queries pre-analyzed data and returns genes or features with shared biological characteristics; a private Galaxy workspace for analyzing and viewing user data in context with public data already integrated into EuPathDB. These free resources easily merge evidence from diverse data and across species to place the power of bioinformatics with every scientist. Our planned future growth includes a merger with VectorBase, home to genome, Omic and population data for many vector species. Our active user support offers an email help desk, social media, video tutorials and a worldwide program of workshops.

420

ONDE É O GARIMPO? A MOBILE APPLICATION TO MAP CASES OF MALARIA ON CLANDESTINE GOLD MINING SITES IN FRENCH GUIANA

Yann Lambert[1], Muriel Galindo[1], Louise Mutricy[1], Pierre Joubert[2], Alice Sanna[3], Laure Garancher[4], Antoine Adenis[1], Mathieu Nacher[1], Maylis Douine[1]

[1]*Centre d'Investigation Clinique Antilles-Guyane (Inserm 1424), Cayenne Hospital, Cayenne, French Guiana,* [2]*Parc Amazonien de Guyane, Cayenne, French Guiana,* [3]*Agence Régionale de Santé de Guyane, Cayenne, French Guiana,* [4]*Pan American Health Organization, Barbados Office, Bridgetown, Barbados*

Dealing with malaria in remote areas is today one of the major challenges for the control and elimination of the disease. In French Guiana, South America, the most important reservoir is a population of illegal gold miners living deep in the Amazon forest. These 5,000 to 10,000 men and women mostly originating from Brazil are highly mobile and hard to reach, thus sustaining the diffusion of malaria in the region of the Guyana Shield and preventing the implementation of usual control measures. The Malakit pilot study experiments the distribution of self-diagnosis and self-treatment kits against malaria to gold miners in several rear bases on the borders of French Guiana with Brazil and Suriname. Portuguese-speaking facilitators are trained to interview participants of the study and collect data using Android tablets with the ODK Collect application after training participants how to use a kit. During inclusion and follow-up visits, mapping the

mining sites where participants got infected with malaria is essential but challenging since both participants and facilitators are not familiar with a geographical map of French Guiana. A simple, *ad hoc* Android application was developed with a database of names and coordinates of known gold mining sites and a map of French Guiana displaying the main rivers, villages, cities and main gold mining areas. The name of a mining site can be entered in a search bar while phonetically matching names are listed dynamically and corresponding mining areas are highlighted on the map. In case no matches are found, the new site name is recorded while one or several mining areas can be selected manually according to the explanations given by the participant. Once validated by the facilitator, the data is sent back to the pending ODK Collect survey. At the end of the pilot study, the geographical data collected by Malakit facilitators will be curated and maintained as a reference dataset available for international collaboration in the surveillance and control of malaria in the region of the Guyana Shield. The source code of this application will be available upon request to be adapted to different settings and environments.

421

SYNTHETIC BIOTIN LIGASE ENABLES TO LABEL SURFACE INVASION FACTORS OF CULTURED HUMAN MALARIA PARASITE, *PLASMODIUM FALCIPARUM*

Sayuki Ochiai, Julie Maeda, Makoto Hirai, Toshihiro Mita, Toshiyuki Mori

Juntendo University, Tokyo, Japan

Merozoite, an invasive form of malaria parasites, enters a mature human red blood cell (RBC), where it multiplies asexually. The repetitive rupture of newly-produced merozoites from RBCs causes host inflammatory responses, leading to cyclic fever, characteristic to malaria. Although several invasion factors residing between merozoite and RBC have been reported to date, the merozoite invasion mechanism is still elusive at the molecular level. Of those factors, the merozoite surface protein AMA1 has been known to bind to merozoite-derived RBC surface protein RON2, forming a tight junction between host-parasite membranes for completion of successful invasion. Since elucidating the whole molecular invasion system should contribute to the development of drugs and vaccines against parasite proliferation, we have been trying to detect unidentified factors, using cultured *Plasmodium falciparum*. Recently, a promiscuous protein screening method utilizing proximity-dependent biotin ligases (BioID and BioID2) has been developed. In this study, we prepared a fusion protein composed of RON2, BioID2 and GFP (RBG) as an in-vitro synthesized peptide, and added it to cultured *P. falciparum* to detect proteins surrounding AMA1-RON2 junction. As a result, we succeeded in confirming GFP signal, prevention of merozoite invasion, and biotinylation of proximal invasion factors by the RBG molecules.

422

MACHINE LEARNING IDENTIFICATION OF MALARIA PARASITE CELL DEVELOPMENT AND ANTIMALARIAL DRIVEN PHENOTYPES USING HIGH-THROUGHPUT, HIGH-RESOLUTION FLUORESCENCE IMAGING

George W. Ashdown[1], Michelle Dimon[2], Minjie Fan[2], Fernando S. Teran[1], Katrin Witmer[1], David Gaboriau[1], Jon Hazard[2], Michael D. Ando[2], Jake Baum[1]

[1]*Imperial College London, London, United Kingdom,* [2]*Google Inc., Mountain View, CA, United States*

High-throughput image-based drug screens have the potential to provide details on drug-mediated effect beyond live/dead efficacy and into the realm of characterizing the diversity of compound-dependent phenotypes on the target cells. A key challenge is the unbiased interpretation of complex cell morphology at the population level, determining normal cells versus -potentially- multiple aberrant cellular phenotypes. Here, we apply semi-supervised deep neural network analysis to high-resolution fluorescent images of malaria parasite-infected red blood cells. We demonstrate that this machine learning approach can sequentially sort asexual life-cycle stages from asynchronous *Plasmodium falciparum* parasite cultures with a fine-grain order. Additionally, the technique can successfully separate aberrant, drug-induced changes in cell morphology from mixed populations despite the wide variation seen within untreated populations. Importantly, drug-induced phenotypes showing a similar mechanism of action could be successfully segregated. Applied more broadly, this methodology provides a means to analyse large-scale imaging datasets that can drive the discovery of novel antimalarials and potentially elucidate mechanism of drug action with more sensitivity and much faster than existing methods.

423

GENERATION OF *PLASMODIUM FALCIPARUM* NF135 REPORTER LINES EXPRESSING FLUORESCENT PROTEINS FOR EXAMINING BLOOD-, MOSQUITO- AND LIVER-STAGES

Shinya Miyazaki[1], Annie S.P. Yang[2], Fiona J. A. van Pul[1], Catherin Marin-Mogollon[1], Yukiko Miyazaki[1], Takashi Imai[1], Surendra K. Kolli[1], Jai Ramesar[1], Geert-Jan van Gemert[2], Youri M. van Waardenburg[2], Adrian V. S. Hill[3], Robert W. Sauerwein[2], Chris J. Janse[1], Shahid M. Khan[1]

[1]*Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands,* [2]*Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands,* [3]*The Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom*

Malaria parasites expressing fluorescent or luminescent proteins are valuable tools, increasingly used in drug and vaccine screening assays as well as to interrogate parasite gene function. However, only a limited number of *P. falciparum* reporter lines have been generated and characterized in the laboratory gold-standard NF54/3D7 strain from West Africa. Here we describe the generation of different reporter lines, using CRISPR/Cas9 gene modification, in the recently described South East Asian *P. falciparum* NF135 strain. This strain has different drug sensitivities to a number of anti-malarial drugs and has been shown to have an increased ability to invade and develop in liver cells, compared to NF54. We first used NF54 parasites to characterize the strength and temporal expression of different gene promoters, in comparison to the previously described ef1a promoter, to select for promoters of strongly and constitutively expressed genes. We next generated and characterized three reporter lines generated in *P. falciparum* NF135 strain with the green fluorescent protein (GFP) expression driven by two novel and the ef1a promoters. Data will be presented on the genotype characterization of reporter lines as well as the different timing and strength of reporter GFP expression throughout the life cycle including liver-stages in primary human hepatocytes. We show strong differential GFP expression in the reporter lines throughout the complete life cycle. One novel NF135 reporter line showed significantly higher GFP expression in sporozoites and liver stages compared to the control ef1a-GFP reporter line. The strength of reporter expression in combination with the increased infectivity for hepatocytes by NF135 parasites makes this line a valuable novel tool for the analysis of *P. falciparum* liver stage development.

424

COST-EFFECTIVENESS OF A PUSH-PULL SYSTEM COMPRISED OF SPATIAL REPELLENTS AND ODOR-BAITED TRAPS FOR CONTROL OF MALARIA VECTORS

Matt Worges[1], Adrian Denz[2], Margaret Njoroge[3], Ulrike Fillinger[4], Joop van Loon[3], Fredros Okumu[5], Sarah Moore[5], Mgeni Mohamed[5], Adam Saddler[5], Alexandra Hiscox[3], Nakul Chitnis[2], Joshua Yukich[1]

[1]*Center for Applied Malaria Research and Evaluation, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Swiss Tropical Public Health Institute, Basel, Switzerland,*

[3]*Wageningen University & Research, Wageningen, Netherlands,*
[4]*International Centre of Insect Physiology and Ecology, Kisumu, Kenya,*
[5]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania*

Residual malaria transmission and insecticide resistance are threats against efforts to eliminate malaria using standard vector control interventions; therefore, additional approaches are needed. An entomological study was designed in order to evaluate the impact of a push-pull strategy for vector control implemented under semi-field conditions in Kenya and Tanzania. The system was comprised of a spatial repellent in the form of a transfluthrin-treated eave ribbon (push component) in combination with an outdoor odor-baited trap (pull component). The underlying assumption of the push-pull system is that host-seeking vectors are repelled from their human targets thereby reducing biting rates and subsequently trapped and killed. Cost models were developed for push-pull system component procurement and deployment. Additionally, malaria cases averted were estimated using the OpenMalaria modelling platform parameterized with field trial results. Costs and effectiveness measures were used in a probabilistic sensitivity analysis to estimate uncertainty around expected incremental cost effectiveness ratios under various scenarios (i.e. variable entomological inoculation rates, different dominant Anopheline species, and variable coverage levels for the push-pull system). Efficiency frontiers generally showed that a combination of high spatial repellent coverage and low- to medium-level trap coverage provided the most cost-effective approach across the majority of tested scenarios.

425

EFFICIENT TRANSCRIPTOME PROFILING ACROSS THE MALARIA PARASITE LIFE CYCLE BY FLOW SORTING

Aliou Dia, Catherine Jett, Marina McDew-White, Timothy Anderson, Ian Cheeseman

Texas Biomedical Research Institute, San Antonio, TX, United States

Plasmodium falciparum is the most virulent and widespread of the human malaria species. This parasite has a complex life cycle that involves sexual replication in a mosquito vector and asexual replication in a human host. During the 48-hour intraerythrocytic development cycle (IDC), the parasites develop and multiply through the morphologically distinct ring, trophozoite and schizont stages. The IDC is responsible for all clinical symptoms and has been recognized as an important target for drug and vaccine development making this stage of major interest to malaria investigators. Stage-specific transcriptomic approaches have shown gene expression profiles continually change throughout the IDC. Cultures of tightly synchronized parasites are required to capture the transcriptome specific to a developmental stage. However, the most commonly used synchronization methods require lysis of late stages, potentially perturbing transcription, and often do not result in tightly synchronized cultures. As a synchronous culture requires frequent sampling over a 48-hour period, producing complete transcriptome profiles of the IDC is both time consuming and labor intensive. Here we developed a method to sample the IDC densely by isolating parasites from an asynchronous culture with fluorescence activated cell sorting. We sort parasites in tight windows of IDC progression based on their DNA/RNA abundance. We confirmed the tight synchronization and stage specificity by light microscopy and RNAseq profiling. We optimized our protocol for low numbers of sorted cells allowing us to rapidly capture transcriptome profiles across the entire IDC from a single culture flask. We extend this approach to single cell transcriptomics to assay cell-to-cell variation throughout the life cycle. This methodology will allow any malaria stage-specific study to perform experiments directly from asynchronous cultures with high accuracy and without the need for labor-intensive time-course experiments.

426

NEW APPROACHES FOR THE REMOTE ELECTRONIC DETECTION OF INSECTICIDE TREATED BEDNET USERS

Paul Joseph Krezanoski

University of California San Francisco, San Francisco, CA, United States

Long-lasting insecticide treated bednets (LLINs) have played a large role in improvements in malaria control. However, recent reports suggest that progress is slowing and there are concerns that LLINs may not be as effective as previously. As a result, there is growing interest in maximizing the value of prevention funds by improving our understanding of LLIN effectiveness. Fundamentally, adequate LLIN use comprises two necessary behaviors: 1) unfurling an LLIN and 2) locating one's self under the unfurled LLIN during potential exposure. Existing tools can detect, with high accuracy and over long periods of time, the unfurling of LLINs; multiple studies utilizing these technologies have been published in recent years. Much more challenging, however, is the remote detection of whether one or more individuals is under an unfurled LLIN and identifying that individual. To achieve this goal, our lab has experimented with various sensor modalities (temperature, humidity, accelerometers, etc.). Furthermore, our prototype uses an open platform and consists of existing technologies for detection of LLIN unfurling plus a variety of sensors for detecting who is under an LLIN. We present data suggesting that our approach can solve the first problem of whether an individual is under an unfurled LLIN with high specificity. We also present promising data suggesting that discriminating between one or more individuals, and whether the individuals are children versus adults for example, should be possible by combining available sensors and utilizing machine learning techniques. The ideal remote LLIN adherence monitoring tool is low-cost, inconspicuous (limiting Hawthorne effects), has low power consumption and is robust to environmental conditions faced by a typical LLIN, including periodic washing. We propose using platforms such as ours to determine the optimal combination of sensors to achieve accurate remote LLIN adherence monitoring with the lowest cost and longest battery life. We believe these tools can contribute to re-igniting progress towards malaria elimination by providing researchers the tools for making LLINs as effective as possible.

427

MICROSCALE MAGNETIC LEVITATION FOR MULTIPLEXED ANALYSIS OF MALARIA-INFECTED BLOOD SAMPLES IN RESOURCE-LIMITED SETTINGS

Shreya S. Deshmukh[1], Naside Gozde Durmus[1], Bryan Greenhouse[2], Elizabeth Egan[1], Utkan Demirci[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*University of California San Francisco, San Francisco, CA, United States*

Malaria, which infects over 200 million and kills close to 400,000 people annually, largely affects low- and middle-income countries. Here, accessible and affordable diagnostic devices are critical for effective treatment and surveillance. Antimalarial resistance adds further pressure to *Plasmodium falciparum* elimination, with an emerging artemisinin resistance crisis in Southeast Asia. There is an urgent need to contain this before it spreads to Africa, as chloroquine did in the 1980s, to devastating effect. This problem is exacerbated by the lack of point-of-care tools to rapidly measure patient-specific response to anti-malarial drugs: neither blood smear microscopy, nor antibody-based rapid diagnostic tests, can measure drug resistance. Therefore, there is a significant unmet need for a field-appropriate tool (inexpensive, rapid, operable without laboratory infrastructure or specialised training) to detect and quantify *Plasmodium* infection in blood, for sensitive, specific diagnosis as well as rapid drug response monitoring. We demonstrate an innovative magnetic levitation platform that precisely measures subtle differences in cell levitation heights as inherent biomarkers for magnetic susceptibility and density signatures. *Plasmodium* infection changes these blood cell properties, primarily through haemoglobin digestion, which we can sensitively measure as levitation height changes at single cell resolution. With this, we show

biophysical separation of ring-stage parasites, the earliest stage and the only one found in patient circulation, for the first time. We also observe that drug treatment causes physical changes in the parasites, translating to levitation height changes. This allows us to phenotypically measure drug response in real time, which we are developing to rapidly differentiate drug-resistant from drug-susceptible parasites. This platform is a portable, smartphone-interfaced device that uses less than a drop of blood (<10 μL) without refrigeration or electricity. We envision that this would equip healthcare workers' changing needs with more data-rich, contextually appropriate tools.

428

A NEW NON-HUMAN PRIMATE MODEL FOR TESTING HUMAN MALARIA VACCINE EFFICACY

Melanie J. Shears[1], Chaitra Parthiban[1], Brad C. Stone[1], B. Kim Lee Sim[2], Stephen L. Hoffman[2], Robert A. Seder[3], Sean C. Murphy[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Sanaria, Inc., Rockville, MD, United States,* [3]*Vaccine Research Center, National Institutes of Health, Bethesda, MD, United States*

Rhesus macaques are considered the best model of the human immune system, but were previously excluded from human malaria vaccine efficacy testing because they do not support *Plasmodium falciparum* blood stage infection. Using clinical-grade 18S rRNA RT-PCR, we previously demonstrated that *P. falciparum* can robustly develop in the liver of fully immunocompetent rhesus. This discovery suggested that liver stage parasite burden could be used to evaluate vaccine efficacy in rhesus and provide a highly relevant endpoint for testing *P. falciparum* liver stage vaccines. This platform would also allow the reduction or elimination of liver stage parasite burden to be correlated with liver-specific immunological responses in the same animals. This model therefore has potential to provide translationally-relevant data and give mechanistic insight into vaccine efficacy that is currently inaccessible in other systems. In this study, we present preliminary data evaluating rhesus as a platform for testing *P. falciparum* liver stage vaccine efficacy and exploring tissue-specific immunological responses. We assess the safety, tolerability and efficacy of a two-step prime-and-trap vaccine composed of a priming DNA vaccine encoding three known malaria antigens followed by a single intravenous trapping dose of PfSPZ cryopreserved, radiation-attenuated sporozoites. This vaccine strategy was previously demonstrated to induce liver resident memory T cells and elicit long-lasting sterile protection in mice. As controls, we compare to mock-immunized animals and those given three doses of radiation-attenuated sporozoites – a regimen known to protect mice, non-human primates, and humans. We give particular focus to the relationship between vaccine efficacy and the magnitude of antigen-specific T cell responses in the rhesus liver. These data provide the first demonstration of this platform for testing the efficacy of vaccines prior to use in humans, and give key insight into how rhesus may be further used to accelerate malaria vaccine development.

429

THE EFFECT OF THE RTS,S/AS01E VACCINE FOURTH DOSE ON CELL MEDIATED IMMUNE RESPONSES TO CSP AND HBSAG ANTIGENS IN AFRICAN CHILDREN

Chenjerai Jairoce[1], Robert A. Mitchell[2], Ruth Aguilar[2], Akshayata Naidu[2], Ana Chopo[2], Maxmillian Mpina[3], Matthew B.B. McCall[4], Miquel Vázquez-Santiago[2], Gemma Ruiz-Olalla[2], Núria Díez-Padrisa[2], Nana A. Williams[2], Joseph J. Campo[2], Pedro Aide[1], Benjamin Mordmüller[4], Selidji T. Agnandji[5], Claudia Daubenberger[6], Gemma Moncunill[2], Carlota Dobaño[2]

[1]*Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique,* [2]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [3]*Ifakara Health Institute. Bagamoyo Research and Training Centre, Bagamoyo, United Republic of Tanzania,* [4]*Institute of Tropical Medicine, German Center for Infection Research, University of Tübingen, Tübingen, Germany,* [5]*Centre de Recherches Médicales de Lambaréné (CERMEL), Albert Schweitzer Hospital, Lambaréné, Ghana,* [6]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

RTS,S/AS01 is the most advanced malaria vaccine worldwide, conferring moderate protection of limited duration in African children. However, the immunogenicity of the vaccine has not been characterized in detail, and its mode of action is not known. The magnitude, quality, and type of antibody responses are thought to be the major correlates. Cell-mediated immune responses to CSP are also involved in RTS,S-induced immune mechanisms against *Plasmodium falciparum* malaria but their role remains inconclusive. We have previously investigated cellular immune responses following the primary 3-dose immunization and found that T helper (T_H)2 cytokines, particularly IL-5, correlated with lack of vaccine efficacy, while T_H1 cytokines, particularly IFN-γ, correlated with malaria protective immunity. As part of the multicenter phase 3 trial, a fourth dose was administered in one of the study groups to increase the longevity of the RTS,S efficacy. The present study aimed to evaluate the cellular immune responses from infants and children in supernatants following ex vivo stimulation of blood cell samples with vaccine CSP and HBsAg antigens using the Luminex technology. We will present cytokine and chemokine antigen-specific data associated with the immunological benefit of the booster dose of RTS,S vaccine and their kinetics. Data will be evaluated in relation to age, clinical malaria, baseline and peak responses, as well as the type and magnitude of antibody responses to the booster dose measured in parallel projects. We observed a positive correlation between the concentrations of IL-2 with IgG concentrations and avidity (R^2=0.53-0.89, $p < 0.001$) at peak response, suggesting the supportive role of IL-2 on the quality of the antibodies response. We expect that the overall levels of cytokines, particular those promoting memory T_H1 responses, will be higher in the booster group, as a result of higher frequency to antigen stimulation and quick response of memory T-cell subpopulations. Elucidation of protective $CD4^+$ T helper memory responses and their mediators is essential to design second-generation longer-lasting effective malaria vaccines.

430

GENETIC DIVERSITY, NATURAL SELECTION AND HAPLOTYPE GROUPING OF *PLASMODIUM VIVAX* VACCINE CANDIDATE, DUFFY BINDING PROTEIN OF CHINA-MYANMAR BORDER AND WESTERN MYANMAR ISOLATES

Yubing Hu

China Medical University, Shenyang, China

Border area with Myanmar is the major *Plasmodium vivax* endemic regions in China. To evaluate the genetic diversity of *pvdbp*-IIgene in isolates from the China-Myanmar border (CM) and Western Myanmar (WM), and compared it with global *P. vivax* populations, a total of 85 and 82 *pvdbp*-IIsequences from CM and WM were used, respectively. The highest peaks of nucleotide diversity were identified between 1078-1332 bp for CM and WM isolates. An annotated R391C mutation was unique to WM isolates. Evidence of positive selection was detected by Fu and Li's *F** test and Tajima's *D*test of *pvdbp*-IIgene from CM isolates ($P<0.05$). A slight genetic differentiation was detected among CM, WM, Central Myanmar, and Thailand population. Clustering analysis grouped the accessions from these four populations into three genetic groups and indicated a high degree of mixed ancestry. High levels of nucleotide diversity were found in epitopes 45 (ϖ=0.029) and Ia (ϖ=0.028) of PvDBP-IIfrom CM and WM isolates. These results will be useful for understanding the nature of the *P. vivax* population in GMS area and for development of PvDBP-II-based vaccine.

431

NATURALLY ACQUIRED TRANSMISSION-BLOCKING ANTIBODIES AGAINST *PLASMODIUM VIVAX*

Sataporn Thongpoon[1], Wanlapa Roobsoong[1], Sadudee Chotirat[1], Takafumi Tsuboi[2], Eizo Takashima[2], Liwang Cui[3], Tomoko Ishino[2], Mayumi Tachibana[2], Jetsumon Sattabongkot[1]

[1]*Mahidol University, Bangkok, Thailand,* [2]*Ehime University, Matsuyama, Japan,* [3]*University of South Florida, Tampa, FL, United States*

Plasmodium vivax is the human malaria species responsible for the majority of cases and widely dispersed in sub-tropical region including South East Asia. Thailand and main countries in GMS region have planned to eliminate malaria by 2030. Transmission-blocking vaccine (TBV) is established for transmission prevention by blocking the development of parasites inside the mosquitoes and has been proposed as a potential tool to eliminate malaria. Transmission-blocking immunity (TBI) can be induced after episodes of malaria. This study aimed to identify and characterize the naturally acquired TBI in malaria endemic population. Transmission-blocking immunity in individuals were identified by feeding their infected blood to *Anopheles dirus* mosquitoes using membrane feeding assay. Patients' blood was prepared in three different conditions (1) whole blood, (2) replace patients 'plasma with AB naïve serum, and (3) immunoglobulins (Ig)-depleted autologous plasma mixed with infected blood. Mosquitoes were examined for oocysts of the parasites on 7-9 days post-feeding. Total 47 *P. vivax* patients' blood were used and 39 of them infected the mosquitoes. Among these cases 25 individuals showed transmission blocking activities which reduced from 10%-100% of the oocyst formation when compared to the feeds that replace patients' plasma with AB naïve serum. Plasma with blocking activity were tested against 1-5 heterologous *P. vivax* isolates. The blocking activity against heterologous isolates were identified in 20 out of 25 plasma samples, although the blocking activity was not always the same as against the autologous isolates. Plasma with blocking activity reacted to gametocyte antigens examined by IFA. These plasma were also tested against *P. vivax* protein library produced by wheat germ cell-free system to screen for the target antigens using AlphaScreen technology. Potential target antigens examined by AlphaScreen will be presented. This study has identified the target antigens that induce naturally acquired TBI against *P. vivax* in population at risk. Further study to characterize these antigens as new candidates for TBV will be performed.

432

CAUSES OF SCREENING FAILURE DURING EXPERIMENTAL VACCINE TRIALS IN A MALARIA VACCINE TESTING SITES OF BURKINA FASO

Alphonse Ouedraogo[1], Daouda Ouattara[1], Maurice Ouattara[1], Moise Kabore[1], Sam Coulibaly[1], Aissata Barry[1], Aissata Barry[1], Desire W. Kargougou[1], Noely Henry[1], Issa Nebie[1], Alfred B. Tiono[1], Sodiomon B. Sirima[2]

[1]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [2]*Groupe de Recherche Action en Santé, Ouagadougou, Burkina Faso*

A safe and effective vaccine is a necessary tool to improve disease control. During early clinical development phase of vaccines, volunteers with non-inclusion criteria are excluded from participating to the trials due to safety concerns and the need to minimize any interference with the outcomes measures. This study aimed to assess screening failure patients' profiles and the reasons in the context of vaccines study sites characterization. All patients screened at the research clinics of Saponé (rural area, 35 km south east of Ouagadougou) and Banfora (suburban area, 500 km south of Ouagadougou) from 2016 to 2017 were reviewed retrospectively. Clinical and laboratory assessments were undergone to evaluate the frequency of different diseases. Among 468 subjects, 191 (40.8%) were screening failure cases; 31.2% (75/240) from suburban area and 50.8% (116/228) from rural area (p<0.000). Proportion of female screen failure was 48% (36/75) vs 51.7% (60/116) at suburban and rural area respectively. The most common causes of screening failure were due to abnormal laboratory value 37.2% (71/191), the infection of viruses 24.6% (47/191) and withdrawal of consent before the enrollment 9.9% (19/191). The main abnormal laboratory values were anemia (17.3%), elevated creatinine (16.7%) and liver disorder. The different viruses were hepatitis B (12.57%), HIV (7.85%) and hepatitis C (4.19%). The others reasons of screening failure were generally sick, heart diseases, pregnancy and refuse to use any contraception method during the study period. Sickle cell trait carriage was associated to other diseases in 6.28% of cases. In term of causes of screening failure, there were no difference according to the study area. The results are consistent with the country's reports in general population in semi urban and rural area. The eligibility rate is relatively low. These findings should be considered when planning for vaccine trials in our context.

433

THE 'ACUTE CHALLENGE' MODEL: SENSITIVE MODERATE-THROUGHPUT ASSESSMENT OF MALARIA T CELL VACCINE ANTIGENS IN MICE

Irene Cruz Talavera, Brad C. Stone, Sean C. Murphy

University of Washington, Seattle, WA, United States

Identification of novel protective *Plasmodium* antigens is thought to be crucial for developing a vaccine that can induce a broad immune response and attain durable protection against this intracellular parasite. Pre-erythrocytic vaccine development for *Plasmodium falciparum* is faced with the complex problem of selecting genuinely protective T cell antigens out thousands of candidates. There are no proven methods for efficiently testing and selecting protective antigens *in vivo*. Furthermore, there are many antigens that are highly immunogenic but ultimately non-protective. To define protective antigens, we developed an "Acute Challenge" model that rapidly and sensitively detects protective T cell responses. In this model, multiple DNA vaccines targeting individual *Plasmodium yoelii* antigens are built in parallel using codon-optimized, 500 bp synthetic fragments. DNA vaccines are delivered twice by gene gun to induce $CD8^+$ T cell responses in BALB/c mice. At the peak of the boosted immune response, we challenge the mice with $2\text{-}5x10^4$ wild-type luciferase-expressing *P. yoelii* sporozoites and measure parasite burden using IVIS imaging 40-44 hours later. The Acute Challenge model gives rapid and sensitive results and allows for moderate throughput (35 days from priming to liver burden endpoint). Our focus is to assess *P. yoelii* candidate antigens of unknown protective potential that are known or putatively involved in hepatocyte invasion or exported from the parasitophorous vacuole into the host cell cytoplasm. Protective antigens are also assessed for their intracellular localization. Data from multiple Acute Challenge screens will be presented. This protection-forward approach contrasts with prior efforts at immunogenicity screening that have often led to identification of immunogenic but non-protective T cell responses. Using the Acute Challenge strategy, we expect to be able to identify protective $CD8^+$ T cells targeting liver-stage antigens that are processed and presented by infected hepatocytes.

434

LESSONS LEARNED FROM A VACCINE EPITOPE DISCOVERY EFFORT TARGETING MALARIA BLOOD-STAGE ANTIGENS

Vinayaka (Vin) Kotraiah[1], Timothy Phares[1], David S. Peabody[2], Manpreet Singh[3], Francis Galaway[4], Cheryl Lobo[3], David Whitacre[5], Bryce Chackerian[2], David R. Milich[5], Jayne M. Christen[1], Federica Pericle[6], Gavin Wright[4], Robin Miller[7], Lorraine Soisson[7], Carter Diggs[7], Susan Youll[7], Amy R. Noe[1]

[1]*Leidos Inc., Frederick, MD, United States,* [2]*Department of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, NM, United States,* [3]*Blood-Borne Parasites, New York Blood Center, New York, NY, United States,* [4]*The Wellcome Sanger Institute, Hinxton, Cambridgeshire, United Kingdom,* [5]*VLP Biotech Inc., San Diego, CA,*

United States, [6]Agilvax Inc., Albuquerque, NM, United States, [7]Malaria Vaccine Development Program, United States Agency for International Development (USAID), Washington, DC, United States

One of the biggest hurdles in developing a blood-stage vaccine is that malaria parasites have developed exceedingly effective mechanisms to circumvent otherwise lethal immune responses, particularly to blood-stage antigens. Blood-stage vaccine development is also compounded by antigen complexity, making recombinant expression and correct folding more difficult. In order to circumvent some of these issues and target critical epitopes of multiple antigens, an epitope-based blood-stage vaccine discovery project was initiated. This project focused on blood-stage antigens: RH5, RIPR, CyRPA, P113, EBA175 and AMA1. We began by selecting blood-stage monoclonal antibodies (mAbs) with high Growth Inhibition Activity (GIA). These mAbs were used for screening and identification of their epitope / mimotope sequences. Several mAbs selected multiple sequence motifs and exemplar epitope sequences were chosen for mouse immunization trials. Forty nine virus-like particles (VLPs) displaying exemplar epitopes were produced and used in mouse immunization trials. Ten of these VLPs elicited antisera that recognized the native antigen by ELISA. The IgG was purified from these ELISA-positive sera and tested in the GIA assay; however, none of these IgG showed sufficiently high levels of inhibition in the GIA assay to warrant moving them into confirmatory assessment. Several lessons learned through this effort will be highlighted including polyspecificity of mAbs, quality of immune response, and the need to employ adjuvants appropriate for human-use, even in preliminary vaccine evaluations. We will also present data on new mAbs targeting RIPR and P113 that were developed as part of this project.

435

FUNCTIONAL CHARACTERIZATION OF A POTENTIAL BLOOD-STAGE MALARIA VACCINE CANDIDATE.

Ojo-ajogu Akuh, Philip Ilani, Grace Opoku, Gordon A. Awandare, Emmanuel Amlabu

West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana

The search for a potent malaria vaccine continues in the face of various challenges. The completion of the genome sequence for *Plasmodium falciparum* has simplified the identification of invasion-related proteins that could be promising targets of protective immunity. We have conducted data-mining analysis for over 3,500 proteins using existing reports on the parasite transcriptome, proteome and predicted substrates for *P. falciparum* subtilisin like protease-1 (PfSUB1). Eighteen (18) top hits emerged from these analyses and most of them were already characterized blood-stage malaria vaccine candidates and a hypothetical protein. The sequence of the hypothetical protein was submitted to several bio-informatics portals and the results show that the protein possesses the structural characteristics of a potential vaccine target during merozoite invasion. We observed by immunofluorescence assays that the protein is localized on the parasite surface. Time-course imaging analysis from initial attachment to internalization shows that a portion of the protein is internalized post-invasion along with $MSP1_{19}$. Invasion inhibition assays showed that antibodies against the protein inhibits erythrocyte invasion of both laboratory strains and clinical isolates. Also, the data obtained from proteolytic processing assays, merozoite invasion inhibition assays and schizont arrest assays presents new insights on the possible mechanism of erythrocyte invasion inhibition by the target antibodies.

436

CYSTEINE MOTIF 1 DOMAIN IN PFS230 MOLECULE IS CRUCIAL TO MAINTAIN THE CORRECT CONFORMATION OF PFS230-BASED TRANSMISSION-BLOCKING VACCINES

Kazutoyo Miura[1], Mayumi Tachibana[2], Eizo Takashima[3], Masayuki Morita[3], Hikaru Nagaoka[3], Thao P. Phama[1], Carole A. Long[1], C. Richter King[4], Motomi Torii[2], Tomoko Ishino[2], Takafumi Tsuboi[3]

[1]*Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Division of Molecular Parasitology, Proteo-Science Center, Ehime University, Toon, Japan, [3]Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Japan, [4]PATH's Malaria Vaccine Initiative, Washington, DC, United States*

Pfs230 is a leading transmission-blocking vaccine (TBV) candidate against *Plasmodium falciparum,* but the size and cysteine-rich nature make it difficult to express the entire Pfs230 molecule as a TBV. However, the locations of the transmission-reducing (TR) epitopes, i.e., the epitopes recognized by the functional antibodies, have not been investigated systematically. In the previous study, we produced a total of 27 proteins (either single-, 2-, or 4-CM (cysteine motif)-domain), which covered the entire Pfs230 molecule, using a wheat germ cell-free expression system, and determined the biological activity of antibodies by standard membrane-feeding assay (SMFA). The antibodies against all five proteins containing amino acid (aa) 543-730 region (a part of prodomain plus CM domain 1, called "pCM1") displayed strong inhibition in SMFA, while antibodies against constructs without pCM1 showed no inhibition. It remained an open question whether (1) all TR epitopes exist within pCM1, or (2) pCM1 supports correct folding of other domains. In this study, we further investigated the four SMFA-active antibodies; antibodies to aa 443-730 ("PCM1"), 443-904 ("PCM1-2"), 443-1132 ("PCM1-3") and 443-1274 ("PCM1-4") proteins. Each of the original antibodies was separated into anti-pCM1-specific and non-pCM1 antibodies using a pCM1-affinity column. All four anti-pCM1-specific antibodies showed significant inhibition in SMFA, but anti-pCM1-specific IgG from anti-PCM1-4 IgG showed a weaker inhibition. The result suggested that the conformation of pCM1 in the four Pfs230 constructs might not be the same. All four non-pCM1 antibodies showed weaker than the original total IgGs, but significant inhibition (>48% inhibition, $p<0.03$ for all). On the other hand, the antibodies against Pfs230 constructs containing CM2, 3 or 4, but not pCM1, did not show any activity in SMFA. Taken together, it is speculated that there are TR epitopes within aa 443-542 and 731-1274 regions, but it is crucial to include the pCM1 of Pfs230 to maintain the correct conformation of the TR epitopes in the recombinant proteins.

437

DEMONSTRATION OF A BLOOD-STAGE CONTROLLED HUMAN MALARIA INFECTION MODEL FOR *PLASMODIUM VIVAX* VACCINE EFFICACY TESTING

Angela M. Minassian[1], **Yrene Themistocleous**[1], Sarah E. Silk[1], Jordan R. Barrett[1], Carolyn M. Nielsen[1], Doris Quinkert[1], Ian D. Poulton[1], Fernando Ramos Lopez[1], Celia H. Mitton[1], Thomas A. Rawlinson[1], Megan Baker[1], Raquel Lopez Ramon[1], Nick J. Edwards[1], Katherine J. Ellis[1], Jee-Sun Cho[1], Florian Bach[2], Wiebke Nahrendorf[2], Alison C. Kemp[3], Philip Spence[1], Andrew M. Blagborough[4], Iona J. Taylor[1], Fay N. Nugent[1], Kimberly J. Johnson[1], Alison M. Lawrie[1], Julian C. Rayner[3], Wanlapa Roobsoong[5], Jetsumon Sattabongkot[5], Sumi Biswas[1], Simon J. Draper[1]

[1]*The Jenner Institute, University of Oxford, Oxford, United Kingdom, [2]School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom, [3]Wellcome Sanger Institute, Wellcome Genome Campus, University of Cambridge, Cambridge, United Kingdom, [4]Infection and Immunity Section, Sir Alexander Fleming Building, Imperial College of*

Science, Technology and Medicine, London, United Kingdom, [5]Mahidol Vivax Research Unit (MVRU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Plasmodium vivax, one of the five *Plasmodium* species to cause human malaria, is the most widespread geographically. Although viewed for many decades as a "benign" parasite, current estimates suggest *P. vivax* accounts for 7.5 million cases each year, among 2.5 billion people living at risk of infection, with a significant burden of morbidity and associated mortality. However, efforts to develop interventions against this parasite lag far behind those for its cousin, *P. falciparum*, in large part because of critical bottlenecks in the vaccine development process. A key problem is limited access to *P.vivax* parasite inocula for use in a controlled human malaria infection (CHMI) model to provide an early indication of vaccine efficacy. To date, only groups in Colombia and the United States have routinely performed sporozoite challenges in human volunteers and the only blood-stage challenges have been performed in Australia. In 2018/19, we undertook the first proof-of-concept studies in Europe of *P. vivax* CHMI (ClinicalTrials.gov Identifiers: NCT03377296 and NCT03797989), demonstrating that we could safely infect two healthy UK adult volunteers with *P. vivax* by the bite of infected mosquitoes transported from southern Thailand. We obtained 250mL of blood from the volunteers at threshold parasitaemia/clinical criteria, and generated a viable, sterile GMP-like bank of cryopreserved *P. vivax*-infected erythrocytes for use in blood-stage CHMI studies. We tested the safety and feasiblity of blood-stage infection at three different dilutions of the inoculum in six malaria-naive volunteers. Infectivity was robust, with even a 1:20 dilution resulting in blood-stage infection. All volunteers were diagnosed within 12-16 days of infection and all had detectable gametocytaemia by qPCR within 1-3 days of peak parasitaemia. This successful model provides a platform for progression of *P. vivax* vaccine candidates to Phase I/IIa efficacy testing. We will also report on a pilot efficacy trial of the high priority blood-stage vaccine candidate *P. vivax* Duffy Binding Protein (ChAd63-MVA PvDBP_RII), scheduled to be undertaken in Oxford in September 2019.

438

EPITOPE-BASED SIEVE ANALYSIS OF *PLASMODIUM FALCIPARUM* SEQUENCES TO ASSESS FMP2.1/AS02$_A$ IS CONSISTENT WITH DIFFERENTIAL VACCINE EFFICACY AGAINST IMMUNOLOGICALLY RELEVANT AMA1 VARIANTS

Amed M. Ouattara[1], Amadou Niangaly[2], Matthew Adams[1], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Karim Traore[2], Matthew B. Laurens[1], Youssouf Tolo[2], Bourema Kouriba[2], Dapa A. Diallo[2], Ogobara K. Doumbo[3], Christopher V. Plowe[4], Mahamadou A. Thera[2], Shannon Takala-Harrison[1], Miriam K. Laufer[1], Joana C. Silva[1]

[1]*University of Maryland, Baltimore, MD, United States,* [2]*Malaria Research and Training Center, University of Sciences, Techniques and Technology, Bamako, Mali,* [3]*Malaria Research and Training Center, University of Sciences, Techniques and Technology, Baltimore, Mali,* [4]*Duke Global Health Institute, Durham, NC, United States*

Highly polymorphic candidate malaria vaccine antigens can induce allele- or strain-specific immunity in response to monovalent vaccines, limiting vaccine efficacy in the field. To prevent premature elimination of promising vaccine programs, it is critical to determine if lack of efficacy in the field is due strain specific-efficacy, rather than to the lack of immunogenicity of the candidate antigen. Here we use samples collected during a field trial of the AMA1-based FMP2.1/AS02$_A$ malaria vaccine, which incorporates the AMA1 variant encoded by the reference *Plasmodium falciparum* 3D7 strain, to assess the usefulness of epitope-based sieve analysis for the detection of vaccine-induced allele-specific immune responses. The samples used are from volunteers who received the malaria vaccine FMP2.1/AS02$_A$ or a control (rabies vaccine), during a vaccine efficacy field trial, and who later developed malaria. Samples were collected during the first six months of follow-up, starting 15 days after the last immunization. In a previous study, *P. falciparum* DNA was extracted from all samples, and the *ama1* locus amplified and sequenced. Here, a sieve analysis was used to measure T and B-cell escape, and difference in 3D7-like epitopes in the two treatment arms. Overall, no difference was observed in mean amino acid distance to the 3D7 AMA1 variant between sequences from vaccinees and controls in B-cell epitopes. However, we found a significantly greater proportion of 3D7-like T-cell epitope that maps to the AMA1 cluster one loop (c1L) region in the control *vs*. the vaccinee group (p=0.02). In addition, AMA1 3D7-type epitopes in infections from vaccinees had mean estimated binding affinity higher than epitopes generated from the control group (p=0.01). These findings are consistent with a well-established vaccine-derived sieve effect on the c1L region of AMA1, and suggest that sieve analyses of malaria vaccine trial samples targeted to epitopes identified *in silico* can help identify protective malaria antigens that may be efficacious if combined in a multivalent vaccine.

439

CELTOS DOMAINS EXPOSED: FROM LIPID BINDING, CONFORMATIONAL FLEXIBILITY TO REGULATORY REGIONS

Hirdesh Kumar[1], John R. Jimah[2], Francis B. Ntumngia[3], Samantha Barnes[3], John H. Adams[3], Paul H. Schlesinger[4], Niraj H. Tolia[1]

[1]*Host-Pathogen Interactions and Structural Vaccinology Section, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [2]*Structural Cell Biology Section, Laboratory of Cell and Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States,* [3]*Center for Global Health and Infectious Diseases Research, University of South Florida, Tampa, FL, United States,* [4]*Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO, United States*

Nearly half of the world population is at the risk of malaria. *Plasmodium* parasite, the causative agent of malaria, requires a vertebrate host and an arthropod vector to complete its life cycle and therefore an effective malaria vaccine should control both the infection and the transmission stages. In the mosquito gut and the human liver, *Plasmodium* traverses several cell types before it commits to the intracellular development. Cell traversal protein for ookinete and sporozoites (CelTOS) is an essential *Plasmodium* traversal protein and a potential malaria antigen. We recently solved the structure of *Plasmodium vivax* CelTOS and showed that it forms pore in the host cell membrane. However, the mechanism of CelTOS mediated pore formation is poorly understood. To determine the role of different CelTOS domains in pore formation, we performed structure-function analyses, accelerated molecular dynamics and membrane pore-forming assays. CelTOS is secreted as a soluble protein and we hypothesized that soluble CelTOS must undergo significant conformational changes to form a pore in lipid membranes. To test this hypothesis, we engineered disulfide bridges in CelTOS to prevent conformational flexibility and showed that the disulfide-locked CelTOS lost pore-forming activity compared to wild-type protein. Additional single point mutations to limit CelTOS flexibility also reduced pore-formation. We truncated distinct domains of CelTOS and observed loss of activity for a majority of mutants. Strikingly, one mutant resulted in a significant 2.5-fold gain in activity suggested CelTOS contains regulatory regions that negatively impact function. These results demonstrate that CelTOS must undergo highly regulated conformational changes to incorporate into cell membranes. These studies form the foundation for structural vaccinology of CelTOS.

440

SYNCHRONIZATION OF A MASS BEDNET DISTRIBUTION CAMPAIGN ACROSS INTERNATIONAL BORDERS: THE EMERGING MODEL FROM THE GAMBIA AND SENEGAL

Libasse Gadiaga[1], Balla Kandeh[2], Michelle Kouletio[3], Vanessa Rouselle[4], Doudou Sene[1], Moustapha Cisse[1], Mamadou Lamine Diouf[1], Florence Penard[4], Fatou Ba Fall[1], Marcy Erskine[5], Mame Birame Diouf[3]

[1]*National Malaria Control Program, Dakar, Senegal,* [2]*National Malaria Control Program, Banjul, Gambia,* [3]*United States Agency for International Development (USAID) and U.S. President's Malaria Initiative, Dakar, Senegal,* [4]*The Global Fund, Geneva, Switzerland,* [5]*The Alliance for Malaria Prevention (AMP), Geneva, Switzerland*

In December 2018, the Health Ministers of The Gambia and Senegal put into place the Senegal-Gambia Malaria Elimination Initiative in recognition that scaling up evidence-based practices and aligning interventions was necessary to speed up progress in the high transmission cross-border region. This year, the National Malaria Control Programs (NMCPs) of Senegal and The Gambia are joining forces in the first known cooperative effort by two governments to synchronize mass bednet distribution campaigns on both sides of an international border. Mass distribution campaigns, providing an average of one net to every two people in all households nationwide, are critical for reducing mortality and malaria illness in affected areas. The NMCPs have successfully aligned the timing of their mass distribution campaigns to deliver nearly 10 million bed nets in Senegal, and another 1 million in The Gambia, between March and July 2019. This required considerable efforts, including moving The Gambia's regularly scheduled campaign one year earlier to align with Senegal's. Both countries had to align the timing of the campaign activities of household registration and distribution, ensure that similar net types were being provided, and map the massive cross-border area, defined as the entire 749 km land boundary of The Gambia, extending 2-5km into both sides of the border. The Global Fund and U.S. President's Malaria Initiative collaborated closely to support the joint planning coordination, implementation and monitoring. Geospatial mapping of health facilities, settlements and geographical landmarks along this cross-border area identified over 30 settlements with difficult access for which the neighboring country could assist to improve coverage as well as efficiency. In addition to setting a common goal and political will of neighbor countries, effective collaboration across multilateral and bilateral donors is important to optimize financial and technical resources. Further, the lessons learned from this joint campaign will help other countries that could benefit from similar cross-border malaria control initiatives.

441

INSECTICIDE RESISTANCE IN INDOOR AND OUTDOOR-RESTING *ANOPHELES GAMBIAE S.L.* IN NORTHERN GHANA

Majidah B. Hamid-Adiamoh[1], Davis Nwakanma[2], Umberto D'Alessandro[2], Alfred Amambua-Ngwa[2], Yaw Afrane[1]

[1]*West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Accra, Ghana,* [2]*Medical Research Council Unit at London School of Hygiene & Tropical Medicine, Banjul, Gambia*

Selection pressure from continued exposure to insecticides seems to drive resistance and changes in behavior of malaria vectors. We examined the association between insecticide resistance and concurrent indoor and outdoor resting behavior within members of An. gambiae s.l. in Northern Ghana. Live adult mosquitoes were collected indoors and outdoors from two communities. F1 progenies were reared from a subset of fed mosquitoes and exposed to dichloro diphenyl trichloroethane (DDT), deltamethrin, malathion and bendiocarb using WHO insecticide susceptibility tests to determine phenotypic resistance. The specific mutations to the four insecticides were analyzed using molecular assays. A statistically significant difference was observed in susceptibility to DDT and deltamethrin between indoor and outdoor mosquito populations with a 24-hour post-exposure mortality of 0% (indoor) and 9% (outdoor) for DDT; 5% (indoor) and 2.5% (outdoor) for deltamethrin [DDT: X2 = 7.58, P =0.006. Deltamethrin: X2 = 5.44, P=0.02]. Mosquitoes were found with suspected resistance to bendiocarb but the difference in mortality between indoor (90%) and outdoor (95%) populations was not significant (X2 = 2.02, df=1, P =0.16). However, mosquitoes were fully susceptible to malathion: 100% (indoor) and 98% (outdoor) mortality. Acetylcholinesterase (Ace)1-119S mutation is associated with more than two times higher odds of resistance to bendiocarb (OR =2.22, P= 0.29). Frequencies of voltage-gated sodium channel (Vgsc)-1014F and Vgsc-1575Y mutations were significantly higher in DDT and deltamethrin-resistant outdoor mosquitoes than the indoor population [Frequencies: 0.54-0.63 (indoor) and 0.66-0.68 (outdoor); X2 = 7.09, p =0.008]. However, glutathione-s-transferase epsilon 2 (GSTe2)-114T was more significantly abundant in indoor deltamethrin-resistant mosquitoes (frequency= 0.59) than the outdoor mosquitoes (frequency =0.08) [X2= 8.06, P = 0.01]. Vgsc-1014F associated strongly with deltamethrin resistance (OR =5.46, P= 0.001). Continued monitoring of vector behavior with evaluation of intra-species behavioral variations is recommended.

442

IMPACT OF INDOOR RESIDUAL SPRAY PROGRAM ON THE PREVALENCE OF *PLASMODIUM* INFECTIONS AND ANEMIA IN WESTERN KENYA: AN OPEN COHORT STUDY

Collince J. Omondi[1], Harrysone Atieli[2], David Odongo[1], Andrew K. Githeko[3], James W. Kazura[4], Guiyun Yan[5]

[1]*University of Nairobi, Nairobi, Kenya,* [2]*Maseno University, Kisumu, Kenya,* [3]*Kenya Medical Research Institute, Kisumu, Kenya,* [4]*Case Western Reserve University, Cleveland, OH, United States,* [5]*University of California, Irvine, CA, United States*

Kenya relied on long lasting insecticide treated nets, but in February 2018 indoor residual spray program was implemented in two Counties with the highest malaria prevalence in Western Kenya. Actellic insecticide was used for indoor residual spray. The objective of this study is to evaluate the impact of indoor residual spray on malaria prevalence and anemia using data from Homa Bay County where indoor residual spray was implemented and Kisumu county where there was no indoor residual spray intervention. Indoor residual spray was implemented in February 2018 and February 2019. Cross-sectional studies in open cohorts with randomly selected residents aged 6 months and above were conducted. In Homa Bay county, during January 2018 (dry season) prior to indoor residual spray intervention, overall malaria prevalence by microscopy was 12.7%, it was increased to 15.7% in the long rainy season in June 2018. Interestingly, malaria prevalence was dramatically reduced to 2.9%, 10 months after indoor residual spray. qPCR results from Homa Bay were consistent with the changes in malaria prevalence detected by microscope: 18.3% in January 2018, 17.0% in June 2018, and 10.2% in January 2019. Overall parasite density was significantly reduced in the rainy season, in comparison to dry season in 2018 (geometric mean 1,674 parasite/µl blood versus 274.8, P = 0.016), likely due to the indoor residual spray campaign in the area. In Kisumu county where no indoor residual spray intervention was implemented, malaria prevalence was not significantly changed. Indoor residual spray campaign did not significantly change anemia prevalence (38.3% in June 2018 versus 37.8% in January 2019). This data suggests a highly significant impact of indoor residual spray on malaria prevalence at least in the first year of implementation, but little impact on anemia.

443

COST PER YEAR OF EFFECTIVE LIFE CAN BE ESTIMATED FROM WHO PHASE III EVALUATION OF LONG LASTING INSECTICIDAL NETS: EVIDENCE FROM A FIELD TRIAL THAT IS WORTH SHARING!

Olukayode Ogo Odufuwa, Sarah Sjm Moore

Ifakara Health Institute (IHI), Bagamoyo, United Republic of Tanzania

Listing of Long Lasting Insecticidal Nets (LLINs) by World Health Organization Pre-Qualification Team (WHO-PQT) is based on longitudinal field studies that measure ABCD: Attrition, Bio-efficacy, Chemical content, and Damage (fabric integrity) of LLINs for 3 years under user conditions. There is a growing body of evidence to show that LLIN durability varies with long-lasting products giving better cost-effectiveness. However, it is currently assumed that all PQ-listed LLINs have a 3-year lifespan, so procurement decisions are based on LLIN unit cost. Data collected in WHO-PQT durability studies may be combined to calculate LLIN median functional survival, and allow ranking of the equivalent annual cost of each product. A double blinded household randomized prospective longitudinal cohort study was conducted for 3 years to compare the durability of two new brands of LLINs. Primary endpoint was median functional survival and secondary endpoints were LLIN bioefficacy and chemical content. Median functional survival of product A was 2.29 (1.47 - 4.30) years compared to 2.65 (1.79 - 4.34) years for Product B. Both products remained insecticidal up to the last sampling point three years (38 months) after distribution, despite having less than 50% of the initial loading dose of insecticide remaining. The proportion of nets that were in serviceable condition after 3 years was around 55% by WHO methods and 75% if the damage in the part of the net that is tucked in (and unavailable for mosquito entry) was not considered. Useful life years of LLINs is < 3 years by WHO methods and longer if the effect of tucking is considered. Procurement of LLINs should be based on cost per year of effective life using median functional survival time and this may be done using existing WHO guidelines. Adequately powered studies to measure net survival from A and D components are important with consideration of LLIN use patterns (tucking) with confirmatory bioassay testing to ensure that nets continue to kill and deter mosquitoes from feeding. Evaluation of chemical content of nets is costly and does not add to information on LLIN performance.

444

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *MORINGA OLEIFERA* LEAVES EXTRACT AND THEIR EFFECT ON MALARIA VECTOR CONTROL

Agnes Ntoumba, Francois Eya'ane Meva, Loick Kojom, Wolgang Ekoko, Philippe Belle Ebanda, Leopold Gustave Lehman

University of Douala, Douala, Cameroon

Malaria, dengue, chikungunya, Japanese encephalitis, and lymphatic filariasis are amongst parasitic deseases caused by mosquitoes . *Anopheles gambiae* is the principal vector of malaria in sub-Saharan Africa.[1] An approach for the control of mosquito populations have used silver nanoparticles for their properties and the potential to be use as simple, inexpensive, biocompatible, eco-friendly method. In the present case, we observe the effect of silver nanoparticles mediated leaf extract of *Moringa oleifera* against fourth instar larvae of *Anopheles gambiae*. as stipulated by standard methods for testing toxicity and susceptibility of mosquito larvae to insecticides of WHO. Fresh leaves of *Moringa oleifera* metabolites are water extracted and used as reducing and capping agents. We were mixed Silver nitrate ($AgNO_3$) solution with the plant extract for nanoparticle synthesis. The synthesized silver nanoparticles formation mechanism, characteristic plasmon resonance, nature, size and interface composition is discussed as key parameters affecting the activity. LD_{50} values and appreciable percentage mortality is recorded after 24 and 48h. This method is an innovative device using nanoparticles generated by plants to vector control of and the first report of *Anopheles gambiae* larvicidal activity using silver nanoparticles mediated *Moringa oleifera* leaf extract.

445

PRESENCE OF THE ZOONOTIC MALARIA PARASITES, *PLASMODIUM KNOWLESI* AND *PLASMODIUM CYNOMOLGI* IN MEMBERS OF SEVERAL *ANOPHELES* SUBGROUP IN TWO DISTRICTS OF SARAWAK, MALAYSIAN BORNEO

Joshua Ang Xin De[1], Khamisah Abdul Kadir[1], Dayang Shuaisah Awang Mohamad[1], Asmad Matusop[2], Khatijah Yaman[1], Balbir Singh[1]

[1]*Universiti Malaysia Sarawak, Sarawak, Malaysia,* [2]*Sarawak Department of Health, Sarawak, Malaysia*

The zoonotic malaria parasite, *Plasmodium knowlesi*, has caused all the 2,470 indigenous human malaria cases reported in Sarawak, Malaysian Borneo, in 2017 and 2018. Previously identified vectors of the parasite in nature in Malaysia and Vietnam all belong to the Leucosphyrus Group. In Sarawak, only *An. latens* was incriminated in one study conducted in the Kapit District. This project was therefore undertaken to identify malaria vectors in other locations of Sarawak. Human landing catches were conducted in forested sites of the Betong and Lawas Districts. Genomic DNA was extracted from the salivary glands of anophelines and screened with nested PCR assays for the presence of *Plasmodium*. The sequences of the SSUrRNA genes of *Plasmodium* spp. and the ITS2 and CO1 sequences of the mosquitoes were derived from the *Plasmodium*-positive samples. Collectively, 238 anophelines and 2,127 culicines were caught. *An. letifer* s. l.(44.5%, n=77) and *An. balabacensis* (47.7%, n=31) were found to be the predominant anophelines in Betong and Lawas, respectively. Phylogenetic analyses of the SSUrRNA genes confirmed the presence of *P. knowlesi, P. coatneyi, P. cynomolgi, P. fieldi, P. inui,* and other unidentified malaria parasites in 3 *An. barbirostris*, 6 *An. balabacensis*, 6 *An. latens* and 7 *An. letifer* s. l. Phylogenies inferred from the ITS2 and CO1 sequences of *An. balabacensis* and *An. barbirostris* indicate that the former is genetically indistinguishable from *An. balabacensis* sequences from Borneo while the latter is closely related to but distinct from the other described members of the Barbirostris Subgroup. The CO1 sequences of *An. latens* were separated into two different clades while those from *An. letifer* s. l. failed to form clades according to its corresponding morphological identification. This suggests that there are more potentially transmissible parasites and vectors in the forest setting than is known and that vector study is imperative, especially when nuclease-based gene drive which is sensitive to nucleotide-level variation is being considered as the novel silver bullet to tackle malaria.

446

BEDNET USE, DISPOSAL PRACTICES AND ASSOCIATED FACTORS IN RURAL WESTERN UGANDA

Alex Ndyabakira[1], Emmanuel Arinaitwe[2], Joaniter I. Nankabirwa[3], Gabriel Chamie[4], Rhodah Namubiru[3], Tobius Mutabazi[3], Daslone Kwarisiima[2], Kara Marson[4], Jaffer Okiring[2], Harsha Thirumurthy[5], Sarah G. Staedke[6], Charles Ssemugabo[7], Moses R. Kamya[3], Yeka Adoke[7]

[1]*Department of Disease Control and Environmental Health, School of Public Health, Makerere University College of Health Sciences, Kampala, Uganda,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda,* [4]*Department of Medicine, Division of HIV, Infectious Diseases and Global Medicine, University of California San Francisco, San Francisco, CA, United States,* [5]*Division of Health Policy, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States,* [6]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [7]*Department of Disease Control and Environmental Health, School of Public Health, Makerere University College of Health Sciences, Kampala, Uganda*

Bednets, in particular, Long Lasting Insecticidal Nets (LLINs) are the most cost-effective malaria control intervention, however effectiveness of LLINs depends on high coverage and consistent use. Malaria burden in Uganda remains high despite free distribution of LLINs through universal

and routine campaigns. We assessed bednet use, disposal practices and associated factors in rural Western Uganda. We conducted a household survey in 252 households from February-March 2018, approximately 4years after the last universal coverage campaign (UCC). Households were randomly sampled from a household census list. Household heads were interviewed and nets were physically observed. Association between variables were determined using prevalence ratios and factors associated with net ownership, use and disposal practices were assessed using modified Poisson regression. Households had a total of 1,206 residents and 384 bednets. A total of 187 (74.2%) households owned at least one bednet and 95.1% (365/384) of the bednets were LLINs. Only 465 (38.6%) of residents slept under a bednet the night before the survey. About 86 (34.1%) households had universal net coverage. Majority (65.7%) of nets were disposed by burning at home. Residents from households with universal net coverage were more likely to use nets (aPR=2.85, 95%CI: 2.51-3.24). Household members were less likely to use bednets if they were males (aPR=0.78, P5%CI: 0.68- 0.91), were aged between 5-17 (aPR=0.65, 95%CI: 0.52-0.81) and 18-35 (aPR=0.71, 95%CI= 0.57-0.89) years, and were unrelated to the household heads (aPR=0.61, 95%CI: 0.44-0.84). Four years after a LLIN campaign, bednet ownership and use were found to be low and bednets were improperly disposed. The country should have more frequent UCC and promote proper disposal of nets.

447

LOCALIZED VARIATIONS IN LLIN USE IN MYANMAR AND CAMBODIA

Ye Kyaw Aung[1], May Me Thet[1], Sochea Phok[2], Si Thu Thein[1]

[1]*Population Services International Myanmar, Yangon, Myanmar,* [2]*Population Services International Cambodia, Phnom Penh, Cambodia*

To enhance use of Long lasting insecticide nets (LLIN) for malaria prevention, it is important to understand the factors that determine the LLIN use at population level. This study aimed to identify the factors associated with population level LLIN use in rural areas of campaign LLIN distribution in Bago Region of Myanmar and Battambang and Siem Reap Provinces of Cambodia. A cross-sectional household survey was conducted in the areas of recent distribution campaigns in both countries. The survey covered households which received at least one LLIN during the distribution campaigns of 2017 and 2016 in Myanmar and Cambodia. A total of 1414 households in Myanmar and 1362 households in Cambodia were recruited though single-stage cluster sampling method. Population level LLIN use and under-five children LLIN use were calculated and potential associations were explored using multivariate logistic regression models in STATA 14.2. Overall LLIN use among the population living in the study area of Myanmar was 61.7% while that was only 27.7% in Cambodia. LLIN use of under-five children followed the same pattern, 65.0% in Myanmar and 23.4% in Cambodia. Coverage of LLINs (HH had a least 1 LLIN per 2 persons) was higher in Myanmar (77.8%) than Cambodia (42.8%). In both countries, population level use of LLIN was significantly higher in households with enough coverage of LLINs, (Odd Ratio (OR) 1.76, 95% CI 1.49 - 2.07 in Myanmar and OR 1.83, 95% CI 1.64 - 2.04 in Cambodia). However, other determinants varied by locality. LLIN use was lower among male household members, households with higher socioeconomic status, and households with practice of prevention to net tear in Myanmar (p>0.001). In Cambodia, these factors were associated with higher use of LLINs (p>0.001). Despite almost the same distribution time in both countries, overall population level use of LLINs was higher in the study area of Myanmar than that of Cambodia, mainly determined by high distribution coverage of LLINs. However, other determinants of LLIN usage varied by locality, and such contextual variations should be taken account in LLIN distribution activities to ensure optimal usage.

448

MOSQUITO AND PARASITE GENETIC DETERMINANTS OF THE EXTRINSIC INCUBATION PERIOD OF *PLASMODIUM FALCIPARUM*

Robert Shaw[1], Kristine Werling[1], Maurice A. Itoe[1], Duo Peng[1], Douglas G. Paton[1], Naresh Singh[1], Roch K. Dabiré[2], Abdoulayé Diabaté[2], Liliana Mancio-Silva[3], Allison R. Demas[3], Sandra March[3], Sangeeta N. Bhatia[3], Thierry Lefèvre[2], Flaminia Catteruccia[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,* [3]*Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, United States*

The extrinsic incubation period (EIP) of *Plasmodium* parasites, the time taken for their sporogonic development within the *Anopheles* mosquito, is a key determinant of malaria transmission; faster developing parasites are more likely to be transmitted to subsequent hosts given the relatively short lifespan of mosquitoes. However, despite its relevance, relatively little is experimentally determined regarding factors that can influence the EIP and could be manipulated in novel transmission-blocking strategies. Here we show that in *Anopheles gambiae*, the major African malaria vector, *P. falciparum* parasites accelerate their growth and shorten their EIP in response to the disruption of steroid hormone signaling following a blood meal. When reproductive investment is reduced via several mechanisms, physiological levels of circulating lipids, transported by the lipid transporter lipophorin, are elevated and exploited by the parasite to develop more quickly. Faster parasites that reach the salivary glands sooner maintain full infectivity to human hepatocytes, as determined by *in vitro* quantification of exoerythrocytic forms. Using multiple gametocyte carriers in a *P. falciparum*-endemic region we confirmed and extended these observations to field-derived colonies of *An. gambiae* and the closely related sibling species *An. coluzzii*. In these field infections we found evidence of both parasite and mosquito genetic determinants controlling the growth rate of parasites, and identified parasite isolates showing remarkably high developmental rates after perturbations of steroid hormone signaling. Combined, these data reveal that parasites have a plastic interaction with mosquito metabolism, with profound implications for vector control strategies aiming to lower mosquito fecundity.

449

INTRAZONAL DIFFERENCE IN MALARIA PREVALENCE AND FACTORS ASSOCIATED WITH MALARIA INFECTION AMONG CHILDREN AGED 0 TO 10 YEARS IN KISANTU HEALTH ZONE

Gillon Ilombe Kaounga[1], Junior Matangila[2], Baby Mabanzila[3], Sylvie Linsuke[1], Emile Manzambi[1], Eric Mukomena[2], Francis Watsenga[1], Pascal Lutumba[2], Seth Irish[4]

[1]*Institut National Recherche Biomedical, Kinshasa, Democratic Republic of the Congo,* [2]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [3]*Clinic University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [4]*Centers for Disease of Control, Atlanta, GA, United States*

The Democratic Republic of Congo (DRC) is the most malaria affected country. The Kongo Central (KC) Province has higher malaria cases than the national average. There is a direct relationship between malaria risk and geo-climatic environment of people. Within the same epidemiological settings, malaria transmission is not homogeneous; this contributes to the complexity of malaria and its elimination. The main of this study was to determine intra-zonal prevalence and factors associated with the malaria infection in children aged 0-10 years in the Kisantu health zone in KC. In this area, a community based cross-sectional study was conducted from October to November 2017. A multi-stage sampling was used, 30 villages from Kisantu health zone were randomly selected. Malaria prevalence was measured by thick blood smear (TBS) and rapid diagnostic test (RDT). Data were analyzed in Stata 14.0. Data were summarized using proportion and mean (with standard deviation). The Student t test and χ^2 test were used respectively for mean and proportion comparison. Logistic regression analysis identified determinants of malaria infection. The prevalence

according to the TBS and RDT was respectively 14.8% and 78.3%. The results in the different village were a heterogeneous distribution of Malaria prevalence using TBS, varying from 0 to 52.6 %. In bivariate analysis, the main type of wall, the type of ground, the main type of domestic roof (straw and thatch) and the malnutrition were factors associated at the risk of the arisen of malaria infection, respectively 2.1 (1.5-2.8); 15.7 (8.5-32.2); 4.5 (3.0-6.7) and 1.9 (1.3-2.6). In multivariate analysis, the fact of having the sand or the wood as the main type of ground 17.3 (8.86-33.76) and the fact of having a moderate or severe insufisance 1.53 (1.13-2.07) were factors associated to one arisen the malaria infection. Generalization of malaria prevalence is not a good approach; local variation impacts malaria control strategies. Malaria control programme has to go in hand with improvement in social economy, considering housing and nutrition. This call for a multi-sectorial approach to control diseases.

450

CHARACTERIZING THE MOLECULAR AND METABOLIC MECHANISMS OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE*S.L. IN FARANAH, GUINEA

Caleb J. Stica[1], Claire L. Jeffries[1], Seth R. Irish[2], Yaya Barry[3], Denka Camara[3], Ismael Yansane[4], Mojca Kristan[1], Thomas Walker[1], Louisa A. Messenger[2]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*Programme National de Lutte contre le Paludisme, Conakry, Guinea,* [4]*StopPalu, Conakry, Guinea*

The scale-up of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has greatly reduced malaria transmission. However, malaria remains a global health problem with the majority of the disease burden in sub-Saharan Africa. Improved understanding of increasing insecticide resistance among *Anopheles* populations is essential for continued effectiveness of control interventions. *An. gambiae* s.l. were collected from villages in the Faranah Prefecture, Guinea and CDC resistance intensity bioassays were used to measure susceptibility to seven insecticides. Vector species, presence of target site mutations (L1014F *kdr*, N1575Y and G119S *Ace-1*), *Plasmodium falciparum* infection, and relative expression of three metabolic genes (*CYP6M2, CYP6P3,* and *GSTD3*) were determined. Intense resistance to permethrin and deltamethrin was observed, with developing resistance to bendiocarb. Vectors were fully susceptible to alpha-cypermethrin, pirimiphos-methyl, clothianidin and chlorfenapyr. *P. falciparum* infection rate was 7.3% (37/508). The L1014F *kdr* mutation was found in 100% of a sub-sample of 60 mosquitoes, supporting its fixation in the region. The N1575Y mutation was identified in 20% (113/561) of individuals, with ongoing selection evidenced by significant deviations from Hardy-Weinberg equilibrium. The G119S *Ace-1* mutation was detected in 62.1% (18/29) of mosquitoes tested and was highly predictive of bendiocarb bioassay survival. When compared to a susceptible *An. gambiae* s.s. G3 laboratory colony, *CYP6M2, CYP6P3* and *GSTD3* were found to be significantly over expressed in wild resistant and susceptible *An. gambiae* s.s. populations. Furthermore, *CYP6P3* was significantly over expressed in bendiocarb survivors, implicating its potential role in carbamate resistance. The intense resistance to permethrin and deltamethrin, identified in the Faranah Prefecture, is of considerable concern, as the Guinea National Malaria Control Program relies exclusively on the distribution of pyrethroid-treated LLINs. Study findings will be used to guide current and future control strategies in the region.

451

NATIONAL MALARIA VECTOR SURVEILLANCE: A REGIONAL ANALYSES AGAINST RECOMMENDED ACTIVITIES

Tom Burkot[1], Tanya Russell[1], Min Myo[2], Abraham Mnzava[3], Effie Espino[2], Robert Farlow[4]

[1]*Australian Institute of Tropical Health and Medicine, Cairns, Australia,* [2]*Asian Pacific Malaria Elimination Network, Singapore, Singapore,* [3]*African Malaria Leaders Alliance, Dar es Salaam, United Republic of Tanzania,* [4]*Robert Farlow Consulting LLC, Burkville, TX, United States*

Malaria vector surveillance is being increasing recognized as critical to the success of national malaria control programs. The World Health Organization recently published the objectives for malaria vector surveillance and provided guidance on core vector surveillance activities by transmission scenarios and vector control interventions deployed. In order to assess the status of vector surveillance, an online survey gathered information from 35 national malaria control programs or their partner organizations. This presentation is one of three providing detailed analyses related to this survey. In this presentation an analyses of national program vector surveillance activities by transmission scenarios and interventions deployed are summarized by countries in Africa and the Asia-Pacific regions including a comparison of vector surveillance in countries attempting elimination to countries with higher transmission rates. Data on vector parameters monitored, the techniques used for their quantitation and methods to assess intervention access and use will be described. Analyses identified strengths across the regions as well as regional differences including significant limitations by comparison to the benchmarks established by the Global Malaria Programme. Additional detailed analyses from the same data set of the limitations to programmatic vector surveillance and will be presented by Dr. Tanya Russell. A complementary study defining next generation vector surveillance tools to address technical limitations of the presently deployed tools to define vector parameters will be presented by Dr Robert Farlow.

452

EFFECTIVENESS OF SELECTED CATTLE-ADMINISTERED ENDECTOCIDES TO REDUCE MALARIA-VECTOR MOSQUITOES

Takalani Irene Makhanthisa, Leo Braack, Heike Lutermann

University of Pretoria, Hatfield, South Africa

Malaria control primarily depends on two vector strategies; indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLIN). Both IRS and LLIN are indoor strategies and some of the major malaria vectors have developed resistance against them. Vector behavioural changes such as outdoor feeding on zoophagic hosts including cattle also limit the effectiveness of these strategies. Novel malaria control strategies must therefore be implemented to complement IRS and LLIN. A promising tool is the use of endectocides in alternative hosts for malaria vectors. Endectocides are broad-spectrum systemic drugs against a range nematodes and arthropods. The aim of this study was to investigate the effect of two endectocide drugs; ivermectin and fipronil on the survival and fecundity of zoophilic *Anopheles arabiensis.* Mosquitoes were reared in an insectary at a constant temperature of 25°C±2°C, 75%±5% humidity and 12 h light: 12 h darkness photoperiod. In total, six cattle individuals were used, each of them got three treatments; ivermectin® (1 ml/50 kg), fipronil® (1 ml/10 kg) and saline (control). Batches of *A. arabiensis* female mosquitoes (n=30) that were allowed to mate first were placed in cups and allowed to feed from the animals. Feeding experiments were conducted at day 0, 1, 4, 7, 13, 21 and 25 post-treatment; mortality and egg production were recorded. Preliminary results show that both ivermectin and fipronil decrease the survival and egg production of mosquitoes compared to the control group. Ivermectin was most effective at day 1 post-treatment with the highest effect at day 4 post-feeding with up to 60% higher mortality compared to control groups. The effect of ivermectin attenuated over a period of two weeks down to 12% and no differences were apparent any longer from day 21 post-treatment

onwards. For both drugs, the treated groups produced up to 70% fewer eggs than the control from day 1-13 post-treatments. Our results suggest that ivermectin may be more effective than fipronil to control zoophagic mosquitoes.

453

OPERATIONAL IMPLICATIONS OF PYRETHROID RESISTANCE AND PIPERONYL BUTOXIDE SYNERGIST ASSAYS ON VECTOR CONTROL DECISIONS IN NIGERIA

Petrus U. Inyama[1], **Lazarus M. Samdi**[1], Adedayo O. Oduola[1], Jesse C. Uneke[2], Andrew B. Yako[3], Atting A. Inyang[4], Kehinde O. Popoola[5], Auwal A. Barde[6], Yahaya M. Abdullahi[7], Manaseh Manyi[8], Joseph I. Okeke[1], Bala Mohammed[9], Okefu O. Oyale[9], Aklilu Seyoum[10], Kelley Ambrose[10], Bradford Lucas[10], Uwem Inyang[11], Jose Tchofa[11], Mark Maire[12], Jennifer S. Armistead[13], William A. Hawley[14]

[1]*PMI Vectorlink Project, Abuja, Nigeria,* [2]*Ebonyi State University, Abakaliki, Nigeria,* [3]*Nasarawa State University, Keffi, Nigeria,* [4]*University of Uyo, Uyo, Nigeria,* [5]*University of Ibadan, Ibadan, Nigeria,* [6]*Abubakar, Tafawa Balewa University, Bauchi, Nigeria,* [7]*Usmanu Danfodiyo University, Sokoto, Nigeria,* [8]*Federal University of Agriculture, Makurdi, Nigeria,* [9]*Federal Ministry of Health, Abuja, Nigeria,* [10]*U.S. President's Malaria Initiative VectorLink Project, Abt Associates, Bethesda, MD, United States,* [11]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Abuja, Nigeria,* [12]*U.S. President's Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Abuja, Nigeria,* [13]*U.S. President's Malaria Initiative, United States Agency for International Development, Washington, DC, United States,* [14]*Entomology Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States*

Deployment of long-lasting insecticidal nets (LLINs) remains the main strategy for malaria vector control in Nigeria. Until recently, LLIN procurement decisions were not guided by local insecticide susceptibility evaluations. This study reports the outcome of insecticide susceptibility tests conducted in 2018 on *Anopheles gambiae* s.l. collected from sentinel sites representing the five ecological zones of Nigeria. Larvae of *An. gambiae* s.l. were collected in 60 Local Government Areas (LGAs) in Akwa Ibom, Bauchi, Benue, Ebonyi, Nasarawa, Oyo, Plateau, Sokoto, and Zamfara states. Adult, female *An. gambiae* s.l. reared from larvae and between 2 and 5 days old were exposed to permethrin, deltamethrin, and alpha-cypermethrin using CDC bottle bioassays. Insecticide resistance tests were done with different doses (2x, 5x, and 10x) to determine the intensity of pyrethroid resistance. Resistant *An. gambiae* s.l. were subjected to synergist assays with piperonyl-butoxide (PBO) to determine mechanisms of resistance. Pyrethroid resistance was detected in *An. gambiae* s.l. from all nine states. *An. gambiae* s.l. were susceptible to permethrin in two LGAs in Sokoto and one LGA in Zamfara. Permethrin resistance at 5x diagnostic concentration was recorded at Nasarawa and at both 5x and 10x in Akwa Ibom, Benue, Ebonyi, and Oyo. PBO restored susceptibility to permethrin in all LGAs in Akwa Ibom and Sokoto, and in five out of the six LGAs in Ebonyi. Susceptibility to deltamethrin at 2x was seen in all LGAs in Benue and Nasarawa. Deltamethrin resistance at 5x was recorded in Akwa Ibom and at 10x concentration in Ebonyi. Exposure to PBO restored susceptibility to deltamethrin in Ebonyi, Nasarawa, Plateau, and Sokoto. *An. gambiae* s.l. was also susceptible to alpha-cypermethrin in select LGAs in Bauchi, Benue, Sokoto, and Zamfara. Alpha-cypermethrin resistance at 5x or 10x was detected in Oyo and at 2x in Ebonyi. In Benue and Oyo, PBO restored susceptibility to alpha-cypermethrin. In the 2020 mass campaign, alpha-cypermethrin LLINs are planned for Zamfara, deltamethrin or alpha-cypermethrin LLINs for Benue, and alpha-cypermethrin+PBO LLINs will be procured for Oyo.

454

BIONOMICS OF *ANOPHELES* MOSQUITOES IN MALARIA ENDEMIC SENTINEL SITES IN GRACIAS A DIOS DEPARTMENT, HONDURAS

Allan Armando Reyes Garcia[1], Erik Alvarez[1], Fredy Zalasar[1], Jose Orlinder Nicolas Zambrano[2], Marissa Bordas[1], Efrain Burgos[1], Diana Nuñez Azzad[2], Neila Julieth Mina[3], Daragh A. Gibson[4], Sheila B. Ogoma[5], Tara Seethaler[4], Wilberto Montalvan[2]

[1]*Honduras Secretary of Health, Health Region N⁹ Gracias a Dios, Puerto Lempira, Honduras,* [2]*Honduras Secretary of Health, Central Level, Tegucigalpa, Honduras,* [3]*Clinton Health Access Initiative, Honduras Office, Tegucigalpa, Honduras,* [4]*Clinton Health Access Initiative, Boston, MA, United States,* [5]*Clinton Health Access Initiative, Kenya Office, Nairobi, Kenya*

The Department of Gracias a Dios (GAD) in Honduras is malaria endemic and has historically registered the greatest number of cases in the country. In 2018, the region reported 259 malaria cases, of which 82.2% were *Plasmodium vivax* and 17.8% were *P. falciparum*. In GAD, the municipalities of Puerto Lempira, Villeda Morales, and Brus Laguna reported an estimated 92.7% of these cases. Efficacy of vector control interventions is largely influenced by malaria vector bionomics. In order to reach the goal of malaria elimination in Honduras by 2020, it is critical to determine the entomological aspects that are driving transmission to best target appropriate interventions. Mosquito collections were conducted in three sentinel sites across the three municipalities: Kaukira (Puerto Lempira), Raya (Villeda Morales), and Brus Laguna (Brus Laguna). Vector species composition, their spatial and temporal distribution, and biting behavior and rates were measured using human landing catches (HLCs). Larval sampling, habitat characterization, and outdoor aspirations in animal sheds were also conducted. Sentinel sites were surveyed monthly for a week from October 2017 through December 2018. A total of 131 potential larval habitats were identified, and about 1,169 and 2,245 *Anopheles* mosquitoes were captured using HLCs and outdoor aspirations in animal sheds, respectively. Of the 1,169 mosquitoes captured via HLCs, 66.3% were morphologically identified as *Anopheles albimanus,* 29% *An. gabaldoni,* 4.5% *An. crucians*, and 0.2% identified as other species. Additionally, 74% of the 1,169 mosquitoes were captured outdoors. Across the three sites, the months with the greatest densities were November 2017, June 2018, and October 2018. The most predominant species across all sites each month was *An. albimanus* with the exception of Kaukira, where *An. gabaldoni* was more prevalent from November 2017 to January 2018. The seasonal and temporal distribution of vectors will help determine indoor residual spray timing for upcoming spray rounds and to measure impact, while additional studies are needed to understand the role of species type on transmission dynamics in GAD.

455

IDENTIFICATION OF ENTEROTOXIGENIC *ESCHERICHIA COLI* FROM INFANTS ATTENDING HEALTHCARE CENTERS IN IBADAN NORTH

Olabisi Comfort Akinlabi[1], Stella Ekpo[1], El-shama Queen Nwoko[1], Catherine O. Ladipo[1], Akinlolu Adepoju[2], Gordon Dougan[3], Iruka N. Okeke[1]

[1]*University of Ibadan, Ibadan, Nigeria,* [2]*Department of Paediatrics, College of Medicine, University of Ibadan, Oyo, Ibadan, Nigeria,* [3]*Cambridge University, Cambridge, United Kingdom*

Enterotoxigenic *E. coli (*ETEC) can cause profuse, watery diarrhea by release of heat labile (LT) and/ or heat stable (ST) enterotoxins. This study hypothesized that ETEC is a predominant cause of infantile diarrhoea in northern Ibadan, Oyo State, Nigeria. We performed a case control study among children under 5 years of age. Stool specimens were collected from 99 children with diarrhoea and 315 controls attending primary health clinics in Lagelu and Egbeda local government areas of Ibadan between November 2015 and January 2019. *E. coli* was isolated and identified using standard laboratory protocols and ETEC were identified by multiplex

PCR. ETEC were isolated from 24 (24.2%) cases and 70 (22.2%) controls. ETEC strains carrying both heat-labile (LT) and heat-stable (ST) enterotoxin-encoding genes were significantly associated with diarrhoea, being recovered from 12 (12.1%) and 14 (4.4%) cases and controls respectively (p=0.01). ETEC bearing only LT genes were isolated from 17(17.2%) cases and 49(15.5%) controls while strains with ST genes only were recovered from 5(5%) cases and 9(2.9%) controls (p>0.05). There was clustering of ETEC recovery in March 2016 and again in August to September 2016. Molecular profiling of ETEC isolates within these clusters points to possible ETEC outbreaks. In conclusion, our data suggest that ETEC strains, especially those that carry both LT and ST genes, may be important causes of diarrhoea. We also found high rates of carriage among apparently healthy children. The study further reveals that outbreaks caused by ETEC could be commonplace but beyond routine detection. Interventions that target ETEC pathotypes may have the potential to reduce the morbidity from infantile diarrhoea in Ibadan.

456

INVESTIGATING VITAMIN A IN TREATING DISSEMINATED INFECTIONS WITH MULTIDRUG RESISTANT NON-TYPHOIDAL *SALMONELLA*

Annica Stull-Lane[1], Kristen Lokken[1], Vladimir Diaz-Ochoa[1], Nicoel White[1], Ariel Muñoz[1], Gregory Walker[1], Daniela Hampel[2], Xiaowen Jiang[2], Charles Stephensen[2], Renée Tsolis[1]

[1]*Department of Medical Microbiology and Immunology; University of California Davis, Davis, CA, United States,* [2]*US Department of Agriculture; Western Human Nutrition Research Center, Davis, CA, United States*

Non-typhoidal *Salmonella* (NTS) serovars are common causes of gastroenteritis; however, factors including young age, malnutrition and concurrent malaria infection underlie susceptibility to disseminated disease. Annually, there are approximately 3.4 million cases and 680,000 deaths due to disseminated NTS infections, as the mortality rate is ~20%. Non-typhoidal *Salmonella* serovar Typhimurium sequence type 313 (ST313) has evolved to become more capable of causing invasive disease in the human host and is more common in sub-Saharan African countries. Our lab focuses on studying a ST313 clinical isolate that is multidrug resistant (D23580). As micronutrient deficiencies overlap with malnourished states, we have modeled in mice how vitamin A deficiency compromises the immune response to systemic NTS infection. We investigate the efficacy of therapy with antibiotics, vitamin A or co-treatment to decrease bacterial burden of *Salmonella* at systemic sites and improve survival in vitamin A deficient (VAD) mice. Our results show that VAD mice are unable to control bacterial replication at systemic sites. Importantly, vitamin A supplementation improves control of infection, and vitamin A treatment increases survival. Ultimately, our goal is to inform clinical studies assessing vitamin A as adjunctive therapy in patients suffering from disseminated NTS infection with the objective of decreasing mortality rate and improving human health outcomes globally.

457

GUT RESISTOME AFTER ORAL ANTIBIOTICS IN PRESCHOOL CHILDREN IN BURKINA FASO: A RANDOMIZED CONTROLLED TRIAL

Catherine Oldenburg[1], Armin Hinterworth[1], Ali Sié[2], Boubacar Coulibaly[2], Lucienne Ouermi[2], Clarisse Dah[2], Charlemagne Tapsoba[2], Susie Cummings[1], Lina Zhong[1], Cindi Chen[1], Samarpita Sarkar[1], Till Barnighausen[3], Thomas Lietman[1], Jeremy Keenan[1], Thuy Doan[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Centre de Recherche en Sante de Nouna, Nouna, Burkina Faso,* [3]*University of Heidelberg, Heidelberg, Germany*

Antibiotic selects for resistance at the individual and community level. We used data from a randomized controlled trial of pediatric antibiotic administration to evaluate the relationship between antibiotic use and the gut resistome, defined as the collection of resistance gene determinants in a given environment. Children aged 6-59 months were enrolled in two rural communities in Nouna District, Burkina Faso. Households were randomized in a 1:1:1:1 fashion to amoxicillin (25 mg/kg/day in twice-daily doses), azithromycin (10 mg/kg on day one and then 5 mg/kg once daily for four days), cotrimoxazole (240 mg once daily), or placebo (powdered milk and sugar in bottled water). All children were treated for 5 days. Rectal samples were collected 5 days after the last antibiotic treatment and sequenced using DNA sequencing to identify antimicrobial resistance determinants, which were classified at the class and gene level. Of 124 children randomized, 120 had a rectal swab collected post-treatment. Beta-lactam resistance did not differ across study arms. Azithromycin samples were more than twice as likely to have genetic resistance determinants to macrolides (risk ratio, RR, 2.61 95% CI 1.55 to 4.42, *P*=0.0003) compared to placebo. Sulfonamide resistance was higher in samples from children in all antibiotic arms compared to placebo. Samples from children randomized to cotrimoxazole were more than 3 times as likely to have genetic resistance determinants to trimethoprim (RR 3.29, 95% CI 1.08 to 9.95, *P*=0.04) compared to placebo. Mean richness (number of unique genes) of antimicrobial resistance determinants was higher in amoxicillin-treated (42.6 versus 23.9, *P*=0.02) and cotrimoxazole-treated (40.1 versus 23.9, *P*=0.049) children compared to placebo-treated children. Richness was not significantly different between azithromycin-treated children and placebo (*P*=0.15). In conclusion, a single course of antibiotic can cause changes in the gut resistome. Regional antimicrobial resistance surveillance will be important to inform antibiotic use policy.

458

CHOLERA VACCINAL IN DRC

Sandra Musungayi,T.S Musungayi

URF/INRB, Kinshasa, Democratic Republic of the Congo

Cholera is major public health problem because of it sever mortality on the affected persons ;in epidémic regions 80% of cases notify by OMS in the world plan comes from sub_sahara africa . in DRC, the profil of infected people with cholera is less known; and strategies to prevent it by vaccine campaign is wrongly done. To determine different profiles of people affected by cholera. A retrospective analytical study was conducted in 17 health zones of the city of Kinshasa Province. Active data collection of suspected cholera cases identified between 2000-2016 was made based on national registries available in the 17 selected areas. These data were organized in excel spreadsheets with variables year, week, health zone, age, sex, occupation for the comparison between the groups and proportion; the statistics of the tests (chi square and Fisher test) were carried out using the SPSS software version 21 for each evolutionary period. The proportions of each variable were calculated with their 95% fixed confidence interval. Les commerçants, les pêcheurs, les élèves, les étudiants, les agriculteurs, les voyageurs et les conducteurs sont les plus touchés dans les zones d'endémie, avec une proportion de 78,5% (IC à 95%: 75,9-80,8). Le groupe d'âge le plus touché est celui des 5-14 ans, avec 30% (IC 95% : 29,5-31,1) dans les zones d'endémie et 25,7% (IC 95%: 22,9% à 28,8%) dans les zones non endémiques. La proportion était respectivement de 28,7% (IC à 95%: 27,3-307), de 20% (IC à 95%: 27,1 à 29,3) et 25,9% (11,5 à 44,7) dans les phases évolutives de l'immobilité, du pic de l'épidémie et de la recrudescence des zones endémiques dans la groupe d'âge de 5-14 ans. Les résultats de cette étude restent un défi car la stratégie sera appliquée en termes d'âge et d'autres aspects. Une approche multidisciplinaire est essentielle pour prévenir le choléra.

459

PATHOGEN BOX CONTAINS INHIBITORS OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* GROWTH WITH ANTIBACTERIAL POTENTIAL

David Ajigasoko Kwasi[1], Adeola Oluwaseyi Bamisaiye[1], Olujide Oludayo Olubiyi[2], Olabisi Comfort Akinlabi[1], Emmanuel Oladayo Irek[2], Chinedum Peace Babalola[1], Ikemefuna Uzochukwu[3], Oladipo Aaron Aboderin[2], Iruka Nwamaka Okeke[1]

[1]*University of Ibadan, Ibadan, Nigeria,* [2]*Obafemi Awolowo University, Ile-Ife, Nigeria,* [3]*Nnamdi Azikiwe University, Awka, Nigeria*

Enteroaggregative *Escherichia coli* (EAEC) are important causes of infantile diarrhoea and often cause persistent diarrhoea, for which antibiotics are indicated. However, EAEC are commonly antibiotic resistant and therefore new and effective therapies are needed. We screened the Medicines for Malaria Venture›s (MMV) Pathogen Box for EAEC growth inhibitors that could be antibacterial forerunners. We performed a medium throughput screen of MMV›s 400-compound Pathogen Box to identify inhibitors of EAEC growth in Dulbecco›s Modified Eagle›s Medium. Hits are systematically being tested for activity against a broader range of Gram negative clinical isolates, including multiply-resistant strains. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations of ‹hit› compounds are measured by broth microdilution. Seven compounds inhibited growth of EAEC strains 042 and MND005E in the screen. Of these compounds, rifampicin and levofloxacin had previously documented anti-Gram negative activity. (Doxycycline, another antibacterial agent present in Pathogen Box was not retrieved in the screen because both strains are tetracycline-resistant). Our screen identified five compounds with no previously documented Gram negative activity. Compounds MMV675968 and MMV002817 exhibited greater growth inhibition activity than the known antibacterials tested. MMV002817 was bactericidal against a broad range of Gram negative bacteria. For this compound, MICs against *Pseudomonas* spp, including multiply-resistant clinical isolates ranged between 4 and 32 μg/ml compared with ciprofloxacin MICs of ≤0.125 to ≥ 64 μg/ml. This study identified novel small molecules with antibacterial activity, one of which demonstrated broad spectrum Gram negative activity against multidrug-resistant Pseudomonas. MMV002817 has the potential for optimization or repurposing for EAEC-based interventions and as last line Gram-negative antibacterial.

460

CHARACTERIZATION OF *SHIGELLA* SPECIES CAUSING DISEASE IN CHILDREN ADMITTED TO KILIFI COUNTY HOSPITAL, KENYA

Anne Amulele[1], Michael Ooko[1], Alfred Mwanzu[1], Sam Kariuki[2], Anthony Scott[1], Nicola Claire Gordon[1]

[1]*KEMRI Wellcome Trust Research Programme, Kilifi, Kenya,* [2]*KEMRI Centre for Microbiology Research, Nairobi, Kenya*

Shigella spp is one of the leading causes of bacterial diarrhoea and related mortality worldwide especially in young children under five years in developing countries. Though childhood mortality attributed to the pathogen has reduced significantly in recent years, most of the deaths occur in Africa and Asia. Infections can be prevented by improving sanitation and hygiene practices, however, an affordable and efficacious vaccine would accelerate disease reduction in resource limited countries. At present there is no licensed vaccine though several potential candidates are in research and development. It is important to understand the epidemiology and characteristics of *Shigella* circulating in our region to estimate the impact of a potential vaccine. We undertook a descriptive retrospective study of *Shigella* gastroenteritis and bacteremia cases occurring in children presenting to a public hospital in Kilifi County from 1994 to 2016. The demographics and clinical characteristics of the patients were obtained while the antibiogram profile, species identification, *S. flexneri* serotypes and virulence diversity of stored isolates was determined by disc diffusion, agglutinating sera and PCR. We identified 200 patients from whom *Shigella spp* was cultured: 183 (91.5%) with gastroenteritis, 13 (6.5%) with bacteremia, and 4 (2%) with both. Children aged 12-59 months were the most affected accounting for 62% (124) of all cases. 11% (21) of the patients died in hospital. *S. flexneri* was the dominant species identified in both gastroenteritis and bacteremia with serotypes 2a, 3a and 6 accounting for 68% of *S. flexneri* serotypes. We observed high rates of resistance to tetracycline (96%), cotrimoxazole (92%) and ampicillin (83%) and no resistance to cephalosporins and ciprofloxacin. The most frequently identified virulence genes were *sen*, *set1A* and *sepA*, while shiga toxin gene was present in half of *S. dysenteriae*. *S. flexneri* is therefore an important pathogen and a vaccine that includes *S. flexneri* serotypes 2a, 3a, 6 and *S. sonnei* could have potentially prevented 55% of all *Shigella* disease cases and 71% of the related deaths in Kilifi County.

461

ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF ENTEROPATHOGENIC *ESCHERICHIA COLI* AND *PSEUDOMONAS* SPECIES OBTAINED FROM MEAT SAMPLES FROM DIFFERENT AREAS OF DHAKA CITY

Rabeya Tafsire Rudhy

BRAC University, Dhaka, Bangladesh

Meat is one of the main source of protein now a days all over the world. From the people living under boarder line of poverty to high maintenance society, beef, mutton and chicken are taken as important source of proteins. Although beef and mutton are the most desirable ones; because of the availability and low price chicken is consumed even more than the rest. Due to improper handling and poor hygiene at the time of preparing the food, meat related foods are extremely unhealthy in Bangladesh. In this study 43 samples were taken from different areas of Dhaka city, the results supported the mentioned concern. Most of the meat samples were cheap and on the list of regular intake of students studying in these areas. Some of the samples showed almost 80-90% contamination with Escherichia coli that is a coliform bacterium and found in human excreta mostly. Most of the samples were cooked and processed; nonetheless they did not lack any less of organisms or contamination. Chicken samples were collected in three states, which were cooked, semi-cooked and raw. Some of the cooked and most consumed samples showed presence of 6-7 organisms. The organisms identified so far are *Escherichia coli, Enterobacteor aerogens, Salmonella spp, Staphylococcus spp, Pseudomonas* and others. The purpose of this study is to make the authorities regulating food safety aware of such contamination and take necessary steps to avoid this sort of disorientation towards food related business. In our research biochemical tests such as streaking on EMB and MacConkey agar, MRVP, TSI etc. were done, in addition morphological characteristics of the single colonies of isolated microorganisms were also examined and interpreted. DNA from samples positive for *E. coli* were isolated and by gel electrophoresis their bands were examined where most of them gave positive bands for STEC, meaning positive for pathogenic *E. coli* strains.

462

IDENTIFYING GEOGRAPHIC PRIORITIES FOR CHOLERA CONTROL INTERVENTIONS

Elizabeth C. Lee[1], Mohammad Ali[1], Ahmed A. Mohamed[2], Ali Nyanga[3], Chimwaza Wiseman[4], Joseph Wamala[5], Justin Lessler[1], Andrew S. Azman[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Tanzania Field Epidemiology and Laboratory Training Program, Dar es Salaam, United Republic of Tanzania,* [3]*Tanzania Ministry of Health Community Development Gender Elderly and Children, Dodoma, United Republic of Tanzania,* [4]*Ministry of Health, Malawi, Lilongwe, Malawi,* [5]*WHO South Sudan, Juba, South Sudan*

In 2018, the 71st World Health Assembly adopted a resolution aimed at reducing global cholera mortality by 90% by 2030. Achieving progress towards this goal requires efficient delivery of cholera control measures,

such as targeted water, sanitation, and hygiene (WASH) interventions and oral cholera vaccination (OCV) campaigns, particularly in the context of limited resources, OCV supply, and political capital. While stakeholders, including the Global Taskforce for Cholera Control, have promoted the idea of targeting control measures to cholera "hotspots," a delimited area with high historical burden of disease, there is little consensus about the methods and quantitative measures that should be used to identify and rank specific locations. Moreover, hotspot identification serves different needs at different spatial scales; ministries of health may be interested to identify areas that have relatively high risk of disease within their own countries, while decision makers at organizations such as the World Health Organization and Gavi may use hotspot identification to inform equitable and data-driven decisions about global allocation of cholera-related resources, like vaccines. In this study, we compare multiple approaches that may be used to prioritize cholera hotspots for intervention such as spatial clustering of the relative risk of incidence and a multidimensional incidence-based index. We first perform this exercise with simulated cholera data illustrating how each metric performs in settings with different cholera dynamics seen across the world (e.g., high historical incidence with endemic seasonality, low historical incidence with sporadic epidemics, etc.). Next, we apply these different prioritization methods to incidence data from Tanzania, Malawi and South Sudan to highlight the strengths and weaknesses of each approach. As more countries work towards cholera elimination, it is critical to develop nuanced guidelines for prioritizing cholera hotspots that meet country goals and global needs.

463

CLINICAL CHARACTERISTICS AND RISK FACTORS FOR *CAMPYLOBACTER SPP* GASTROENTERITIS IN THE FIRST YEAR OF LIFE IN A NICARAGUAN BIRTH COHORT

Denise T. St. Jean[1], Roberto Herrera[2], Lester Gutierrez[2], Nadja A. Vielot[3], Fredman Gonzalez[2], Yaoska Reyes[2], Margarita Paniagua[2], Natalie Bowman[4], Filemon Bucardo[2], Samuel Vilchez[2], Sylvia Becker-Dreps[3]

[1]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Universidad Nacional Autónoma de Nicaragua, León, León, Nicaragua,* [3]*Department of Family Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [4]*Department of Infectious Diseases, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Acute gastroenteritis (AGE) is one of leading causes of morbidity and mortality in children in Nicaragua. Between June 12, 2017 and July 31, 2018, we recruited a prospective birth cohort of 444 newborns in León, Nicaragua to assess the burden of AGE. Fieldworkers conducted household surveys on a weekly and monthly basis to identify risk factors for AGE. Fieldworkers also collected routine monthly stool and stool samples from infants experiencing AGE, which were tested for *Campylobacter spp* (*Campylobacter)* by real time polymerase chain reaction. We first described the clinical characteristics of AGE episodes in which *Campylobacter* was detected. Next, we conducted a nested case-control study to assess risk factors for *Campylobacter* AGE. For each case of *Campylobacter* AGE (restricted to first episodes), we matched 2:1 to infants from the cohort with no history of *Campylobacter*, within 3-month age groups. Finally, we estimated the population attributable fraction of *Campylobacter* among infants experiencing AGE, matching infants with AGE 1:1 to infants who had not experienced AGE within 28 days of the case. Of the 287 AGE stool samples that were collected and tested, 61 (21.3%) were positive for *Campylobacter*. 55 cases (90.2%) presented with diarrhea, which lasted a median 3.4 days. 6 cases presented with vomiting only and bloody stool was observed in only 3 (5.0%) episodes. Cases were significantly more likely than controls to have experienced a prior AGE episode (p<0.001), have recent contact with a person experiencing AGE (p=0.002), and have soap at all household sinks (p=0.014). In a conditional logistic regression model adjusting for predictors of *Campylobacter* AGE, a chicken in the home (OR: 3.64, 95% CI: 1.38, 9.60), soap at all sinks (OR: 4.60, 95% CI: 1.49, 14.23), sharing a bottle in the past week (OR: 3.19, 95% CI: 1.06, 9.63), and having had a prior episode of AGE (OR: 3.82 95% CI: 1.61, 9.03) were independent significant predictors of *Campylobacter* AGE. Comparing 90 infants experiencing AGE to 90 healthy controls, an estimated 22.3% (95% CI: 11.2, 32.1) of AGE episodes in the first year of life can be attributed to *Campylobacter* exposure.

464

CHOLERA OUTBREAKS IN SUB-SAHARAN AFRICA: 1996-2016

Qulu Zheng, Joshua Kaminsky, Heather S. McKay, Andrew S. Azman, Justin Lessler

Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Cholera remains a major public health threat worldwide. Sub-Saharan Africa has a high cholera burden and, in some locations, frequent epidemics. However, comprehensive studies of the overall epidemiology of cholera outbreaks in the region are lacking. The aim of this study is to provide comprehensive descriptions of epidemic features and the diversity of outbreaks across sub-Saharan Africa. We collected and combined surveillance data from multiple data sources (WHO, ProMED, ReliefWeb, UNICEF, MSF, ministries of health, and publications). We identified outbreaks based on local incidence occurring above a locally defined threshold and classified them into three spatial scales, including the country level, the province level ,which was defined as the first subdivision of the country, and the sub-province level, which was defined as the second or lower subdivision of the country. Descriptive analysis was performed separately for outbreaks at each spatial scale. We identified a total of 1,860 cholera outbreaks from multiple spatial scales between 1992-2016 in 25 countries in sub-Saharan Africa. We conducted in-depth analysis of 338 outbreaks with at least 100 suspected cases between 1996-2016 in 22 countries. These included 22 country-level outbreaks, 16 province-level outbreaks and 300 sub-province level outbreaks. The average attack rate for country-level outbreaks was 0.51 per 1,000 (range, 0.01-4.69), for province level was 8.14 per 1,000 (range, 0.03-63.16), and for sub-province level was 9.04 per 1,000 (range, 0.05-185.30). The average case fatality rate (CFR) was 5.48% at the country level (range, 0.39%-20.07%), 3.15% at the province level (range, 0.46%-16.07%) and 4.16% at the sub-province level (range, 0-26.29%). We also observed that the maximum CFR usually occurred during the first months of outbreaks and the estimated basic reproductive number (R_0) varied between outbreaks (mean, 1.56; range, 0.59-3.67). These results show the diversity of cholera outbreaks transmission across sub-Saharan Africa and highlight the importance of early outbreak response and timely case management in the control of cholera outbreaks.

465

A QUANTITATIVE ANALYSIS USING TAQMAN ARRAY CARD TESTING OF SELF-COLLECTED STOOL SMEARS ON WHATMAN FTA ELUTE CARDS TO DETERMINE THE PATHOGEN-SPECIFIC EPIDEMIOLOGY OF TRAVELERS' DIARRHEA

Michele D. Tisdale[1], Indrani Mitra[2], Jamie Fraser[2], Eric Houpt[3], Jie Liu[3], Mark S. Riddle[4], Drake H. Tilley[5], Anjali Kunz[6], Charla Geist[7], Heather Yun[8], Christa Eickhoff[9], David Tribble[2], Michael Price[10], Tahaniyat Lalani[2]

[1]*Infectious Disease Clinical Research Program, Portsmouth, VA, United States,* [2]*Infectious Disease Clinical Research Program, Bethesda, MD, United States,* [3]*University of Virginia, Charlottesville, VA, United States,* [4]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [5]*Naval Medical Center San Diego, San Diego, CA, United States,* [6]*Madigan Army Medical Center, Tacoma, WA, United States,* [7]*Landstuhl Regional Medical Center, Landstuhl, Germany,* [8]*Joint Base*

San Antonio, San Antonio, TX, United States, [9]Naval Medical Center Portsmouth, Portsmouth, VA, United States, [10]Tripler Army Medical Center, Honolulu, HI, United States

PCR based detection of diarrheal pathogens is increasingly used, but its application in field studies is limited by operational challenges (i.e. stool collection and transportation in austere environments) and clinical interpretation of data (i.e. attribution of travelers' diarrhea (TD) to detected pathogens). Self-collection of diarrheal smears on FTA Whatman Elute Cards® (FTA card) by subjects during travel, and mailing to a central testing location in a multi-barrier pouch, is a practicable alternative to conventional methods and is supported by the performance characteristics of the TaqMan Array Card PCR (TAC) on smeared FTA cards vs. stool. We performed a case-control analysis, using a customized TD TAC on self-collected stool smears from adult travelers and deployed military personnel enrolled in a prospective observational cohort study (TravMil). Subjects collected a stool smear on an FTA card during a TD episode (cases) or towards the end of travel if they did not experience any diarrhea (controls), and mailed the card to a central testing site. FTA cards from 231 cases (101 mild acute watery diarrhea [AWD], 65 moderate-severe AWD, 54 febrile TD and 11 dysentery) were matched with controls based on region and duration of travel, age group and time from collection to PCR extraction and testing. Odds ratios (OR) were used to determine the association between detected pathogens and TD. The overall detection rate (60% vs. 21%) and co-pathogen detection rate (21% vs. 3%) was significantly greater in cases vs. controls. Diarrheagenic *E coli* (enterotoxigenic *E coli* (ETEC) (26%), enteroaggregative *E coli* (EAEC) (23%), and enteropathogenic *E coli* (13%) were the most common pathogens in TD cases, and EAEC was detected most in controls (15%). Detection of most bacterial pathogens was significantly associated with TD: ETEC OR: 11.7 [95% CI: 5.2-26.3]; Shigella: 8.3 [95% CI: 1.0-66.5], Campylobacter: 6.8 [95% CI: 2.0-23.3]; EAEC: 1.72 [95% CI: 1.1-2.8]). Higher ORs were observed at cycle threshold (Cq) values < 30. TAC testing of self-collected FTA stool cards is a practical alternative to standard collection and testing methods in TD field studies.

466

ISOLATION, IDENTIFICATION AND *HIL*A GENE CHARACTERIZATION OF *SALMONELLA TYPHI* ISOLATES FROM BLOOD SAMPLES

Sandeep Thapa[1], Nilam Thakur[1], Ajaya Kunwar[1], Govardhan Joshi[1], Kumar Shrestha[2], Junu Budhathoki[2]

[1]*Kathmandu Center for Genomics and Research Laboratory, Gwarko, Nepal,* [2]*St. Xavier's College, Department of Microbiology, Kathmandu, Nepal*

Salmonella enterica serovar Typhi is a gram-negative, rod-shaped facultative anaerobe which is a major causative agent of typhoid fever in the impoverished population with poor sanitation and food habits. Enteric fever is a systemic infection of the reticuloendothelial system caused by the human-adapted pathogens *Salmonella enterica* serotype Typhi and *Salmonella enterica* serotype paratyphi A, B. However, the occurrence of *Salmonella enterica* B and C infection have not been reported in Nepal so far. A cross-sectional study was designed to collect blood samples from patients with typhoid fever after inform consent form. *Salmonella enterica* serovar Typhi was isolated and identification was confirmed using a standard microbiological technique. Further, it was subjected to antibiotic susceptibility test through Kirby-Bauer technique. Moreover, amplification of the *hil*A gene was employed for the molecular diagnosis using PCR. A total of 141 (78 male and 63 female) samples were subjected for diagnosis of typhoid. In this study, PCR has high sensitive (100%) and specificity (62.5%) for *S.* Typhi followed by Widal test (26.9%) and blood culture (14.9%).Additionally, PCR positive were high prevalence in a male with incidence rate higher in age groups 0-15 (14.8%) years followed by 15-30 (12.7%) and 30-45 (9.1%). Twenty-one samples were found to be culture positive with 100% sensitive to tetracycline, ampicillin, cotrimoxazole, and chloramphenicol. Likewise, 71.4 % were sensitive to ofloxacin and azithromycin, 57.4 % to nitrofurantoin. Subsequently, 9.5% and 23.8% of isolates were intermediate to ofloxacin and nitrofurantoin respectively. In contrast, all the isolates were 100% resistance to nalidixic acid. None of the isolates were found MDR. In conclusion, it can be affirmed that PCR is a sensitive, rapid and better alternative than conventional method which can be used in clinical diagnosis that may allow early detection of the causative organism and facilitate initiation of prompt treatment.

467

ANTIBODY GLYCOSYLATION AND THE CORRELATION TO FUNCTIONAL ASSAY ACTIVITY

Anthony R. Vortherms, Hailey P. Weerts, Robert W. Kaminski

Walter Reed Army Institute of Research, Silver Spring, MD, United States

While traditional ELISA quantitates the level of antibodies, these assays do not evaluate the functionality of these antibodies. The *Shigella*-specific bactericidal assay (SBA) is an immunoassay that measures direct bacterial killing by antibody-mediated complement activation. Antibody activity in the SBA requires a surface exposed target and the ability to interact with complement. While antibody isotype plays a major role in immunological function, glycosylation of the antibody also contributes to determining antibody function. Glycosylation of the antibody heavy chain is an important modification that modulates the recognition of the antibody by the immune system. Glycosylation changes can occur in response to external signaling during both *in vivo* and *in vitro* production of the antibody. Monitoring antibody glycosylation may provide insight into the functional activity of the humoral immune response. To develop this relationship, glycan profiles of anti-*Shigella* LPS monoclonal antibodies (mAbs) were correlated to their effectiveness in the SBA. The correlation suggests the best mAb to use in the assay as a control and can be used to compare the lot-to-lot variability of mAb produced to ensure consistency in the SBA. Furthermore, by understanding glycosylation patterns of functionally active antibodies, inducing comparable responses after vaccination can be monitored, which could inform the rational design of Shigella vaccines and be used as a parameter to up or down select candidates for future development.

468

ROLE OF GEOSPATIAL MAPPING TO COMPARE *SALMONELLA TYPHI* CULTURE RESISTANT CASES PRE- AND POST-VACCINATION

Abdul Momin Kazi, Ayub Khan, Mohammad Tahir Yousafzai, Hussain Kaleem, Zabin Wajidali, Farah Naz Qamar

Aga Khan University, Karachi, Pakistan

Pakistan is facing one of the largest epidemiological outbreaks of ceftriaxone-resistant *Salmonella typhi* with outbreak isolates reported mainly in Hyderabad and Karachi; however, cases are being reported in all districts of Sindh and spreading all over Pakistan. The WHO Strategic Advisory Group of Experts has recommended use of typhoid conjugate vaccine (TCV) in endemic countries, prioritizing use in high disease burden areas. Since Nov 2016 to March 2019, 1801 *S. typhi* resistant culture positive cases have been confirmed by Aga Khan University Hospital main lab and collection point and based on this information location based GIS maps stratified according to all 17 towns of Karachi using geospatial mapping techniques have been plotted. The catchment area of Saddar town according to geospatial map and number of *S. typhi* resistant culture positive cases (124) showed highest burden of disease in Saddar town, and hence a TCV vaccination campaign is being conducted as part of outbreak response in high risk Saddar town of Karachi. We aim to use the location of cases along with regional demographic data to project location based geospatial maps before and after this immunization campaign in Saddar town. Depending on the resolution of the data, we may be able to examine whether there are any changes in the spatial distribution of incidence over time. We can additionally extend this analysis to Hyderabad, where TCV is being distributed through an emergency campaign, ongoing since last year. Finally, we will use spatio-temporal analysis to examine the spatial distributions of typhoid before and during the vaccine roll-out, to

assess the need for responsive vaccination strategies in outbreak scenarios. This is work in progress but will be completed by August/September 2019. In conclusion we will show difference between pre and post vaccination cases in high XDR epidemic areas.

469

A LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE DETECTION OF *TREPONEMA PALLADIUM*

Laud Anthony Wihibeturo Basing

Komfo Anokye Teaching Hospital, Kumasi, Ghana

The eradication of yaws caused by *Treponema pallidum subsp. pertenue*, is constrained by the lack of rapid, accurate diagnosis. We sought to develop a molecular point-of-care test for the diagnosis of yaws. A Loop-mediated isothermal amplification (LAMP) assay with primers targeting the conserved gene, *tp0967,* with visual detection by lateral flow test strip was developed and optimized. The limit of detection was evaluated while 63 samples from clinical cases of yaws and 5 samples with PCR-confirmed syphilis were used to determine the sensitivity and specificity of the assay compared to the current molecular testing protocol. The developed LAMP assay was found to be optimal when run at 65^{o}C for 30 minutes. The limit of detection was $2.7*10^4$ DNA copies/ml. The sensitivity of the LAMP assay using unextracted and DNA extracted samples were 0.67 and 1.00 respectively. None of the syphilis samples tested positive in any of the assays. In conclusion, we show the development of a fast and sensitive LAMP assay for *yaws* detected by lateral flow test strip. Using extracted DNA, the assay sensitivity is at par with gold standard detection. The assay can be adapted to minimal sample processing required for in-field detection without DNA extraction.

470

RIFAMPIN-OFLOXACIN-MINOCYCLINE (ROM) FOR THE TREATMENT OF PAUCIBACILLARY LEPROSY: A SYSTEMATIC REVIEW

Michael A. Klowak[1], Shareese Clarke[1], Shveta Bhasker[1], Olamide Egbewumi[1], Celine Lecce[1], Alexandra Stoianov[1], Samed Asmer[1], Sharmistha Mishra[2], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada*

Leprosy is a complex tropical infection from a diagnostic and management perspective, as patients with leprosy are at risk of numerous related complications from the disease itself and its treatment. Standard WHO multi-drug treatment (MDT) consists of medications that are potentially harmful and cause a range of adverse systemic effects. Monthly- or single dosing of ROM has emerged as a potential treatment option for leprosy, however, a synthesis of the evidence supporting ROM does not exist. Paucibacillary leprosy, characterized by limited skin lesions and a low bacillary load, may be most amenable to a fluoroquinolone-based treatment protocol. We performed a systematic review of relevant literature to evaluate the safety and efficacy of ROM-based treatment for paucibacillary leprosy. Various databases were searched from inception to March 2019, using a combination of search terms "leprosy", "rifampin", "ofloxacin", "minocycline", and "ROM", while also accounting for alternative disease and chemical identifiers. The systematic review will focus on assessing and reporting on the efficacy, and safety of monthly ROM in the treatment of paucibacillary leprosy within a human population. 1139 records were retrieved for title and abstract screening, however, after a multi-step de-duplication pipeline, 568 articles remained. Subsequent title screening yielded 288 studies that were eligible for final inclusion. Main outcome measures to be assessed are lesion clearance, treatment failure, relapse, side effects and reversal reactions. A cursory review of relevant abstracts suggests that important determinants of health in the treatment of leprosy are: social environments, patient education, health services, gender and income. Synthesizing the current evidence discussing the efficacy of monthly ROM, will strengthen the current body of knowledge surrounding the treatment of paucibacillary leprosy, and may allow for the development of standardized fluoroquinolone-based treatment protocols.

471

THE TREATMENT OF MULTIBACILLARY LEPROSY UTILIZING RIFAMPIN-OFLOXACIN-MINOCYCLINE (ROM): A SYSTEMATIC REVIEW

Shareese Clarke[1], Michael A. Klowak[1], Shveta Bhasker[1], Olamide Egbewumi[1], Celine Lecce[1], Alexandra Stoianov[1], Samed Asmer[1], Sharmistha Mishra[2], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada*

From a diagnostic and management perspective, leprosy is a complex tropical infection. Patients who are affected by leprosy are at risk of several complications associated with the disease itself and its treatment. Standard WHO multi-drug treatment (MDT) is comprised of medications that are potentially harmful and can induce a variety of adverse systemic effects. Alternative options for potential treatment have emerged such as monthly dosing of Rifmapin-Ofloxacin-Minocycline (ROM) combination therapy, however, there is limited synthesized evidence of efficacy. Multibacillary leprosy, characterized by numerous skin lesions and a high bacillary load, requires more prolonged daily treatment compared to paucibacillary disease. Monthly ROM-based protocols may enable reduced pill burden and translate to fewer adverse effects associated with the clofazimine and dapsone components of standard MDT, in particular. To assess the safety and efficacy of monthly ROM treatment and to determine how this may be affected by determinants of health, we conducted a systematic review of relevant literature. Various databases were searched from inception to March 2019, using a combination of search terms "leprosy", "rifampin", "ofloxacin", "minocycline", and "ROM", while also accounting for alternative disease and chemical identifiers. 1139 records were retrieved for title and abstract screening, after which 288 studies were eligible for final inclusion. Primary outcome measures to be evaluated are lesion clearance, treatment failure, relapse, side effects and reversal reactions. A perfunctory review of relevant abstracts proposes that the major determinants of health to be considered in the treatment of leprosy are: social environments, education level of the patient, access to health services, gender and income. By synthesizing the current evidence discussing the efficacy of monthly ROM in treating multibacillary leprosy, we will map the current body of knowledge that exists with the ultimate goal of enabling more simplified standardized treatment protocols.

472

RISK FACTORS FOR BACTEREMIA IN SEVERELY MALNOURISHED UNDER-FIVE PNEUMONIC CHILDREN AND THEIR OUTCOME

Abu sadat mohammad sayeem Bin Shahid, Tahmeed Ahmed, Km Shahunja, Mohammod Jobayer Chisti

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Bacteremia is quite common in severe acute malnourished (SAM) children with pneumonia, who often experience a fatal outcome, especially in developing countries. There is limited information in the medical literature on the risks of bacteremia in SAM children with pneumonia. We have examined the factors associated with bacteremia and their outcome in under-five children who were hospitalized for the management of pneumonia and SAM. In this unmatched case-control study, SAM children of either sex, aged 0-59 months, admitted to the Dhaka Hospital of the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) with cough or respiratory distress and radiological pneumonia during April 2011 to July 2012 were enrolled (n=405). Those with pneumonia as well as bacteremia constituted the cases (n=18), and randomly selected SAM children with pneumonia without bacteremia constituted controls

(n=54). A wide range of bacterial pathogens were isolated among the cases of which 13 (72%) were Gram negatives. Death rate was higher among the cases than the controls (28% vs. 9%) but the difference was not statistically significant (p=0.111). In logistic regression analysis, after adjusting for potential confounders, such as the lack of DPT/oral polio/HIV/hepatitis vaccination, measles vaccination, vomiting, and clinical dehydration (some/severe) the SAM children with pneumonia as well as bacteremia more often had the history of lack of BCG vaccination (95% CI=1.17-29.98) and had diastolic hypotension (<50 mm of Hg) (95% CI=1.01-12.86) not only after correction of dehydration but also in its absence.The results of our study suggest that history of lack of BCG vaccination and presence of diastolic hypotension in absence of dehydration on admission are the independent predictors of bacteremia in SAM children with pneumonia. The results indicate the importance of continuation of BCG vaccination to produce benefits beyond the primary benefits.

473

SEROEPIDEMIOLOGY OF *BURKHOLDERIA PSEUDOMALLEI*, ETIOLOGIC AGENT OF MELIOIDOSIS, IN THE OUEST AND SUD-EST DEPARTMENTS OF HAITI

Thomas A. Weppelmann[1], Michael H. Norris[2], Michael E. von Fricken[3], Bernard A. Okech[2], Anthony P. Canella[2], Herbert P. Schweizer[2], Apichai Tuanyok[2]

[1]*Herbert Wertheim College of Medicine, Miami, FL, United States,* [2]*University of Florida, Gainesville, FL, United States,* [3]*George Mason University, Fairfax, VA, United States*

Burkholderia pseudomallei, the etiological agent of melioidosis, has been hypothesized to be endemic throughout the Caribbean, including Haiti. Due to the protean clinical manifestations, presence of asymptomatic infections, and limited diagnostic capacity, the identification of active melioidosis cases remains challenging. A seroepidemiological study was conducted using a novel enzyme-linked immunosorbent assay (ELISA) to detect antibodies toward *B. pseudomallei* in the native population. The performance of an indirect ELISA with purified lipopolysaccharide (LPS) from *B. pseudomallei* was evaluated using serum collected from rhesus macaques exposed to aerosolized *B. pseudomallei*. After optimization, serum collected from asymptomatic population members (n=756) was screened for polyvalent (IgM/IgG/IgA) and monoclonal (IgG or IgM) immunoglobulins against *B. pseudomallei* LPS. The population seroprevalence was 11.5% (95% CI: 9.2, 13.8) for polyvalent immunoglobulins, 9.8% (95% CI: 7.7, 11.9) for IgG, and 1.7% (95% CI: 0.8, 2.6%) for IgM. The seroprevalence was not significantly different by gender (P =0.16), but increased significantly (P <0.001) with age, yielding an estimated annual seroconversion rate of 1.05% (95% CI: 0.81, 1.3). In conclusion, the detection of both recent (IgM+) and previous (IgG+) exposure to *B. pseudomallei* provides serological evidence that melioidosis is endemic in Haiti.

474

MELIOIDOSIS AND SEROLOGICAL EVIDENCE OF EXPOSURE TO *BURKHOLDERIA PSEUDOMALLEI* AMONG PATIENTS WITH FEVER, NORTHERN TANZANIA

Michael J. Maze[1], Mindy Glass Elrod[2], Alex Hoffmaster[2], Holly M. Biggs[3], Philoteus Sakasaka[4], Wilbrod Saganda[5], Bingileki F. Lwezaula[5], Blandina Mmbaga[4], Venance P. Maro[4], Matthew P. Rubach[3], John A. Crump[6]

[1]*University of Otago, Christchurch, New Zealand,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*Duke University, Durham, NC, United States,* [4]*Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania,* [5]*Mawenzi Regional Referral Hospital, Moshi, United Republic of Tanzania,* [6]*University of Otago, Dunedin, New Zealand*

Prediction models indicate that melioidosis may be common in parts of East Africa, but there are few empiric data. We evaluated the prevalence of melioidosis among patients presenting to hospital with fever in northern Tanzania. Patients with fever were enrolled at Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital 2007-08 and 2012-14. Participants had aerobic blood culture performed and bloodstream isolates were identified by conventional methods. Following testing on the API20NE (BioMérieux, Marcy-l'Étoile, France) biochemical identification system, non-glucose fermenting gram-negative bacilli were further tested by *B. pseudomallei* latex agglutination (Mahidol University, Thailand) test. In addition, acute serum was collected at enrolment and convalescent serum 4-6 weeks later. The convalescent serum of a subset of participants considered at high epidemiologic risk of melioidosis: those admitted within 30 days of rainfall was tested using *B. pseudomallei* indirect haemagglutination assay serology (IHA). We performed *B. pseudomallei* IHA on acute and convalescent sera of participants with a reciprocal titer ≥40 on their convalescent serum. We defined a confirmed case of melioidosis as isolation of *B. pseudomallei* from blood culture; probable melioidosis as a ≥4-fold rise in antibody titers between acute and convalescent sera; and seropositivity as a single antibody titer ≥40. We enrolled 2,663 participants and isolated 4 (0.2%) non-enteric gram-negative bacilli from blood culture; none were *B. pseudomallei*. We tested 323 participants by IHA. Of participants, 142 (44.0%) were male, the median (range) age was 27 (0-70) years, and the median (range) duration of fever was 4 (1-60) days. We identified no cases of confirmed melioidosis; 2 (0.6%) of probable melioidosis; and 57 (17.7%) were seropositive. The high proportion of seropositive participants may suggest that either *B. pseudomallei* or an antigenically similar organism is present in northern Tanzania. Further research into the presence of *Burkholderia* spp. as a cause of illness and in the environment is warranted.

475

ETIOLOGY OF NEONATAL SEPSIS IN SUB-SAHARAN AFRICA: PATHOGENS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS

Elias Impens[1], Piet Cools[2], Ann Impens[3], Guy Mulinganya[4], Steven Callens[2]

[1]*Pennsylvania Hospital, Philadelphia, PA, United States,* [2]*Ghent University, Ghent, Belgium,* [3]*Midwestern University, Downers Grove, IL, United States,* [4]*Université Catholique de Bukavu and Hôpital Provincial Général de Référence de Bukavu, Bukavu, Democratic Republic of the Congo*

Neonatal sepsis is the leading cause of neonatal mortality in sub-Saharan Africa (SSA), but data on the etiology of neonatal sepsis in developing countries are limited in both quality and quantity. Current recommendations for empiric treatment of neonatal sepsis are primarily based on data from high-income countries, where Group B *Streptococcus* and *Escherichia coli* are the predominant pathogens. Better knowledge about pathogens causing neonatal sepsis in SSA, as well as their antibiotic susceptibility patterns, is needed to design and implement strategies based on pathogens that locally predominate. Therefore, a systematic literature review was conducted, with the objective of providing an overview of these pathogens and their antibiotic susceptibility patterns. A protocol was submitted to PROSPERO, an international prospective register of systematic reviews, prior to starting the review. Studies were identified using the stepwise approach specified in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement. MEDLINE/Pubmed, EMBASE, Web of Science, Cochrane Library, African Index Medicus, African Journals Online and Hindawi journals were searched. An exhaustive grey literature search was conducted as well. All studies were screened by two independent reviewers based on eligibility criteria, and approximately 150 original studies will be included for analysis. To our knowledge, we are conducting the first and most comprehensive systematic review and meta-analysis on this topic. This information on pathogens and their susceptibility patterns by region will be useful for local guideline development and appropriate treatment of neonatal sepsis.

476

DESIGN AND EVALUATION OF NOVEL LIPOSOME-BASED PEPTIDE VACCINES FOR IMPROVED EFFICACY AGAINST GROUP A STREPTOCOCCAL INFECTIONS OF MUCOSA AND SKIN

Victoria Ozberk[1], Mehfuz Zaman[1], Emma Langshaw[1], Sharareh Eskandari[1], Ainslie Calcutt[1], Jessica Powell[1], Michael R. Batzloff[1], Jes Dietrich[2], Manisha Pandey[1], Michael F. Good[1]

[1]*Institute for Glycomics, Griffith University, Southport, Australia,* [2]*Statens Serum Institut, Copenhagen, Denmark*

Victoria Ozberk *Streptococcus pyogenes* (Group A Streptococcus; GAS) infections and their sequelae are responsible for significant morbidity and mortality worldwide. A vaccine that can prevent GAS infection at the primary sites of infection is urgently needed to block the onset of potentially life threatening GAS-associated diseases. We demonstrated that intranasal administration of a liposomal delivery system, incorporating GAS peptide antigens, J8 and S2, protects against upper respiratory tract (URT) infection with wild-type and hypervirulent GAS strains. Addition of an immunostimulatory glycolipid to the mucosally active liposomal vaccine significantly enhanced vaccine efficacy to protect at both mucosal and systemic compartments of immunity. We show that secretory IgA is not necessary in vaccine-mediated protection against URT GAS infection but that a vaccine-specific IL-17A response is associated with protection. Furthermore, we show that prime-pull immunisation with J8-DT and K4S2-DT formulated with another novel liposomal delivery system promotes high and sustained antibody levels in the airway mucosa and in the serum. The same vaccine also leads to the establishment of cellular immunity and protection against skin and URT hypervirulent GAS infections. In conclusion, the findings reported here represent a significant advancement in overcoming many obstacles impeding the development of GAS vaccines to prevent infection at mucosal and systemic sites.

477

BACTERIAL MENINGITIS IN MALAWIAN CHILDREN, 2000-2018

Markye Nielsen[1], **Stephen TJ Ray**[1], Kondwani Kawaza[2], Brigitte Denis[1], Stephen B. Gordon[1], Michael J. Griffiths[3], Enitan Carrol[3], Neil French[3], Queen Dube[2], Pui-Ying Iroh Tam[1]

[1]*Malawi-Liverpool-Wellcome Trust, Blantyre, Malawi,* [2]*University of Malawi, College of Medicine, Blantyre, Malawi,* [3]*Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom*

Bacterial meningitis represents a significant burden of infectious disease in hospitalised children in sub-Saharan Africa, where antimicrobial resistance (AMR) is a growing problem. We describe trends of bacterial meningitis infections in children ≤5 years admitted to a tertiary referral hospital in Blantyre, Malawi between 2000 and 2018. We determined the total number of cerebrospinal fluid (CSF) culture pathogens and associated AMR profiles. A total of 2,375 pathogens (48.5% Gram-positive, 51.5% Gram-negative) were identified in children ≤5 years. There was an absolute decrease in number of CSF pathogens identified across three periods (2000-2014), most notably in *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and nontyphoidal Salmonella. However, compared to the third period (2010-2014), the last period (2015-2018) has seen an absolute increase in CSF pathogens, 8% for Gram-positives and 97% for Gram-negatives. Children ≤60 days of age accounted for 35% of positive isolates overall, but 45% of positive isolates in the last period. An increase in AMR was noted across four periods, with resistance to ceftriaxone increasing from 20.0% to 95.8% for *Klebsiella pneumoniae* and 0% to 62.8% for *Escherichia coli*. Absolute numbers of culture proven bacterial meningitis cases in hospitalised children at our tertiary referral hospital decreased overall during the last 19 years. Rollout of conjugate vaccines and improvement in hygiene/sanitation are in large part responsible for the overall decrease. However, that decreasing trend has recently been reversing, with an increase in drug resistant pathogens, particularly larger numbers of Gram-negative CSF pathogens resistant to first-line antimicrobials. These data mirror the worrisome emergence of AMR in childhood blood stream infections in our setting.

478

ASSESSING THE ROLE OF POSTMORTEM MICROBIOLOGY IN DETERMINING THE CAUSE OF FATAL FEBRILE ILLNESS, KILIMANJARO, TANZANIA

Cristina Costales[1], Matthew P. Rubach[1], Alex Mremi[2], Patrick Amsi[2], Manuela Carugati[1], Ann M. Nelson[3], Venance P. Maro[4], John A. Crump[1]

[1]*Division of Infectious Diseases and International Health, Duke University, Durham, NC, United States,* [2]*Department of Pathology, Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania,* [3]*Department of Pathology and Laboratory Medicine, Duke University, Durham, NC, United States,* [4]*Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania*

Understanding infectious cause of death (COD) is critical, yet the role of postmortem (PM) microbiology is debated. To evaluate the contribution of PM microbiology to identify causes of fatal febrile illness in northern Tanzania, from November 2016 through October 2018 families of all inpatient febrile deaths at two referral hospitals were offered autopsy with multi-site aerobic and mycobacterial cultures. Culture of venous blood, bone marrow (BM), cerebrospinal fluid (CSF), liver and lung tissue was done to assess the yield and concordance of the different specimen sites. COD was assigned by panel adjudication based on review of medical charts, autopsy gross and histologic findings, and culture results. Microbiology was 'contributory' if there was clinical or histologic evidence to support infection by the isolated organism and the organism was a pathogen associated with intermediate or immediate COD diagnoses. Among 179 PMs, 119 (67%) had ≥3 aerobic cultures collected. The most common aerobic isolates were *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus*. Of samples, 38 (56%) of 68 blood, 16 (52%) of 31 BM, 12 (71%) of 17 CSF, and 22 (37%) of 60 liver cultures were contributory to COD. When considering only febrile deaths where the same organism was isolated from ≥2 sites, 24 (71%) of 34 blood, 13 (59%) of 22 bone marrow, 11 (79%) of 14 CSF and 18 (72%) of 25 liver cultures were contributory to COD. Of 122 febrile deaths with mycobacterial cultures, 116 (95%) had ≥2 specimen collection sites. Of PMs with tuberculosis as COD, *Mycobacterium tuberculosis* grew in 11 (65%) of 17: 4 (7%) of 52 CSF, 8 (7%) of 115 liver, and 5 (4%) of 113 lung, and all were contributory. We found that when the same organism was isolated from ≥2 sites, PM aerobic cultures from blood, CSF, and liver tissue, provided contributory information to determine COD in over two-thirds of febrile deaths. Mycobacterial cultures were infrequently positive but were universally contributory.

479

CHANGING PATTERN OF STREPTOCOCCAL INFECTIONS

Don W. Kannangara, Dhyanesh Pandya

St Luke's University Health Network, Phillipsburg, NJ, United States

Group A Streptococcal infections (GAS) are usually a problem in developing countries. In a study of 296 isolates of hemolytic streptococci in India 74% were group A and only 3.7% belonged to group B. Worldwide, an estimated 18 million people are believed to suffer from GAS related illnesses. Recently there was a resurgence of scarlet fever in England with over 19,000 cases reported in 2016. In Ireland there was an upsurge of GAS in 2012-2015 period. An outbreak of GAS was reported in a homeless community in Anchorage, Alaska (2016-2017) and other outbreaks in Hong Kong (2012-2015) and South Korea (2011-2016). Also, there were reports of resurgence of GAS in injection drug users (New Hampshire) and a mental health facility (Singapore). Group B *Streptococcus* (GBS) is classically considered a problem in neonates and puerperal sepsis. In 2018, Los Angeles county reported 62% Clindamycin resistance in GBS. A 32% reduction in GBS incidence in infants was

recently reported from Germany. We studied 1321 isolates of Streptococci reported by our network labs from adults. GBS was the predominant isolate (481). Blood cultures were positive in 199. 175 were from urine and others were from wounds, tissue and body fluids. Group A was found in 80 blood cultures (BC), followed by *S. anginosus* 66, Group G 54, Group C 51, S. intermedius 31, *S. constellatus* 11 and *S. dysgalactiae* 3. Source of the blood cultures for majority of the isolates was lower extremity cellulitis. The commonest Streptococcal isolate from BC, urine, Soft tissue and joint fluid in adults was GBS. *S anginosus* incidence was higher than expected. A detailed analysis of all isolates will be presented.

480

MOLECULAR PROFILING OF SEPSIS PATIENTS FROM AUSTERE ENVIRONMENTS USING TOPOLOGICAL DATA ANALYSIS

Joost Brandsma[1], Kevin Schully[1], Deborah Striegel[1], Josh G. Chenoweth[1], Anissa Elayadi[1], Subramaniam Krishnan[1], Dennis Faix[2], Amitha Fitkariwala[2], Te Vantha[3], Anne Fox[4], Andrew Letizia[4], Alex Owusu-Ofori[5], George Oduro[5], Daniel Ansong[5], Ephraim L. Tsalik[6], Christopher W. Woods[6], Danielle V. Clark[1]

[1]*Henry M. Jackson Foundation, Bethesda, MD, United States,* [2]*Naval Medical Research Unit Number* [2]*, Phnom Penh, Cambodia,* [3]*Takeo Provincial Referral Hospital, Takeo, Cambodia,* [4]*Naval Medical Research Unit Number* [3] *Ghana Laboratory, Accra, Ghana,* [5]*Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [6]*Duke University, Durham, NC, United States*

Expeditious and accurate information for clinical decision-making is critical for improving outcomes for patients with sepsis, particularly in low- and middle-income countries, and can be enhanced using host-response biomarkers indicative of sepsis type and prognosis. The Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO) is a consortium of military medical and academic research institutes aiming to improve early recognition, diagnosis and treatment of sepsis in low-resource settings. The aim of this study was to identify gene and protein expression phenotypes in sepsis patients enrolled in an observational study from sites in Cambodia, Ghana and the USA (n=586). Topological data analysis (TDA) was used as an unsupervised method for identifying clusters of patients with similar molecular phenotypes, as well as broader trends in gene and protein expression across the TDA network. Differences in clinical presentation and clinical laboratory measurements between TDA clusters were tested for statistical significance to inform on sepsis endotypes associated with the molecular expression phenotypes. Our analysis highlighted two major trends in the protein data and identified six distinct but partially overlapping patient clusters. Protein concentrations along the primary TDA axis were predictive of mortality within the first 28 days of disease, representing a two-fold increase in mortality risk for patients at either end of the spectrum, independent of enrollment site. At least six distinct patient clusters were identified in the gene expression TDA, with corresponding differences in clinical presentation and a five-fold difference in 28-day mortality. In summary, TDA of protein and gene expression in patients with sepsis identified multiple host-biomarker phenotypes with distinct clinical presentations and significant differences in sepsis outcome. The ability to accurately stratify patients by mortality risk at presentation would inform decisions regarding the urgency of care required, direct medical resources to patients with the greatest need, and would do so early when the impact is greatest.

481

BACTERIOPHAGE THERAPY: RENEWED STRATEGY TO TARGET DRUG RESISTANT *STAPHYLOCOCCUS AUREUS*

Rutu M. Dave[1], Robert P. Fort[1], Ricardo Abadie[2], Patrick Schukraft[1], Jose Chu Luo[1], Gillian Naro[1], David W. Craft[1], James Regeimbal[2]

[1]*Pennsylvania State College of Medicine, Hershey, PA, United States,* [2]*Naval Medical Research Unit No.* [6]*, Lima, Peru*

As antibiotic overuse and misuse is increasing the incidence of drug resistant bacterial species worldwide, the value of bacteriophage as a bactericidal agent has become increasingly important. Revisiting this strategy from the annals of history may serve as an opportunity for targeting drug resistant bacteria. We sought to identify and isolate bacteriophages with infectivity specific to drug resistant *Staphylococcus aureus* (SA) isolated from clinical samples including wound cultures in Iquitos, Peru. Ten strains of drug resistant SA were used to isolate phages. Each strain was incubated with sewage water and TSB overnight to amplify phages specific to the drug resistant SA strain. Serial dilutions of the supernatant were subsequently plated on the corresponding strain of drug resistant SA. Individual plaques were isolated, purified and tested for cross-infectivity against a panel of 20 drug resistant SA clinical strains. Five phages were isolated on five of the ten drug resistant SA strains used for phage harvesting. Here we present the infectivity profile of these five potential phages. Each phage infected the same 16 across the panel of 20 drug resistant SA strains, suggesting that all the phages were the same virus. These findings raise the possibility of homogeneity among drug resistant SA surface receptors or a lack of diversity among SA specific bacteriophages within the environmental samples used. While challenges remain in the development of bacteriophage therapy, phages isolated in this and partnering studies will contribute to a greater library of bacteriophages which has the potential to revolutionize existing antimicrobial therapy as greater resistance to standard therapies emerges.

482

A FIELD PORTABLE THERMOCYCLER T-COR 8™ FOR MULTIPLEX DETECTION AND DIFFERENTIATION OF *BURKHOLDERIA PSEUODOMALLEI* AND *BURKHOLDERIA MALLEI*

Neeraja Venkateswaran, Tracy Fecteau, Patricia Valencia, Kodumudi S. Venkateswaran, William M. Nelson

Tetracore, Inc., Rockville, MD, United States

CDC classifies *Burkholderia pseudomallei* (Bp), and *Burkholderia mallei* (Bm) as Tier 1 select agents and they cause melioidosis and glanders respectively in humans and solipeds such as horses. Clinical diagnostics currently use isolation and culture of these two organisms as the gold standard which is painstakingly slow. Phylogenetic similarities between Bp, Bm and *B. thailandensis* (Bt) which is a very similar saprophyte make the accurate detection and differentiation of these organisms very challenging. We have developed a multiplex assay that uses two different Burkholderia genomic targets and an internal control that acts as an amplification control. The first target is a flagellin *fliC* gene variant that is positive for both Bp and Bm and negative for Bt, and the second target is a unique sequence that was designed by subtractive hybridization and is present on chromosome II of both Bp and Bt and not Bm. Thus, a sample is identified as Bp if it is positive for both markers, a sample positive only for fliC assay is Bm, and a sample positive for the second marker only is identified as Bt. Analytical sensitivity of this multiplex assay was determined by titration of genomic DNA isolated from Bm strain ATCC 23344 and Bp strain ATCC 11668. We tested each target individually and in the multiplex format to determine the assay characteristics and assure that the addition of multiple targets does not negatively affect the performance of the test. Next, we tested the heat inactivated cultures directly without any genomic DNA extractions in the assay to determine the robustness of the multiplex assay. We tested 20 different strains of Bm, Bp, Bt, and 4 near neighbors with this multiplex assay. We found that this assay was able to detect accurately and differentiate between Bp, Bm, and Bt. All the near neighbors tested here were found to be negative for both markers. No false positive with the high concentration of Bm sample for Bp-specific assay were observed. Either Bp / Bt assay did not detect one out of 7 Bt. The 4 near neighbors tested were negative for both the targets. We conclude that this multiplex assay can successfully detect and differentiate between Bm and Bp.

483

RISING AWARENESS OF LEPTOSPIROSIS IN PANAMA

Laia Jimena Vazquez Guillamet[1], Ana Belen Arauz[2], Jose Antonio Suarez[3], Emma Gonzalez[4], Jose Domingo Obaldía[3], German Henostroza[1], Blas Armien[3]

[1]*University of Alabama at Birmingham, Birmingham, AL, United States,* [2]*Hospital Santo Tomas, Panama City, Panama,* [3]*Instituto Conmemorativo Gorgas de Estudios de La Salud, Panama City, Panama,* [4]*Hospital Aquilino Tejeira, Cocle, Panama*

Global leptospirosis burden is significant; however, inadequate diagnosis has affected the awareness of the disease. Despite favorable environmental transmission conditions, Panama has a significantly lower incidence of cases and alerts than its neighbor countries. We would like to expand the knowledge of leptospirosis in Panama and discuss diagnostic yield of current tests available. A cohort of patients admitted from January 2013 to December 2018 to Santo Tomas Hospital (STH) with suspected leptospirosis was retrospectively reviewed. Patients were selected if a serology test for leptospirosis was requested. Demographic data, clinical signs and symptoms, laboratory profile and treatment regimens were collected. Microagglutination assay results were used for confirmation. We retrieved 107 medical records from a total of 188 cases with suspected leptospirosis (56.9%). Mean age was 45.2 years (SD 17.2), 68.2%(73/107) were male, and 67.6%(71/107) were from Panama district. The most prevalent signs and symptoms were fever (75.7%, 81/107), nausea/vomiting (57%,61/107), abdominal pain (53.3%, 57/107), and jaundice (43%, 46/107). First serology for Leptospira was obtained in 91.6%(98/107), and second serology was obtained in 34.8%(34/98). Time from admission to first IgM was in average 7 days. Leptospirosis was ruled out in 34.8%(34/98) and confirmed in 14.3%(14/98), leaving 55.1%(59/107) probable cases. Antibiotics were initiated in average 2.1 days after admission and were appropriate in 59.6%(53/89) of cases. Confirmed cases happened mostly during wet season 92.9%(13/14) and 50%(7/14) had severe disease. Mortality was 28.6%(4/14) among confirmed cases and 23.8%(14/59) among probable cases. Features of leptospirosis in STH were like those reported in other series. High prevalence of severe disease among confirmed and probable cases correlates with tertiary nature of the hospital and highlights the need of early diagnosis. Cost-effectiveness of PCR analysis should be considered in this setting.

484

EFFECTIVENESS OF COMMUNITY VERSUS FACILITY DELIVERY OF DIHYDROARTEMESININ-PIPERAQUINE FOR POST-DISCHARGE MALARIA CHEMOPREVENTION IN MANAGEMENT OF SEVERE ANEMIA IN MALAWIAN CHILDREN: A CLUSTER RANDOMIZED TRIAL

Thandile Nkosi-Gondwe[1], Bjarne Robberstad[2], Mavuto Mukaka[3], Richard Idro[4], Robert Opoka[4], Feiko Ter Kuile[5], Saidon Banda[1], Bjorn Blomberg[6], Kamija Phiri[1]

[1]*University Of Malawi, College Of Medicine, Blantyre, Malawi,* [2]*University of Bergen, Department of Global Public Health and Primary Care, Centre for International Health, Bergen, Norway,* [3]*Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand,* [4]*Department of Pediatrics and Child Health, Makerere University College of Health Sciences, Kampala, Uganda,* [5]*Kenya Medical Research Institute (KEMRI), Centre for Global Health Research (CGHR), Kisumu, Kenya,* [6]*Department of Health Promotions and Development, University of Bergen, Bergen, Norway*

Evidence shows that provision of post-discharge malaria chemoprevention (PMC) for a child with severe malarial anemia reduces the risk of death and hospital re-admissions. We aimed to evaluate the most effective delivery method of PMC. We conducted a 5-arm, cluster-randomized trial at Zomba Central hospital in Southern Malawi. Severely anemic children aged less than five years were randomized to receive dihydroartemisinin-piperaquine (DHP) within the community or facility at 2, 6 and 10 weeks after discharge. In community arms, all DHP doses were given at discharge and caregivers collected from hospital in facility arms. Trial arms were: 1) community without a short-text message service (SMS) reminder; 2) community with an SMS; 3) community with a CHW reminder; 4) hospital without an SMS or 5) hospital with an SMS. Factorial design analysis for pooled effect of delivery method was utilized and a Poisson regression model was fitted. The primary outcome was adherence. Between March 2016 and October 2018, we recruited 375 children and followed them up for 15 weeks. When PMC was delivered through the community; adherence was higher compared to facility-based methods (IRR=1.24; 95%CI1.06, 1.44 p=0.006) and this was observed in both the SMS recipients (IRR=1.41;CI1.21, 1.64,p<0.001) and in the non-SMS recipients (IRR=1.37;CI1.18, 1.61,p<0.001). However, there was no evidence that SMS reminders resulted in greater adherence (IRR=1.03;CI0.088, 1.21,p=0.677). When compared to the standard of care, delivery utilizing CHWs resulted in higher adherence (IRR=1.32;CI1.14, 1.54,p<0.001). But there was no difference in adherence between facility delivery with SMS and the standard of care (IRR=1.16;0. 99,1.37,p=0.066). Delivery of DHP for PMC resulted in higher adherence when delivered using community-based methods compared to facility-based methods with or without SMS.

485

THE CLINICAL MANIFESTATIONS AND HEPATIC INVOLVEMENT OF DENGUE INFECTION IN ADULTS

Bo Bo Thet Ko[1], Weerapong Phumratanaprapin[2], Benjaluck Phonrat[2], Jittima Dhitava[2], Vorada Choovichian[2], Maleerat Sutherat[2]

[1]*University Research Co.LLC, Dawei, Myanmar,* [2]*Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

A retrospective study was conducted in the Hospital for Tropical Diseases, Bangkok, Thailand to describe the hepatic involvement in dengue infection, describe the clinical manifestation of dengue, determine the risk factors for DHF and describe the outcome of dengue infection. After reviewing 534 patients records during January 2007 to September 2010 were reviewed and 315 patients who fulfilled the inclusion criteria were enrolled in this study. Out of 315 patients, 250 were DF and 65 were DHF. Hepatitis was found in 132 (42.9 %) during hospitalization. The mean level of peak AST and ALT level during hospitalization were 262.8 (±257.4) U/L and 212 (±214.4%) U/L. Severe hepatitis was found in 10 patients and the percentage of severe hepatitis on DF group was higher than that of DHF group. Eighty percent of the patients who had increased ALT and AST levels returned to normal at follow up within 4 weeks duration. Moreover, ALT and AST levels of female population were higher than that of male. Common clinical presentation of dengue infection in this study were malaise (98.7 %), anoraexia (93.5%), headache (92.7 %), nausea (77.7 %), chills (75.2 %), vomiting (66.4 %) and diarrhoea (37.6 %). Two risk factors for developing DHF found by this study were platelets count < 50 (x 10^3 cells/μL) on admission and gum bleeding. The mean duration of fever in this study was 5.9 (±1.5) days. The mean duration hospital stay was 3.6 (± 1.5) days.

486

COLONIZATION AND INFECTION WITH ANTIBIOTIC-RESISTANT ORGANISMS IN A VIETNAMESE INTENSIVE CARE UNIT

Duong Bich Thuy[1], James Campbell[2], Chung The Hao[2], Stephen Baker[2], Nguyen Van Vinh Chau[1], Ronald Geskus[2], Le Thanh Hoang Nhat[2], Guy Thwaites[2], Louise Thwaites[2]

[1]*Hospital for Tropical Diseases, Ho Chi Minh, Vietnam,* [2]*Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam*

Little is known about colonisation and subsequent infections with antibiotic-resistant organisms (AROs) in Vietnam, particularly from intensive care units (ICUs). There are several reasons for this, but include the unregulated antibiotic use, lack of infection control, and limited funding. A prospective longitudinal study was conducted in Adult ICU,

Hospital for Tropical Diseases, Vietnam from 10th November 2014 to 14th January 2016 to characterize colonisation, and understand its relationship with infections by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp.. In the first 48 hours of ICU admission, 63.1% (529/838) of patients were colonised with AROs. The colonisation proportion with AROs among patients admitted from the community was comparable to those transferred from other hospitals (62.2% vs 63.8%). During ICU stay, 61.3% (223/364) of patients with a ICU stay >48 hours acquired AROs colonisation. The proportion of patients with hospital-acquired infections (HAIs) was 23.4% (85/364), and AROs accounted for 41.5% (44/106) of pathogens causing HAIs. Multilocus sequence typing analysis indicated matching sequence types of infecting and previously colonizing isolates in 90% (9/10) and 94.4% (17/18) patients with HAIs by *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively. Phylogenetic analysis confirmed that ICU patients became infected with their previously colonizing *Staphylococcus aureus* ST188 (4 patients) or *Klebsiella pneumoniae* ST17, ST23 and ST86 (6 patients). It is also noteworthy that some hypervirulent *Klebsiella pneumoniae* clones with the acquisition of siderophore and *rmpA* are circulating in Vietnam. This study provides strong evidence of the high burden of antimicrobial resistance in Vietnam along with high rate of colonisation and infections with AROs not only in ICU but also in the community. Therefore, the implementation of infection control measures and antimicrobial stewardship programs is urgently needed in Vietnam.

487

ARE WE CLINICALLY MISSING LEPROSY IN ERA OF ELIMINATION?

Neena Khanna

AIIMS, New Delhi, India

With elimination of leprosy from most parts of the World including India, its diagnosis is becoming challenging. We retrospectively reviewed case files of 1543patients registered during 2009-2015 in leprosy clinic of All India Institute of Medical Sciences, New Delhi, India to determine number of patients in whom diagnosis of leprosy had initially been missed resulting in delay in starting antileprosy treatment (ALT). We also identified the spectrum of diseases diagnosed in these patients. Of the 1543files reviewed, we observed that 156 (1.01%) patients had been misdiagnosed at presentation to the doctor and institution of ALT had been delayed for periods varying from 1- 36 months. In 27 (17.3%) of these patients the diagnosis of leprosy was missed at the primary health center, in 46 (29.5%) by the general practitioner, in 34 (21.8%) by practitioners of alternate systems of medicine, in 39 (25%) by a dermatologist, in 9 (6.4%) by an internist and in 1 (0.6%) by a surgeon. In 92 (59.0%) patients, the delay in the diagnosis had led to development of disability (grade I: 60; grade II: 32). In 110 (70.5%) patients, the patient's prescription did not have any diagnoses and the patient had been treated variously with topical steroids, vitamin supplements and antihistamines. Of the 36 patients in whom a diagnoses of dermatological conditions was made, 22 (14.1%) were diagnosed to have a fungal infection and in other 14 (9.0%) a spectrum of dermatological diseases were diagnosed including orofacial granulomatosis (2 patients), post kala azar dermal leshmaniasis (3), granuloma annulare (2), erythema annulare centrifugum (1) and sarcoidosis (2). One patient each with erythema nodosum leprosum (ENL) was misdiagnosed as erythema nodusum and Sweet's syndrome. Nine (5.8%) patients with ENL presented to the internist due to the presence of conspicuous constitutional symptoms. Three of these were misdiagnosed as lymphoma, and 6 as different connective tissue disorders. The patient who presented to the surgeon was misdiagnosed as neurosarcoma. In conclusion, leprosy remains a great mimicker and clinicians really need to think of leprosy, even in era of elimination.

488

SPECTRUM OF CLINICAL PRESENTATIONS OF *FASCIOLA* INFECTION AMONG CHILDREN IN A COMMUNITY-BASED STUDY

Karen E. Neira[1], Maria L. Morales[2], Martha Lopez[2], Clinton White[3], Andrés G. Lescano[1], Miguel M. Cabada[3]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Universidad Peruana Cayetano Heredia-University of Texas Medical Branch Collaborative Research Center, Cusco, Peru,* [3]*University of Texas Medical Branch, Galveston, TX, United States*

Three presentation patterns were recognized in our community-based studies on fasciolasis: acute (no eggs in stools eggs, a positive Fas2 ELISA test and increased eosinophils and/or transaminases (E/T), chronic with inflammation (eggs in stools, Fas2 ELISA positive and increased E/T) and chronic (eggs in stools with normal E/T). However, not all diagnosed cases match these presentations. We analyzed data from a cross-sectional study of 2473 children from Anta - Cusco to determine the characteristics of *Fasciola* infections. To identify subjects matching the above presentations and classify unmatched subjects we compared signs and symptoms between the different groups using latent class analysis (LCA). We identified two groups of unmatched subjects: UM1 (4%: eggs in stools but negative Fas2 ELISA and increased E/T) and UM2 (32%: no eggs in stools, a positive Fas2 ELISA test, and normal E/T). UM1 mean age was 10.2 ± 3.5 years, their most frequent symptoms were headache (70%), nausea/vomiting (50%) and cough (50%). In general, UM1 presented a higher symptom frequency and had less differences with chronic cases with negative Fas2 ELISA (31, 13%) results. UM1 may include chronic cases with Fas2 negative and altered markers values near the cutoff. UM2 mean age was 11.2 ± 3.8 years and the most common symptoms were right upper quadrant pain (29%) and headache (23%). UM2 had similar frequencies of signs and symptoms with the acute cases (18, 7%) and chronic with positive Fas2 ELISA (84, 34%). UM2 can be chronic with positive Fas2 ELISA with a false negative stools test or can be acute cases with normal markers values near the cutoff to be considered altered. The LCA identified four classes: Class 1 (42%, "subclinical cases"), Class 2 (24%, "cases with mild hepatic symptoms)", Class 3 (17%, "cases with early symptoms") and class 4 (18%, "symptomatic cases"). UC1 were more frequent in classes 3 and 4 (30% on each), while UC2 were most frequent in class 1 (60%). Results suggest that Fas2 ELISA sensibility and specificity may lead to unclassified cases.

489

TOPICAL SUNFLOWER SEED OIL THERAPY IN THE MANAGEMENT OF SEVERE ACUTE MALNUTRITION IN CHILDREN UNDER TWO YEARS OF AGE: A RANDOMIZED CONTROLLED CLINICAL TRIAL IN BANGLADESH

Km Shahunja[1], Tahmeed Ahmed[1], Md Iqbal Hossain[1], Mustafa Mahfuz[1], Lindsay Kendall[2], Xinyi Zhu[2], Krishan Singh[3], Jonathan M Crowther[4], Sunita Singh[2], Rachel A Gibson[2], Gary L Darmstadt[5]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*GlaxoSmithKline R&D, Stevenage, United Kingdom,* [3]*GlaxoSmithKline R&D, Collegeville, PA, United States,* [4]*JMC Scientific Consulting Ltd, Egham, Surrey, United Kingdom,* [5]*Stanford University School of Medicine, Stanford, CA, United States*

Children with severe acute malnutrition (SAM) often present with complications, including infections, due in part to compromised skin barrier function. Sunflower seed oil (SSO) which contains high quantity of essential fatty acid (EFA) including linoleic acid, can augments skin barrier function, reduces transepidermal water loss (TEWL) and risk of bloodstream infection, and promotes weight gain in preterm infants by its topical application. We hypothesized that topical treatment of hospitalized children with SAM using SSO, in addition to standard-of-care for SAM, would improve weight gain, skin barrier function, reduce risk of nosocomial infection, and accelerate clinical recovery. We conducted a randomized, two-arm, controlled, unblinded clinical trial in 212

participants aged 2 to 24 months admitted to icddr,b hospital, Dhaka, Bangladesh during January 2016 to November 2017 with SAM (weight for length <-3 SD). Enrollment was age-stratified into 2 to <6 and 6 to 24 months age groups in a 1:2 ratio. All children received SAM standard-of-care, and the SSO group was also treated with 3 g of SSO per kg body weight three times daily for 10 days. At day 10 we found, rate of weight gain was higher in the SSO than the control group [adjusted mean difference (AMD) 0·90 g/kg/day, 95% CI: -1·22 to 3·03] in the younger age stratum, but was not statistically significant. Nosocomial infection rate was significantly lower in the SSO group in the older age stratum (adjusted odds ratio 0.41, 95% CI: 0·19 to 0·85). Skin condition score improved (AMD: -14·88, 95% CI: -24·12 to -5·65) and TEWL was reduced (AMD: -2·59, 95% CI: -3·86 to -1·31) significantly in the SSO group compared to the control group at day 10. Reduction in CRP level was also significantly greater in the SSO group (median: -0·28 mg/dl) than the control group (median 0·00 mg/dl) in the younger age stratum at day 10. Topical therapy with SSO was beneficial for children with SAM when applied as an adjunct therapy. However, a community-based trial with a longer intervention period is recommended to validate these results.

490

EVALUATION OF SUSCEPTIBILITY OF CLINICAL BACTERIA ISOLATES TO HONEY, GINGER AND MUSHROOM

Evangelyn Chiemerie Agochukwu[1], Sampson Tonye[1], Edward Chieke Nwanegbo[2], Renner Renner Nrior[1]

[1]*Rivers State University, Porthacourt, Nigeria,* [2]*Nnamdi Azikiwe University Awka, Awka, Anambara State, Nigeria*

Applications of honey in treatment of diseases were reported in the world's oldest medical literatures. It was reported to possess antimicrobial as well as wound healing properties. In addition, edible Mushroom has also been deployed in traditional medicine as antibacterial and antiviral therapies. Equally used in traditional medicine is Ginger, a perennial plant that has been deployed for human and animal ailments. In this study, the antibacterial properties of these products will be investigated using clinical bacteria isolates from wound care unit in a General Hospital in Southern Nigeria. The antimicrobial effect of honey, ginger and mushroom extracts was tested against each 10 identified samples of S. aureus, S. Pyogenes, P. aeruginosa and E. coli wound isolates from patients. Isolates were harvested from culture media when organisms were at their exponential phase of growth and susceptibility evaluated using serially diluted purified extracts of ginger, mushroom or honey. Inhibition of bacterial growth of the various agents was analyzed and compared. Study is ongoing, and data is being collated. Data from this study may provide rationale for further development of honey, mushroom or ginger for clinical applications in wound care units in developing countries where antibiotics are not readily available and antibiotics resistant remain a major public health concern.

491

A SYSTEMATIC REVIEW OF SOLID ORGAN TRANSPLANTATION IN ACUTE PRESENTATIONS OF TROPICAL INFECTIOUS DISEASES

Shveta Bhasker[1], Emma Hagopian[1], Celine Lecce[1], David Harris[1], Shareese Clarke[1], Priyanka Challa[1], Michael A. Klowak[1], Eric Shao[1], Kimberley Marks - Beaubrun[1], Katherine Faith Tan[1], Mofe Adeosun[1], Osaru Omoruna[1], Christian Lecce[1], Avinash N. Mukkala[1], Rachel Lau[2], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario, Toronto, ON, Canada*

Fulminant life-threatening presentations of acute tropical infections such as yellow fever, dengue, malaria, hepatitis E, and leptospirosis, may occur, and the degree of end-organ impairment may qualify patients for solid-organ transplantation (SOT) in centres with such capacity. However, due to a paucity of synthesized data, there is a knowledge gap around indications for and outcomes in SOT for severe acute tropical infectious diseases. We therefore aim to synthesize such knowledge, focusing on patient outcomes in order to inform triage and treatment protocols in centres where acute tropical infectious diseases and SOT capacity may intersect. Five electronic databases were searched (PubMed, Embase, Scopus, Cochrane, and LILACS) using combinations of search terms such as the following: "liver" or "hepatic" "transplant," "yellow fever" "dengue" and "*Plasmodium* spp.," from database inception to March 4, 2019. A total of 6317 articles were retrieved: 2324 articles on PubMed, 3839 on Embase, 244 on Scopus, 43 on Cochrane, and 108 on LILACS. After eliminating duplicates using Mendeley software, a total of 4944 articles remained for title screening. Titles, abstracts, and full-text articles will be systematically double screened by two reviewers with a tertiary arbitrator. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) will be implemented. Data extraction will be performed by two reviewers and the quality of the articles will be critically evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. The data will be summarized to systematically map published literature that will illuminate the frequency, indications for, and health outcomes of SOT recipients in the treatment of acute tropical infectious diseases. Where SOT capacity exists alongside the occurrence of endemic or imported tropical infectious diseases, such synthesized information, particularly in the form of a clinical resource, is essential for appropriate resource allocation and informed clinical decision-making.

492

EPIDEMIOLOGICAL UPDATE ON FEVER IN RETURNING TRAVELERS TO ONTARIO FROM THE 'RAPID ASSESSMENT OF FEBRILE TRAVELERS' (RAFT) PROGRAM

Aisha Khatib[1], Shareese Clarke[1], Michael A. Klowak[1], Emma Hagopian[1], Farah Jazuli[2], David Harris[1], Ruwandi Kariyawasam[3], Rachel Lau[4], Stefanie A. Klowak[1], Evan Belsky[1], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Department of Emergency Medicine, McMaster University, Hamilton, ON, Canada,* [3]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [4]*Public Health Ontario, Toronto, ON, Canada*

Fever in returning travelers may indicate a life-threatening infection, such as malaria. However, most cases are due to more benign, self-limited etiologies such as traveler's diarrhea. In the absence of a priori predictors of which febrile travelers will develop severe clinical sequelae from their imported infection, which remains undifferentiated pending confirmatory diagnostics, close follow-up and monitoring of travelers during the initial few days of illness is required. The rapid assessment of febrile travelers (RAFT) programme was implemented to standardize the evaluation and disposition of febrile returned travelers in Toronto. We herein provide an epidemiological update on travelers assessed via RAFT from 2016 to 2018, and the illnesses with which they returned from travel. Criteria for RAFT referral include: presentation to participating EDs, reported fever, and travel outside of Canada within the past year. Exclusion criteria include *Plasmodium falciparum* malaria, and fulfilment of admission criteria such as unstable vital signs or major lab derangements. Demographic, clinical, and travel-related data were collected, and analyzed using descriptive statistics. From January 2016 to December 2017, 302 ill returned travelers were evaluated via RAFT: 147(49%) were men and 155(51%) were women. Median age was 34 years (range 16-93 years). Travelers returned from 82 countries with the most represented countries being: India (25, 8%), Mexico (21, 7%), Thailand (18, 6%), Cuba (17, 6%), and Costa Rica (13, 4%). Common diagnoses included: viral syndrome (78, 26%), traveler's diarrhea (34, 11%), viral respiratory infection (27, 9%), dengue (16, 5%), lab-confirmed influenza (16, 5%), and typhoid fever (10, 3%). Among lab-confirmed cases of influenza evaluated in RAFT, off-season transmission accounted for a quarter. Cases of Zika virus (6, 2%) only occurred in 2016. An additional 207 travelers referred in 2018 will also be reported. Understanding the range of illnesses imported by febrile returned travelers will inform pre-travel counseling and both clinical and laboratory algorithmic approaches to care of such travelers.

493

BURDEN OF NON-COMMUNICABLE DISEASES AND THEIR RISK FACTORS IN A KENYAN CASUALTY DEPARTMENT

Thomas Kedera[1], Christine Ngaruiya[2], Mbatha Wambua[3], Mugane Mutua[4], Frances Ogudebe[2], Morgan Muchemi[4], Daniel Owambo[4], Kipkoech Rop[4], Benjamin Wachira[5]

[1]*Kakamega County Referral Hospital, Kakamega, Kenya,* [2]*Yale University, Stamford, CT, United States,* [3]*Kenyatta National Hospital, Nairobi, Kenya,* [4]*University of Nairobi, Nairobi, Kenya,* [5]*Aga Khan University Hospital, Nairobi, Kenya*

It has been noted with some concern that in LMIC, there is little information on NCDs, and even less so I the a&e settings. It is necessary to determine the burden of disease for the main NCDs (diabetes, CVS disease, cancer and chronic respiratory disease) affecting the population. It is a Cross sectional Pilot Study, conducted at the casualty department of the Kenyatta National Hospital. Ethical approval for the study was obtained from the UoN-KNH Ethics Committee. A little under 23990 patients are seen at the outpatient department monthly. Our sample size was calculated as 10% of the general estimated visitors to KNH Casualty Department. This is a standard number as recommended for pilot studies, which for us translated to a target of 2400 patients. Mini-STEPs & SQR - 20 were the validated survey tool used to collect date from the patients. The questionnaires were self-administered electronically. Convenience sampling was employed, recruiting willing participants soon after triage. The data was analysed using STATA version 14.1. 12.7% of respondents smoke tobacco with a mean starting age of 20.4yo; 42.3% of the respondents indicated smoke exposure at work. 53.1% of participants reported engaging in alcohol use; 5.25% had stopped drinking due to health reasons or medical advice. 19.44% reported having been told that they have raised blood pressure but only 9.3% reported receiving treatment. 6.8% reported being told that they have elevated blood sugar; only 1.5% reported being treated with insulin. 8.64% reported having had chest pain or suffered a stroke with; <1% receiving treatment with statins. 16.5% reported history of suicidal ideation. A high burden of NCDs exists among patients in the Casualty Department, one that is comparable to, or supersedes, the general population. However, treatment targeting NCDs or NCD risk factors in Casualty Department patients is minimal. This highlights the importance of developing interventions including clinical policies, education for practitioners on NCDs in Casualty, and other interventions unique to this population.

494

EXAMINING MALNUTRITION AS A CAUSE AND CONTRIBUTOR TO DEATH IN CHILDREN AGED 6-59 MONTHS WITHIN THE CHILD HEALTH AND MORTALITY SURVEILLANCE (CHAMPS) NETWORK

Kasthuri Sivalogan[1], Shams El Arifeen[2], Victor Akelo[3], Quique Bassat[4], Beth A. Tippett Barr[3], Richard Chawana[5], Emily Gurley[6], Karen Kotloff[7], Shabir Madhi[5], Inacio Mandomando[8], Dickens Onyango[9], Rebecca Philipsborn[10], Samba Sow[11], Parminder Suchdev[1], Dianna Blau[12], Robert Breiman[1]

[1]*Emory Global Health Institute, Emory University, Atlanta, GA, United States,* [2]*International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh,* [3]*United States Centers for Disease Control and Prevention, Kisumu, Kenya,* [4]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [5]*Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa,* [6]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [7]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [8]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique,* [9]*Kisumu County Public Health Department, Kisumu, Kenya,* [10]*Emory Global Health Institute, Atlanta, GA, United States,* [11]*Center for Vaccine Development, Bamako, Mali,* [12]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Up to 50% of all under-5 deaths in low and middle-income countries may be associated with malnutrition, characterized by protein energy malnutrition or micronutrient deficiencies. The relationship between malnutrition and morbidity is complex and bidirectional; severely ill children are more susceptible to malnutrition, and poor nutritional status increases risk of severe morbidity and mortality. The Child Health and Mortality Surveillance Network (CHAMPS) actively identifies under-5 deaths and uses demographic and clinical information, post-mortem tissue specimen testing, and verbal autopsy to determine the underlying cause and sequence of events leading to death in catchment areas with high child mortality. Malnutrition was assessed in 148 cases aged 6-59 months with cause of death determined from five surveillance sites in South Africa, Mozambique, Kenya, Mali and Bangladesh from July 2017-March 2019. Nutritional status was assessed by postmortem anthropometric measurements, photographs obtained during the tissue sampling procedure, histopathology results, clinical records and VA responses. Malnutrition was listed as the underlying cause or in the sequence of events leading to mortality in 41.2% (n=61) of cases. Among all 61 cases, 78.7% of cases were considered moderately or severely underweight (weight-for-age z-score ≤ -2), 62.3% of cases were considered moderately or severely stunted (height-for-age z-score ≤ -2), and 73.8% of cases were considered moderately or severely wasted (weight-for-height z-score ≤ -2). Malnutrition was considered present in 60.7% of cases according to data abstracted from clinical records and 63.9% of cases according to VA responses. The most common underlying causes of death were malnutrition (57.4%), congenital birth defects (11.5%), lower respiratory infections (8.2%), and HIV infection (6.6%). The most common immediate causes of death were sepsis (42.6%), lower respiratory infections (37.7%), and malaria (8.2%). Early data suggests that malnutrition, frequently associated with infections, is an important cause of under-5 mortality in high child mortality areas.

495

SEQUENTIAL MIXED METHODS STUDY ON NOMA, NORTHWEST NIGERIA 2017 AND 2018

Elise Farley[1], Modupe Juliana Oyemakinde[1], Jorien Schuurmans[1], Aisha Abubakar[1], Fatima Saleh[2], Gloria Uzoigwe[3], Karla Bil[4], Bukola Oluyide[5], Nma Muhammed Jiya[6], Simba Tirima[5], Adolphe Fotso[5], Mohana Amirtharajah[4], Jorieke Vynke[4], Raphael Brechard[4], Adeniyi Semiyu Adetunji[7], Koert Rietmeijer[4], Saskia van der Kam[4], Denise Baratti-Mayer[8], Ushma Mehta[9], Shafiu Isah[7], Chikwe Ihekweazu[2], Beverley Stringer[10], Cono Ariti[11], Annick Lenglet[4]

[1]*Medecins Sans Frontiers, Sokoto, Nigeria,* [2]*Nigerian Centre Disease Control, Abuja, Nigeria,* [3]*Department of Dentistry, Nigerian Ministry of Health, Abuja, Nigeria,* [4]*Medecins Sans Frontiers, Amsterdam, Netherlands,* [5]*Medecins Sans Frontiers, Abuja, Nigeria,* [6]*Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria,* [7]*Department of Clinical Services, Noma Children's Hospital, Sokoto, Nigeria,* [8]*GESNOMA (Geneva Study Group on Noma), Service of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospitals, Geneva, Switzerland,* [9]*University of Cape Town, Cape Town, South Africa,* [10]*Medecins Sans Frontiers, London, United Kingdom,* [11]*Centre for Medical Education, Cardiff University School of Medicine, Cardiff, United Kingdom*

Noma, a rapidly progressing infection of the oral cavity affecting children <5 years, has a 90% mortality rate. In 2017-2018 we conducted a three stage sequential mixed methods study on noma to guide prevention and health programming for this disease in NW Nigeria. Stage 1 was a qualitative study exploring language and beliefs through 12 in-depth interviews with hospital staff and five focus group discussions with caretakers of noma patients. Stage 2 was a case control study to estimate risk factors for diagnosed noma. Consenting caretakers of noma patients (<15 years) and matching controls (age, sex and village) answered questionnaires. Risk factors were estimated with odds ratios (ORs) using

logistic regression. Stage 3 was a two stage cluster survey in Kebbi and Sokoto states. We conducted oral screening on all persons <15 years using WHO noma classification:S0:simple gingivitis;S1:necrotizing gingivitis;S2: oedema;S3:necrosis;S4:healing; S5:sequela. A weighted analysis of noma prevalence with 95% confidence intervals (CI) was completed. Caretakers referred to noma as "ciwon daji" and staff as "noma". Participants believed noma was caused by spirits, insects, birds or previous illness. The case control study (74 cases and 222 controls) identified the following risk factors for diagnosed disease: the mother not being the primary caretaker (OR 0.1; CI 0.05-0.4), the caretaker being unmarried (OR 0.01; CI 0.004-0.04), colostrum not being given (OR 0.3; CI 0.1-1.1) and the child being fed maize porridge every day (OR 5.0; CI 1.2-20.8). In the prevalence survey we included 7120 children; 181 children presented as S0 (3.11%; CI 2.6, 3.8%), 10 children as S1 (0.1%; CI 0.1, 0.3%) and three children as S2 (0.04%; CI 0.01, 0.1%). No S3-5 was detected. Noma is a poorly understood disease, social conditions and infant feeding practices are associated with it. We demonstrated that in this part of Nigeria, precursor stages of noma are prevalent. Prevention efforts for noma must target nutrition, oral hygiene and improved access to care.

496

ASSESSMENT OF THE DATA QUALITY AND STANDARD OF OUT PATIENT REGISTRATION FOR DETECTION OF CHOLERA CASES IN CAMEROON

Ayok M. Tembei[1], Martin Yakum[1], Maurice Ebode[1], Anthony Chebe[2], Sonia Nafack[3], Pascal Goura[1], Manualla Cheugeu[4], Nelson Guemechio[4], Joliette Azakoh[3], Anne Cecile Bissek[5], Jerome Ateudjieu[1]

[1]*Meilleur Accès aux Soins de Santé (M.A. SANTE), Cameroun, Yaoundé, Cameroon,* [2]*Meilleur Accès aux Soins de Santé (M.A. SANTE), Cameroun, Kousserri, Cameroon,* [3]*Meilleur Accès aux Soins de Santé (M.A. SANTE), Cameroun, Douala, Cameroon,* [4]*University of Dschang, Dschang, Cameroon,* [5]*Division of Health Operations Research, Ministry of Public Health, Yaoundé, Cameroon*

Data from out-patient units are expected to provide information for monitoring health program effectiveness and disease distribution. Reliable epidemiological data from active surveillance is crucial for efficient implementation of prevention and preparedness measure for epidemic prone diseases. Data quality and the standardization of out-patient registration tools at health facility level was assessed to determine whether external consultation registers can be used to detect cholera cases in Cameroon. A descriptive cross sectional study was conducted in 22 health facilities selected from Far North and Littoral regions of Cameroon. External consultation registers were reviewed using a grid. In each current register, the 100 recently consulted patient records and the last 10 diarrhoeal consulted cases were reviewed for presence, completeness, adequacy and standardization of data available. Descriptive statistics was used to summarize and describe data in SPSS. A mixture of locally adapted and nationally provided registers often not standardized is in place. Of 20 variables assessed, level of data completeness was very low with only 4.3% of 47 registers reported as complete. All variables assessed had at least 01 missing patient record. Of 427 diarrhoeal patient records reviewed, 87% of variables present in registers are useful for cholera detection. But, with an exception of age (0.9%) and symptoms (1.4%), other variables had high levels of missing records for key cholera parameters reporting 96.3%, 93.9% and 70.7% non-completeness for duration of diarrhoea before consultation, number of stools within 24hours and type of diarrhoea patients were experiencing respectively. The Cameroon national health information system is hindered with poor data quality and standardization of data tools for out-patient registration. Data provided in registers are not adequate to effectively inform cholera surveillance system for prompt response in case of an epidemic. A discussion is thus needed to standardized and contextualized registers and make sustainably available for all external consultation purposes per health facility.

497

A DOCTOR'S EXPERIENCE: THE DILEMMA FACED USING PERSONAL PROTECTIVE EQUIPMENT WHILE WORKING IN AN EBOLA TREATMENT UNIT

Mohamed Boie Jalloh[1], May C. Chu[2]

[134] *Military Hospital, Republic of Sierra Leone Armed Forces, Freetown, Sierra Leone,* [2]*Colorado School of Public Health, Aurora, Colorado, CO, United States*

We have a daunting task to design a piece of personal protective equipment (PPE) ensemble to improve safety and comfort for the health workers at the frontline. During the 2014-2015 Ebola outbreak, I was tasked to set up the Ebola Holding Unit at the 34 Military Hospital. I had not set eyes on PPEs or even heard the term but was aware of the Ebola outbreak that was ravaging the Eastern districts. The very first time I was trained to use PPE, it gave me so much sense of security and confidence to provide clinical care to Ebola patients. It made me feel beyond the reach of the ominous hand of Ebola. I even wondered: Can we not end the outbreak by providing PPE to all and requiring each to put them on for at least 4 weeks, far beyond the incubation period? However, it was in the first 20 minutes of using PPE for the first time to assess an Ebola case that I realized the complexity of PPE and the fact that my thinking earlier was impractical. The enthusiasm I had for PPE turned into doubt and I started asking questions like" was this PPE made for us?", and by us, I meant, clinical care providers in Africa whose climate is hot and humid. Finally, I struggled with the very basic question: Is PPE that covers with no bit of exposed skin necessary? Or am I safer with a limited level of PPE? Why do we have to put on full PPE for all Ebola patients regardless of their clinical profile (wet or dry)? To date, I am of the view that we do not need the current prescribed full PPE for patients who are not wet. I know there is no data that can help decide where to draw the line on the level of exposure of skin. In the end, though, I chose safety over comfort where I could, even though at times the two are inseparable. Comfort for me could mean less safety for me or the patients. I followed the infection prevention control requirements of PPE-use religiously. However, this reduced my reaction time to emergencies in the ETU, my contact time with patients and inevitably may have reduced the survival rate of my patients. For how much care can I provide to a patient when on an 8-hour shift, I can only withstand working for 135 minutes? Compare this to 400 minutes contact time expected in an 8-hour shift. We need a science-based discussion and solution.

498

PREVALENCE AND ASSOCIATED FACTORS OF ANEMIA IN WOMEN CHILDBEARING AGE IN POPOKABAKA, DEMOCRATIC REPUBLIC OF CONGO

Gisele M. Mvumbi, Marc M. Bosonkie, Vitus L. Okitolonda, E. W. O

ESP-KINSHASA/DRC, Kinshasa, Democratic Republic of the Congo

l'anémie continue à être une barrière majeure à la santé maternelle et au développement socio-économique particulièrement dans les pays sous-développés. Popokabaka est un territoire de la province de Bandundu qui est dans un contexte de faible disponibilité en aliments riches en fer, faible connaissance des mères sur les aliments riches en fer et monotonie alimentaire. L'étude avait pour objectif de déterminer la prévalence et les facteurs associés à l'anémie chez la femme en âge de procréer à la cité Popokabaka. Une étude transversale a été menée dans les ménages de la cité Popokabaka pendant la période allant du 2 au 30 avril 2017. Les potentiels facteurs sociodémographiques, gynécologiques et cliniques, la connaissance des femmes sur l'anémie, l'état nutritionnel et la fréquence de consommation d'aliment riche en fer étaient recherchés. La régression logistique était utilisée pour déterminer les facteurs associés à l'anémie. Un total de 393 femmes en âge de procréer a constitué notre échantillon. Le taux moyen d'hémoglobine était de 1.8 ± 1.2 g/dl. La prévalence de l'anémie était de 50.6% (95% CI: 45.7- 55.6). Environ deux tiers de nos sujets (64.3%) avait un score moyen de diversité alimentaire. Plus

de la moitié 58.5% (95% CI 53.6-63.4) avait une faible connaissance sur l'anémie. Environ 38.1 % et 6.1% avaient consommé les aliments riches en fer deux fois la semaine. Le fait d'avoir 2 ou 3 enfants [Odds Ratio Ajusté (ORa) =2.06, 95 % CI: 1.07–3.97] et la consommation des fruits riches en vitamine c au moins une fois la semaine (ORa=0.56, 95 % CI: 0.33–0.98) étaient les facteurs indépendamment associés à l'anémie.L'anémie chez la femme en âge de procréer constitue un problème majeur de Santé Publique à la cité Popokabaka. Le fait d'avoir 2 ou 3 enfants; et la consommation des fruits riches en vitamine c au moins deux fois la semaine étaient révélés comme facteurs associés à l'anémie à Popokabaka. L'éducation nutritionnelle basée sur la consommation régulière des fruits et la supplémentation en fer seraient donc des interventions efficaces pour la prévention de l'anémie chez la femme en âge de procréer à cité Popokabaka.

499

THE INCIDENCE OF ACUTE FEBRILE ILLNESS AMONG CHILDREN IN BUTAJIRA, SOUTH-CENTRAL ETHIOPIA

Mekonnen Teferi Mekonnen[1], Mulualem Desta[2], Biruk Yeshitela[1], Tigist Beyene[1], Ligia Maria Cruz Espinoza[3], Justin Im[4], Hyon Jin Jeon[4], Jong-Hoon Kim[4], Frank Konings[4], Soo Young Kwon[4], Gi Deok Pak[4], Jin Kyung Park[4], Se Eun Park[4], Melaku Yedenekachew[1], Stephen Baker[5], Won Seok Sir[6], Florian Marks[4], Abraham Aseffa[1], Ursula Panzner[4]

[1]*AHRI, Addis Ababa, Ethiopia,* [2]*Science and Technology Information Center (STIC), Addis Ababa, Ethiopia,* [3]*International Vaccine Institute, Seoul, Republic of Korea,* [4]*IVI, Seoul, Republic of Korea,* [5]*The Department of Medicine, The University of Cambridge, Cambridge, United Kingdom,* [6]*Graduate School of Public Health, Yonsei University, Seoul, Republic of Korea*

A clear differentiation of causes of febrile illnesses is challenging for health professionals in locations were diagnostic capacities are limited. A lack of an etiological diagnosis often results in poor patient management. Here, we aimed to investigate the incidence of pediatric acute febrile illnesses in Butajira, Ethiopia during the Typhoid Fever Surveillance in Africa Program (TSAP). Febrile children aged ≤15 years were enrolled at primary/secondary healthcare facilities. Aerobic blood culture, malaria microscopy, and complete blood count were performed on samples of patients' blood. Microbiological, biochemical, species/sub-species, and antimicrobial susceptibility testing of positive cultures were performed. A scheme for classifying children with acute febrile illness, malaria, acute respiratory tract, gastrointestinal, and urinary tract infections was developed. Incidence rates were calculated and a multivariate logistic regression analysis was performed to determine predictors for febrile illnesses. Results Among 513 children recruited during January 2012 to January 2014, we found that hospitalization was low (4.1%, 21/513),as was stunting(0.8%, 4/513), being underweight(0.2%, 1/513) and wasting(0.2%, 1/513).The blood culture positivity for true pathogens was 1.6% (8/513); the malaria prevalence was13.5% (69/513).Temporal and spatial incidences, derived from the case classification scheme, revealed a higher incidence of febrile disease in children aged ≤5 than >5 to ≤15 years. The average annual incidence per 100,000 population were694.3 (95%CI: 644.7-747.7) for acute febrile illness, 301.3 (95%CI: 269.2-337.2) for malaria, 1,860.1 (95%CI: 1778.0-1946.0) for acute respiratory tract infections, and 379.9 (95%CI: 343.6-420.0) for gastrointestinal infections in children aged ≤5 years. In conclusion, we find that fever is common in this location but its origin is often unknown. Findings from this study may prompt healthcare personnel and decision makers in the development of revised policies and frameworks for the diagnosis & management of febrile illnesses in children.

500

A RANDOMIZED PILOT CLINICAL STUDY ON THE USE OF HERBAL PRODUCT HB01, IN TREATMENT OF HIV POSITIVE PATIENTS IN ANAMBRA STATE, NIGERIA

Uchenna C. Ogwaluonye[1], Joseph I. Ikechebelu[1], Ngozi N. Ikechebelu[2], George Eleje[1], Izuchukwu Ibeagha[3], Charles O. Esimone[1], Edward C. Nwanegbo[1]

[1]*Nnamdi Azikiwe University, Awka, Awka, Nigeria,* [2]*Chukwuemeka Odimegwu Ojukwu University Teaching Hospital, Awka, Nigeria,* [3]*University of Nigeria Teaching Hospital, Enugu, Nigeria*

In Nigeria, herbal medicines are heavily used but poorly documented, lacking safety and efficacy data, therefore presents a liability in orthodox practice. This study was designed to clinically evaluate the efficacy and safety of an anti-HIV herbal formulation, HB01 in treatment of HIV positive adults in Anambra, South eastern Nigeria. A randomized double-blind clinical study was carried out with 7 consenting, HIV positive adults for a duration of three months. Following recruitment, each patient was sequentially assigned a number which was randomized into treatment and control group using the online research randomizer, resulting in 4 persons in treatment group and 3 persons in the control group. Primary outcome parameters, including viral load and CD4 count, were measured at the beginning and end of the study while microbial safety studies, Liver function test and Electrolyte concentration were performed on a monthly basis using specialized test kits. The clinical study revealed a statistically insignificant decrease in the viral load (20,609 to 1,323 copies/ml) and increase in CD4 count (565 to 640 cells/mm3) when compared to the control. There was no negative change in the liver function test and electrolyte levels of participants between the test and control groups. Side effects recorded among participants included increased appetite and micturition, self-limiting diarrhea, vomiting and menstrual pains. Antimicrobial evaluation of HB01 revealed no activity against test bacteria, yeast and mould cultures. HB01 was generally well-tolerated, showing no negative outcome on HAART goals for HIV infection among the study participants and complied with BP limits for microbial load. Our findings also indicate positive changes in the viral load and CD4 count but being insignificant, would require further studies in order to ascertain its potential as a viable adjunct to HAART.

501

CLINICAL PROFILE AND OUTCOME OF TETANUS AMONG ADULT PATIENTS IN ETHIOPIA

Godana Jarso

Adama Medical College, Adama, Ethiopia

Tetanus is a major health problem in developing countries as it is associated with high morbidity and mortality. There is scarcity of data regarding patterns of disease and outcomes in our Ethiopia and the study area. This study was undertaken to assess treatment outcome and predictors among adult patients admitted with tetanus. A cross sectional study design was conducted based on secondary data recorded over five year from January 2014 to December 2018. Records and charts of all adult patients admitted with tetanus at Adama hospital were included in the study. Accordingly out of 80 patients, 66 with complete data were included. Data was collected using structured checklist, then entered and analyzed using SPSS 22.0. The patients' characteristics were explored using descriptive statistics. The association was analyzed using binary logistic regression and magnitude were estimated using odds ratio with 95%CI. The significance of association was declared at p-value < 0.05. About 57 (86.40%) of patients were male and 44(66.7%) were under 40 years of age. In about 42(63.6%) patients the site of injury was lower limbs. The median incubation period was 12.50(IQR: 8-18.5) days. Sixty (91%) of the patients had generalized tetanus and 38(57.6%) had severe forms of tetanus. Complications were reported in 41(62.1%) of patents. Death occurred in 38 patients with case fatality of 57.6% (95%CI: 45.5, 69.7). The predictors of death were severe tetanus [AOR=16.41; 95%CI 2.20, 122.21] and presence of complication [AOR=6.23; 95%CI: 1.05,

36.81] and being admitted to ward [AOR=33.95; 95%CI: 3.12, 369.07] compared to ICU. In conclusion, tetanus still remains to be major health problem in our setup with unacceptably high mortality. Even though majority present had severe diseases and complications, only few patient were managed in intensive care unit (ICU) due to lack of such facility earlier. The burden and mortality of tetanus can be reduced via effective immunization, identification of severe cases and proper management in ICU.

502

RETROSPECTIVE REVIEW OF BLOOD STREAM INFECTIONS IN A GOVERNMENT HOSPITAL IN MAHARASHTRA, INDIA

Kananbala Avinash Yelikar[1], Jyoti Anil Iravane[1], Anil Gaikwad[1], Poonam Korpe[2]

[1]*Government Medical College, Aurangabad, Aurangabad, India,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Antimicrobial resistance (AMR) has become a global crisis. In India, efforts to study AMR have been hindered by lack of public health infrastructure and lack of a robust national surveillance system. This study was conducted to identify the most common pathogens involved in blood stream infections and to characterize their AMR. This was a retrospective chart review of all positive blood cultures at the Government Medical College Aurangabad hospital in 2016. Blood specimens were collected and cultured as per standard protocol. Antibiotic susceptibility testing was performed by disk diffusion testing using modified Kirby Bauer methods. From January 1, 2016 to December 31,2016, a total of 543 blood cultures returned positive. Of these 189 were due to *Staphylococcus aureus* (34.8%), 192 due to *Klebsiella pneumoniae* (35.4%), 56 from *Pseudomonas aeruginosa* (10.3%), and 45 due to *Escherichia coli* (8.3%). Of *Staph aureus* isolates, 77 were tested for cefoxitin resistance and 57 (74%) returned as methicillin-resistant *Staph aureus*. Of the *Klebsiella* isolates, 81.6% met CLSI criteria for extended-spectrum beta-lactamase producers (ESBL) and 57.9% met criteria for carbapenem-resistant Enterobacteriaceae (CRE). 42.2% of the *E. coli* isolates were ESBL, and 60.0% were CRE. Resistance testing for the *Pseudomonas* isolates revealed 62.5% were ESBL and 57.9% were carbapenem resistant organisms. The most common pathogens involved in blood stream infections were *Klebsiella, Staph aureus, and Pseudomonas*. The majority of *Staph aureus* BSIs were attributed to MRSA. There was a high level of cephalosporin and carbapenem resistance among the gram-negative organisms,with rates consistent with other studies from Maharasthra, India. Routine surveillance of microbiology results in Indian hospitals is necessary to understand trends in antibiotic resistance and to prevent spread of resistant organisms.

503

ECONOMIC BURDEN OF ANTIMICROBIAL RESISTANCE IN LOW AND MIDDLE-INCOME COUNTRIES: A SYSTEMATIC REVIEW AND EXPERT CONSULTATION

Kyaw Zay Ya

International Rescue Committee, Yangon, Myanmar

Antimicrobial resistance (AMR) is a global challenge which is associated with the utilization of more healthcare resources as result of high medication costs, prolonged hospital stay, and decreased in productivity. Therefore, it is expected to have a major negative impact on economic sector especially for low and middle income-countries (LMICs). To assess the economic burden, it is great of importance to find out comprehensive definition of costs and cost items attributable to AMR. Additionally, it is crucial to identify current evidence related to the costs of AMR in LMICs. The methodology for this study was a systematic review from Pubmed and Embase in line with PRISMA guidelines and expert consultations. Defining AMR cost is a complex methodology involving a series of assumptions and estimation methods. The systematic review found that additional length of hospital (LOS) was used to calculate AMR direct cost in hospital settings (n=19), and the attributable cost for AMR was estimated by comparing infections with non-resistant strains. Few studies tried to estimate societal cost to some extent (n=5) while most studies only concentrated on direct medical costs (n=18). Expert consultations highlighted the costs for basic health infrastructure and supply chain management are new items to be considered. Experts suggested to estimate financial consequences of losing the efficacy of existing therapies. There is a significant gap in the development of methodology for cost estimation in LMICs. Theoretical framework is needed to categorize and measure the costs associated with AMR but those are not being used in LMICs due to methodology limitation and lack of expertise. Cost identification is one of the requirements for subsequent economic evaluations. New additional cost items were identified with this study, but it is still an issue how to estimate those items. These findings suggest for methodology development is needed in LMICs.

504

URINARY TRACT INFECTION IS A COMMON REASON FOR POTENTIALLY INAPPROPRIATE ANTIMICROBIAL USE AMONG INPATIENTS IN SRI LANKA

Tianchen Sheng[1], Gaya B. Wijayaratne[2], Thushani M. Dabrera[3], Richard J. Drew[1], Ajith Nagahawatte[2], Champica K. Bodinayake[2], Ruvini Kurukulasooriya[4], Truls Østbye[1], Kristin J. Nagaro[1], Cherin De Silva[4], Hasini Ranawakaarachchi[4], Arambegedara Thusitha Sudarshana[3], Deverick J. Anderson[1], Christopher W. Woods[1], L. Gayani Tillekeratne[1]

[1]*Duke University, Durham, NC, United States,* [2]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka,* [3]*Sri Lanka Ministry of Health, Colombo, Sri Lanka,* [4]*Duke-Ruhuna Collaborative Research Centre, Galle, Sri Lanka*

Urinary tract infection (UTI) is a common reason for inappropriate antimicrobial use globally. Reducing inappropriate antimicrobial use is important for tackling the current crisis in antimicrobial resistance. We determined the prevalence of UTI and associated inappropriate antimicrobial use among inpatients in Sri Lanka. A point-prevalence study of antimicrobial use was conducted using one-day cross-sectional surveys at five public hospitals in Southern Province, Sri Lanka from Jun-Aug 2017. Inpatients' medical records were reviewed for clinical data including antimicrobials prescribed. Inappropriate antimicrobial use was identified as 1) antimicrobial use discordant with the Sri Lanka College of Microbiologists' guidelines, and 2) redundant combinations of antimicrobials. Of 1,709 surveyed patients, 935 (54.7%) received antimicrobials, of whom 779 (83.3%) had a specified or inferred indication for antimicrobial use. Among these patients, UTI was the third leading indication for antimicrobial use (91 patients, 11.7%). Commonly used antimicrobials for UTI included amoxicillin & clavulanic acid (34.9%), fluoroquinolones (31.4%), and third-generation cephalosporins (23.2%). Antibiotic combinations were used in 28.6% of UTI patients. Urine cultures were obtained in only 26 (28.6%) of UTI patients, with 11 of UTI patients (12.09%) having positive cultures. Potentially inappropriate antimicrobial use was observed in 36.3% of UTI patients, with redundant antibiotic therapy observed in 6.6% of UTI patients. Patients in pediatric compared to medical/surgical wards (81.82% versus 29.11%, p<.001) were more likely to receive inappropriate therapy. UTI was a common reason for antimicrobial use, with one-third of patients receiving potentially inappropriate antimicrobial therapy. We identified targets for future antimicrobial stewardship efforts.

505

PROFILE OF ADVERSE EVENTS FOLLOWING ROUTINE IMMUNIZATION IN 5 HEALTH ZONES OF KINSHASA, THE DEMOCRATIC REPUBLIC OF CONGO

Trésor Bodjick Muena Mujobu, Gaston Tona Lutete

Clinical Pharmacology and Pharmacovigilance Unit , University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Millions of doses of vaccines are regularly administered to Congolese infants each year as per a schedule inspired from the World Health

Organization (WHO). But the safety profile of routine vaccines is unknown. Reporting adverse events following immunization maintains the safety of vaccines and the confidence of the community. We initiated a project to collect adverse events occurring in the context of routine immunization in 5 health zones of Kinshasa, the capital city of the Democratic Republic of Congo. Mothers reported the events they noticed after the previous session of vaccination and the nurses recorded all the relevant information in forms that were submitted to the National Pharmacovigilance Center. The collected data were reviewed and uploaded into the Vigiflow® system (a web-based safety report system supported by the World Health Organization). The reports entered from October 2017 to December 2018 were extracted for statistical analysis using Excel 2016. A total of 1075 vaccinated children developed 1216 adverse events that were reported by their mothers. The most common events according to crude incidence rates were fever (66.9%), persistent crying (11.6%), injection site pain (6.9%) and injection site swelling (3.9%). There were also 11 other reactions that were reported at a lesser rate: injection site induration (3.1%), injection site erythema (1.9%), Asthenia or fatigue (1.3%), myalgia (1.15%), Diarrhea (1.15%), Vomiting (0.8%), decreased appetite (0.3%), injection site abscess (0.3%), rash (0.25%), injection site irritation (0.2%) and vaccination site inflammation (0.1%). The subjects were predominantly males (51%). Injection site pain, swelling and induration (local reactions) were mostly reported in females (52.35%). None of the reported adverse events was serious. The vaccines routinely administered to Congolese infants have a good safety profile, comparable to what is published internationally. This is the kind of information that needs to be shared with the general public to maintain and improve confidence in the health system.

506

THE ROLE OF NUTRITIONAL STATUS IN THE EFFICACY OF AZITHROMYCIN TO REDUCE CHILD MORTALITY IN NIGER: A SUBGROUP ANALYSIS OF THE MORDOR TRIAL

Kieran S. O'Brien[1], Ahmed M. Arzika[2], Ramatou Maliki[2], Sun Y. Cotter[1], Elodie Lebas[1], Catherine Cook[1], Kathryn J. Ray[1], Sheila K. West[3], Robin L. Bailey[4], Catherine E. Oldenburg[1], Jeremy D. Keenan[1], Thomas M. Lietman[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*The Carter Center, Niamey, Niger,* [3]*Johns Hopkins University, Baltimore, MD, United States,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

A large simple cluster-randomized trial demonstrated that biannual azithromycin distribution to children 1-59 months old reduces child mortality. Although the mechanism of effect remains to be determined, the likely pathway involves reducing the infectious disease burden. The mortality effect might be more pronounced in malnourished children, who face an increased risk of infectious mortality compared to well-nourished peers. Indeed, targeting distributions to malnourished children could maximize the mortality benefit while minimizing selection for antimicrobial resistance. To determine whether biannual azithromycin has a differential effect on mortality by nutritional status, we used data from children 1-11 months old with weight recorded for azithromycin dosing from the Niger site in the MORDOR trial. We calculated weight-for-age Z-scores (WAZ) using the World Health Organization's Child Growth Standards, categorizing children as malnourished (WAZ < -2) or not (WAZ ≥ -2) at a child's entry into the study. We used a Cox proportional hazards model with a shared frailty for cluster and an interaction between treatment arm and baseline nutritional status to assess effect modification on the multiplicative scale. There were 26,242 children 1-11 months in Niger with baseline weight recorded, and 1,152 deaths in this group over the 2-year study. Comparing children in azithromycin-treated communities to placebo-treated communities, the hazard of death was 33% lower (HR 0.67, 95% CI 0.53 to 0.84) in malnourished children and 24% lower (HR 0.76, 95% CI 0.65 to 0.89) in non-malnourished children within communities. The interaction was not statistically significant ($P = 0.33$). As mortality is a rare event even in high mortality settings, it is plausible that this study was not powered to detect an interaction. On the other hand, even if the effect was stronger in malnourished children, the absolute number of deaths prevented will be greatest in the overall group. To maximize the societal impact of biannual azithromycin to reduce child mortality, these results support treatment of all children 1-59 months, regardless of nutritional status.

507

RICKETTSIA FELIS ASSOCIATED WITH FATAL CENTRAL NERVOUS SYSTEM INFECTION IN INDONESIA

Arthur Mawuntu[1], Edison Johar[2], Riane Anggraeni[1], Feliana Feliana[1], Janno Bernadus[1], Dodi Safari[2], Frilasita Yudhaputri[2], Rama Dhenni[2], Yora Dewi[2], Cecilia Kato[3], Ann Powers[3], Ronald Rosenberg[3], **Amin Soebandrio**[2], Khin Saw Myint[2]

[1]*Universitas Sam Ratulangi, Manado, Indonesia,* [2]*Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [3]*Centers for Disease Control and Prevention, Fort Collins, CO, United States*

Rickettsia felis has recently emerged worldwide as a cause of human illness. Typically causing mild, undifferentiated fever, it has been implicated in several cases of neurological diseases. Although rickettsial etiology of acute febrile cases is well documented in Southeast Asia, there is limited information on central nervous system (CNS) rickettsioses due to limited laboratory capability and low clinical awareness. Cerebrospinal fluid (CSF) specimens from patients over 15 years of age admitted to adult wards at Kandou General Hospital in Manado, North Sulawesi, Indonesia from August 2015-February 2017 with presumed CNS infections were screened for *O. tsutsugamushi and Rickettsia spp.* DNA using real-time PCR targeting 47-kDa and 23S rRNA genes. *Rickettsia spp.* positive specimens were further characterized using semi-nested PCR targeting the 17-kDa, *ompB*, and *gltA* genes. In addition, sera and CSF specimens were tested for rickettsial antibodies with scrub typhus, spotted fever group and typhus group *Rickettsia* IgM ELISA. Two out of 38 available CSF samples were positive for *Rickettsia* DNA as determined by genus-specific qPCR, both from fatal cases. Amplicons were subsequently confirmed to be *R. felis* by Sanger sequencing. The 17-kDa and *ompB* sequences shared 100% similarity with reference strain *R. felis* upon BLAST analysis whereas *gltA* sequences of both cases matched 99% with the *R. felis* sequences. In addition, both cases were negative for Rickettsial IgM antibodies. *R. felis* usually associated with non-specific febrile illness, was found to cause a fatal CNS infection in our study. Further studies on this undocumented syndrome are urgently needed as morbidity and mortality could be considerably reduced through recognition, timely and available pharmacotherapy.

508

WHEN MALIGNANCY AND INFECTION INTERTWINE: A DIAGNOSTIC CHALLENGE IN THE TROPICS

Jessica Tuan[1], Janvier Murayire[2], Alain Prince Kubwayo[2], Thomas Havanabakize[2], Felix Manirakiza[2], Leway Kailani[2], Menelas Nkeshimana[2], Florence Masaisa[2]

[1]*Yale University, New Haven, CT, United States,* [2]*University of Rwanda, Kigali, Rwanda*

A 38-year-old Rwandan male presented to an urban Rwandan hospital with a painful, 6/10 left axillary mass and chest pain for 2 weeks. He had fevers and weight loss for 1 month. For 1 year, he had pain with swallowing solids initially, then liquids. He had 3 days of emesis. On exam, he was cachectic. T was 40°C, HR 106 bpm, BP 91/50 mmHg, RR 20 bpm, and O_2 92% on RA. He had a tender, fixed, 4-cm left axilla mass. He had a white, coated tongue. He had WBC of 48,800 cells/μL with 68% eosinophils, 23% monocytes, 3% neutrophils. Hb was 9.1 g/dL, MCV 101 fL, and PLT 88,000 cells/μL. HIV was negative. On day 1, he started antibiotics in the setting of neutropenic fever and fluconazole for thrush. Fine needle aspiration (FNA) cytology of the mass revealed lymphocytes, granulomatous formations, and giant cells in a protein-necrotic background. On day 3, Auramine stain of FNA cytology of the mass showed acid-fast bacilli, supporting the diagnosis of *Mycobacterium*

tuberculosis lymphadenitis. He started rifampicin, isoniazid, pyrazinamide, ethambutol with pyridoxine. On day 4, peripheral blood smear showed 95% myeloblasts, consistent with M4 acute myeloid leukemia (AML). On day 8, CT chest showed enlarged left axilla and subpectoral lymph nodes, some necrotic and bilateral pulmonary emboli. Enoxaparin was started. CT abdomen demonstrated mild splenomegaly. Endoscopy revealed ulcerative esophagitis. On day 10, he transferred to Butaro Cancer Center in Rwanda, for bone marrow biopsy which confirmed acute monomyelocytic leukemia. TB lymphadenitis typically occurs due to reactivation of TB at a hematogenously seeded site during primary TB. AML, a myeloid blood cell line cancer, is the most common adult acute leukemia. Few case reports describe TB lymphadenitis and concurrent AML. Hematologic malignancies increase relative risk of TB by 2-40 fold. Clinical suspicion for malignancy should be high in a patient with leukocytosis, constitutional symptoms, and persistent fevers. An important lesson to highlight in this case is to not overlook concurrent opportunistic infections, such as TB, particularly in endemic regions in Africa, in the tropical clinical setting of Rwanda.

509

LAUNCH OF A NEW FECAL MOLECULAR EXTERNAL QUALITY ASSESSMENT (EQA) SCHEME BY UK NEQAS PARASITOLOGY

Jaya Shrivastava, Peter L. Chiodini, Agatha C. Saez

Public Health England, London, United Kingdom

Protozoan infections are responsible for a large proportion of infectious gastroenteritis world-wide. The common pathogenic protozoa are *Giardia intestinalis*, *Cryptosporidium hominis* or *C. parvum* and *Entamoeba histolytica*. Because of the clinical importance of these parasites, recent years have seen increased use of molecular methods for their detection with current clinical algorithms relying on qualitative detection of these parasites using molecular methods. Thus, the need for a fit-for-purpose qualitative EQA or Proficiency testing scheme for these parasites is very clear. In February, 2019 UK NEQAS Parasitology sent out a questionnaire to all participants within its existing microscopy-based Faecal Parasitology EQA scheme in order to determine whether the need for such an EQA scheme exists. The survey was sent to about 550 laboratories and 87 responded. 52 participants confirmed existing or planned capability in molecular testing for faecal parasites, with 43 labs confirming plans to use molecular diagnostic methods in near future. 65 labs expressed an interest in participating in a UK NEQAS scheme for molecular detection of faecal parasites. Responding to the results of this survey, UK NEQAS parasitology is developing a fit-for-purpose qualitative EQA scheme for the faecal parasites *G.intestinalis*, *Cryptosporidium* spp and *E.histolytica* using freeze dried faecal specimens. The *Giardia* and *Cryptosporidium* specimens were created using clinical samples obtained from patients attending the Hospital for Tropical Diseases in London; whereas the *E. histolytica* samples were prepared using cultured amoebae mixed in negative faeces. The specimens have been successfully freeze-dried and they are working well in molecular assays. The homogeneity and stability of these specimens at various temperatures and time points is currently being tested, with initial results showing very high homogeneity and stability for up to 12 weeks. We shall send the first pilot distribution to the labs that expressed a definite interest by the end of May, 2019.

510

A PROTEOMICS APPROACH TO IMPROVE THE DIAGNOSIS OF NEGLECTED TROPICAL DISEASES

Markus Winterberg, Joel Tarning

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

The challenge for the management of tropical diseases is often not the treatment, but the diagnosis. In many cases, patients have nonspecific symptoms like fever, headache or rash, which can have their origin in bacterial or viral infections. Although the symptoms are similar, the treatment differs between pathogens. Conventionally used diagnostic tools like serology or microscopy may deliver reliable results, but are often only possible in later stages of the infection. Time consuming cultures of the pathogen and sophisticated equipment may be required to deliver a clear identification. Examples of these difficult to differentiate diseases are typhus fevers caused by intracellular bacteria of the genus *Rickettsia* or *Orientia*, leptospirosis, melioidosis and various viral diseases like dengue and chikungunya. To simplify the diagnosis of these diseases, a rapid diagnostic test (RDT) is needed. This would provide point-of-care identification of the causative pathogen without the need for elaborate laboratory tests, allowing for a rapid and targeted treatment of the patients. The bases of such RDTs is an easily accessible, pathogen-specific biomarker. Using a proteomics approach, we screened the urine of patients with undefined fevers and patients with confirmed differential diagnosis. We were able to identify specific protein biomarkers for some of the most prevalent febrile illnesses in South-East Asia giving rise to the possibility of developing non-invasive RDTs for these diseases.

511

SEROLOGICAL INFERENCE OF PAST PRIMARY AND SECONDARY DENGUE INFECTION: IMPLICATIONS FOR VACCINATION

Ha Minh Lam[1], Huynh Thi Phuong[1], Nguyen Ha Thao Vy[1], Nguyen Thi Le Thanh[1], Pham Ngoc Dung[2], Thai Thi Ngoc Muon[3], Nguyen Van Vinh Chau[4], Isabel Rodríguez-Barraquer[5], Derek A. Cummings[6], Bridget A. Wills[1], Maciej F. Boni[7], Maia A. Rabaa[1], Hannah E. Clapham[1]

[1]*Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,* [2]*An Giang Central General Hospital, Long Xuyen, An Giang Province, Vietnam,* [3]*Quang Ngai General Hospital, Quang Ngai City, Quang Ngai Province, Vietnam,* [4]*Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam,* [5]*University of California, San Francisco, CA, United States,* [6]*University of Florida, Gainesville, FL, United States,* [7]*Pennsylvania State University, University Park, PA, United States*

Due to the finding that Dengvaxia® (the only licensed dengue vaccine to date) increases the risk of severe illness among seronegative recipients, the World Health Organisation has recommended screening individuals for serostatus prior to vaccination. To decide whether and how to carry out screening, it is necessary to estimate the transmission intensity of dengue and to understand the performance of the screening method. In this study, we inferred the annual force of infection (FOI, a measurement of transmission intensity) of dengue virus in three locations in Vietnam: An Giang (FOI=0.04 for the below-ten-years age-group and FOI=0.20 for the above-ten-years), Ho Chi Minh City (FOI=0.12), and Quang Ngai (FOI=0.05). In addition, we show that using a quantitative approach to IgG levels (measured by indirect enzyme-linked immunosorbent assays, ELISAs) can help to distinguish individuals with primary exposures (primary-seropositive) from those with secondary exposures (secondary-seropositive). We found that primary-seropositive individuals - the main targets of the vaccine - tend to have a lower IgG level, and thus a higher chance of being misclassified as seronegative, as compared to secondary-seropositive cases. Nevertheless, we demonstrated that screening performance can be improved by incorporating patient age and transmission intensity into the interpretation of IgG levels.

512

SELECTION AND VALIDATION OF LABORATORY INSTRUMENTATION TO SUPPORT CLINICAL RESEARCH INVOLVING HIGH-CONSEQUENCE PATHOGENS DURING OUTBREAKS

Willy Kayondo[1], Sharon Kagabane[1], Sharon Atukunda[1], Lydia Tumubeere[1], Daniel Kibombo[2], Brenda Kusiima[2], Joseph Wandege[1], Prossy Naluyima[1], Karen Martins[3], Chi Ritchie[3]

[1]*Makerere University Walter Reeed Project Uganda, Fort Portal, Uganda,* [2]*Infectious Diseases Instititute Uganda, Fort Portal, Uganda,* [3]*US Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, United States*

The 2014-2016 Ebola outbreak in West Africa exposed the dire need for medical interventions for high-consequence pathogens. The Joint Mobile Emerging Infectious Disease Intervention Clinical Capability (JMEDICC) was established in Fort Portal, Western Uganda, with an objective to train a local team ready to conduct clinical research of Investigational Drug Interventions in an outbreak setting. As part of the laboratory capability development, instruments were selected based on the following: size, to enable appropriate biocontainment as well as movement to remote sites; availability of consumables; and US FDA approval status so as to capitalize on the minimized validation requirements and maximize reliability. Good clinical laboratory practices (GCLP) were introduced and instruments were validated at the study hub site in Fort Portal by assessing accuracy, precision (within day and between day), and linearity. Instrument verification was unfamiliar to the study team, which increased the complexity of the validation process and the time required for verification. Overtime, following training and repeated execution as new instruments were brought on board, the time of method validation was greatly shortened. The team then developed a mobile verification plan to re-verify the assays and instruments after they are moved to the field. From the perspective of outbreak preparedness, strict adherence to GCLP may not be feasible in a mobile, rapid-response environment. Achieving instrument validation in an outbreak response is a challenge because a rapid response is required yet GCLP requires time-consuming validation of test methods prior to testing of study participants. Defining a minimum re-verification procedure and the samples required to execute that procedure will assist with project execution in an outbreak setting. The lessons learned from the JMEDICC laboratory team may assist other outbreak preparedness teams in establishing high quality laboratory support for high consequence pathogen clinical trials in remote settings.

513

COMBAT AND NON-COMBAT MORTALITY AMONG U.S. MILITARY ADVISORS IN SOUTHEAST ASIA: 1956-1964

David Adams, Valerie Adams

Point University, Midway, GA, United States

The French presence in Southeast Asia came to an ignominious end at the surrender of Dien Bien Phu in spring of 1954. Their defeat came despite millions of dollars of materiél—not ground troops--supplied by the United States. When asked why he refused to commit manpower to aid the French with manpower, the American President Eisenhower famously remarked that, when he looked at Southeast Asia, he saw it swallowing division after division of his troops. Prophetic words, indeed, in light of the next two decades that would do exactly that, killing more than 58,230 military personnel in all. But Eisenhower—and later Kennedy—sent several thousand erstwhile American military "advisors" well before the arrival of the Marines at Da Nang in 1965. Their job? To assist South Vietnamese troops (Army of the Republic of Vietnam) in their struggle against the communist North Vietnamese. But what of so-called American military advisors prior to 1965? Utilizing archival data from the Southeast Asia Combat Casualty File located at the U.S. National Archives, this presentation will analyze cause-specific mortality among advisory military personnel. The results will suggest that "combat" deaths, although numerous, were accompanied by substantial mortality from disease and, perhaps most importantly, non-combat causes such as suicide.

514

CARDIAC EVALUATION IN ADULTS WITH DENGUE INFECTION BY SERIAL ECHOCARDIOGRAPHY

Chayasin Mansanguan[1], Weerapong Phumratanaprapin[1], Borimas Hanboonkunupakarn[1], Sant Muangnoicharoen[1], Arun Huntrup[1], Akkapon Poolcharoen[2]

[1]*Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [2]*Medical Eepartment, Bhumibol Adulyadej Hospital RTAF, Bangkok, Thailand*

Background: Dengue viral infection has been a major health problem worldwide. The clinical spectrum of dengue infection is broad ranging from asymptomatic to dengue infections to a severe disease. Although cardiac involvement has been reported in dengue infection, the incidence and cardiac involvement are not well established. A better understanding of cardiac involvement in dengue infections is required. Methods: From Jan 2016 to Dec 2018, patients hospitalized at Bangkok Hospital for Tropical Diseases with dengue confirmed by positive NS1 antigen or positive dengue IgM. We characterized the incidence and change in cardiac function by serial echocardiography, troponin T and CK - MB level at the day of admission, day of defervescence, day of hypotension (if the patient develop hypotension) and two – weeks follow up. Results: Seventy – three patients were evaluated and 5 patients (%) presented with elevated biomarker levels. There was no difference in clinical presentation between dengue (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Cardiac involvement were found 24.65% in this present study including left ventricular systolic dysfunction 4(5.5%), transient diastolic dysfunction 3(4.1%), increased levels of at least 1 cardiac biomarker (Troponin T, CK-MB) 5(6.85%) and small pericardial effusion6(8.2%), respectively. Only one case that suspected myocarditis in DHF patient. Three patients develop DSS during admission and transfer to intensive care unit (ICU). We found that dengue hemorrhagic fever was the associate risk factor to develop cardiac involvement with clinically significant ($p < 0.006$). Conclusion: Cardiac involvement in adults with dengue infection was found 24.65% ranging from elevated cardiac biomarker, transient left ventricular systolic and diastolic dysfunction and pericardial effusion. The functional abnormality spontaneously resolved at the day of follow up without specific treatment. Myocarditis in dengue infection patients were uncommon.

515

EFFICACY AND TOLERABILITY OF MILTEFOSINE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

JeanAnne M. Ware[1], Elise M. O'Connell[1], Kawsar R. Talaat[2], Thomas B. Nutman[1], Theodore E. Nash[1]

[1]*Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [2]*Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States*

Miltefosine is an oral drug approved for the treatment of cutaneous leishmaniasis (CL), but its efficacy in varied species and its associated side effects have not been well studied. We reviewed the treatment courses of 18 patients (2 not yet assessable for cure) seen at the National Institutes of Health with CL due to the following species: *Leishmania braziliensis* (Lb; n=6); *L. aethiopica* (La; n=3); *L. panamensis* (Lp, n=2); *L. infantum (n=1)*; *L. donovani chagasi (n=1)*; *L. tropica (n=1*); *L. major* (n=1) ; and unspeciated (n=3). Among the 8 patients given miltefosine (with or without topical imiquimod) at the time of presentation, 6 (75%) healed, 1 (12.5%) failed to heal, and 1 (12.5%) was intolerant of treatment. In 8 additional patients who had been treated previously with other therapies, miltefosine resulted in 4 healed, 3 failed to heal, and 1 was intolerant of treatment. All patients who failed miltefosine were effectively treated with alternative

medications, thermal therapy or self-healed. Three of those who failed to heal despite optimal therapy (1 immunosuppressed patient with *La* and 2 with *Lb*-like organisms) were cured with combined miltefosine and Ambisome treatment. An additional patient with complicated severe disseminated CL and mucosal disease due to *Lp* responded to 56 days of miltefosine after failing liposomal amphotericin B. Overall, miltefosine resulted in cure in 62.5% (10/16), 12.5% (2/16) failed to cure, and 25% (4/16) remained unhealed. Nausea, vomiting, anorexia, and/or diarrhea occurred varyingly in all patients and frequently required decreased dosing. Two patients (11.1%) stopped drug prematurely, 8 (44%) required reduced dosing, and 6 (33%) were started on a lower dose in an attempt to prevent symptoms. Gout occurred in one patient during 2 separate miltefosine courses. Miltefosine either alone or in combination with Ambisome cured 81.3 % and was effective for most species encountered. Side effects were frequent and led to decreased dosing. Further evaluation is needed to determine the efficacy and optimal dosing of miltefosine.

516

PATIENT CENTERED MANAGEMENT OF BURULI ULCER IN CAMEROON: INTEGRATING LOCAL DIAGNOSIS, MENTAL HEALTH AND TRADITIONAL CONSIDERATIONS

Lucrece Eteki[1], Radhika Sundararajan[2], Rodrigue Ntone[1], Joel Djatche[3], Franck Wanda[4], Yves Hako[5], Jacques Minyem[6], Earnest Njih[7], Sara Eyangoh[8], Alphonse Um Boock[9], **Yap Boum**[1]

[1]*Epicentre, Yaoundé, Cameroon,* [2]*Cornell University, New York, NY, United States,* [3]*UNIPSY, Yaoundé, Cameroon,* [4]*Cires, Yaoundé, Cameroon,* [5]*HRRA, Yaoundé, Cameroon,* [6]*FAIRMED, Yaoundé, Cameroon,* [7]*CNLP²LUB, Yaoundé, Cameroon,* [8]*Centre Pasteur Cameroun, Yaoundé, Cameroon,* [9]*Fairmed, Yaoundé, Cameroon*

Early diagnosis and adherence to treatment are crucial to control chronic wounds including Buruli Ulcer (BU). However, diagnosis and treatment are frequently delayed, resulting in significant morbidity and mortality. This study sought to improve clinical diagnosis of BU through use of a clinical diagnosis score (CDS), describe the patients' mental health status, and understand current perceptions about the disease. Our mixed methods study was conducted in three BU endemic Districts in Cameroon: Akonolinga, Ayos, Bankim, where patients were enrolled at district hospitals and health centers. Health care workers (HCW) administered a CDS that is compared to Polymerase Chain Reaction (PCR) for detection of BU. BU patients also completed psychological scales (MINI and QSCPGS) and questionnaires. Current perceptions regarding BU were assessed through Focus Group Discussions (FGDs) and interviews with patients, community traditional healers and HCWs. The CDS was administered to 162 patients; 73 (45%) of them were BU patients confirmed by PCR. Positive Predictive Values (PPV) of physicians' clinical diagnosis compared to PCR was 62% without CDS, and 86% with the use of CDS. Psychometric testing (N=73) demonstrate that 36% (N=26) of patients suffered from depression and 25% (N=18) of them were suicidal. The majority (75%, N=55) of patients reported feeling of stigmatization and discrimination. Qualitative data (N=58) also showed 1) general belief in the mystical origin of the disease, 2) patients combine both traditional and biomedical treatment to heal faster and 3) traditional healers are more open to collaboration than physicians. Our preliminary data show that the CDS increases PPV of the clinical diagnosis of BU from 62% to 86%, beyond the 70% WHO recommendation for BU. Given the elevated rates of psychological pathologies among patients and the impact of local beliefs in the choice of treatment, it is crucial to implement holistic culturally competent programs that integrate accessible and effective diagnostic tools, patients' cultural beliefs, and psychological effects of BU.

517

GENE EXPRESSION ANALYSIS OF ANTI-FILARIAL ACTIVITY OF NATURAL AND SYNTHETIC SESQUITERPENE LACTONES FROM THE PLANT *NEUROLAENA LOBATA*

Lizzette Perez-Perez, Jessica Grant, Susan Haynes, Kevin M. Shea, Steven A. Williams

Smith College, Northampton, MA, United States

Lymphatic filariasis (LF) is a common vector-borne disease in many regions of the tropics and the leading cause of permanent and long-term disability worldwide. Current drugs used to treat LF are given annually for about five years due to poor activity in killing the adult worms. However, because of the prolonged administration, therapeutic resistance to current antihelminthics has become increasingly important. Therefore, there is a need to develop novel, safe and short course micro- and macrofilaricide treatments that can help accelerate the elimination and eradication of LF. Our work has focused on studying the bioactivity of natural sesquiterpene lactones, available from *Neurolaena lobata,* and synthetic analogs against the lymphatic filarial parasite *Brugia pahangi.* These analogs were created by modification of the neurolenin scaffold including esterification at the reactive secondary alcohol position. The bioactivity of natural neurolenin and the synthetic analogs was measured *in vitro* against male and female adult nematodes by adding one dose (3μg/mL) of the respective drug and then monitoring worms loss of motility as the read-out over a period of 100 hours. Interestingly, the activity of the sesquiterpene lactones varies between male and female nematodes, indicating the presence of some mechanism resulting in gender selectivity. The basis of the bioactivity of neurolenin B, one of the neurolenins from *N. lobata* to exhibit a strong activity against the motility of adult worms, was studied using RNA-seq analysis. In this study, transcription profiles were compared between drug-treated and untreated *B. pahangi* female adults. Studying the effect of neurolenin B on the transcriptome will provide us with insight into the molecular target and mechanism of action of the compound in killing adult worms. These results will provide further useful information on the impact of sesquiterpene lactones as promising alternatives for the treatment of LF and perhaps other diseases caused by parasitic nematodes.

518

ANTI-FILARIAL DRUGS INHIBIT EXTRACELLULAR VESICLE RELEASE FROM PARASITIC NEMATODES

Hannah J. Loghry, Wang Yuan, Michael J. Kimber

Iowa State University, Ames, IA, United States

Strategies to control parasitic nematode infection rely on mass administration of anthelmintic drugs in endemic areas. For Lymphatic filariasis, a disease caused by filarial nematodes, these drugs are ivermectin (IVM), albendazole (ABZ) and diethylcarbamazine (DEC). Despite their widespread use, the therapeutic mode of action of these drugs is not clear. For example, IVM binds parasite glutamate-gated chloride channels but how this molecular activity results in the rapid clearance of larval stage parasite *in vivo* is obscure. There is a clear need to better understand anti-filarial drug mechanisms of action. Extracellular vesicles (EVs) are membrane-bound vesicles secreted into the extracellular environment by eukaryotic and prokaryotic cells and are important mediators of cell-to-cell signaling. We have previously reported that filarial nematodes secrete EVs and that their cargo has immunomodulatory properties. We propose that nematode EVs are essential for parasitism and hypothesize that effective anti-filarial drugs inhibit their release. To test this hypothesis, we used Nanoparticle Tracking Analysis to quantify the effect of IVM, ABZ and DEC treatment on EV release from a panel of filarial nematode species and life stages. We included *Ascaris*, a gastrointestinal nematode, and the nicotinic agonist levamisole (LEV), for comparison. We show that effective anti-filarial drugs inhibit EV release but with marked variations in efficacy between species, sex and life stage. IVM exerted the most broadly effective inhibitory effects, followed by DEC and LEV. Where testable, females were the life stage most sensitive to drug treatment whereas males were not affected by any drug. ABZ had little effect on EV release,

except in *Brugia pahangi.* Drug effects on *Ascaris* supported those seen in filarial nematodes suggesting broadly conserved processes. Our data show release of immunomodulatory EVs by parasitic nematodes is inhibited by some anti-filarial drugs, shedding new light on the mechanism of action of these drugs and suggesting a novel phenotype in screening strategies for novel anti-filarial drugs.

519

GENOME SEQUENCES OF THE *WOLBACHIA* ENDOSYMBIONTS FROM THE FILARIAL PARASITES *MANSONELLA PERSTANS* AND *MANSONELLA OZZARDI*

Zhiru (Liz) Li[1], Amit Sinha[1], Catherine B. Poole[1], Richard D. Morgan[1], Laurence Ettwiller[1], Nathalia F. Lima[2], Marcelo U. Ferreira[2], Samuel Wanji[3], Clotilde K. Carlow[1]

[1]*New England Biolabs, Ipswich, MA, United States,* [2]*University of Sao Paulo, Sao Paulo, Brazil,* [3]*University of Buea, Cameroon, Cameroon*

Wolbachia, an alpha-proteobacterium closely related to Rickettsia, is a maternally transmitted, intracellular symbiont of arthropods and most filarial nematodes. *Wolbachia* have also been observed in *Mansonella ozzardi* and occasionally in *Mansonella perstans*, using PCR to detect 16S rDNA, *ftsZ* and *wsp* markers. These analyses have placed these *Wolbachia* into the supergroup F, while other filarial *Wolbachia* are members of supergroup C, D and J of *Wolbachia* phylogeny. The supergroup F is unique as it contains both arthropod and nematode *Wolbachia*. In the current study, we have obtained the first draft genome of the *Wolbachia* from *M. perstans* (*w*Mpe) as well as *M. ozzardi* (*w*Moz). The *Wolbachia* draft genomes are both ~ 1Mb in size. These draft genomes contain ~500 contigs, probably due to the low sequencing coverage, reflecting the low *Wolbachia* titers in *Mansonella* microfilariae. The completeness of these genomes was evaluated using BUSCO, a software that measures the fraction of single copy orthologs expected to be conserved within a taxa. The BUSCO score for *w*Moz is ~80%, similar to the scores of other *Wolbachia* with complete genome sequences, indicating the high quality of these *Wolbachia* genomes. The *w*Mpe and *w*Moz genomes show ~97% average nucleotide identity (ANI) over their entire genome, and are most similar to the *Wolbachia* present in the bed bug *Cimex lecturalius* (*w*Cle), with ANI scores ~ 93%. These results are particularly interesting, as they represent the first draft genomes from nematode-associated supergroup F *Wolbachia*. The availability of genomes sequences from *w*Mpe and *w*Moz will provide further insight into the evolution and phylogenetic relationships of these *Wolbachia*. They will also enable further biochemical, molecular and genomic studies on the symbiotic relationship, and facilitate the development of new drugs targeting *Wolbachia*.

520

DEVELOPMENT OF A QUANTITATIVE PCR ASSAY FOR THE DETECTION OF *MANSONELLA PERSTANS* IN HUMAN BLOOD

Tamara S. Thomas, Nils Pilotte, Lori J. Saunders, Steven A. Williams

Smith College, Northampton, MA, United States

Here we describe a novel, species-specific, quantitative, real-time PCR assay for the detection of the parasite *Mansonella perstans*. *M. perstans* is closely related to *Wuchereria bancrofti*, the causative agent of lymphatic filariasis in Africa, and has the potential to cause false positive results when using antigen detection-based tests. To combat this problem, we capitalized on the sensitivity and specificity of PCR-based assays to develop a new quantitative PCR (qPCR) assay for *M. perstans* based on the detection of species-specific repetitive DNA sequences using our previously published genomics/bioinformatics-based platform. *M. perstans* was sequenced using Illumina next-generation sequencing and the raw sequence reads were ported to the bioinformatic tool RepeatExplorer to screen for the most highly repeated DNA sequences. These sequences were used to design qPCR primer and probe sets to optimize the sensitivity and specificity of the qPCR assay. The primer and probe set that produced the best sensitivity and specificity was selected for further study and tested using *M. perstans* DNA, human DNA and the DNA of several related filarial parasites. Results from the real-time amplification plots showed that only the *M. perstans* DNA was amplified and that attogram levels of DNA could be detected. Following these laboratory-based studies, field samples collected in several countries in Africa will be tested and results will be compared to the standard antigen detection-based method for lymphatic filariasis (FTS strip test). One goal is to determine the proportion of LF positive tests that are due, in fact, to the presence of *M. perstans*.

521

POPULATION GENOMICS AND MOLECULAR PHYLOGENETIC RELATIONSHIPS OF *SIMULIUM* VECTORS OF ONCHOCERCIASIS IN GHANA: LARGE INTERBREEDING SYMPATRIC POPULATIONS AND LARGE TRANSMISSION ZONES

Warwick Grant, Ernest Gyan, Shannon Hedtke

La Trobe University, Bundoora, Australia

Onchocerca volvulus, the causative agent of onchocerciasis, is transmitted by anthropophilic flies of the genus *Simulium*. Taxonomic identification of African *Simulium* vectors is challenging, relying on a combination of cytogenetics derived from banding patterns of polytene chromosomes in the salivary glands of aquatic larvae and/or on a suite of overlapping morphometric characters. Several attempts have been made to construct molecular phylogenies of the *Simulium* vectors of *O. volvulus* but in general there has been poor concordance between the molecular phylogenies and the cytogenetic/morphometric phylogenies. The low level of polymorphism observed in the mitochondrial barcode amplicons that have been used is the most likely cause of the poor resolution achieved to date using molecular methods. We show here that relatively low depth whole genome sequencing of single flies yields sufficient data to reconstruct the entire mitochondrial genome of individuals at >50X depth, and a nuclear genome that is >90% complete by CEGMA and BUSCO criteria. We have constructed molecular phylogenies using (a) conventional, relatively short barcode amplicons (b) a single amplicon spanning >3kb of the mitochondrial genome and (c) whole mitochondrial genomes of >60 flies reconstructed from low-depth whole genome sequences, and (d) partial CEGMA and BUSCO genes. The flies were collected from 3 river basins across an approximately 250km east-west transect in central Ghana (the "transition zone" between forest and savannah) during the dry season. Even with whole mitochondrial genome sequence, there was no evidence of significant population structure between river basins. This implies a history of frequent long-range fly movement and is consistent with a parallel lack of population structure in parasites sampled from the same locations. Phylogenetic analysis also suggested either the flies are most likely a single species or an unusual case of sympatric species between whom there is still gene flow.

522

EFFECT OF DIFFERENT ALBENDAZOLE-BASED TREATMENT REGIMENS ON *LOA LOA* MICROFILARAEMIA IN AN ENDEMIC REGION OF GABON PRELIMINARY RESULTS OF AN OPEN-LABEL RANDOMIZED CONTROLLED CLINICAL TRIAL

Rella Zoleko-Manego[1], Ghyslain Mombo-Ngoma[1], Ruth Kreuzmair[2], Wilfrid Ndoumba[1], Michael Ramharter[3]

[1]*CERMEL, Lambarene, Gabon,* [2]*Institute of Tropical Medicine, University of Tuebingen and German Centre for Infectious Diseases (DZIF), Tuebingen, Germany,* [3]*Bernhard Nocht Institute for Tropical Medicine, World Health Organization Collaborating Centre for Arbovirus and Hemorrhagic Fever Reference and Research, Hambourg, Germany*

Loiasis is a parasitic infection caused by the filarial worm *Loa loa*. Despite its wide range of clinical symptoms, loiasis is not even considered a neglected tropical disease and its importance was only highlighted when the implementation of mass drug administration programs for lymphatic filariasis and onchocerciasis had to be stopped in Loa Loa co-

endemic areas. However, with more than 10 Mio people affected, there is a need for a safe and effective treatment of loiasis. Currently, there is no sufficiently safe treatment option for mass drug administration for the interruption of *L. loa* transmission available. Potent drugs such as ivermectin or diethylcarbamazine have led to severe post-treatment adverse events in patients with high *Loa Loa* microfilaremia. While albendazole treatments seem to be safe, it is unclear if this leads to complete suppression of microfilaremia. Here we present an open label clinical trial evaluating different albendazole based regimen alone or in combination with ivermectin for loiasis in Gabon. Forty-two adults of both sexes with initial filarial count between 7000-50000 were randomized in four arms and followed up to six months. Arm 1 (6 subjects) untreated controls; arm 2 (12 subjects): albendazole 400 mg twice daily for 21 days; arm 3 (12 subjects): albendazole 400 mg twice daily for 21 days followed by additional albendazole 400 mg twice daily for 14 days; arm 4 (12 subjects): albendazole 400 mg twice daily for 21 days followed by a single dose of 150 μg/kg of ivermectin. L. loa microfilaraemia was measured before each treatment and twice weekly for three weeks and then weekly follow-up visits took place from week 4 through week 8; thereafter, twice monthly visits took place until the end of follow-up. Post treatment adverse events were similar in the three treatment arms, one adverse event recorded was considered probably treatment related. In all treatment arms, microfilarial levels decreased significantly ($p<0.05$) following the first round of albendazole. More detailed results of the respective treatment arms will be presented at the congress.

523

POTENTIAL CROSS REACTIVITY OF *MANSONELLA PERSTANS* WITH *WUCHERERIA BANCROFTI* BY FILARIASIS TEST STRIPS

Yaya Ibrahim Coulibaly[1], Lamine Soumaoro[1], Benoit Dembele[2], **Mary Hodges**[3], Yaobi Zhang[4]

[1]*International Center for Excellence in Research, Bamako, Mali,* [2]*Helen Keller International, Bamako, Mali,* [3]*Helen Keller International, Freetown, Sierra Leone,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Lymphatic filariasis is considered endemic where mapping using Immunochromatographic test (ICT) cards or night blood films find antigenemia or microfilaria (mf) prevalence of *Wuchereria bancrofti* to be ≥1% in persons over 15 years of age. Elimination is considered achievable by annual mass drug distribution (MDA) with ivermectin and albendazole for a minimum of 5 years. In recent years, Filariasis Test Strips (FTS) have been used to replace ICT cards to detect *W. bancrofti* antigen with the advantage of being cheaper than ICT, with greater sensitivity and simplicity than mf (day versus midnight sampling). However, concerns over the specificity of ICT or FTS were raised where *Loa loa* or *Mansonella perstans* co-exists. In Mali, stored day and night blood samples from a study on the impact of high dose of albendazole and ivermectin on *W. bancrofti* mf clearance supported by NIH were examined by microscopy and by Trop-Bio ELISA for *W. bancrofti* in Bamako. Three thick smear slides were made per sample, and microfilaremia positives for either *W. bancrofti* or *M. perstans* were confirmed by another technician. Ninety (90) confirmed positives samples for *W. bancrofti* and/or *M. perstans* were subsequently selected and tested by FTS: 30 samples with *W. bancrofti* positive only (Wb+Mp-), 30 samples with *M. perstans* positive only (Wb-Mp+, Wb- confirmed by microscopy and ELISA/ICT), and 30 samples with both positive (Wb+Mp+). The results showed that 25/30 Wb+Mp- samples and 21/30 Wb+Mp+ samples were FTS positive. However, 3/30 Wb-Mp+ samples were also FTS positive. Although the sample sizes tested were relatively small, it highlights potential cross reactivity between *W. bancrofti* and *M. perstans* antigens by FTS. *M. perstans* infection is often asymptomatic, but reported manifestations include transient subcutaneous swellings, urticaria, pruritus, arthralgia, abdominal pain, and fatigue. The presence of this parasite may have significant implication in assessing LF MDA impact by FTS in areas where both parasites co-exist, such as in Sierra Leone. Further research is needed to clarify this.

524

EVALUATION OF THE THERAPEUTIC COVERAGE OF MASS TREATMENT CAMPAIGN AGAINST LYMPHATIC FILARIASIS IN A CONTEXT OF PERSISTENT TRANSMISSION OF THE DISEASE

Roland Bougma[1], **Mamadou Serme**[1], Christophe Nassa[1], Micheline Ouedraogo[2], Appolinaire Kima[1], Clarisse Bougouma[1], Dieudonné Nare[2], Jean-Paul Djiatsa[2], Fanny Yago-Wienne[2], Amy Veinoglou[3], Yaobi Zhang[4]

[1]*NTD Control Program, Ministry of Health, Ouagadougou, Burkina Faso,* [2]*Helen Keller International, Ouagadougou, Burkina Faso,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Burkina Faso has been implementing preventive chemotherapy against lymphatic filariasis since 2001. While 61 health districts (HDs) have stopped mass drug administration (MDA), transmission persists in nine HDs despite good MDA coverage reported. To validate the reported coverage, an independent post-MDA coverage survey was conducted in Tenkodogo and Fada N'Gourma HDs in September 2018. The study population consisted of all persons in the communities. The Coverage Survey Builder (CSB) tool was used to calculate the sample size and to conduct the random selection of households. A total of 30 villages per HD were selected. The investigators were Ministry of Education agents and health workers not involved in MDA. Data were collected on smartphones through the KoBo Collect application on age, sex, ingestion of the drugs (ivermectin + albendazole), adverse events, and appreciation of MDA and strategies. Stata Version 14 and QGIS 3.4 were used for data analysis. A total of 3,741 individuals were surveyed, 53.3% were female and the average age was 20.4 years. Surveyed epidemiological coverage was 73.9% [95% CI: 72.6-76.5] in Fada N'Gourma and 79.3% [95% CI: 77.8-81.5] in Tenkodogo, compared to reported coverages of 82.6% and 82.0%, respectively. Village-level coverage ranged from 32.9% to 100% in Fada N'Gourma and from 56.7% to 93.3% in Tenkodogo. In total, 99% of those treated said they had swallowed the drugs in front of the community drug distributor (CDD) and confirmed the use of dose poles. The main reasons for non-treatment were CDD did not come (54%) and absence during MDA (43%). A total of 98 (0.34%) people reported developing adverse events including breathing difficulties, drowsiness, diarrhea and nausea. Results showed that surveyed coverage was lower than reported coverage in both HDs, yet both were above the 65% threshold recommended by WHO. However, major variations among villages raised alarm for attention. Directly observed treatment appeared to have been well respected. The main challenges to increase coverage will be the systematic revisit of households with absentees and the targeting of all households in each village.

525

STANDARDIZATION OF DIAGNOSTIC METHODS FOR THE DETECTION OF MICROFILARIAEMIA IN BLOOD FOR LYMPHATIC FILARIASIS: A REVIEW AND META-ANALYSIS

Natalie Vivian Vinkeles Melchers, Luc E. Coffeng, Sake J. de Vlas, Wilma A. Stolk

Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

Active lymphatic filariasis (LF) infection is generally diagnosed through parasitological identification of microfilariae (mf) in the blood. Blood samples can be tested through various diagnostic techniques. We have derived transformation factors to standardise mf prevalences as measured by a wide variety of diagnostic tests, blood volumes, and time of sampling (day versus night blood). This way, we can express any mf prevalence as obtained by the historically most commonly used night thick blood smears (TBS) in 20 μL blood volume. We first performed a systematic review to identify studies that reported on comparative mf prevalence data as measured by more than one diagnostic test for the same study population. Results from different diagnostic tests were then related to mf prevalences as obtained by TBS 20 μL/blood using a meta-regression

model based on odds-ratios (OR). Resulting ORs were translated to transformation factors as a function of prevalence measured by the more sensitive diagnostic tool. We identified 607 articles matching our search strategy and included 17 surveys, all conducted in the period 1971-1998. The mf prevalences as measured by the more sensitive nuclepore filtration (1 mL/blood) should be multiplied with a range in transformation factors of 0.45-0.69, depending on the transmission setting (here 10% and 40% mf prevalence as measured by TBS 20 μL respectively), to standardise to mf prevalences as obtained by TBS in 20 μL blood. For TBS in >40 μL/blood this corresponding range of transformation factors was 0.59-0.82; for Knott's technique 0.63-0.83; and for counting chamber (≥50 μL/blood) it was 0.67-0.87. Our results allow comparison of LF survey data through mf prevalence standardisation to reflect mf prevalences as detected by TBS 20 μL blood volumes. This will facilitate use of more datasets in e.g. meta-analyses and geographic mapping initiatives.

526

CYTOKINE SIGNATURES ASSOCIATED WITH MICROFILARIA CLEARANCE FOLLOWING SINGLE DOSE OF IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE THERAPY FOR LYMPHATIC FILARIASIS IN COTE D'IVOIRE

Charlene Aya Yoboue[1], Sarah Frischmann[2], Claudia Daubenberger[1], Juerg Utzinger[1], Benjamin Guibehi Koudou[3], Christopher Lee King[2]

[1]*Swiss and Tropical Public Health, Basel, Switzerland,* [2]*Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States,* [3]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Univerité Nangui Abrogoua, Abidjan, Côte D'Ivoire*

The combination of Albendazole, Ivermectin and Diethylcarbamazine (IDA) constitutes the new therapy against Lymphatic Filariasis for mass drug administration outside sub-Saharan African. A single dose of IDA demonstrated sustained clearance in ~95% of individuals for up 3 years in Papua New Guinea and similar efficacy was observed at 1 year in other areas (Indonesia, Haiti). However, initial results of IDA in Africa showed less success; the case of Côte d'Ivoire with 50% of microfilariae clearance 24 months post treatment. This failure sustain Mf clearance may be due to reinfection, regional differences in drug absorption and metabolism, less susceptibility of parasite to the drugs, or immune suppression by chronic infection. It is well established that host immune response is required for IDA to work effectively *in vivo*. Here we examined the hypothesis that individuals that showed greater immune modulation by increased production of immunoregulatory cytokines (e.g. IL-10 and TGF-β), and reduced production of pro-inflammatory cytokines (e.g. IFNg, TNF, IL-17) prior to treatment and 24 hours following treatment would be associated with impaired clearance of Mf 1 year after treatment. We examined both ex vivo, BMA and mitogen induce cytokine release in whole blood from participants who cleared Mf (N=15) and those that did not (N=6) 1 year following IDA administration because re-infection is unlikely in 1 year. We examined both plasma cytokines supernatants (IL-2, IL-13, IL4, IL-5, IL-10, IL12p70, GMCSF, IFNg and TNFa) from whole blood at 16 and 72 hours in cultures. Our initial results failed to show significant differences in plasma cytokine levels in culture supernatant before or after treatment with exception of TNFa which was consistently higher plasma and culture supernatants from individuals that cleared Mf compared to those that did not (P=0.02 to <0.003). Thus, these preliminary results failed to show clear evidence of immune suppression by individuals who failed to clear Mf at 12 months, but further assays are underway examining the frequency of antigen-specific lymphocytes, antibody levels and immune responses at later time points.

527

ASSESSING THE IMMUNOGENICITY OF ADJUVANT-ANTIGEN FORMULATIONS IN A NATURAL BOVINE - *ONCHOCERCA OCHENGI* INFECTION MODEL FOR HUMAN ONCHOCERCIASIS

John Graham-Brown[1], Lisa Luu[1], Catherine Hartley[1], Bin Zhan[2], Maria-Elena Bottazzi[2], David Abraham[3], Nikolai Petrovsky[4], Nicholas Bayang[5], Germanus Bah[5], Vincent Tanya[5], Sara Lustigman[6], Benjamin Makepeace[1]

[1]*Infection and Global Health, Liverpool, United Kingdom,* [2]*Baylor College of Medicine, Houston, TX, United States,* [3]*Thomas Jefferson University, Philadelphia, PA, United States,* [4]*Flinders University, Adelaide, Australia,* [5]*L'Institut de Recherche Agricole Pour le Développement, Yaounde, Cameroon,* [6]*New York Blood Center, New York, NY, United States*

Human onchocerciasis (river blindness) is a neglected tropical disease affecting 15.5 million people in sub-Saharan Africa. The first-stage larvae (microfilariae) of the filarial worm, *Onchocerca volvulus*, cause severe dermatitis and keratitis, with the latter leading to irreversible blindness. In Africa, efforts to eliminate *O. volvulus* via mass administration of the microfilaricidal drug ivermectin over 30 years has seen a 31% reduction in prevalence. Disease elimination is complicated by both the emergence of ivermectin-resistant *O. volvulus* and the restricted use of this drug in areas where *O. volvulus* and another filarial nematode, *Loa loa,* are co-endemic. Development of new control methods, including prophylactic vaccines, are therefore indicated. To determine optimal formulation for vaccines in a bovine - *Onchocerca ochengi* natural infection model for *O. volvulus*, twelve immunologically naïve calves were split into four groups of three. Three groups were immunised with recombinant versions of the *O. volvulus*-derived antigens RAL-2 and 103 formulated with one of three adjuvants: Alum, Advax-2 or Montanide ISA 206VG. Immunisations consisted of a 500 μg primary and 250 μg 4 week booster dose of each antigen administered separately via intra-muscular injection. The fourth group served as an unvaccinated control. Weekly serum collection to measure antibody responses revealed detectable levels of IgG and IgE against both RAL-2 and 103 antigens in all immunised animals by week 6 post-primary immunisation. Additional results comparing the humoral and cellular responses to the different adjuvant formulations and unvaccinated controls will be presented, with discussion on how these results have informed formulation for a full-scale bovine - *O. ochengi* vaccine efficacy trial.

528

IMMUNE ACTIVATION IN PATIENTS WITH FILARIAL LYMPHEDEMA BEFORE AND AFTER TREATMENT WITH DOXYCYCLINE

Inge Kroidl[1], Anja Feichtner[1], Sacha Horn[1], Upendo Mwingira[2], Abdallah Ngenya[3], Godfrey Chotta[4], Ute Klarmann-Schulz[5], Janina Kuehlwein[5], Achim Hoerauf[5], Jubin Osei-Mensah[6], Linda Batsa Debrah[6], Alexander Y. Debrah[6]

[1]*Medical Center of the University of Munich (LMU), Munich, Germany,* [2]*National Institute for Medical Research, Dar es Saalam, United Republic of Tanzania,* [3]*National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania,* [4]*Sokoine Regional Hospital, Lindi, United Republic of Tanzania,* [5]*Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany,* [6]*Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana*

Lymphatic filariasis is a mosquito-transmitted helminth infection caused by *Wuchereria bancrofti* and *Brugia* spp., characterized by lymphangitis, lymphedema (LE) and hydrocele. Disabling pathology affects about 30% of the 68 million infected people worldwide. Former studies have shown improvement or halt of progression of filarial lymphedema after treatment with Doxycycline (DOX) 200mg/d for 6 weeks. Double-blind, randomized, placebo-controlled trials (TAKeOFF-LEDoxy) are being conducted in Ghana and Tanzania to confirm the effect of DOX 200mg/d and to test the effect of a dose reduction to DOX 100mg/d for 6 weeks on filarial LE. Potential changes in pathology will be recorded at several time points

over a 24-month period. In addition we address immunological aspects both before and after treatment with DOX or placebo. Enrolment is still ongoing, however the first baseline, 6 weeks and 6 month data are available. Peripheral blood mononuclear cells were characterized for the presence of memory CD4 or CD8 T cells (CD45, CD27), regulatory CD4 T cells (FoxP3, CD25), transcription factors for TH1 and TH17 cells (Tbet, RORyt) and immune activation markers (CD38, HLADR). When comparing individual baseline and 6 weeks data in the first analyzed 43 participants, we saw stable values for various T cell subgroups (central and effector memory CD4+ T cells, regulatory T cells). However, when looking at a immune activation parameter there was a significant decline for HLADR+, p =0.03 and HLADR+/CD38+ CD4 T cells, p=0.0015, (median 7.15% before and 6.1% after Doxycycline treatment, relative reduction of 14.7%) between pre- and post-treatment paired values. Since these are double blinded studies, clear conclusions can only be drawn after unblinding of the study participants, which will be done 24 months after treatment onset. However, as two thirds of the study group were treated with DOX, we anticipate that the measured decline in immune activation parameters might be a result of the larger group of DOX treated participants.

529

DEVELOPMENT OF A TWO-STEP FLOW CYTOMETRY METHOD FOR A FIELD SETTING USING WHOLE BLOOD FOR THE DESCRIPTION OF PHENOTYPIC MARKERS ON PERIPHERAL BLOOD CELLS

Sacha Horn, Mohamed I. Ahmed, Christof Geldmacher, Michael Hölscher, Inge Kroidl

Medical Center of the University of Munich (LMU), Munich, Germany

Neglected tropical diseases are often found in remote areas. In resource poor settings, research activities are limited because local laboratories lack equipment or trained personnel. For immunological evaluations, usually a flow cytometer is needed, if possible with 2 or 3 lasers, which is not available in many areas. To circumvent the problem, peripheral blood mononuclear cells can be processed and preserved; measurements can then be later performed in specialized laboratories. However, this usually requires a larger blood draw which is not possible in small children and not agreed to by many adults. We have developed and optimized a method for measuring up to 9 phenotypic markers using 100 µl of blood, which can potentially be taken via finger prick. Blood was collected with EDTA or heparin collection tubes. Extracellular antibodies (b7-PE, CD4-PerCpC5.5, HLADR-PeCy7, CD38-APC, CD27-APC-H7, CD45RA-Bv421, CCR5-APC, CD8-V500, CD25-Bv605) were added and after a 30 minute incubation time, cells were lysed, frozen and stored in liquid nitrogen for transport and further processing. After a period of up to 6 months, cells were thawed, permeabilized, and stained with intracellular antibodies (CD3-ECD, FoxP3-FITC, Tbet-PE, Eomes-PeCy7) before imaging on the Cytoflex and analyzing with FlowJo software. Initial testing showed highly comparable results between freshly drawn and cryopreserved samples. This method has now been performed in two studies, one conducted in Ghana and one in Tanzania, focusing on the immunology of individuals infected with *Wuchereria bancrofti.* Over 200 participant samples have been analyzed with promising results. The participant samples that were preserved for up to 6 months before analysis are comparable to those that were freshly processed and analyzed. Due to the minimal amount of blood required and the robustness, we believe that the method is useful for both evaluating immunological parameters in children and field studies in remote settings.

530

IMPROVED PERFORMANCE OF A SEROLOGY RAPID DIAGNOSTIC TEST FOR ONCHOCERCIASIS BY USING DRIED BLOOD SPOTS

Guilherme Maerschner Ogawa[1], Allison Golden[2], Jui A. Bhingarde[3], Andreas Nshala[4], Eugene Liu[1], Austin Newsam[1], Ryan Wiegand[1], Paul Cantey[1], Vitaliano Cama[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*PATH, Seattle, WA, United States,* [3]*Emory, Atlanta, GA, United States,* [4]*Tanzania Onchocerciasis Program, Dar el Salam, United Republic of Tanzania*

The SD Bioline™ Onchocerciasis IgG_4 Rapid Test (RDT) was developed to facilitate serological assessments for onchocerciasis. It has not been used as widely as expected due to concerns about sensitivity in low prevalence areas compared to ELISA. To determine if RDT sensitivity could be boosted, we compared the performance of the RDT using whole blood (RDT-WB) to performance using blood eluted from dried blood spots (RDT-DBS) in the CDC laboratory. We evaluated samples from Tanzania (n=931) and Togo (n=999), where transmission had been suppressed after >15 years of ivermectin treatment. Sampling occurred across 5 predefined age groups in villages close to *Simulium* breeding sites. Data were analyzed for agreement with OV-16 ELISA. Overall seroprevalence rates by RDT-WB, RDT-DBS and ELISA were 4%, 8.5% and 8.6% respectively for the specimens from Tanzania. Positive and negative agreement with OV-16 ELISA were 40% and 98.9% for RDT-WB and 75% and 97.5% for RDT-DBS, respectively. Among the specimens from Togo, sero-prevalence rates for RDT-WB, RDT-DBS and ELISA were 2.5%, 5.3% and 8.8% respectively. Positive and negative agreement with OV-16 ELISA were 28.4% and 99.9% for RDT-WB, and 55.7% and 99.6% for RDT-DBS. Using eluted DBS resulted in improved agreement between RDT and ELISA, suggesting improved sensitivity, though the magnitude was not predictable. Using eluted DBS may produce results from RDTs that are similar to those obtained by ELISA. Further evaluation of RDT sensitivity using DBS might facilitate RDT use in low prevalence settings (e.g. for mapping hypoendemic zones).

531

DEVELOPMENT OF AN EXPERIMENTAL NEUROTOXOCARIASIS IN A PORCINE MODEL

Luis A. Gomez-Puerta[1], Katherine Robles[1], Ana Vargas-Calla[1], Gianfranco Arroyo[2], Armando Gonzalez[1], Hector H. Garcia[2], Alessandra Nicoletti[3]

[1]*Universidad Nacional Mayor de San Marcos, Lima, Peru,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Universita' Degli Stdudi di Catania, Catania, Italy*

Human toxocariasis is a parasitic zoonosis caused by larval stages of *Toxocara canis*, a common parasite of dogs, and probably also by *Toxocara cati*, a parasite of cats. *Toxocara* larvae can cross the blood-brain barrier, invade the central nervous system and produce neurotoxocariasis. However, the frequency and clinical significance of neurotoxocariasis remains unknown until now. In the present study cerebral toxocariasis was produced in a porcine model by intraperitoneal inoculation of *T. canis* larvae. Ten pigs of 8 weeks of age from a technified farm were used. *T. canis* eggs were collected directly from the uterus of adult nematodes obtained from naturally infected dogs. The eggs were placed in a 2.5% potassium dichromate solution for 30 days until the larvae developed. Then, egg hatching was induced and the infectivity of the larvae was evaluated in 20 Balb/C mice. Mice were inoculated intraperitoneally with 500 larvae, and toxocariasis was confirmed at 5 days post infection (PI). After this, all pigs were infected intraperitoneally with 3000 larvae. Pigs had brain magnetic resonance imaging exams taken immediately before necropsy, at days 7 (two pigs), 12 (two pigs), 19 (three pigs) and 49 (three pigs). Neurotoxocariasis development in pigs was confirmed by presence of larvae in brain tissue. No abnormalities were detected by magnetic resonance imaging. While no larvae were detected on microscopic

examination in the pigs sacrificed at day 7 post infection, one pig from day 12 was infected with three larvae in the brain (one in the right posterior lobe and two in the right anterior lobe). All three pigs from day 19 had neurotoxocariasis with 1, 3 and 9 larvae, respectively. Finally, one pig from day 47 was infected with 1 larva in the right posterior lobe. Intraperitoneal infection of *Toxocara* larvae results in neurotoxocariasis in the pig model. Unfortunately, magnetic resonance is not sensitive to detect *Toxocara* larval infection in the brain of pigs.

532

RWANDA NEGLECTED TROPICAL DISEASE CONTROL PROGRAM: WHAT IS THE IMPACT OF DEWORMING OF SCHOOL-AGE CHILDREN 6 YEARS AFTER IT STARTED?

Eugene Ruberanziza[1], Denise Mupfasoni[2], Aimable Mbituyumuremyi[1], Jamie Tallant[3], Jean Bosco Mbonigaba[1], Ursin Bayisenge[1], Michee Kabera S.[1], Innocent Turate[1]

[1]*Rwanda Biomedical Center/Ministry of Health, Kigali, Rwanda,* [2]*World Health Organization, Geneva, Switzerland,* [3]*The END Fund, New York, NY, United States*

Soil–transmitted helminth (STH) infections are among the most important Neglected Tropical Diseases (NTDs) that affect a high number of population in 102 tropical countries. Rwanda launched the NTD control program in 2007 with initial national mapping survey completed in 2008, followed by bi-annual preventive chemotherapy (PC) targeting pre-school and school-age children (pre-SAC, SAC). From 2008 to 2014, nine rounds of PC for pre-SAC and eight rounds for SAC were implemented at national level. In line of evaluating the morbidity of STH infection after 6 years of PC, a national morbidity assessment survey was conducted in 2014. STH data were collected from 186 schools and 9,252 pupils. Stool samples were collected and analysed using Kato-Katz method. The overall prevalence of STH decreased from 65.8% in 2008 to 45.2% in 2014. Hookworms and *T. trichiura* prevalence decreased from 31.7% to 4.5% and from 27.0% to 22.8% respectively while *A. lumbricoides* did not decrease much. The proportion of moderate or heavy intensity of STH infection increased from 11.5% in 2008 to 12.2% in 2014 but the difference was not statistically significant. The main species causing the morbidity were *A. lumbricoides* while *T. trichiura and* hookworm intensity were mostly light across the districts. Northern and Western provinces bearded most of the burden in 2008 and remained the more severely affected in 2014. These settings were also the ones showing a high proportion of moderate or heavy intensity of STH infections. In conclusion, the present analysis suggests to adapt the control strategy to the different epidemiological situations: i) in areas where STH prevalence was not reduced, PC can be extended to all at-risk groups which include pre-SAC, SAC, women of reproductive age and adults at high risk occupation, and if resources are available PC every 3 to 4 months can be considered; ii) in areas where the present intervention reduced the STH prevalence but transmission is still present the intervention could be maintained; iii) in areas in which STH prevalence is very low, the frequency of intervention can be reduced.

533

HELMINTH MEDIATED MODULATION OF THE SYSTEMIC AND MYCOBACTERIAL ANTIGEN - STIMULATED CYTOKINE PROFILES IN EXTRA-PULMONARY TUBERCULOSIS

Gokul Raj Kathamuthu[1], Saravanan Munisankar[1], Baskaran Dhanaraj[2], Subash Babu[1]

[1]*NIH-ICER-NIRT, Chennai, India,* [2]*NIRT, Chennai, India*

Helminth infections are known to regulate cytokine responses in both pulmonary and latent tuberculosis infection. Whether helminth infections also modulate cytokine responses in extra-pulmonary tuberculosis, specifically tuberculous lymphadenitis (TBL), has not been studied thus far. Hence, our study examines the cytokine profile in helminth-TBL co-infection. We measured the systemic and mycobacterial-antigen stimulated levels of Type 1, Type 2, Type 17, regulatory and pro-inflammatory cytokines in TBL individuals coinfected with (n=44) or without (n=44) *Strongyloides stercoralis*(Ss) infection by multiplex ELISA. Chi-square and Mann-Whitney U test were used to measure the significance. TBL-Ss+ individuals have significantly higher bacterial burdens in the affected lymph nodes in comparison to TBL-Ss- individuals. TBL-Ss+ individuals exhibit significantly enhanced plasma levels of Type 2 (IL-5 and IL-13), Type 17 (IL-17 and IL-22) and regulatory (IL-10) cytokines in comparison to TBL-Ss- individuals. In contrast, TBL-Ss+ individuals exhibit significantly diminished plasma levels of pro-inflammatory cytokines (IL-1$\alpha$and GM-CSF) in comparison to TBL-Ss- individuals. TBL-Ss+ individuals also exhibit significantly diminished unstimulated or mycobacterial - antigen stimulated levels of Type 1 (TNFα), Type 17 (IL-17 and IL-22) or pro-inflammatory (IL-1$\alpha$and IL-1β) cytokines in comparison to TBL-Ss- individuals but no differences seen in mitogen stimulated cytokine levels. In contrast to the cytokine profile in plasma and peripheral blood, helminth coinfection had no significant impact on the unstimulated or mycobacterial - antigen stimulated expression of Type 1, Type 2, Type 17, regulatory and pro-inflammatory cytokines at the site of infection i.e. the affected lymph nodes in TBL individuals. Therefore, our data reveal a profound influence of Ss infection on the bacteriological profile of TBL and suggest that the underlying modulation of cytokine responses might be a mechanism by which this helminth infection could impart a detrimental effect on the pathogenesis of TBL disease.

534

THE IMPACT OF FIFTEEN YEARS OF IVERMECTIN AND ALBENDAZOLE TREATMENT FOR THE CONTROL OF LYMPHATIC FILARIASIS ON SOIL-TRANSMITTED HELMINTHS

Dziedzom K. de Souza[1], Edward Dumashie[1], Joseph Otchere[1], Collins S. Ahorlu[2], Irene Ayi[1]

[1]*Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana,* [2]*Department of Epidemiology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana*

Ivermectin and albendazole, the drugs used for lymphatic filariasis (LF) control in areas co-endemic for onchocerciasis, also have an impact on soil-transmitted helminths (STH). However, the importance of this on achieving the 2025 STH goals has not been effectively assessed. In the Western Region of Ghana, yearly mass treatment of entire communities for LF control has been on-going since the year 2000. This presents the opportunity to assess the impact of ivermectin and albendazole treatment on STH. In this study, 1042 participants from 18 communities were assessed for STH infections using the Kato-Katz method. Infections were observed in individuals 5 to 80 years old (mean 34.1 ± 18.8). The community mean egg count for Hookworms, *Ascaris lumbricoides*, *Trichuris trichiura* and *Hymenolepis nana* were 1.64 eggs per gram (epg) (CI: -0.09 - 3.38; range: 120 - 720 epg; n= 5), 119.3 epg (CI: 40.25 - 198.3; range: 24 - 29676 epg; n = 36), 197.9 epg (CI: -47.38 - 443.1; range: 12 - 123396 epg; n = 56) and 0.01 epg (CI: -0.01 - 0.03; egg count of 12 1 epg; n = 1), respectively. Majority of infections were reported in females, with 83%, 54% and 62% for Hookworm, *A. lumbricoides* and *T. trichiura*, respectively. The analysis of variance revealed the results of *A. lumbricoides* and *T. trichiura* infections were significant ($P < 0.0001$). These results point to a very low infection prevalence for *H. nana* (0.1%) and hookworm (0.5%), and not so much impact on *A. lumbricoides* (3.5%) and *T. trichiura* infections (5.4%). The results suggest that while ivermectin and albendazole may have an impact on Hookworms, other strategies will need to be adopted for the control of other STH.

535

STAKEHOLDER SURVEY ON 2030 WORLD HEALTH ORGANIZATION TARGETS FOR SOIL-TRANSMITTED HELMINTHIASIS

Jasmine L. Irish, Girija Sankar, Michael R. Diaz, Sanjaya Dhakal, Alexander H. Jones, Rubina Imtiaz

Children Without Worms, Decatur, GA, United States

The World Health Organization (WHO) sets global monitoring targets for soil-transmitted helminthiasis (STH) control. Existing targets intend to measure progress toward the goal of eliminating STH as a public health problem by 2020. As 2020 approaches, it is apparent that the goal will not be met. WHO has drafted 2030 targets for STH in conjunction with the 2018 STH Advisory Committee (STHAC) in October 2018. From November 9 to December 15, 2018, we surveyed STH stakeholders using a commercial online survey platform for data collection. Data were collected on proposed 2030 targets and the need for updating existing WHO guidance. The response rate for this survey was 17% (34/202), low because of the wide variance in targeted respondents' engagement in STH activities. Responders represented non-governmental development organizations (44%), researchers (20%), donors (9%), NTD Program implementing partners (9%), National NTD/STH programs (6%), and others (12%). Overall, respondents (52%) agreed with the proposed STH target of elimination of morbidity by 2030, but a majority (59%) expressed concern around the lack of a clear definition of "elimination of morbidity" within the target. Respondents were given space to provide text feedback on each of the targets. The 73 total comments collected were grouped into four categories: feasibility of target (37%); unclear definitions (37%), needs more evidence (16%), and generally positive (10%). Respondents (41%) indicated that changes were needed in the deworming guidance that is available to country programs. The majority (59%) indicated their satisfaction with the "timeliness and content of policy and guidance" issued by WHO. Respondents also indicated the need for additional clear and consistent guidance, including a clear and measurable definition of "elimination of STH morbidity". Of particular note in the responses is the expressed (30%) need for changes to guidance and forms to include deworming for women of reproductive age as an additional risk group beyond 2020. The survey results were shared with WHO to help inform the 2030 targets finalization process by 2020.

536

DEVELOPING NEW THERAPIES FOR SOIL TRANSMITTED NEMATODE INFECTIONS

David M. Gazzola, Hanchen Li, Ambily Abraham, Yan Hu, Kelly Flanagan, You-Mie Kim, Anand Sitaram, Tasia Kellogg, Florentina Rus, Martin Nielsen, Anne Zajac, Joe Urban, Jennifer Ketzes, Gary Ostroff, Raffi V. Aroian

University of Massachusetts Medical School, Worcester, MA, United States

Soil-transmitted nematodes (STNs), most notably, hookworms, whipworms, and *Ascaris*, infect over 1.5 billion of the poorest people and are amongst the leading causes of morbidity and financial constraint worldwide. New anthelmintics, particularly drugs with novel mechanisms of action, are urgently needed to overcome emerging resistance to currently used drugs and to produce higher cure rates. An enormous challenge for STN infections, in contrast to other diseases is the requirement that therapies be very cheap, scalable and have a long shelf life in potentially harsh environments. Our lab has pioneered development of anti-nematode Crystal (Cry) proteins, in particular Cry5B, made by *Bacillus thuringiensis (Bt)*, as promising new therapy for STNs. Cry5B has excellent *in vitro* activity against parasitic nematodes as well as *in vivo* efficacy against multiple STN infections in rodents, pigs, and dogs. We will present the development efforts to produce a deployable, formulated version of Cry5B and other Crystal proteins that are cheap, safe, scalable, and stable. We will also provide updates on additional investigations to discover new crystal protein cures for STN infections using microbiology and bacterial engineering, including efforts to develop quantitative high through-put drug screening for novel anthelmintic candidates.

537

GLOBAL EPIDEMIOLOGY OF STRONGYLOIDIASIS: FILLING THE KNOWLEDGE GAP

Donal Bisanzio[1], Dora Buonfrate[2], Antonio Montresor[3], Micheal French[1], Richard Reithinger[1], Giovanni Giorli[4], Zeno Bisoffi[2]

[1]*RTI International, Washington, DC, United States,* [2]*Centre for Tropical Diseases, IRCCS Sacro Cuore Don Calabria Hospital, Negrar, Verona, Italy,* [3]*Department of Control of Neglected Tropical Diseases, Geneva, Switzerland,* [4]*Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London, United Kingdom*

Strongyloidiasis is a common neglected tropical disease (NTD) in tropical and sub-tropical climatic zones. At the worldwide level, there is high uncertainty about strongyloidiasis burden. The range of estimated infected people varies from 30 to 370 million. This is an important knowledge gap which affects the planning of intervention to reduce the burden of strongyloidiasis in endemic countries. Similar to other soil-transmitted helminths, strongyloidiasis can be prevented and treated by preventive chemotherapy (PC) through mass drug administration (MDA). However, due to the limited knowledge on strongyloidiasis epidemiology no endemic country is conducting any infection or disease prevention and control efforts. Having an estimate of the global strongyloidiasis burden is crucial to plan prevention and control programming and reduce its toll on the global population. This study aimed to estimate the global strongyloidiasis burden and calculate the population of school-age children needed to be covered with MDA in 2018 Epidemiological data on strongyloidiasis were gathered by literature review and merged with data on country characteristics obtained from public data sources. Prediction of strongyloidiasis prevalence for each country was performed using a spatio-temporal statistical modeling approach. The country prevalence obtained from the model was used to calculate the number of infected people and the fraction of school-age children to be covered by MDA. We estimate the global prevalence of strongyloidiasis in 2018 to be 8.1% (95% CI: 4.2-12.4%), corresponding to 613.9 M (95% CI: 313.1-910.1 M) people infected; SEARO, AFRO, and WPRO regions accounted for 76.1% of the global infections. Moreover, we estimated that at least 238.8 M (95% CI: 134.1-317.4 M) school-age children live in areas where strongyloidiasis prevalence is over 5% and therefore need MDA. The results of our study highlighted that global strongyloidiasis prevalence has been underestimated. Our results could be used to identify those countries in which strongyloidiasis prevalence is highest and MDA should be deployed for its prevention and control.

538

SYSTEMATIC LITERATURE REVIEW OF GLOBAL SOIL-TRANSMITTED HELMINTHIASIS PREVALENCE AND INTENSITY STUDIES: IDENTIFYING KNOWLEDGE GAPS, METHODOLOGICAL CONCERNS AND GUIDING FUTURE RESEARCH

Michael R. Diaz, Jasmine L. Irish, Zena Belay, Stacy L. Davlin, Sanjaya Dhakal, Alexander H. Jones, Rubina Imtiaz

The Task Force for Global Health, Decatur, GA, United States

To understand the existing evidence on global soil-transmitted helminthiasis (STH) prevalence and intensity, we undertook a systematic literature review of related peer-reviewed journal articles. The review included all published articles with data collected between January 1st, 2006 and February 26, 2018, to align with the start of the global drug donation for deworming children. Eligible studies were from countries requiring preventive chemotherapy for STH, in English, from cross-sectional surveys, and provided basic demographic information such as age or age

groupings, sample size, type of laboratory testing performed, and parasites tested. This presentation focuses on the status of the recent published literature, in terms of survey design methodology, surveyed risk groups, and the geographical distribution of studies. Overall, 209 studies from 54 countries met the inclusion criteria and were fully reviewed by two independent reviewers. The geographic distribution of studies revealed significant gaps in available data, exemplified by no published studies from Bangladesh but 17 from neighboring India. In total, 140 (67%) of the studies came from just 15 (of 103 endemic) countries. Of the studies, 127 (61%) employed school-based sampling while the remaining 82 (39%) employed community-based sampling. A majority of studies (86%) included school-age children, while 117 (56%) included preschool-age children. Adults were included in slightly less than half the study populations (45%). Of concern, only 118 (56%) studies used random sampling designs that were clearly explained in the methods section. In conclusion, this review identified critical knowledge gaps, specifically the lack of parasitologic data among preschool-age children and adults, research inefficiencies, and methodological concerns. Clear and actionable guidance is needed to direct global STH research to link with the known, critical knowledge gaps. This would facilitate policy and operational decisions to increase the efficiency of resource allocation and generate comparable results across studied populations.

539

DIFFERENT SOIL-TRANSMITTED HELMINTHS INFECTIONS AMONG TWO NEIGHBORING INDIGENOUS POPULATIONS FROM PUERTO IGUAZÚ, MISIONES, ARGENTINA

Ernesto Candela-Senti[1], Carolina Goizueta[2], Marta Cabrera[3], Carla Muñoz-Antolí[1], **Maria V. Periago**[4]

[1]*Departamento de Biología Celular y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain,* [2]*Fundación Mundo Sano, Puerto Iguazú, Misiones, Argentina,* [3]*Departamento de Parasitología. Instituto Nacional de Enfermedades Infecciosas. ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina,* [4]*Fundación Mundo Sano/CONICET, Buenos Aires, Argentina*

Soil-transmitted helminths are a serious health problem for indigenous communities in Argentina, studies from Misiones Province have documented a high prevalence of STHs. Most of these studies usually report indigenous populations as a homogeneous group, without considering the different cultural and hygienic habits that could predispose them to different infections. The aim of this study was to determine the STH prevalence of two neighboring indigenous populations from Puerto Iguazú (Misiones), namely Fortín Mbororé and Yryapú, both belonging to the Mbyá-Guaraní ethnicity and separated by only two km. Fortín is composed of around 200 families while Yryapú is composed of around 100. The main income for both communities comes from guided tours through the villages, crafts and social government plans. Both populations were characterized and georeferenced and 128 households were included (63 from Fortin and 65 from Yryapú). In total, 327 fecal samples (218 and 109 samples, respectively), were obtained and analyzed. Samples were processed through sedimentation and Baermann techniques. The age range was from 1 to 87 years old (mean 20 yrs) in Fortín and from 1 to 54 (mean 10 yrs) in Yryapú, with females being 53.2% and 55.9% of the population, respectively. The prevalence of STH infection was 72.9% in Fortín and 75.2% in Yryapú, this difference was not statistically significant (p=0.55). Moreover, a similar prevalence of hookworm infection was detected in both communities (63.8% vs 68.8%). However, results revealed statistically significant differences of other species of STHs between both communities, showing a prevalence of 11.4% for *Strongyloides stercoralis* in Fortín vs 32.1% in Yryapú (p<0.001) and a prevalence of 0.9% of *Ascaris lumbricoides* in Fortín vs 18.4% in Yryapú (p<0.001). Although the study detects both villages as endemic for hookworm, the differences of prevalence for *S. stercoralis* and *A. lumbricoides* can be appreciated. This could probably be explained by the different hygienic habits among both communities. Future studies would be necessary to elucidate the reasons for the different transmission patters of STHs observed.

540

IMPACT OF ASSORTATIVE MIXING ON STABILITY OF TRANSMISSION AND FEASIBILITY OF CONTROL OF SOIL-TRANSMITTED HELMINTH INFECTIONS

Luc E. Coffeng

Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

Soil-transmitted helminths (STH) are currently targeted for control by 2020, defined as less than 1% of moderate-to-heavy intensity of infection in high-risk groups. Mathematical models suggest that this goal can be achieved by preventive chemotherapy (PC), if effectively targeted at these high-risk groups and implemented at sufficiently high coverage. However, an important limitation of existing models is that they assume homogeneous mixing, meaning that individuals in a community are assumed to mix perfectly by being exposed to one central reservoir of infection. In reality, eggs are distributed throughout the environment in a patchy manner and individuals contribute and are exposed to different patches in a differential manner. Most likely, individuals with higher infection loads share specific patches because they are members of the same social unit (e.g. household, school), meaning that they are more likely to infect one another and themselves than individuals in other social units. This process of assortative mixing is known to have an important stabilising effect on transmission and to impede the impact of interventions that do not necessary reach all of the population at risk (like PC). To investigate the impact of assortative mixing on feasibility of achieving the STH control target, we developed a new individual-based transmission model that incorporates a bipartite network as a representation of humans and environmental reservoirs of infection, and quantify it to represent households as social units within a larger community. Model predictions suggest that correlation between exposure and an individual's household membership has a strong stabilising effect on transmission, which in some settings may mean that the 1% target cannot be met. The net effect of PC was further predicted to depend strongly on patterns in individual participation in PC and clustering of individual participation by household. These findings stress the need to investigate who typically infects whom in endemic communities, and to better quantify and understand participation patterns at both the individual and household level.

541

SOCIO-CONTEXT INTERLINKAGES OF NEGLECTED TROPICAL DISEASES AND WATER SANITATION AND HYGIENE INTERVENTIONS IN TWO RURAL DISTRICTS IN AFRICA: POLICY IMPLICATIONS OF CONTROL AND ERADICATION OF NEGLECTED TROPICAL DISEASES IN GHANA

Martin Amogre Ayanore

University of Health and Allied Sciences, Ho, Ghana

Ghana is one of the countries with the highest number of reported cases of Neglected Tropical Disease globally. Ghana is ranked as second to Cote D'Ivore with regards to the prevalence of Buruli Ulcer in sub-Saharan Africa. Effective Water Sanitation and Hygiene interventions are known to be cost-effective in supporting the eradication of Neglected Tropical Disease in some settings. This study examined socio-context interlinkages for Neglected Tropical Disease and Water Sanitation and Hygiene interventions in two rural districts of Ghana. Data was collected between February-March 2019 among persons identified to have Neglected Tropical Disease conditions, health staff, and community opinion/change leaders, health policy actors and staffs working with local Non-Governmental Organization on Water Sanitation and Hygiene projects for the control of Neglected Tropical Diseases. Surveys (n=240), document reviews (3), in-depth interviews (n=30) and focus group discussions (n=8) were tools applied to illicit information from respondents. Low self-esteem and poor health seeking behaviours was significantly associated with poor Neglected Tropical Disease outcomes in both study districts. Social safety nets and social systems such as faith-based organizations play mediating

roles in Neglected Tropical Disease control efforts. Travel distance and cost, unsustainable community water systems, ineffective behaviour change interventions led by local community actors and systemic challenges in clinic level referrals for persons suffering from Neglected Tropical Disease impacts negatively on Neglected Tropical Disease control strategies. Continuum of clinic-community care and sustained Water Sanitation and Hygiene behaviour change interventions that address cultural barriers are effective for community level control on Neglected Tropical Disease A health system policy that prioritizes clinic-community knowledge sharing on Water Sanitation and Hygiene improvements and delivers holistic primary health care services in an integrated approach is foundation to reducing Neglected Tropical Disease burden in Ghana

542

DO COMMUNITIES PERCEIVE THE NEED FOR A COMMUNITY-WIDE DEWORMING PROGRAM? QUALITATIVE RESULTS FROM THE DEWORM3 STUDY, INDIA

Kumudha Aruldas[1], Angelin Titus[1], Yesudoss Jacob[1], Rajeshkumar Rajendiran[1], Surya Kulasekaran[1], Jabaselvi Johnson[1], Saravanakumar Puthupalayam Kaliappan[1], Marie-Claire Gwayi-Chore[2], Mira Emmanuel-Fabula[2], Judd Walson[3], Sitara Swarna Rao Ajjampur[1], Arianna Rubin Means[2]

[1]*Christian Medical College, Vellore, Vellore, India,* [2]*University of Washington, Seattle, WA, United States,* [3]*Natural History Museum, University of Washington, Seattle, WA, United States*

Interest in moving Neglected Tropical Disease programs from disease control towards elimination has prompted the evaluation of interventions that may interrupt the transmission of specific infections. Recent mathematical modeling studies suggest that community-wide deworming programs targeting individuals of all ages may interrupt transmission of soil-transmitted helminths. In the context of India's school deworming program, we conducted a qualitative study in 2018 in two blocks of Tamil Nadu, India to evaluate community members' perceptions of community-wide deworming programs and identify opportunities to optimize community-wide deworming. Eight focus group discussions, guided by the Consolidated Framework for Implementation Research, were conducted among 65 purposively selected adult men and women. Data were analyzed in ATLAS.ti 8.0 using primarily *a prior thematic* coding. Participants were aware of the effectiveness of medications to treat intestinal worm infections and the current national deworming program. The participants noted that adults can also be infected with intestinal worms but as the treatment for adults is not supported by the current program, they must purchase deworming medications from medical shops or consult a doctor for treatment. There was confusion as to whether adults can take the same deworming tablets that are given to children and whether they may have side effects. Participants also acknowledged that people who think they are not at risk for intestinal worms may be hesitant to be treated. Many participants were aware of how these infections are transmitted and knew that hygiene behaviors may help in preventing spread. Participants stated that family members who defecate in the open or those who walk around where people had defecated can be infected and transmit the infection to others, making everyone in the community susceptible. The study suggests that adults may be amenable to a policy shift towards adult deworming programs, particularly where sanitation practices are poor. The study also suggests the importance of informing adults about their risk of intestinal worm infections.

543

MASS DEWORMING CAMPAIGN: ACHIEVEMENTS, PROSPECTS AND CHALLENGES IN THE CONTEXT OF NIGERIA PROGRESS TOWARDS THE WHO 2020 GOAL

Obiageli O. Nebe[1], Chukwuma C. Anyaike[1], Evelyn E. Ngige[1], Ima Ip Chima[2], Ima Ip Chima[2], Francisca F. Olamiju[3], Francisca F. Olamiju[3], Rita R. Oguntoyinbo[4], Ikenna I. Nwoye[1], Rita R. Urude[1]

[1]*Federal Ministry of Health, Abuja, Nigeria,* [2]*Deworm The World Initiative/ Evidence Action, Abuja, Nigeria,* [3]*Mission To Help The Helpless, Jos, Nigeria,* [4]*Amen Health Care and Empowerment Foundation, Abuja, Nigeria*

Nigeria has the highest burden in terms of the number of preschool and School-Age Children (SAC) requiring preventive chemotherapy against schistosomiasis and Soil Transmitted Helminthiasis (STH) within the continent of Africa. The national baseline mapping report (FMOH 2017) shows that among the total of 582 schistosomiasis endemic districts, 10 districts are high-risk (&gt 50% prevalence), 293 districts moderate-risk category (&gt 10% but &lt 50% prevalence), while 279 districts are low-risk (&lt 10% prevalence). Nigeria has a total of 429 endemic districts for STH (&gt 20% prevalence), of which 107 can be classified as high-risk STH LGAs &gt 50% prevalence) and 322 as moderate-risk districts (&gt 20% to 50% prevalence). School and Community based approaches have been leveraged to implement cost-effective preventative chemotherapy in Nigeria. The recent data analysis initiated by the Federal Ministry of Health and World Health Organization showed that of the 450 schistosomiasis implementing districts, 305 (67.8%), districts have achieved 75% effective therapeutic coverage at least once. Out of this number, 68 (22.3%) districts have maintained at least 3 effective treatment rounds. As for STH, 353 (87.3%) of the 404 implementing districts have reached 75% therapeutic coverage at least once and of the 353; 196 of them (55.5%) districts have successfully maintained 75% effective therapeutic coverage for at least three rounds of MDA. Achievements and prospects of the National deworming Programme include: availability of policy and guidelines specific to NTDs, established coordinating mechanisms, completion of Schistosomiasis and STH baseline mapping and availability of donated medicines. These modest achievements notwithstanding, the National Programme contends with such challenges as highly populated and difficult terrain; huge treatment gaps and insufficient funding remains a critical barrier to program ownership and sustainability. Given the new global momentum to control / eliminate NTDs the foregoing situates a clarion call for Nigeria to urgently scale up interventions in all the endemic districts.

544

MASS DRUG ADMINISTRATION COVERAGE SURVEY IN THREE DISTRICTS THAT FAILED A REPEAT PRE-TRANSMISSION ASSESSMENT SURVEY IN 2017

Ibrahim Kargbo Labour[1], Habib I. Kamara[2], Benoit Dembele[3], Mohamed Turay[2], Abdul Conteh[1], Abdulai Kandeh[2], Victoria Redwood-Sawyer[2], Amy Veinoglou[4], **Mustapha Sonnie**[2], Mary H. Hodges[2], Yaobi Zhang[5]

[1]*Neglected Tropical Disease Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, Freetown, Sierra Leone,* [3]*Helen Keller International, Bamako, Mali,* [4]*Helen Keller International, New York City, NY, United States,* [5]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Four health districts (HDs) in Sierra Leone had high *Wuchereria bancrofti* microfilaremia (mf) prevalence at baseline. Despite effective reported epidemiological coverage (≥65%) with mass drug administration (MDA) with ivermectin and albendazole, these HDs failed a pre-transmission assessment survey (pre-TAS) with night blood test after five rounds of MDA and a re-pre-TAS using Filarial Test Strips (FTS) after 3 more rounds of MDA. To investigate potential reasons for repeated failure a coverage survey was performed in 2017 according to the WHO guidelines within 3 months of MDA in the 3 HDs with the highest FTS prevalence. In each HD, 30 subunits were selected with probability proportional to the number of

segments in the subunits, assisted by the Coverage Survey Builder. These were segmented, households randomly selected, and heads of households interviewed. Data were collected on smartphones and uploaded using Open Data Kit, then extracted to excel and analyzed using SPSS. A total of 6016 respondents were interviewed with 1977 in Bombali, 2668 in Kailahun and 1371 in Koinadugu. Proportion of those reported receiving ivermectin or albendazole and was 79.0% and 79.1% in Bombali, 76.1% and 76.4% in Kailahun and 61.8% and 62.2% in Koinadugu. Less than 1% admitted they had not swallowed either drug. Koinadugu did not reach effective epidemiological coverage. Top reasons cited for not taking the drugs were: CDDs did not come (472), unaware (172), afraid of side effects (142), distance too far (111), busy (54), medicines do not work (51), breastfeeding (33), etc. Some respondents (376) were ineligible at the time of the MDA: underage (204), sick (62), pregnant (95), very old (13), below the minimum height (2). Mild adverse events ranged from 22.2% in Koinadugu to 45.6% in Kailahun. Responses such as 'CDD did not come' or 'distances too far' accounted for 40% of non-compliance. Koinadugu has the lowest number of 75 health facilities compared to Bombali (111) and Kailahun (88). The catchment area covered by health facility is greatest in Koinadugu 162 km^2 versus Bombali 71 km^2 and Kailahun 44 km^2 making all forms of public health interventions challenging.

545

STUDIES ON SCABIES IN IMO STATE SOUTH EASTERN NIGERIA

Chinyere N. Ukaga, Ann I. Ogomaka, Betram E. Nwoke, Lillian C. Chimechefulam, Evangeline C. Uwalaka

Imo State University, Owerri, Nigeria

Prevalence of scabies among boarding schools in Orlu L.G.A. of Imo State Scabies being included to the list of neglected tropical disease as well as associated with resource-poor conditions, informed this study to investigate the prevalence of scabies among boarding school students in Orlu L.G.A. of Imo State. This study was conducted through the months of June to October 2018. A total of 2639 students from 5 out of the 8 boarding schools visited, took part in the screening. The sample comprised of three age ranges 9-11, 12-14 and 15-17 years as well as male and female genders from four different locations in Orlu L.G.A. Data on scabetic parameters such as pruritus status, periodicity/frequency of scabies occurrence and morbidities associated with scabies were collected using an interview based questionnaire. 384 (14.6%) students were infected with scabies. Using chi-square analysis, more female students 341 (16.1%) were infected than male students 43 (8.3%) with significant difference at ($P<0.05$). Age range 9-11 years recorded the highest disease prevalence 135 (17.4%) followed by 12-14 years 164 (14.2%) and then 15-17 years 85 (11.9%); ($P<0.05$). With regard to schools having highest scabies prevalence represented by location, Okporo-Orlu had 96 (16.8%) infected students closely followed by Ihioma-Orlu 101 (16.6%) infected students then Amaifeke-Orlu 74 (14.3%) infected and the least being Umudioka-Orlu 113 infected (11.9%). Using SPSS analysis pruritus showed that out of 384 infected students, 361 (96.3%) had constant severe pruritus while 14 (3.7%) had infrequent mild pruritus. 144 (37.5%) were experiencing scabies for the first time, 135 (35.2%) were having it for the second time while 105 (27.3%) have had the disease severally. This study thus established that scabies has a high prevalence among students in boarding schools, recommends that overcrowding and poor personal hygiene should be curbed while improved health services should be made available to students in hostels.

546

INTERRUPTING TRANSMISSION OF SOIL-TRANSMITTED HELMINTHS AND SCHISTOSOME PARASITES IN ETHIOPIA - THE GESHIYARO PROJECT PROTOCOL

Kalkidan Mekete[1], Alison Ower[2], Julia C. Dunn[2], Heven Sime[1], Gemechu Tadesse[1], Ebba Abate[1], Nebiyu Nigussu[3], Fikreselasie Seife[3], Emily McNaughton[2], Anna Phillips[2], Roy M. Anderson[2]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*Imperial College London, London, United Kingdom,* [3]*Federal Ministry of Health, Addis Ababa, Ethiopia*

Soil-transmitted helminths (STH) and schistosomiasis are neglected tropical diseases (NTDs) that are estimated to affect over 1.4 billion people globally. Recent research, including longitudinal epidemiological studies and analyses of transmission dynamics, have suggested that mass drug administration (MDA) should be community-wide to interrupt transmission of STH in areas of medium to high transmission (as measured by the basic reproductive number R_0) instead of solely targeted at school aged children (SAC). This is especially the case where hookworm is the dominant infection. The Geshiyaro project seeks to interrupt transmission of STH and SCH in the Wolayita zone of south-western Ethiopia. Two interventions will be implemented and assessed through the project (i) expanded MDA through biannual community-wide treatment with a target of 90% treatment coverage, and (ii) provision of water sanitation and hygiene (WaSH) with complementary behaviour change communication (BCC) in combination with MDA. These interventions should allow the suppression of STH and SCH infection to very low levels that is achieved through MDA, to be sustained once transmission is infrequent. The Geshiyaro project is a five-year project which began in July 2018 and is currently ongoing. The impact of these interventions will be evaluated through epidemiological assessments of infection (prevalence and intensity) across all age groups using sensitive diagnostics at baseline and endline, as well as through the monitoring of longitudinal sentinel site cohorts. Treatment coverage and individual compliance to treatment will be monitored by employing fingerprint biometric technology and barcoded identification cards, and an independent treatment coverage validation survey. The outcomes of this project will help to inform country NTD programmes on steps to be taken once STH and SCH infections are at low prevalence following sustained MDA. The focus on cost-effectiveness and scalability may also provide a sustainable model for country programmes to follow to achieve transmission elimination in defined settings.

547

COMMUNITY-BASED SURVEY FOR PROGRAM MONITORING OF SOIL TRANSMITTED HELMINTHIASIS IN SIERRA LEONE

Ibrahim Kargbo Labour[1], Jusufu Paye[2], Stacy Davlin[3], Abdul Conteh[1], Victoria Redwood-Sawyer[2], Amy Veinoglou[4], Mustapha Sonnie[2], Mary H. Hodges[2], Alexander H. Jones[3], **Yaobi Zhang**[5]

[1]*Neglected Tropical Diseases Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, Freetown, Sierra Leone,* [3]*Children Without Worms, Task Force for Global Health, Atlanta, GA, United States,* [4]*Helen Keller International, New York, NY, United States,* [5]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

World Health Organization currently recommends sentinel site surveys in school-age children (SAC) for impact assessment of soil-transmitted helminth (STH) control programs. The new STH guidelines recommend targeting additional risk groups, e.g. preschool-age children (PSAC), and high-risk adults. The sentinel site survey of SAC alone provides insufficient data for different risk groups. Using a methodology developed by Children Without Worms, in May 2018, we conducted a multi-stage, cluster household survey in two Sierra Leone districts that produced equal-probability samples of all three risk groups: PSAC (1–4 years), SAC (5–14 years), and adults (≥ 15 years). Standardized questionnaires were administered and fresh stool samples (1 slide) examined by Kato Katz. In total, 1,270 persons (M:48.0%, F:52.0%) in Bo and 1,508 (M:54.6%,

F:45.4%) in Kenema were tested. Prevalence of hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* was 5.0% (95%CI: 3.8-6.2), 4.0% (95%CI: 2.9-5.0) and 1.2% (95%CI: 0.6-1.8) in Bo and 10.4% (95%CI: 8.9-11.9), 7.4% (95%CI: 6.2-8.9) and 0.9% (95%CI: 0.4-1.4) in Kenema respectively. Overall prevalence in PSAC, SAC and adults was 7.7% (95%CI: 5.8-11.7), 7.4% (95% CI: 7.7-12.7) and 9.2% (95%CI: 7.7-13.9) for hookworm; 6.5% (95%CI: 3.6-8.3), 4.2% (95%CI: 2.4-5.7) and 9.2% (95%CI: 6.4-10.2) for *A. lumbricoides*; and 0.9% (95%CI: 0.2-2.2), 0.7% (95%CI: 0.2-1.1) and 1.5% (95%CI: 0.2-1.9) for *T. trichiura*, respectively. There was <1% STH infections with moderate-to-high intensity in all risk groups. There were no significant differences by sex and any STH infections in either district. Prevalence for all species was lower among individuals who always wore footwear compared to those who sometimes wore footwear (p<0.0001). Both districts had low prevalence for all STHs in all risk groups and the new protocol was relatively easy to use.

548

PREVALENCE AND INTENSITY OF STH INFECTIONS IN TWO SETTINGS WITH A CONTRASTING HISTORY OF IVERMECTIN MASS DISTRIBUTIONS: INDICATIONS OF COLLATERAL IMPACT

Hugues Nana Djeunga[1], Linda Djune Yemeli[1], Cédric Lenou Nanga[1], André Domche[1], Floribert Fossuo Thotchum[1], Yannick Niamsi Emalio[1], Thérèse Nkoa[2], Joseph Kamgno[1]

[1]*Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaoundé, Cameroon,* [2]*Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon, Yaoundé, Cameroon*

Onchocerciasis and Soil Transmitted helminthiases (STH) are among neglected tropical diseases that have been targeted by the national control programs for several years because of their heavy burdens on infected populations. The control of these diseases relies on preventive chemotherapies (PCT) with ivermectin (IVM) for onchocerciasis and mebendazole (MEB)/albendazole (ALB) for STH. IVM, a broad spectrum endectocide, was found to exhibit antiparasitic activity on STH. IVM-based mass drug administrations (MDA) have led to the interruption of transmission of onchocerciasis in many *foci*. The objective of this study was then to compare the trends in prevalence and intensities of STH infections in two settings with very contrasting history of IVM-PCT. Cross-sectional surveys were carried out in the Yabassi health district where IVM-PCT to fight against onchocerciasis were ongoing for 20 years, and in the Akonolinga health district where no IVM-based MDA was never implemented because of hypo-endemicity of onchocerciasis and high endemicity of loiasis. Stool samples were collected from all volunteers aged ≥2 years and analyzed using the Kato-Katz technique. A total of 799 individuals (52.2% female), aged 2 to 90 years old (Median: 17 years old; IQR: 7- 45), provided stool samples and were enrolled in this study. As expected, school aged children were most infected than the other surveyed participants. Two STH species were found in the study population, *Ascaris lumbricoides* and *Trichuris trichiura*, with respective prevalence of 20.0% (95% CI: 17.4-22.9) and 17.0% (95% CI: 14.6-19.8). Importantly, both prevalence and intensities of infection were significantly lower in the IVM-treated area than in IVM-naïve area ($P \leq 0.0001$). This study revealed that the prevalence and intensity of STH infections were significantly lower in the IVM naïve area compared to areas where repeated rounds of IVM-based MDA have been implemented since two decades. This observation thus confirms the collateral impact of IVM-PCT on STH transmission, supporting an optimal timing of IVM- and MEB/ALB-based PCTs in controlling onchocerciasis and STH in co-endemic areas.

549

THE IMPACT OF ADJUSTED MOBILE TREATMENT POSTS STRATEGY ON MASS DRUG ADMINISTRATION COVERAGE IN AN URBAN SETTING: CASE STUDY OF DAR ES SALAAM, TANZANIA

Alistidia Simon[1], Cecilia Uisso[1], Upendo Mwingira[2], Jeremiah Ngondi[3], Hailey Mableson[1], Andreas Nshala[4]

[1]*NTD Control Program, Dar Es Salaam, United Republic of Tanzania,* [2]*NTD Control Program, Dar es Salaam, United Republic of Tanzania,* [3]*RTI, Washington, DC, United States,* [4]*Uppsala University, Uppsala, Sweden*

Tanzania has been conducting Neglected Tropical Diseases (NTD) control and elimination activities for lymphatic filariasis (LF), schistosomiasis (SCH) and soil transmitted helminths (STH) since 2001. By 2018, 27 districts were still undergoing mass drug administration (MDA) for LF, 56 districts progressed through TAS, and the remaining 65 districts were classified as non-endemic. These 27 districts under MDA have a total of 10.2 million people at risk of infection and 56% of them live in Dar es salaam city. Since 2013, Centre for Neglected Tropical Diseases (CNTD) has supported the national NTD Control Programme (NTDCP) to deliver the MDA in Dar es Salaam city. With the country progressing towards elimination of LF, it is essential to ensure that optimal coverage is attained during MDA especially in urban settings. MDA coverage evaluation survey report of 2017 recommended the use of mixed strategy in order to improve the MDA coverage in urban areas but also to raise awareness and increase demand for MDA. In 2018 MDA various approaches were implemented to improve MDA coverage, including: house to house sensitization combined with mobile treatment teams (drug distributors), mass media campaign through radio and television, Town crier's announcements; and social media platforms such as WhatsApp, twitter, Instagram and Facebook pages. Post MDA coverage evaluation survey was conducted in two districts in 2017 and 2018. Survey results showed increased MDA coverage in 2018 compared to 2017 in Kigamboni (74% vs. 53% and Ubungo (80% vs. 53%). The MDA coverage findings suggest the strategies implemented in 2018 improved MDA coverage and will facilitate elimination of LF in Dar es Salaam. Lessons learned from Dar es Salaam can be implemented in other urban settings experiencing low MDA coverage.

550

AN INNOVATIVE INTEGRATIVE APPROACH FOR NEGLECTED TROPICAL DISEASES (NTD) CONTROL IN ETHIOPIA: SUSTAINED REDUCTION IN PREVALENCE OF SCHISTOSOMA AND SOIL-TRANSMITTED HELMINTHIASIS (STH) INFECTIONS IN RURAL AND URBAN POPULATIONS

Zvi Bentwich, Nala Team

NALA/Ben Gurion University, Tel Aviv, Israel

Ethiopia has one of the highest burdens of neglected tropical diseases (NTD) in Africa, with more than 75 million people at risk of infection from soil-transmitted helminthiasis (STH) and schistosomiasis. To control these diseases, the Ethiopian Federal Ministry of Health (FMoH) has instituted regular mass drug administration (MDA) campaigns. However, these campaigns have not prevented reinfection, which occurs due to poor hygiene practices and a lack of adequate sanitation in affected communities; therefore, the high prevalence of infections has remained high in many parts of the country. Since 2008, the organization NALA has developed an integrated model for controlling schistosomiasis and STH that complements MDA by promoting behavioral change and community-led improvements to water, sanitation, and hygiene (WASH) infrastructure in order to create an enabling environment for sustained disease prevention. In 2015, the urban and rural districts of Adwa were selected as a project area by NALA and the Tigray Regional Health Bureau due to its high prevalence of intestinal worms and schistosomiasis. From 2015-2019, the NALA team provided behavioral change health education to children in nearly 60 schools, trained thousands of community members to be health messengers, and strengthened the capacity of local health workers and government officials. In addition, NALA recruited the community

to be integral partners and owners of the program and also provided small grants for community-led WASH infrastructure projects in selected schools. As a result of this work, stool surveys have shown a significant and sustained decrease in helminth prevalence. Between March 2015 and March 2019, the prevalence of helminth infections in eight intervention schools dropped from 26% to 1.5% (!!) and the prevalence of *Schistosomiasis mansoni* in these 8 schools dropped from 11.8% in 2015 to 0% in 2019. These results demonstrate that an integrative approach can attain remarkable results in the control of these neglected tropical diseases, especially if the approach is strongly focused on community partnership and cooperation with the health authorities.

551

HEALTH EDUCATION AND BEHAVIOR CHANGE OF CHILDREN ARE ESSENTIAL FOR CONTROLLING NTDS: LESSONS FROM A SUCCESSFUL PILOT TRIAL IN ETHIOPIA

Zvi Bentwich, Liat Rennert

NALA, Maccabim, Israel

Neglected tropical diseases (NTD) are a major cause in undermining childrens' potential for both physical and mental development. Soil transmitted helminths (STH), schistosomiasis and trachoma have a direct effect on stunting and on cognitive capabilities and skill learning. That is why their control is so critical in young children, and why it is essential to have a comprehensive program that is focused on children and started at an early age, and that combines behavioral change, improved water sanitation and hygiene (WASH), and mass drug administration (MDA). During the past nine years we have developed a comprehensive integrated model intervention that combines all these elements and has been successfully implemented by now in several locations in Ethiopia where it has achieved a significant and sustained decrease in prevalence of both schistosomiasis and STH and more lately of Trachoma. Since children are at the highest risk to contract these diseases we launched an innovative pilot program in 2017, focused on children attending kindergarden, training educators, parents and community leaders to playfully create healthy hygiene habits for young children in Ethiopia. This program took place in 9 districts and 300 schools focusing on early childhood (ages 3-6) instilling habits that can help prevent the diseases at a younger age. Schools, educators and community leaders were trained in early childhood education methods, focusing on innovative ways to transmit messages and reinforce behaviors and habits in young children and support health and hygiene. We are now scaling up the program and assessing : 1) The effectiveness of our model intervention program 2) The processes through which behavior change interventions influence the practices and understanding of the children leading to lowered disease prevalence primarily by prevention of infection. The results of this study have already given us an insight into the mechanisms that determine behavioral change in children and that may help us expand the program and apply it universally for the control of NTDs in Ethiopia and elsewhere.

552

KNOWLEDGE, ATTITUDE AND PRACTICE (KAP) SURVEY IN PRIMARY SCHOOL STUDENTS IN BENCH MAJI ETHIOPIA: THE URGENT NEED FOR BEHAVIOR CHANGE AND HEALTH EDUCATION IN THE FIGHT AGAINST NTDS

Zvi Bentwich, Asrat Meleko, Dorin Turgeman

NALA, Maccabim, Israel

Water, sanitation and hygiene are essential for preventing diseases such as soil transmitted helminths, trachoma and other related neglected tropical diseases . Access to safe drinking water and adequate sanitation facilities, together with promotion of knowledge, attitude and practice of students on water, hygiene and sanitation related factors are key components for prevention of these diseases. We therefore tried to assess the knowledge, attitude and practice of students at baseline and before the beginning of a behavioral intervention project by NALA. A school based descriptive cross-sectional study was conducted from April to May, 2018 in primary schools of one of 11 districts in Bench Maji zone, Ethiopia. The study population was primary school students from grade 5 to 8 consisting of students able to fill the self-administered questionnaires. Verbal and formal consent for the usage of the questionnaire was obtained from teachers. The main results of the study revealed the following: a) Only 93 (31%) of students knew that walking barefoot and bathing in the river may lead to parasitic infections and schistosomiasis . b) Only one third of students knew that washing or cooking vegetables is important for preventing infection. c) only 120 (40%) knew that flies are carriers of the trachoma pathogen. d) 182 (69%) practice frequently open defecation. e) 172 (57.%), are afraid of taking drugs during mass drug administration campaigns because they fear from side effects. f) 66 (22%), did not wash their hands regularly , and 114 (38%) wash their face only when there is discharge from eyes or nose. g) only 69 (23%) boil water before drinking regularly. This study demonstrates the low awareness of school children about transmission, symptoms and preventive measures for trachoma schistosomiasis and soil transmitted helminths and their poor attitude and practice of water, hygiene and sanitation related preventive behaviors. These findings clearly indicate the essential need for an immediate and comprehensive health education and behavior change intervention in this population as a major condition for controlling NTDs in this region.

553

EVIDENCE FOR GLOBAL HEALTH INTERVENTIONS: PERSPECTIVES FROM THE AFRICAN GREAT LAKES ON EVIDENCE IN MASS DRUG ADMINISTRATION PROGRAMMES FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS

Cristin Alexis Fergus[1], Georgina Pearson[2], Bianca D'Souza[2]

[1]*London School of Economics, London, United Kingdom,* [2]*Firoz Lalji Centre for Africa, London School of Economics, London, United Kingdom*

In many places, public health practitioners are part of a larger global health system, wherein they are responsible for the implementation of disease-specific health interventions, largely financed by external actors through a variety of mechanisms. Emphasis on the need for evidence-informed decision-making often includes rhetoric for the localization of this approach to assist practitioners in resource allocation. In practice its realisation has been challenging. For one, decision-making processes are heterogenous at different localities and the evidence needs of local practitioners are not well understood. Additionally, evidence development and knowledge synthesis pertaining to health interventions are rarely linked in ways that feedback and respond to local implementation and decision-making practices. The goal of this research was to identify local evidence needs and examine acceptability of various forms of evidence, e.g. modeled disease estimates. Decision-making processes around implementation, including political and social factors, were examined to understand how these evidence and decision-making processes interact. Mass Drug Administration (MDA) for schistosomiasis and soil-transmitted helminths was utilized as a representative global health intervention, with a focus on the epidemiologically relevant areas of Kenya, Malawi, Tanzania, and Uganda. We used complementary qualitative approaches to collect data, which included 4 workshops with district- and national-level MoH personnel, and key informant interviews and electronic survey questionnaires from a sample of relevant organisations, including NGOs and International/Global Health Organisations. Data were coded and analyzed thematically. The results provided important insights as to the sources, types, and formats of evidence which local public health practitioners find acceptable and useful. The social and political pressures were found to influence the decision-making around resource allocation and uptake of evidence. These results are intended to assist in the translation and uptake of evidence for decision-making in global health.

554

INTEGRATING SOIL/TRANSMITTED HELMINTHIASES AND SCHISTOSOMIASIS CONTROL PROGRAMS IN PRIMARY HEALTH CARE: A STEP TO FORWARD FOR UNIVERSAL HEALTH COVERAGE

Arancha Amor Aramendia[1], Melaku Anegagrie Mekonen[2], Elena Barrio Miguel[1], Birhanu Tashu[3], Juan Jose de los Santos[1]

[1]*Mundo Sano Foundation, Madrid, Spain,* [2]*Mundo Sano Foundation, Bahir Dar, Ethiopia,* [3]*Amhara National Regional Health Bureau, Zenzelema Health Center, Bahir Dar, Ethiopia*

Soil-transmitted-helminthiases (STH) and schistosomiasis (SCH) are major control programs of WHO agenda for neglected tropical diseases (NTDs). Periodic mass drug administration for at-risk population is the main strategy for reducing their burden. Despite all the efforts made, so far STH-SCH are far from control in Africa endemic areas. Recently, WHO encourages for integrating NTDs into the primary health system, to ensure a universal health coverage (UHC). The aim of this study was to support the improvement of a diagnosis protocol in a primary health center in a rural area of Ethiopia integrating, in an effective and efficient manner, STH-SCH control program in the primary health system. According to the Ethiopian national guidelines, the wet mount technique is used for the diagnosis of STH-SCH infection in the health centres. We compared the results of this technique with the ones obtained by a comprehensive protocol that included three easy to perform techniques: formol-ether concentration, Kato-Katz and Baermann methods. From October to December 2018, 186 patients from the outpatient department of a health center in the rural area of Ethiopia were included in the study. Infections by hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis* and *Schistosoma mansoni* were recorded. 24 (13%) patients were diagnosed by the wet mount technique in the routine laboratory, while with our protocol the number of diagnosis increased until 131 (70.4%) (p=0.0008). The vast majority of patients carrying intestinal helminths are not diagnosed in this health center. This pattern could be similar in other facilities in endemic areas, working with the same diagnosis procedure, as is stated in the guidelines. Integrating a comprehensive management of intestinal helminth infection in primary health facilities, including an accurate diagnosis, will be a step forward to reach UHC. It will also be a turning point for the control of NTDs, as this under-diagnosis (and therefore the lack of treatment) perpetuates these infections in endemic areas. More studies are necessary and make new and more promising steps to achieve UHC.

555

DATA QUALITY ASSESSMENT AS A PROJECT MONITORING TOOL IN MASS DRUG ADMINISTRATION FOR NEGLECTED TROPICAL DISEASES IN GUINEA

André Géopogui[1], Christelly Badila Flore[2], Mamadou Siradiou Baldé[1], Lamine Lamah[2], Bamba Foungotin Ibrahim[2]

[1]*Ministry of Health, Conakry, Guinea,* [2]*Helen Keller International, Conakry, Guinea*

The transfer of quality data from the community level to the central level is important for impactful decision-making for the national NTD program. Yet, data received at the national level are often incomplete, late or of questionable accuracy. The Guinean MOH conducted a data quality assessment (DQA) through the National NTD reporting pathway after the integrated treatment for lymphatic filariasis (LF), onchocerciasis (OV) and soil transmitted helminths (STH) in 2016. Data from two health districts (HDs) in two regions were selected for analysis and these data came 4 health centres (2 from each HD) and 12 communities (3 in each health centre). Availability, accuracy and completeness of MDA data were reviewed and compared with the reported data. The data management and reporting system was also assessed. At the national level, the results show that LF coverage (indicator 1) was 98.7%. compared to the 99.6% reported during the MDA. The number of remaining ivermectin and albendazole tablets (indicators 2 & 3) indicated an accuracy of 94.0% and 84.8% respectively. With respect to the number of SAC treated (indicator 4), the results indicated 90.39% percent of accuracy. No data were available at the regional level. At the HD level, the result show 79.9% accuracy for indicator 1, 55.3% and 168.5% respectively for indicators 2&3 and 109.5% accuracy for indicator 4 (data only available from one HD). At the health centre level the verification percentage varied from 74.8% to 92.4% for indicator 1, no data were available for indicators 2&3 and 74.4% and 109.5% for indicator 4. Completeness and availability of reports were 100% at national and health centre levels. For data management and the reporting system, the DQA showed deficiencies in filling in data at the HD and the health centre level; data are usually transmitted daily by phone and even though templates for MDA reporting exist at all levels, the quality control is not systematic. NTD data are currently not integrated in the national health system information. The recommendations resulting from this evaluation will improve the understanding of reported data and will be used to improve program performance.

556

GETTING OUT OF THE COMFORT ZONE: INTEGRATED APPROACH OF SKIN NTDS

Patrick Suykerbuyk[1], Pierre Umba[2], Osman El Tayeb[3], Tine Demeulenaere[1]

[1]*Damien Foundation, Brussels, Belgium,* [2]*Damien Foundation, Kinshasa, Democratic Republic of the Congo,* [3]*Damien Foundation (DFB) Nigeria, Nigeria, Nigeria*

Damien Foundation (DF) was established in 1964. Since then, it has traveled a long way, from a Belgian Medical development NGO solely focused on leprosy to one dealing also with tuberculosis and further widening its horizon to leishmaniasis and other poverty-related diseases. Through the years, DF had to adapt its way of working and its approach to development aid to fit the new realities of a changing international community: Recently, the WHO is promoting an integrated strategy for skin-related NTDs that require active detection, management and control, and is harnessing the experience of many experts involved in skin diseases to provide guidance to implement the integrated strategy in countries where skin related NTDs are a major burden. Furthermore, the integration of skin diseases should provide an opportunity to build the capacity of primary health care workers to detect and treat a number of diseases using the same resources. However, such an integrated management of skin NTDs, forces organizations as DF to step out of its comfort zone; i.e., to evolve from a historic vertical-oriented, disease-specific approach to an integrated approach whereby strategic partnerships, knowledge transfer and synergies are key to success. Here we provide insights in the internal kitchen of a NGO to respond to this international call to action. We identified 4 main transition zones on the roadmap for integration: the comfort zone, fear zone, learning zone and growth zone. We discuss how this transitions zones have an impact on (i) the internal organizational model of DF; (ii) capacity building of human resources (e.g., training) and infrastructure (e.g., sensitization support, diagnosis and treatment); (iii) funding challenges and opportunities; (iv) research (cost-effectiveness of integration); (v) communication and advocacy; as well as (vi) local and international partnerships. Moreover, we illustrate this transition zones using pilot projects in Nigeria and the Democratic Republic of the Congo and discuss how the Global Partnership for Zero Leprosy could play a catalyzing role in the integrated approach to control skin NTDs.

557

CENTRIN-DEFICIENT *LEISHMANIA MEXICANA* AS A SAFE AND EFFECTIVE VACCINE AGAINST CUTANEOUS LEISHMANIASIS

Greta Volpedo[1], Sanjay Varikuti[1], Wen Wei Zhang[2], Patrick Lypaczewski[2], Sreenivas Gannavaram[3], Ranadhir Dey[3], Subir Karmakar[3], Nevien Ismail[3], Abu Musa[4], Risa Nakamura[4], Shinjiro Hamano[4], Greg Matlashewski[2], Hira L. Nakhasi[3], Abhay R. Satoskar[1]

[1]*The Ohio State University, Columbus, OH, United States,* [2]*McGill University, Montreal, QC, Canada,* [3]*Food and Drug Administration, Silver Spring, MD, United States,* [4]*Nagasaki University, Nagasaki, Japan*

Leishmaniasis is a neglected protozoan disease affecting over 12 million people in more than 90 tropical and subtropical countries. Despite the high morbidity and mortality of this condition, there are no approved vaccines for human use. Using the CRISPR/Cas9 technique, our group has generated live attenuated *Centrin* knock out (*Cen-/-*) *Leishmania* parasites. Centrin is a cytoskeletal protein important for cellular division in eukaryotes and, in *Leishmania*, it is only necessary for amastigote replication. We hypothesize that *Leishmania Cen-/-* parasites are able to infect the host cells and induce a protective immune response, but due to the lack of Centrin, they are not able to establish and spread the infection. Our data shows that *Leishmania (L.) mexicana Cen-/-* amastigotes present a growth defect, which results in significantly lower survival within murine bone marrow-derived macrophages and dendritic cells and in the augmented production of the protective cytokine interleukin (IL)-12, compared to the wild type (WT) strain. *In vivo*, we confirmed that *L. mexicana Cen-/-* parasites are safe in a susceptible murine model and do not lead to clinical symptoms. *L. mexicana Cen-/-* inoculated mice produced higher ratios of protective cytokines, such as interferon (IFN)-γ, vs. disease exacerbating cytokines, such as IL-10 and IL-4, compared to the group infected with *L. mexicana* WT. Additionally, *L. mexicana Cen-/-* parasites are effective in the protection against *L. mexicana* WT challenge, as they elicit a protective immune response while obliterating the clinical symptoms of cutaneous leishmaniasis. Taken all together these results suggest that live attenuated *Cen-/-* parasites are effective candidate prophylactic vaccines for protection against leishmaniasis.

558

DRIVERS OF INFLAMMASOME ACTIVATION IN IMMUNOPATHOLOGIC CUTANEOUS LEISHMANIASIS

Christina K. Go, Fernanda O. Novais, Phillip Scott

University of Pennsylvania, Philadelphia, PA, United States

In cutaneous leishmaniasis, the host immune response is critical for controlling parasite burden as well as for the severity and duration of disease. Although parasite control may be accomplished, many patients continue to exhibit severe disease due to dysregulation of the inflammatory response. We have previously reported that blocking IL-1β signaling in infected mice ameliorated pathology. This supports that IL-1β is a major component of pro-inflammatory signaling in cutaneous leishmaniasis. Cleaving of pro-IL-1β to its mature form is mediated by inflammasome signaling pathways, which can be triggered by a variety of stimuli including DAMPs and PAMPs and can lead to pyroptosis. Therefore, inflammasome signaling mediates both response to and generation of pro-inflammatory factors. In particular, blocking NLRP3 in experimental leishmaniasis or in human skin biopsies of leishmanial lesions reduced IL-1β levels, suggesting its production is downstream of the NLRP3 inflammasome in human disease and mouse models of leishmaniasis. Given the role of IL-1β in dysregulating inflammation, we are investigating how inflammasome signaling is activated in leishmaniasis. Our preliminary results indicate that parasites induce a low level of IL-1β production in LPS-stimulated cells infected with *L. major*. Since we previously reported that CD8+ T cell-mediated cytotoxicity promotes IL-1β production, we also investigated if cytotoxicity induced inflammasome activation. We found that co-culture of cytolytic CD8 T cells and antigen-pulsed target cells induced inflammasome assembly and IL-1β production in an antigen presentation-dependent manner. Thus, our findings have identified leishmania infection and cytotoxicity as pathways for initiating IL-1β and immunopathology in cutaneous leishmaniasis. Understanding these and other mechanisms of IL-1β release will be important for addressing severe disease caused by uncontrolled inflammation in cutaneous leishmaniasis.

560

OVEREXPRESSION OF THE KINASE *JEAN3* FROM *LEISHMANIA MAJOR* MAY ATTENUATE PARASITES INFECTIVITY IN BALB/C MICE BY IMPAIRING THE TH2-TYPE IMMUNE RESPONSE THROUGH THE DOWNREGULATION OF *IL4, IL10,* AND *ARG1*

Andres Vacas[1], Celia Fernandez-Rubio[1], Esther Larrea[1], Jose Pena-Guerrero[1], Miriam Algarabel[1], Fabio Rocha Formiga[2], **Paul Nguewa**[1]

[1]*University of Navarra, Istun Institute of Tropical Health, Pamplona, Spain,* [2]*Departamento de Imunologia Instituto Aggeu Magalhães - IAM Fundação Oswaldo Cruz - FIOCRUZ, Recife/PE, Brazil*

No human vaccine is currently available to prevent the diverse clinical pathologies caused by *Leishmania* parasites. Nevertheless, the evidence that most patients that have suffered from leishmaniasis are resistant to subsequential infections provides the ground for making efforts in this task as it will not be unfruitful. Our laboratory has developed an *L. major* mutant cell line (pXG-*LmJean3*) overexpressing the conserved trypanosomatid kinase *Jean3*. This study outlines *LmJean3*-overexpressing parasites infectivity *in vitro* and *in vivo*. Furthermore, we have examined in these mutants the expression of some known virulence factors such as *SHERP* and *GP63*. *LmJean3*-overexpressing parasites displayed lower phagocytosis rates *in vitro* and a significantly smaller footpad swelling in susceptible BALB/c mice when compared to Controls. Gene expression of Th2-associated cytokines and effectors allowed the observation of a significant reduction in *IL4*, *IL10,* and *ARG1* levels; no expression change was found in Th1 associated cytokines except for *IL12*. Our analysis indicates that the significantly smaller swelling of the infected mice foot pads is probably caused by an impaired Th2 immune response that benefits Th1 prevalence. These studies depict Jean3 as a new putative target for studying the immune responses to *Leishmania* and may allow the improvement of vaccination research.

561

TRYPANOCIDAL AND APOPTOSIS-INDUCING PROPERTIES OF *ANOGEISSUS LEIOCARPUS* AND *PSEUDOCEDRELA KOTSCHYI* ROOT EXTRACTS

Olakunle O. Kassim[1], Hilaire M. Kenguele[2], Oladapo Bakare[3], Kwashie A. Ako-Nai[4], Winston A. Anderson[3], Clarence M. Lee[3]

[1]*Howard University College of Medicine, Washington, DC, United States,* [2]*Universite des Sciences et Techniques de Masuku, Franceville, Gabon,* [3]*Howard University, Washington, DC, United States,* [4]*Obafemi Awolowo University, Ile-Ife, Nigeria*

Years of intensive research have not produced a protective vaccine against Chagas disease, which is caused by *Trypanosoma cruzi*. Current therapeutic drugs are also not very effective therapeutically. It is therefore imperative to find new drugs that are more effective and with minimum side effects. We undertook *in vitro* evaluation of methanol root extracts of *Anogeissus leiocarpus* and *Pseudocedrela kotschyi* against *T. cruzi* epimastigotes for their trypanocidal and apoptosis-inducing properties. Nifurtimox was used as a positive control. Dose response determinations at 96 hrs of culture yielded an IC_{50} of 0.82 μg/ml for *A. leiocarpus,* 8.94 μg/ml for *P. kotschyi* and 7.70 μg/ml for nifurtimox ($p < 0.05$). These results indicate that *A. leiocarpus* extract was significantly more potent than *P. kotschyi* extract and nifurtimox ($p < 0.001$). *T. cruzi* epimastigotes treated with the two plant extracts were also doubled stained with Annexin V and propidium iodide and analyzed by flow cytometry for distribution of epimastigotes that underwent apoptosis and necrosis. The results

showed that the percentages of early and late apoptotic epimastigotes were 2.98 % for *A. leiocarpus,* 9.55% for *P. kotschyi* and 12.37 % for nifurtimox. In contrast, the percentages of epimastigotes that underwent necrosis were 0.10% for *A. leiocarpus*, 14.6% for *P. kotschyi* and 0.48% for nifurtimox. These results suggest different modes of action for the individual plant extracts and nifurtimox. Morphological examination of treated epimastigotes by scanning electron microscopy revealed swelling of the perinuclear region and ruffling of cell membrane as well as impaired flagella growth. The overall results from this study strongly suggest that the two root extracts possess potent trypanocidal properties and can be used as sources of potential antichagasic drugs.

562

PARASITE BURDEN IS ASSOCIATED WITH THERAPEUTIC FAILURE IN HUMAN CUTANEOUS LEISHMANIASIS

Mauricio Nascimento, Rubia Costa, Maíra Saldanha, Sergio Arruda, Paulo Machado, Edgar M. Carvalho, **Lucas P. Carvalho**

Instituto Gonçalo Moniz- Fiocruz, Salvador, Brazil

Ulcer development in cutaneous leishmaniasis (CL) patients is associated with exaggerated inflammatory response with high levels of TNF and IL-1 beta. We have been able to identify CL patients in the very early phase of the disease (before ulcer development). These individuals are known as early cutaneous leishmaniasis (ECL) patients. Pentavelent antimony is the preconized drug to treat leishmaniasis in Brazil. Despite pentavalent antimony treatment, 75% of ECL patients go on to develop ulcer while only 35% of CL will have already ulcerated lesion. Here we worked with two hypothesis for high therapeutic failure rate in ECL: 1) High parasite amounts is associated with treatment failure. 2) Individuals that fail therapy have defects in immunoregulatory mechanisms. This study was conducted in Northeast Brazil and *Leishmania braziliensis* was the causative agent in all patients. We found that the parasite burden is higher in ECL than in CL lesions and that the higher the parasite load the longer the healing time. We also assessed immune response on day 0, day 15 after treatment had been started. Peripheral blood mononuclear cells from patients in the early phase of CL produced high levels of inflammatory cytokines in response to soluble *Leishmania* antigen (SLA). However, non of the cytokines tested were associated with therapeutic failure. Addition of recombinant IL-10 to cells extracted from lesion biopsies decreased the levels of TNF, IL-1 beta, IL-2, IL-15 and IL-17, cytokines known to be involved in the pathogenesis of CL. Together, our data suggest that parasite number may serve as an indicative of therapeutic failure at point-of-care and that induction of IL-10 at lesion site may benefit the patient by decreasing inflammatory response.

563

IMPAIRED TH1 RESPONSE IS ASSOCIATED THERAPEUTIC FAILURE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS AND NEGATIVE LEISHMANIA SKIN TEST

Augusto M. Carvalho[1], Luiz Guimarães[1], Iana Prates[1], Rubia Costa[1], Lucas P. Carvalho[1], Phillip Scott[2], Edgar M. Carvalho[1]

[1]*Federal University of Bahia, Salvador, Brazil,* [2]*Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, United States*

Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by an exaggerated inflammatory response with high systemic production of Th1 cytokines such as IFN-γ and TNF to soluble *Leishmania* antigens (SLA). This inflammatory response contributes for the control of parasite multiplication but is also associated with tissue damage and ulcer development. Moreover, Th1 response is usually observed *in vivo*, by a positive delayed type hypersensitivity to the *Leishmania* skin test (LST). Despite, there are few CL patients with negative LST in the endemic area. Here we compare the clinical presentation, immune response and response to therapy in CL patients with a negative and positive LST. Participants include 13 patients with CL with LST- (cases) and 26 CL patients with LST+ (controls). The diagnosis was performed by the detection of DNA of *L. braziliensis* by PCR. All subjects had a negative test for HIV. Cytokine levels were determined by ELISA in supernatants of mononuclear cells stimulated with SLA and the ability of macrophages to kill *Leishmania* in the presence or absence of autologous lymphocytes was determined by optical microscopy. There was no difference in the size of the lesions in those with LST+ and LST-. However, while failure to antimony (Sb5) therapy was observed 9 of 13 (69,2%) of the cases it was found in 9 of 26 (34,6%) of controls (P< .05). IFN-γ and TNF levels were lower in those with LST-, while IL-1β, IL-6, IL-17 and MMP-9 levels were similar in both groups. There was no difference regarding the *Leishmania* killing by macrophages among the groups. However, while addition of lymphocytes in the culture decreased the percentage of infected cells and the number of intracellular amastigotes in LST+ group, no difference in infection rates was observed in LST- patients after coculture. Our study pointed out that impairment in Th1 immune response is associated with therapeutic failure and high levels of inflammatory cytokines IL-1β, IL-6, IL-17 contributes to o immunopathology in CL.

564

BLOOD MONOCYTES IN HUMAN VISCERAL LEISHMANIASIS ARE SKEWED TOWARDS NON INFLAMMATORY PHENOTYPE AND DISPLAY DEFECTIVE PHAGOCYTOSIS AND OXIDATIVE BURST

Neetu Singh[1], Christian Engwerda[2], Shyam Sundar[1]

[1]*Institute of Medical Sciences, BHU, Varanasi, Varanasi, U.P., India,* [2]*QIMR Berghofer Medical Research Institute, Herston, Australia, Australia*

Monocytes are important effector cells during Leishmania infection, and changes in their functions may impact development of immunity. However, functional characteristics of monocytes in patients with visceral leishmaniasis (VL) remains poorly understood. Peripheral blood monocytes from patients with VL and healthy endemic controls from Muzaffarpur, India, were isolated and compared in an ex vivo setting, using cell-culture techniques, flow cytometry, and reverse transcription quantitative polymerase chain reaction analysis. A blood monocyte population with a gene signature comprising upregulated expression of TGM2, CTLRs, VDR, PKM, SOCS1, and CAMP1 and down regulated expression of NOS2 and HIF1A was observed in patients with VL but not in controls. Monocytes from patients with VL also had impaired expression of pro inflammatory cytokines and their receptors. Importantly, monocytes from patients with VL had a markedly reduced capacity for phagocytosis and killing of engulfed promastigotes. Monocytes from patients with VL express antiinflammatory molecules and lack a classically activated phenotype. They have reduced expression of molecules related to activation and antiparasitic effector functions, indicating that monocytes are skewed toward an anti inflammatory phenotype. These findings provide insights into the functional status of monocytes during VL and advise that therapeutic manipulation of this important cell population may result in favorable patient outcomes.

565

IMPACT OF THE INTRODUCTION OF PCV7/13 ON ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE IN INDONESIA

Ebrima Jobarteh, Mustapha Danso, Dawda Kairaba Jawara, Mam Mass Sey

Universitas Islam negeri Uin syariff hidayatullah jakarta, Jakarta, Indonesia

We describe antimicrobial resistance in invasive pneumococcal disease due to all serotypes and non-vaccine types (NVT) pre and post pneumococcal conjugate vaccine (PCV) implementation in Indonesia in all age groups. We identified, serotyped, and performed antimicrobial susceptibility testing using disc diffusion methods on pneumococcal isolates obtained from invasive samples collected from standardised population-based pneumococcal disease surveillance in the Tangerang Health & Demographic Surveillance System. The study commenced in June 2006. PCV7 was introduced in August 2008 and PCV13 in May 2010. Antibiotic

susceptibility was interpreted using the Clinical Laboratory Standard Institute guidelines. 455 Pneumococcal isolates were screened against five antimicrobial agents. There was a moderate decline in antibiotic resistance in all age groups in invasive pneumococcal disease during vaccine implementation. In the 2-23 month age group, annual counts of oxacillin, chloramphenicol, and tetracycline resistant cases fell from 10-15 in 2009 and 2010 to 6-7 in 2014 and 2015. In the 24 – 59 month age group, there was a large fall in tetracycline resistant cases. In those >5 years, oxacillin, chloramphenicol, and tetracycline fell to zero cases in 2013 and 2014. Resistance fell primarily due to reductions in vaccine-serotypes 1, 5, 14 and 23F. The proportion of resistant NVT cases increased over time, particularly in the 2-23 month age group. Although there is an overall reduction in cases of antimicrobial resistant, we hypothesise that increased transmission of NVT after the introduction of PCV and exposure to antimicrobials facilitates the emergence of resistance in NVT. On-going surveillance is important to determine future trends in resistance as it has both clinical and public health importance in PCV era.

566

EVIDENCE OF EXOGENOUS AND ENDOGENOUS RE-INFECTION WITH *MYCOBACTERIUM TUBERCULOSIS* COMPLEX STRAINS AMONG PULMONARY TB PATIENTS WITH RECURRING TB EPISODES IN GHANA; A CALL FOR INTENSIFYING TB MONITORING

Prince Asare[1], Stephen Osei-Wusu[1], Adwoa Asante-Poku[1], Isaac D. Otchere[1], Dina A. Prah[1], Sonia Borrell[2], Edmund Bedeley[1], Audrey Forson[3], Kwadwo A. Koram[1], Sebastien Gagneux[2], Dorothy Yeboah-Manu[1]

[1]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana,* [2]*Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [3]*Department of Chest Diseases, Korle-Bu Teaching Hospital, Korle-Bu, Accra, Ghana*

This study sought to analyze *Mycobacterium tuberculosis* complex (MTBC) strains obtained from pulmonary TB (PTB) individuals with recurring TB episodes in Ghana to provide necessary data to aid TB control. Following a 3½ year population-based sampling, mycobacterial isolates were obtained from individuals clinically diagnosed of PTB and confirmed as members of the MTBC using PCR targeting IS*6110, rpoB* and/or spacer oligonucleotide typing (spoligotyping) analysis. MTBC isolates obtained from both episodes among individuals with recurring disease after lineage assigning using spoligotyping, were subjected to standard 15-*loci* MIRU-VNTR typing for strain differentiation. Using the combined resolution power of spoligotyping and MIRU-VNTR data, phylogenetic trees were reconstructed with the aid of the miru-vntr*plus* online tool to aid in the mapping of the various strains and also for differentiating endogenous re-infection (classical relapse case and a proxy for treatment failure) from exogenous re-infection (new secondary case). Participants' clinical and socio-demographic characteristics were analyzed together with the molecular data. Sixty (60) out of 2,309 participants (2.6%) were classified as suspected relapse cases and found not to significantly differ from the national average of 3% (p-value > 0.05). We obtained MTBC isolates from both TB episodes for 35 participants. Compared to the primary case, 54% (19/35) were resulting from the same strain (endogenous re-infection) with the remaining being either similar (11/35, 32%) or different (5/35, 14%) strains (exogenous re-infection). Prominent among these cases was a case with three different episodes with the third episode harboring a drug resistant strain. There is substantial evidence of the occurrence of both endogenous and exogenous re-infection of MTBC strains in the country and a clear indication of the predominance of the former probably resulting from treatment failures which can contribute to the development of drug resistant strains thus we advocate for increased TB monitoring. Whole genome sequencing and analysis is underway to confirm these observations.

567

ASSESSMENT OF HEALTH RELATED QUALITY OF LIFE OF ADULTS TREATED FOR PNEUMONIA IN NAIROBI HEALTH FACILITIES

Apollo Odhiambo Maima[1], Faith Apolot Okalebo[2], Dan Owino Kaseje[3]

[1]*United States International University - Africa, Nairobi, Kenya,* [2]*University of Nairobi, Nairobi, Kenya,* [3]*Great Lakes University of Kisumu, Kisumu, Kenya*

Pneumonia is a common cause of hospitalization in Kenya, causing major health and psycho-social impacts, with about 11% of patients dying. In Kenya, there has been little monitoring of the burden of adult pneumonia and, especially, Health-Related Quality of Life (HRQoL) after recovery. We set out to assess the HRQoL of adults treated for pneumonia in health facilities in Nairobi. The study was conducted in 2016 in Nairobi, a densely populated city burdened by chronic urban poverty, slum life, pollution and disease. The over-arching design of the study was descriptive cross-sectional survey. Adult pneumonia patients were surveyed as a census after giving signed consent to the study. The 8 sampled health facilities had both in-patient and out-patient services; and had laboratory and radiological capacity to confirm a pneumonia diagnosis. Basic patient data and demographics were collected on open data kit (ODK) platform using android phones. The Standard RAND 36-Item Health Survey (Version 1.0) questionnaire was used to assess the HRQoL. Data obtained was programmed and coded. It was then entered, decoded and analysed in Windows EXCEL, SPSS and in STATA 13. Descriptive statistics, inferential statistics and Correlation coefficients were generated. The median age for the male participants was 32(IQR: 28-42) and 28(IQR: 24-36) for females, which ages are much lower than those of pneumonia patients elsewhere like Karachi (60); Enugu (53); and Yaounde (40). The 18-29 year age group had an overall HRQoL median score of 32(IQR, 27-40). These rose with age to cap at median score of 62.5(IQR, 50-75) for the 65+ years age group. This is at odds with the findings elsewhere. Overall, women had higher HRQoL scores than men. Both sexes had comparable social functioning, pain, general health and emotional health role limit. But females fared much better in the physical functioning, energy/fatigue, and emotional wellness. Overall women had higher HRQoL scores than men, which is diametrically different from other findings, possibly because of the steeliness of Kenyan women.

568

A PROSPECTIVE STUDY FOR THE TREATMENT OF MULTIDRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* USING CIPROFLOXACIN-CHLORAMPHENICOL COMBINATION THERAPY

Sinthia Kabir Mumu, Akash Ahmed, M. Mahboob Hossain

BRAC University, Dhaka, Bangladesh

Pneumonia has become the leading cause of child death since many decades in developing countries. It is also a form of acute respiratory infections that affect lungs eventually. The purpose of the study was to develop a new approach to treat antibiotic-resistant *Klebsiella pneumoniae* infection. This study aimed in quest of a drug to combine with ciprofloxacin, a broad spectrum antibiotic frequently used to treat lung infections. A total of 23 lung infection bacterial samples were collected and studied against 14 antibiotics of different classes. The combination of ciprofloxacin with several drugs and the synergy screening, MIC value and qualitative toxicity analysis of ciprofloxacin and chloramphenicol combination done by the disk diffusion method. After the primary screening of antibiotic susceptibility, they were categorized into multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan drug-resistant (PDR) pathogens where 9 isolates were MDR, 5 were XDR and 3 isolates were PDR. Furthermore, they were trialed in combination ciprofloxacin along with other 7 drugs in disk diffusion to explore synergistic effect. The combination of ciprofloxacin and moxifloxacin, ciprofloxacin and chloramphenicol were found to be synergic. Then the MIC test was

done for the combination Ciprofloxacin and chloramphenicol. When the MIC result was generated, the MIC of the respective combination was analyzed. Furthermore, the fractional inhibitory concentration (FIC) was calculated and in accordance with the results of FIC index, the ciprofloxacin-chloramphenicol combination has shown value 0.4510 which revealed a synergistic effect against multi-drug resistant *K. pneumoniae*. The qualitative analysis of ciprofloxacin and chloramphenicol combination was done and the result revealed no higher toxicity against eukaryotic cell Saccharomyces spp. All things considered, developing countries should grab the chance to combat antibiotic-resistant *K. pneumoniae* from this combination therapy and reduce the mortality rate from prolonged pneumonia since this pathogen is emerging as a superbug and developing resistant mechanism smartly.

569

STANDARDIZATION OF A GENE COMPLEMENTATION TECHNIQUE PNCA IN MYCOBACTERIUM TUBERCULOSIS PNCA-KNOCKOUT: TOOL FOR THE STUDY OF THE RELATIONSHIP BETWEEN MUTATIONS IN PLAIN AND PHENOTYPIC PARAMETERS

Yudith Cauna Orocollo, Patricia Sheen Cortavarría, Mirko Zimic Peralta

Universidad Peruana Cayetano Heredia, Lima, Peru

Mutations in the pncA gene are the major cause of resistance to pyrazinamide (PZA), but there are alternative mechanisms that also provide resistance. The objective of the study was to standardize the technique of complementing the pncA gene in Mycobacterium tuberculosis H37Rv pncA-KO to evaluate the effect of mutations in the pncA gene on phenotypic parameters, in a scenario controlled by the genetic variability of the clinical isolates from which they originated. the mutant pncA genes. Strains supplemented with pncA WT genes and mutants D49N, H51R, G78C, and F94L were generated through the pNIT-1 expression system to evaluate PZAsa activity by quantitative Wayne test, the expression level of the pncA gene and susceptibility to PZA using concentrations PZA gradients under uninduced and induced conditions.The pNIT-pncA expression system restored the phenotype of the pncA-KO strain and the plasmids remained stable intracellularly during the observation period that lasted 18 weeks. The basal expression levels were constant and similar to that of the H37Rv strain. The complemented pncA WT strain showed PZAsa activity and PZA Minimum Inhibitory Concentration similar to that of the H37Rv strain. However, the pncA complemented mutant strains were resistant to PZA in both conditions and the overexpression of pncA mutant genes allowed to verify the total or partial loss of PZAsa activity.

570

EVALUATION OF SAME, DAY DIAGNOSIS OF TUBERCULOSIS MICROSCOPY IN COMPARISON TO THE SPOT-MORNING-SPOT METHOD IN SELECTED HEALTH INSTITUTION IN ADDIS ABABA, ETHIOPIA

Shemsu Kedir Juhar[1], Sisay Kebede Gebregorgis[2]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*Ethiopian public health institute, Addis Ababa, Ethiopia*

Over six million new cases of tuberculosis (TB) are diagnosed each year throughout the globe, and each year witnesses 1.3 million TB-related deaths. One smear-positive patient can infect 10-15 people in a year, and 10% of them will develop this disease. The need to collect serial sputum specimens over multiple patient visits for pulmonary tuberculosis results in a protected diagnostic process with rates of patients with high rates of patient dropout. Recent studies on spot morning spot (SMS) method of examination PTB reported that the first two specimens have high smear positivity in line with this WHO changed its policy to minimize the number of sputum specimens from three to two. Across-sectional study was conducted in 16 conveniently selected private clinics, governmental health centers, public and private hospitals from September - December 2017. Individuals attending the selected health institutions for the diagnosis of MTB submitted three sputum samples for routine diagnosis (the standard approach). One additional sample was collected 1 h after the first sputum (the same-day approach). One sputum sample was cultured. The diagnosis was performed using ZN sputum smear microscopy and light-emitting diodes fluorescent microscopy (LED-FM) technique. Data were entered and analyzed using SPSS version 16. We used sensitivity, specificity and predictive values for the different methods.A total of 209 participants enrolled, 43(21%) were identified culture positive, 39 (18.7%) were detected by the same day approach and 40(19.1%) by the standard approach.On the other hand, LED-FM and ZN microscopy detected 39(18.1%) and 48(23%) tuberculosis cases respectively. Sensitivity was 88.4% for ZN microscopy and 95.3% for LED-FM and the specificity was 99.4% and 95.9% for ZN and LED -FM microscopy respectively.Using same day approach together with LED-FM would reduce workload, TAT, patient drops out and increases the smear detection rate. Therefore it is necessary to give in-service and off service training for health personnel towards the use of LED-FM, conventional approach and the same day approach in the diagnosis of TB.

571

ASSESSMENT OF EFFLUX PUMPS FROM *MYCOBACTERIUM TUBERCULOSIS* BY CRISPR INTERFERENCE IN *MYCOBACTERIUM SMEGMATIS* IN VIVO MODEL AND ITS EFFECT ON SUSCEPTIBILITY TO PYRAZINAMIDE

Stefany Quinones-Garcia[1], Patricia Sheen[1], Mirko Zimic[1], Jeremy M. Rock[2], Robert H. Gilman[3]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*The Rockefeller University, New York, NY, United States,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). In 2017, 1 400 000 deaths and 100 000 new cases were reported. Among the several therapeutic schemes, pyrazinamide stands out for being the only drug that is used for both types of TB, as it has a bactericidal effect on the latent forms of the tubercle bacillus. However, the mechanism of action of PZA and its active derivative, pyrazinoic acid (POA), remains unknown on the current field. In a previous study of our MTB research group, genes efflux pumps were identified in MDR or XDR strains, which showed a negative correlation with the kinetics of POA efflux, suggesting that such efflux pumps could contribute to the resistance mechanism on POA. The objective of this study is to explore the correlation of MTB of homologous efflux pumps with their respective kinetics of POA efflux. Transcriptional repression by CRISPRi was conducted using the *in vivo* model of *Mycobacterium smegmatis*. Nine genes will be silenced and analyzed with their individual response to POA as well as their collective response in groups of two. So far, genes Msmeg_0250 and Msmeg_3815 have been silenced successfully, to which their kinetics are being evaluated. With regard to the other seven, different gRNAs are currently being cloned in the plasmid pJR962, until the silencing induced with anhydrotetracycline can be corroborated through real-time PCR. Multiple silencing for the collective POA response will be performed in function of the total individual results, which is to be expected by June of 2019.

572

HEALTH CARE UTILIZATION SURVEY OF CHILDREN UNDER FIVE WITH PNEUMONIA IN PERI URBAN COMMUNITIES OF KARACHI, PAKISTAN

Bushra Abid Iqbal Mufti, Salima Kerai, Imran Nasir, Muhammad Ilyas, Sana Qaiser, Khalid Feroz, Azhar Raza, Azhar Raza, Faizan Khalid, Benazir Balouch, Fyezah Jehan

Aga Khan University Hospital, Karachi, Pakistan

Pneumonia is regarded as leading cause of child mortality worldwide, accounting for 15 million deaths every year in children under five years of age.Timely recognition of symptoms, appropriate care seeking and management are key to survival. We assessed health care utilization

pattern among caregivers of children under five with fast breathing pneumonia in urban slums of Karachi, Pakistan. Random sample stratified on age was drawn from an existing line listing obtained from ongoing demographic surveillance. Data on household demographics, pneumonia specific symptoms, care seeking, air quality and knowledge regarding preventive measures for pneumonia was gathered on a standardized questionnaire from caregivers of children by community health workers (CHWs). A weight was assigned to each child in the sample representing the number of children in the population. Predictors of care seeking were estimated using weighted logistic regression. Information on 1152 children was included in the analysis. About 95% of caregivers sought care; 68.5% privately. Care seeking was higher for infants. Primary health center utilization was comparatively higher for non-severe (fast breathing) pneumonia (29%), whereas, private care was sought for conditions like chest indrawing and/or danger sign (70%). About 5% did not seek any care. Reasons identified were clinic too far from home, siblings at home who could not be left alone and high cost of treatment. Odds ratios for predictors of seeking care were younger age of child (OR 3.6, 95% CI 2.65,4.87), caretaker with primary education compared to none (OR 3.4, 95% CI 2.46,4.70), vaccination of child (OR 1.28, 95% CI 1.12,1.46) and presence of symptoms like fever (OR 1.51, 95% CI 1.30,1.76), tachypnea (OR 1.57, 95% CI 1.35, 1.83), chest indrawing(OR 2.56, 95% CI 2.05, 3.18), persistent vomiting (OR 1.69, 95% CI 1.37, 2.09), recurrent illness (OR 2.57, 95% CI 2.23, 2.97) and vaccine (OR 1.65, 95% CI 1.45,1.87) and breastfeeding awareness (OR 1.32, 95% CI 1.13,1.53). The study findings indicate high health care utilization for pneumonia in these communities.

573

AN OUTBREAK OF ADENOVIRUS CAUSING SEVERE RESPIRATORY ILLNESS IN SOUTHERN SRI LANKA, 2018

Weerasinghe M. D. G. B. Wijayaratne[1], Sky Vanderburg[2], Vasantha Devasiri[1], Ajith Nagahawatte[1], Champika K. Bodinayake[1], Eelizabeth Petzold[2], Sunethra Gunasena[1], Nayomi Danthanarayana[3], Bhagya Piyasiri[3], Muhunthan Sellathurai[3], Nayani P. Weerasinghe[1], Chathuranga L. Fonseka[1], Ruvini P. Kurukulasooriya[1], Nishantha C. Gunasekara[1], Brad P. Nicholson[2], Chris W. Woods[2], L. Gayani Tillekeratne[2]

[1]*University of Ruhuna, Galle, Sri Lanka,* [2]*Duke University, Durham, NC, United States,* [3]*Teaching Hospital Karapitiya, Galle, Sri Lanka*

In Sri Lanka, influenza activity peaks during March- June annually. In southern Sri Lanka, paediatricians noted a rapid increase in admissions of children with serious, and in some cases fatal, respiratory illnesses from April to June 2018. Consequently, active surveillance of cases was initiated at Teaching Hospital Karapitiya (THK) the largest tertiary care hospital in the Southern Province, Sri Lanka, to better characterize cases. Patients in all pediatric wards and intensive care units at THK were systematically screened daily from 28/05/2018 to 25/06/2018 using a case definition of fever with one or more features of respiratory tract infection. Nasopharyngeal (NP) and oropharyngeal (OP) swab samples were collected from each case for real time-PCR (RT-PCR) identification of adenovirus, influenza A, and respiratory syncytial virus (RSV). Blood samples were collected from a subset of patients to determine viremia. During the period of active surveillance, 170 pediatric and 12 adult cases were identified, of which 9 (5.3%) paediatric cases and 3 (25%) adult cases were fatal. Fifty-three (31.2%) paediatric patients needed respiratory support by means of oxygen, intubation, ICU care or a combination of these. Adenovirus was detected in 43.7%, RSV in 29.6%, and influenza A in 26.7% of 135 pediatric NP/OP swab samples tested by RT-PCR. Of 11 adult NP/OP swab samples tested, adenovirus was detected in 45.5%, influenza A in 27.2%, and RSV in 0%. In 25 (13.2%) of samples, ≥2 viruses were detected. Adenovirus was detected in blood samples of all cases (N=3) tested, corresponding to adenovirus detection in NP/OP samples from the same individuals. Further serotyping of adenovirus positive subset of samples revealed that these isolates belong to adenovirus type B. This severe outbreak of respiratory viral infection in the Southern Province of Sri Lanka turned out to be due to adenovirus, although clinicians were expecting influenza. This outbreak signifies the importance of continued surveillance of all major respiratory viruses across the year.

574

BIOMARKERS OF PEDIATRIC PNEUMONIA: THE POSSIBILITY OF A FINGERSTICK DIAGNOSTIC TEST

Jack Underschultz[1], Jeremy Soo[1], Ravi Bhargava[1], Robert Opoka[2], Andrea Conroy[3], Sophie Namasopo[4], Michael Hawkes[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Makerere University, Kampala, Uganda,* [3]*Indiana University School of Medicine, Indianapolis, IN, United States,* [4]*Kabale Regional Referral Hospital, Kabale, Uganda*

Chest x-ray (CXR) is commonly used as a diagnostic tool for pediatric pneumonia, but is not available in many resource-limited areas of the world where a large number of pneumonia deaths occur. We hypothesized that plasma biomarkers of inflammation, endothelial activation, and lung injury, which could be adapted to a point-of-care platform, would be associated with CXR consolidation and could be used as a diagnostic test to replace the CXR. We conducted a cross-sectional study of 108 children under 13 years of age who were hospitalized with clinical pneumonia at two resource-limited hospitals in Uganda. Chest radiography was performed locally and interpreted by a Canadian certified (FRCPC) pediatric radiologist. Enzyme-linked immunosorbent assays were performed in order to measure the following serum host response biomarkers: C-reactive protein (CRP), Chitinase 3-like 1 (CHI3L1), Lipocalin-2 (LCN2), Tie-2, Intercellular adhesion molecule-1 (ICAM1), endoglin, Tissue Inhibitor of Metalloprotease-1 (TIMP1), and surfactant protein-D (SP-D). A total of 108 children were included (39% female) with median age 10 months (IQR 3-20 months). Based on x-ray findings, children were categorized as primary end-point pneumonia (n=24), other infiltrates (n=49), or normal chest x-ray (n=35). Compared to children with normal x-ray, children with end-point pneumonia had significantly higher levels of CRP, CHI3L1, LCN2, and SP-D. CRP had the best discriminatory power (AUROC 0.73 (95%CI 0.57, 0.88) p=0.0036). Using the optimal cutoff (>35 μg/mL), CRP had a sensitivity of 71% (95%CI 53-89%) and a specificity of 79% (95%CI 65-93%) to discriminate between end-point pneumonia and normal chest x-ray. The probability of CXR consolidation increased in a step-wise manner with the number of elevated biomarkers (8% if none to 79% in children with all five biomarkers elevated). In summary, among Ugandan children with clinical pneumonia, host biomarkers distinguished between end-point pneumonia and normal chest x-ray. Together, this group of biomarkers may have clinical utility in predicting alveolar consolidation.

575

DETECTION AND MOLECULAR CHARACTERIZATION OF *GIARDIA DUODENALIS* IN PATIENTS WITH CHRONIC AND PERSISTENT DIARRHEA

Sumeeta Khurana, Monika Jangra, Usha Dutta, Rakesh Sehgal, Br Thapa, Nalini Gupta, Ritambhra Nada

Postgraduate Institute of Medical Education and Research, Chandigarh, India

Giardia duodenalis is an important cause of diarrhoeal illness in humans worldwide. The clinical manifestations range from asymptomatic to acute or chronic diarrhoea. This high variability depends on interplay between various factors like inoculum size, host factors and parasitic factors like assemblage. Stool samples were collected from 100 patients with chronic diarrhoea (excluding celiac and inflammatory bowel disease) and 50 patients without gastrointestinal symptoms attending Postgraduate Institute of Medical Education and Research, Chandigarh, India for microscopy (3 consecutive stool samples), Nested PCR (triosephosphateisomerase gene (tpi)) and Assemblage specific PCR for Giardia. Duodenal biopsy samples were available for 30 patients only. The sensitivity and specificity of nested PCR was initially evaluated on 40 Giardia microscopy positive and 50 negative stool samples. Overall prevalence of *G. duodenalis* infection in patients with chronic diarrhoea

was found to be 48%. In children less than 14 years, prevalence of 53.33% was seen which was higher compared to 45.71% prevalence seen in adult patients. Assemblage B (60.60%) was found to be the more common than assemblage A. There was no assemblage specific difference in severity or chronicity of clinical manifestations. In stool samples, sensitivity and specificity of nested PCR was found to be 100% each while that of microscopy was 70.45% and 100%, respectively. Duodenal biopsy PCR additionally detected 4 patients of giardiasis that were negative by stool PCR; however former missed 5 stool PCR positive Giardia infections. Thus, it was found that nested PCR can be used as a new gold standard for detecting giardiasis in chronic diarrhoea patients. However, neither stool sample nor duodenal biopsy alone can exclude giardiasis in all the cases. 26.67% of infections can still remain undetected if only stool samples are tested by PCR. This can be due to intermittent shedding of the parasite. In such situation, PCR of duodenal biopsy samples can help in picking up the remaining cases of giardiasis who are not detected by stool nested PCR.

576

GIARDIA ASSEMBLAGES AND DIARRHEA IN CHILDREN BELOW FIVE YEARS IN SIAYA COUNTY, KENYA

Esther Omuseni[1], Benjamin Ochieng[1], Jane Juma[1], Evans Apondi[1], Richard Omore[1], Irene N. Kasumba[2], Anna Rose[2], Jie Liu[3], Eric Houpt[3], Sharon Tennant[2], Karen Kotloff[2]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States*

Giardia is one of the most common pathogens known to cause diarrhea among children in underprivileged communities. *Giardia duodenalis* is the species affecting humans and is comprised of eight distinct groups, called assemblages. Only A and B are associated with human infection. Asymptomatic infected children persistently contaminate their environment by shedding cysts. We assessed the association of *G. duodenalis* assemblages and diarrhea with the aim to find a common genotype among children with moderate-to-severe diarrhea (MSD) in the case-controlled, prospective Vaccine Impact on Diarrhea in Africa (VIDA) study. 408 stool samples were randomly selected from symptomatic and asymptomatic children positive for *Giardia* by Enzyme Immunoassay. Samples were tested by real-time PCR for *Giardia spp.* and assemblages A and B. Number of cycles required for the fluorescent signal to cross the threshold (Ct) was used to analyze the parasite load (Ct lt 25, abundant target; 25-35; moderate amounts; and, 36-40 indicates minimal amounts of target). Of the 408 stool samples, 293 (71.8%) were positive for *Giardia*, of which 32 (10.9%) and 218 (74.4%) were positive for A and B respectively. There was an increase in *Giardia* with age (16.7%, 34.1% and 49.1% in 0-11, 12-23 and 24-59 months respectively) in both symptomatic and asymptomatic children.. Within age groups, *Giardia* detection in symptomatic children decreased (55.1% to 44.4%) and increased in asymptomatic children (44.9% to 55.6%) from youngest to eldest age group with assemblage B being the most common. Proportion of cases increased as the Ct value increased (44% for CT lt 25 to 64% for CT 30-35). The reverse was observed for controls suggesting a protective mechanism. Our data suggest that B is the most common assemblage in both cases and controls. Asymptomatic infections increase with age hence these children could serve as potential reservoirs of *Giardia* and this parasite could have a protective effect against diarrhea. Future investigations should focus on understanding the potential mechanisms that contribute to carrier status.

577

COMPARATIVE EFFICACY OF DNA ISOLATION FROM RECTAL SWABS AND BULK STOOL FOR MOLECULAR DETECTION OF *GIARDIA INTESTINALIS*

Jacqueline R. Maasch[1], Ahmed M. Arzika[2], Catherine Cook[3], Elodie Lebas[3], Nils Pilotte[1], Jessica R. Grant[1], Steven A. Williams[1], Jeremy A. Keenan[3], Kristen A. Aiemjoy[4]

[1]*Smith College, Northhampton, MA, United States,* [2]*University of California San Francisco, Niamey, Niger,* [3]*University of California San Francisco, San Francisco, CA, United States,* [4]*Stanford University, San Francisco, CA, United States*

Enteropathogen control and elimination efforts require sensitive, cost-effective, and practical diagnostics that yield accurate and reproducible results. While bulk stool remains the gold standard specimen type for enteropathogen diagnosis, evidence suggests that rectal swabs may offer comparable diagnostic sensitivity with greater ease of sample collection for select bacterial and viral pathogens. We sought to evaluate the validity and reproducibility of rectal swabs for the molecular detection of the protozoan parasite *Giardia intestinali*s in a population-based sample of young children. We collected paired rectal swab and bulk stool samples from 86 children ages 0-5 years living in the Boboye and Loga departments of Niger. Duplicate rectal swab and bulk stool samples were collected among a subset of 50 children. Both swabs and stool samples were stored without any media preservative and frozen at -20°C on the day of collection. For detection of *G. intestinalis*, we used an in-house real-time PCR assay targeting a segment of the 16S ribosomal RNA repeat determined to be conserved across three strains that commonly infect humans. *G. intestinalis* was detected in 63.95% (55/86) of bulk stool and 41.86% (36/86) of rectal swab samples. The kappa evaluating test agreement among children with duplicate samples was 0.91 (95% CI 0.63-1.00) for bulk stool and 0.80 (95% CI 0.52-1.00) for rectal swabs. The PCR sensitivity of rectal swabs relative to bulk stool was 62.0% (95% CI 48.0-75.0%) and the specificity was 94.0% (95% CI 79.0-99.0%). The PCR sensitivity and specificity of rectal swab samples were not affected by visible presence of fecal matter, the child's age, or the consistency of the matched bulk stool sample. While rectal swabs may be a viable source of pathogen DNA for select diagnostic targets, these findings suggest that rectal swabs provide less sensitive and reproducible results than bulk stool for the real-time PCR diagnosis of *G. intestinalis*. However, the high specificity and fair reliability of swabs indicate that they may still be of value in situations where bulk stool collection is logistically infeasible or cost-prohibitive.

578

MOLECULAR CHARACTERIZATION OF *ENTAMOEBA* COMPLEX IN HUMAN STOOL SAMPLES FROM CASES AND CONTROLS IDENTIFIES *ENTAMOEBA MOSHKOVSKII* FOR THE FIRST TIME IN KENYA

C. Kyanya[1], F. Eyase[2], E. Odundo[2], E. Kipkirui[2], N. Kipkemoi[2], R. Kirera[2], C. Philip[2], J. Ndonye[2], M. Kirui[2], A. Ombogo[2], M. Koech[2], W. Bulimo[2], A. Flynn[2], C. E. Hulseberg[3]

[1]*Jomo Kenyatta University of Science and Technology, Nairobi, Kenya,* [2]*United States Army Medical Research Directorate-Africa, Nairobi, Kenya,* [3]*United States Army Medical Research Institute of Infectious Diseases, Silver Spring, MD, United States*

Amoebiasis caused by *Entamoeba histolytica* is a leading cause of diarrhea globally. Indistinguishable in morphology from *E. histolytica* are non-pathogenic *E. dispar* and *E. moshkovskii* which all form the *Entamoeba* complex. Microscopy remains the mainstay for diagnosis of *E. histolytica*, especially in low resource settings. WHO recommends treatment of only *E. histolytica*, but this is only possible with an accurate diagnosis. In Kenya, the distribution of *E.* complex is not well-defined. We sought to speciate *E.* complex species from stool samples for an ongoing enteric pathogen surveillance study in Kenya. A total of 46 paired case-control archived stool specimens collected between April 2013 and September 2014 across

seven sites in Kenya were analyzed. Twenty-three had previously been identified as positive for *E.* complex by microscopy. DNA was extracted using the QIAamp DNA Stool Mini Kit. Species detection was done using nested PCR with the resulting amplicons sequenced by Sanger method. Consensus sequences were compared to those on GenBank database and maximum likelihood phylogenies reconstructed using phyML 3.1. Out of the 46 samples, 22 (47.8%) were positive for *Entamoeba* species. Of these, 16 had initially been identified as microscopy positive for *Entamoeba* complex. Among the 22 PCR-positives *Entamoeba* complex species were identified as follows: 9 were *E. dispar* (40.9%), 2 were *E. moshkovskii* (9.1%), and 1 was *E. histolytica* (4.5%). Combinations of *Entamoeba* species detected were: 3 *E. histolytica* and *E. dispar* (13.6%), 2 *E. histolytica* and E. *moshkovskii* (9.1%), 4 *E. moshkovskii* and *E. dispar* (18.2%) and 1 *E. histolytica* and *E. dispar* and *E. moshkovskii*. Sequence analysis revealed 99% identity to *E. dispar* (SAW 760), *E. moshkovskii* (Laredo) and *E. histolytica* (HM-1: IMSS). Reconstruction of phylogenetic relationships revealed distinct species-specific clustering. It's possible that *E. moshkovskii* infections have been in circulation in Kenya for some time and are only now being reported. It is important to establish the molecular epidemiology of *E.* complex, so as to accurately treat amoebiasis in endemic areas like Kenya.

579

PREVALENCE OF *BLASTOCYSTIS SP.* AND ASSOCIATED FACTORS TO INFECTION AND SYMPTOMATOLOGY IN PERIURBAN COMMUNITIES OF AREQUIPA, PERU

Victor Luis Vasquez Huerta[1], Renzo Sadath Salazar Sánchez[1], Elí Martínez Barrios[1], Kasandra Lizzeth Ascuña Durand[1], Ana Leila Maza Santillán[1], Mónica Yauri Huamani[1], Almendra Del Rosario Ascuña Durand[1], Jorge Andrés Ballón Echegaray[1], Ricardo Castillo Neyra[2]

[1]*Universidad Nacional de San Agustín, Arequipa, Peru,* [2]*University of Pennsylvania, Philadelphia, PA, United States*

Entero-parasitic infections are one of the most important causes of acute illnesses in humans. They are mainly present in poor areas with unhealthy environmental conditions. Periurban communities usually experience some of these conditions, including limited access to safe drinking water and inadequate disposal of human feces. Among entero-parasites, *Blastocystis sp.* is the most common protozoa in the human gut with wide distribution around the world. People infected with *Blastocystis sp.* do not show specific symptoms or are asymptomatic, making it difficult to assess its pathogenic potential and determine if and how much zoonotic transmission occurs. The aim of this study was to determinate the prevalence of *Blastocystis sp.* and the factors associated with infection, and symptomatology presentation in periurban communities from Arequipa city. We conducted epidemiological surveys and analyzed stool samples from 189 participants and 144 animals from participants' households using the concentration-spin method and direct stool exam by microscopy. We compared individual-level and household-level covariates between infected and uninfected participants and between symptomatic and asymptomatic cases. 49.2% of participants were infected with *Blastocystis sp.* Among infected, 61.3% had non-specific gastrointestinal symptomatology. We found an association between *Blastocystis sp.* infection and lack of access to safe drinking water (p=0.004) and inadequate disposal of human feces (p=0.03). Other variables such as age, sex, presence of animals or vectors at home, food consumption, and hygienic habits were not associated. Additionally, we found 34.4% of coinfections between *Blastocystis sp.* and other intestinal parasites. Only 8.3% of the animals were infected with *Blastocystis sp*. Our results suggest that *Blastocystis sp.* infection does not present a clear symptomatology and that the main factors associated with *Blastocystis sp.* infections ocurr at the household-level: water supply and the feces final disposition. The implications of these findings on the control and transmission of *Blastocystis sp.* are discussed.

580

HEALTHY COMMUNITY STOOL SCREENINGS IN RURAL NICARAGUA REVEAL HIGH PREVALENCE OF PROTOZOAL INTESTINAL PARASITES AND POLYPARASITISM

Jolie Starling[1], Anna Strasma[2], Reyna Silva[3], **Rebecca S. Fischer**[1]

[1]*Texas A&M University Health Science Center, College Station, TX, United States,* [2]*Baylor College of Medicine, Nephrology, Houston, TX, United States,* [3]*Amigos for Christ, Chichigalpa, Nicaragua*

Individuals in tropical, rural regions of the world are at high risk for infectious diseases, including parasitic diseases. Protozoal and worm parasites are a public health challenge in low-income settings, where prevention, surveillance, detection, and access to treatment are stressed by limited infrastructure and resources. Identifying epidemiologic patterns within high-risk communities can inform vulnerable populations and serve as evidence to develop efficient treatment and prevention measures, such as water sanitation and filtration, education, and targeted surveillance. Stool and urine specimens were collected from individuals attending health fairs in 2 rural, agricultural communities in western Nicaragua. Stool was analyzed by microscopy. We generated descriptive statistics and report prevalence and characteristics with Chi-squared and ANOVA using Stata 15. We analyzed stool from 221 residents, ages 3 months to 89 years (median 21 years). *Endolimax nana* (67%), *Entamoeba coli* (44%), *Entamoeba histolytica* (33%), and *Giardia lamblia* (23%) were most common; *Necatur americanus* eggs were recovered from one 87-year old. One community had higher prevalence of *E. histolytica* (56%;p=0.004) and *E. coli* (51%;p=0.005) than the other. 28% harbored 3 or more species, but *G. lamblia* and *E. histolytica* were negatively correlated (p<0.05). Only 18 individuals (8%) were parasite-free. We document a high prevalence of parasites and polyparasitism, and we suggest widespread exposure or frequent transmission is likely occurring within these and neighboring communities. Although not all organisms identified are perceived as pathogenic, there is clearly a need for health education and interventions to reduce exposure to protozoal parasites in the community-at-large. Geographic differences we found may guide treatment campaigns, interventions to break transmission cycles, and health campaigns to prevent disease. These data can inform targeted public health efforts to reduce morbidity, mortality, and long-term health consequences of parasitic infections in high risk communities.

581

INFLUENCE OF HOST NUTRIOME ON IMMUNOLOGICAL CONTROL OF PROTOZOAL INFECTIONS

Emma Hagopian[1], Shveta Bhasker[1], Ruwandi Kariyawasam[2], David Harris[1], Priyanka Challa[1], Celine Lecce[1], Rachel Lau[3], Andrea Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [3]*Public Health Ontario Laboratories, Toronto, ON, Canada*

Immunologic control of parasitic infections arises from a combination of humoral and cellular mechanisms, both of which may be influenced by host nutritional status. Micronutrient depletion or over-repletion impairs the functioning of the immune system, potentially resulting in increased susceptibility to and poor immunologic control of protozoal infections. We aim to synthesize the knowledge surrounding the interplay between host micronutrient status and tissue-based protozoal infections. Specifically, we will map the literature of how nutrient deficiencies such as zinc, iron, and vitamin A impact immune response and defenses in infectious diseases such as malaria, Chagas disease, and leishmaniasis. Five electronic databases were searched including PubMed, Embase, Medline, Scopus, and LILACS with combinations of search terms such as Parasite* AND (Immunology OR Immunity OR Immune System OR Immune Function OR Immune Impairment OR Immune Response OR Immune Status) from database inception to March 13, 2019. A total of 30 872 articles were retrieved: 15 254 articles on PubMed, 8192 on Embase, 5909 on Medline,

1411 on Scopus, and 106 on LILACS. After eliminating duplicates using Mendeley software, a total of 21 821 articles remained for title screening. Titles, abstracts, and full-text articles will be systematically double screened by two reviewers with a tertiary arbitrator. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) will be implemented. Data extraction will be performed by two reviewers and the quality of the articles will be critically evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. The data will be summarized to systematically map published literature that will illuminate a number of ways in which nutrient deficiencies or abnormal micronutrient status alter and impair immune function in persons with protozoal infections. This synthesized body of information will ultimately inform adjunctive therapeutic decisions in the context of protozoal infections, which has the potential to improve patient prognosis.

582

THE ROLE OF PROTOZOAN PARASITES IN FEVERS OF UNKNOWN ORIGINS IN GHANA

Georgina I. Djameh, Annabella Nkansah, Senyo Botchie, Irene Ayi

Noguchi Memorial Institute for Medical Research, Accra, Ghana

Fever is a major feature of many illnesses and remain one of the most challenging clinical evaluations for pediatric clinicians. In Ghana, parasitic diagnosis of fevers in children have focused on malaria but only half or less the number of such cases are observed to have malaria parasite. This study sought to determine other etiologies of FUO besides *Plasmodium* parasites and profiling cellular immune responses to parasitic infections in children. Children younger than 13 years who reported at the pediatric ward of the Cape Coast Teaching Hospital, Ghana with fever (a high temperature ≥ 38°) were enrolled in the study. Venous blood and stool samples were collected from 143 participants and transported to the Noguchi Memorial Institute for Medical Research for analysis. Differential diagnosis was performed for the presence or absence and further genotyping of *Plasmodium*, *Toxoplasma gondii*, *Babesia*, *Cryptosporidium*, *Giardia lamblia* and *Entamoeba* spp. using Polymerase Chain Reaction (PCR). Enzyme-linked immunosorbent assay was performed to obtain and compare the levels of cytokines with respect to infection status. *Plasmodium falciparum* was detected in 27.3% (39/143) of participants. Out of the 104 *P. falciparum* negative samples, 18 (16.7%) *G. lamblia*, 2 (1.9%) *E. histolytica*, 2 (1.9%) *Toxoplasma gondii* and 1 (0.9%) *Cryptosporidium* spp. were detected. Cytokine analysis revealed that, *Plasmodium* negative detected parasites had lower cytokine level than *Plasmodium* positive samples. Also, the level of cytokines established between *Plasmodium* positive infection and *Plasmodium* negative detected parasites were not significantly different (*P*-value > 0.05). Varying levels of parasites virulence can lead to modulating effect by either aggravating or alleviating immune responses.

583

EPIDEMIOLOGY AND CLINICAL PRESENTATION OF *CRYPTOSPORIDIUM*-ASSOCIATED DIARRHEAL DISEASE IN CHILDREN UNDER FIVE FROM THREE COUNTRIES IN SUB-SAHARAN AFRICA

M. Jahangir Hossain[1], Anna Roose[2], Samba Sow[3], Sanogo Doh[3], Richard Omore[4], Ben Ochieng[4], Joquina Chiquita M. Jones[1], Syed M.A. Zaman[1], Henry Badji[1], Sharon M. Tennant[2], Irene Kasumba[2], Helen Powell[2], Dilruba Nasrin[2], Jie Liu[5], James Platts-Mills[5], Martin Antonio[1], Eric Houpt[6], Karen L. Kotloff[2]

[1]*Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [2]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Center for Vaccine Development-Mali, Bamako, Mali,* [4]*Kenya Medical Research Institute, Kisumu, Kenya,* [5]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States,* [6]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States*

Cryptosporidium causes significant diarrheal morbidity and mortality in children worldwide, including over 40,000 deaths in sub-Saharan Africa. We describe the epidemiology of *Cryptosporidium* in children <5 years of age from the Vaccine Impact on Diarrhea in Africa (VIDA) study in The Gambia, Mali and Kenya (2015-2018). VIDA enrolled healthcare-seeking cases with moderate-to-severe diarrhea (MSD, defined as diarrhea [≥3 loose stools/day] with dysentery or signs of dehydration, IV fluids or hospitalization). Diarrhea-free controls matched for gender, age, time, and community were enrolled at home. Each case and one control provided a stool sample at enrolment to be tested for a panel of enteropathogens, including *Cryptosporidium* species, by TaqMan Array Card quantitative PCR. Using conditional logistic regression, the association between *Cryptosporidium* and MSD status was assessed, adjusting for other pathogens and including interactions with age stratum and site. Episode-specific attributable fractions (AFes) were calculated for each case; cases with an AFe ≥ 0.5 were considered etiologic. Altogether, 4738 cases and 4738 matched controls were enrolled and analysed. *Cryptosporidium* was frequently detected among both groups (23.3% in cases v. 18.3% in controls) and common at all three sites (29.5% of MSD cases in The Gambia, 23.9% in Mali, 16.0% in Kenya). *Cryptosporidium* peaked annually in both The Gambia and Mali coinciding with the rainy season, but was prevalent year-round in Kenya. Among cases, *Cryptosporidium* detection was considered etiologic for 49.9% of detections. MSD cases with etiologic *Cryptosporidium* were younger than non-etiologic cases (median 13 v. 17 months), and 81.5% of etiologic cases were less than 24 months of age. *Cryptosporidium* cases had a longer duration of illness compared to watery diarrhea cases attributable to other causes (Median (IQR): 6 days (4-9) vs. 5 days (3-8), p<0.01) and had higher mean modified Vesikari severity scores (10.0 vs. 9.3, p<0.01). Our results suggest that *Cryptosporidium* infection is prevalent and pathogenic in the populations examined in VIDA.

584

ENVIRONMENTAL EXPOSURES ARE A RISK FACTOR FOR *TOXOPLASMA GONDII* INFECTION IN AN URBAN SLUM IN SALVADOR, BRAZIL

Arnau Casanovas-Massana[1], Joyce Wang[1], Elsio A. Wunder[1], Ridalva D. Felzemburgh[2], Renato B. Reis[2], Nivison Nery[2], Guilherme S. Ribeiro[2], Federico Costa[2], Peter J. Diggle[3], Mitermayer G. Reis[2], Claudia Munoz-Zanzi[4], Albert I. Ko[1]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*Oswaldo Cruz Fundation, Salvador, Brazil,* [3]*University of Lancaster, Lancaster, United Kingdom,* [4]*University of Minnesota, Minneapolis, MN, United States*

Toxoplasmosis, caused by the parasite *Toxoplasma gondii*, is one of the most common zoonotic diseases globally. Urban slum communities in tropical developing countries are especially vulnerable to the disease because of low socioeconomic status, increased exposure to contaminated environments, and the lack of sanitation infrastructure. Yet, our understanding of the transmission of the disease in these high-risk settings is very limited. Here, we performed a retrospective longitudinal study and an environmental survey of the pathogen in an urban slum in Salvador (Brazil). We enrolled a cohort of 728 young residents (aged 5-18) and followed them for 5 years. Serum samples were tested annually for *T. gondii* IgG antibodies with an enzyme immunoassay. We collected information on demographic and social status, household environment, and household related behaviors. We also measured the occurrence of *T. gondii* by qPCR in sewage from the precarious open sewer system. The overall prevalence of *T. gondii* antibodies was 49.0% (95% CI 44.3-51.5) with a cumulative incidence of 2.9% infections (95% CI 1.9-6.5) per 1,000 follow-up events. We used binomial regression multivariate analysis to evaluate risk factors for *T. gondii* antibodies and found that males were at greater risk than females (OR 1.9, 95% CI 1.4-2.6) and seroprevalence increased with age from 23.4% (95% CI 15.0-30.2) in the 4-6-year-old

group to 64% (95% CI 54.8-69.1%) in the 16-18-year-old group. Low per capita income was an independent risk for infection and contact with sewage near the house was associated with a 96% increased risk of antibody acquisition. A one-meter increase in household elevation was associated with a 2% decrease in infection odds (95% CI 0%-3%), indicating higher risk in the lower elevation areas, which are more prone to flooding during seasonal rainfall. Furthermore, 9 of 72 (13%) sewage samples were positive for *T. gondii* with an average concentration of 3.5 ± 1.9 oocysts/L. Overall our findings indicate that urban slum dwellers are at high risk for *T. gondii* infection at young ages and that environmental exposures at the household level are a key risk factor for infection.

585

THE FECAL MICROBIOME ASSOCIATED WITH *CRYPTOSPORIDIUM*-INFECTION AND DIARRHEAL SYMPTOMS IN BANGLADESHI CHILDREN

Maureen A. Carey[1], Gregory L. Medlock[1], Sultan Uz Zaman[2], Md Jashim Uddin[1], Emtiaz Ahmed[2], Masud Alam[2], Shahnawaz Ahmed[2], Mamun Kabir[2], Jason Papin[1], A. S. G. Faruque[2], Rashidul Haque[2], William A. Petri, Jr.[1], Carol A. Gilchrist[1]

[1]*University of Virginia, Charlottesville, VA, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh*

Diarrhea is a leading global cause of morbidity and mortality in young children, and the *Cryptosporidium* parasites are the 5th leading cause of diarrhea in this population. Acute *Cryptosporidium* infection results in 50,000 deaths annually, primarily in children. Infection early in life is also associated with long term consequences like growth stunting, even in the absence of diarrheal symptoms. Here, we sought to identify the role of the microbiome in cryptosporidiosis using samples from a longitudinal birth cohort in Bangladesh. Infants were enrolled at birth and were actively monitored for diarrhea for two years. Both monthly surveillance and diarrheal stool samples were collected and tested for enteric pathogens, including *Cryptosporidium*. We conducted 16S ribosomal RNA gene sequencing on matched stools samples (time-of-detection *Cryptosporidium*-positive sample and the surveillance stool immediately prior) from 157 children infected with *Cryptosporidium*. Using univariate statistics, principal component analysis, and random forest analysis, we interrogated the relationship between the members of the microbiota, infection, and clinical outcomes. We observed high variability from child-to-child with most sequence variants in fewer than 50% of all samples. No individual bacterial sequence variant discriminated between the pre-detection and time-of-detection samples and, accordingly, no consistent microbial signature was associated with the development of cryptosporidiosis. Additionally, no members of the microbiome differentiated subclinical from diarrheal infection. Thus, the gut microbiota is highly variable in this cohort and we hypothesize that host or pathogen genetics explain variability in symptoms, not a conserved difference detectable in the fecal microbiota.

586

EVOLUTION OF THE *THEILERIA PARVA* REPEAT (*TPR*) GENE FAMILY IS CONSISTENT WITH ADAPTATION TO MAMMALIAN HOST SPECIES

Nicholas C. Palmateer[1], James B. Munro[1], Roger Pelle[2], Lucilla Steinaa[2], Vish Nene[2], Richard P. Bishop[3], Donald P. Knowles[3], Ine De Goeyse[4], Dirk Geysen[4], Ivan Morrison[5], Joana C. Silva[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*International Livestock Research Institute, Nairobi, Kenya,* [3]*Department of Veterinary Microbiology and Pathology, Pullman, WA, United States,* [4]*Institute of Tropical Medicine, Antwerp, Belgium,* [5]*The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom*

The most rapidly evolving genes in *Theileria parva* are those that form the *T. parva* repeat (*Tpr*) gene family. Each *Tpr* gene typically contains one to three complex domain structures, reminiscent of a system that has evolved because of its role in the generation of protein sequence diversity. This gene family is comprised of a tandem array of highly conserved open reading frames on chromosome 3, termed the *Tpr* locus, along with several other *Tpr* genes distributed throughout the other three nuclear chromosomes. The function of the *Tpr* genes is currently unknown. Here we characterized the genomic and taxonomic distribution of *Tpr* gene family members, under the hypothesis that their evolution will contribute to our understanding of the function of *Tpr* genes. The *Tpr* gene family is found in *T. parva* strains isolated from both cattle and the African Cape buffalo, as well as in a closely related but undescribed *Theileria* species, also found in the buffalo, called *Theileria* sp. (buffalo). Novel sequencing approaches have allowed us to generate whole genome assemblies for several *Theileria* isolates and obtain representatives of their *Tpr* gene families. In this study, we analyzed 93 nearly complete *Tpr* sequences from 12 genome assemblies, four each from three sets of *Theileria* strains: *T. parva* from cattle, *T. parva* from buffalo and *Theileria* sp. (buffalo). We found that the *Tpr* genes are organized in three main subclasses, each characterized by a different number of complex domains. All subclasses were found in the three sets of *Theileria* strains analyzed. Phylogenetic analyses showed that *Tpr* sequences often cluster based on their genomic location, with all genes that fall within the *Tpr* locus forming a monophyletic clade, suggestive of concerted evolution. Interestingly, *Tpr* alleles from *T. parva* from buffalo clustered with those from *Theileria* sp. (buffalo) rather than with those from *T. parva* from cattle, consistent with a role in host adaptation. We are currently analyzing the composition and structure of the motifs encoded by *Tpr* genes, as well as their relative transcript abundance, with the intent to better understand the protein function of the *Tpr* gene.

587

BEI RESOURCES: A BIOLOGICAL RESOURCE CENTER SUPPORTING NEGLECTED AND EMERGING PARASITIC DISEASES

Robert E. Molestina, Biniam Hagos, Ioana Brasov

ATCC, Manassas, VA, United States

Neglected parasitic diseases represent a major cause of morbidity and mortality across the developing world. To succeed as parasites, organisms such as *Toxoplasma gondii*, African trypanosomes, *Trypanosoma cruzi*, *Leishmania*, and *Giardia*, have evolved a series of complex strategies to evade the host immune system and establish chronic infections. In this setting, the accessibility of reference parasite strains and specialized reagents is critical to the generation of studies focused on new targets for therapeutic intervention or vaccine development. For 16 years, BEI Resources has served as a centralized repository for the advancement of infectious disease research. Our primary mission is the acquisition, authentication, preservation, and distribution of cultures and related reagents to the scientific community. Parasite strains deposited at BEI Resources are characterized using a variety of tests, including: (i) verification of viability and purity; (ii) phenotypic properties (morphology, antibiotic susceptibility); and (iii) genotypic analysis (PCR-based tests, RFLP, sequencing). Establishment of seed and distribution stocks for every strain ensures that cultures distributed to researchers are minimally passaged from the material provided by the depositor. Over the last decade, parasite resources have expanded to include *Babesia* sp., an emerging tick-borne parasite in the United States, as well as a variety of recombinant parasites expressing transgene reporters and knockout strains. Specialized reagents for parasite research include polyclonal and monoclonal antisera, purified genomic DNAs, and expression vectors. This presentation will provide an overview of the biological resources available to the parasitology researcher, the benefits of registering and depositing with BEI Resources, methods of characterization and future perspectives.

588

INTESTINAL PARASITES CAUSING DIARRHEAL ILLNESS IN KENYA

Cliff Odhiambo Philip[1], Nancy Kipkemoi[1], Janet Ndonye[1], Margaret Koech[1], Abigael Ombogo[1], Mary Kirui[1], Ronald Kirera[1], Erick Kipkirui[1], Elizabeth Odundo[1], Brook Danboise[2], Christine Hulseberg[3], Stacey Bateman[4], Alexander Flynn[1], Brett Swierczewski[5]

[1]*Kenya Medical Research Institute/U.S Army Medical Research Directorate-Africa, Kericho, Kenya,* [2]*University of Michigan Medical School, Michigan, MI, United States,* [3]*US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States,* [4]*Madigan Army Medical Center, Tacoma, WA, United States,* [5]*Walter Reed Army Institute of Research, Silver Spring, MD, United States*

Kenya is among the developing countries where diarrheal illness is a significant source of morbidity and mortality. We identified intestinal parasites circulating in Kenya through an ongoing case-control study titled "Surveillance of Enteric Pathogens Causing Diarrheal Illness in Kenya" to determine incidence of parasites among cases presenting with diarrhea and asymptomatic controls of all ages as well as co-infection with other enteric pathogens. Stool samples collected from 6,252 subjects enrolled from September 2009 to February 2019 were concentrated using Mini Parasep® SF Fecal Parasite Concentrators (Apacor). Microscopy was conducted by wet preparation for identification of intestinal parasites. Modified acid-fast staining was used for detection of *Cryptosporidium* spp, *Cyclospora* spp and *Isospora* spp. Parasites were detected in 29% of the subjects. The most common parasite detected was *Blastocystis hominis* (29%) followed by *Giardia lamblia* (25%), *Entamoeba coli* (15.6%), *Ascaris lumbricoides* (8.3%) *Endolimax nana* (6.2%), *Cryptosporidium parvum* (5.3%), *Entamoeba histolityca/dispar* (3.6%), *Chilomastix mesnili* (3.5%) and *Iodomoeba bustchlii* (3.0%). Of these positive identifications, 57% were from asymptomatic controls as opposed to 43% from symptomatic cases (p value <0.0001) except for *C. parvum and I. bustchili*, which were detected significantly more in cases than in controls (p-value <0.0001). *A. lumbricoides* and *G. lamblia* were also detected more in cases than in controls though the difference was not significant (p value = 0.748, p value = 0.6288 respectively). Most parasites were identified more in female subjects than in male subjects (55% vs 45%, p value <0.0001). Parasites were detected in 51% subjects over 5 years and 49% in those under 5 years (p value <0.0001). Co-infections were 13% with bacterial-parasite (43% and 57%), 15% two parasites (58% and 42%) and 11% viral-parasite (66% and 34%) among controls and cases respectively. This information will add to the public health knowledge on intestinal parasites circulating in Kenya, help in placing mitigation measures to curb infection and reduce disease burden.

589

COMPARISON OF CONVENTIONAL AND IT MOLECULAR METHODS OF DETECTION OF HAEMOPARASITES FROM NIGERIAN CATTLE

Anise Nkenjop Happi[1], Olawale Osifade[1], Paul E. Oluniyi[2], Bamidele N. Ogunro[1]

[1]*University of Ibadan, Ibadan, Nigeria,* [2]*Redeemer's University, Ede, Nigeria*

Current diagnostic practices for haemoparasites are restricted to light microscopy and clinical examinations in most sub-Saharan African countries. This study was designed to investigate the haemoparasites of cattle in Ibadan, Nigeria with a comparative evaluation of both LM and PCR methods. Blood samples from 100 cattle slaughtered at Ibadan abattoirs, revealed a total infection rate of 34% including *Hemoplasma spp* (17%), *Anaplasma spp* (16%), microfilaria (5%) and *Trypanosoma spp* (12 %) by LM. While, 86% positivity was recorded with PCR amplification targeting the 16S and 18S rRNA genes of selected haemoparasites DNA, such as *Hemoplasma spp* (64%), *Babesia/Theleiria spp* (46%) and *Anaplasma/Ehrlichia spp* (5%). Comparison of LM and PCR analyses showed that no LM *Anaplasma spp* positive samples and 7 out of the 17 LM hemoplasma positive cows were confirmed by PCR. In addition, LM led to misdiagnosis of 46 *Babesia/Theleiria spp* positive samples. Amplicon sequencing with phylogenetic analysis of *Babesia/Theileria spp* positive samples revealed *Theileria velifera Theileria annulata* and for the first time in Nigerian cattle, *Theileria lestoquardi*. While in the *Anaplasma/Ehrlichia spp* positive samples, only *Anaplasma marginale* was characterized. *Mycoplasma wenyonii,* and for the first time, "*Candidatus* Mycoplasma haemobos" and *Pseudomonas fluorescens* were chracterized among the hemoplasma-infected cattle. The first report of "*Candidatus* Mycoplasma haemobos", *Pseudomonas fluorescens* and *Theileria lestoquardi* in Nigerian cattle is herewith documented. The alarming LM misdiagnosis of haemoparaites during this study shows its limitations as it fails to identify many parasites and emphasizes the need for inclusion of molecular techniques in their detection. The study also shows for the first time the high prevalence of haemotropic mycoplasma in Nigerian cattle via molecular diagnostic techniques.

590

USE OF A NEW TRICHROME STAIN FOR RAPID IDENTIFICATION OF CYSTS AND TROPHOZOITES OF *COLPODELLA* SP. (APICOMPLEXA)

Tobili Y. Sam-Yellowe, Kush Addepalli

Cleveland State University, Cleveland, OH, United States

Colpodella sp. are free-living protists, closely related to parasites of the apicomplexan phylum. Recently, *Colpodella*-like parasites infecting human red blood cells were reported in a patient with low natural killer cells and anemia. An additional tick borne *Colpodella* sp. human infection was reported in a patient with neurological symptoms. *Colpodella* sp. DNA was identified in the patient's cerebrospinal fluid (CSF) and in "host-seeking" *Ixodes persulcatus* ticks. *Colpodella* sp. although free-living may have the potential to be an opportunistic human pathogen transmissible by different routes. Clear differentiation of cysts belonging to each protist could not be achieved in previous studies using Giemsa staining. The life cycle stage initiating infection in humans is unknown. In this study, we developed a trichrome staining technique that differentiates cysts of both *Colpodella* sp. (ATCC 50594) and its prey *B. caudatus*. Mature *Colpodella* sp. cysts with developing trophozoites were identified by staining, revealing encystation steps in the life cycle not previously described. Morphological features of the different stages of the encystment process were identified by staining. Mitotic processes resulting in two-stage and four-stage juveniles within cysts were identified in *Colpodella* sp. Developing cysts of *B. caudatus* possessd small cytoplasmic vesicles unlike in *Colpodella* cysts. Calcofluor White staining also showed cyst differentiation between *Colpodella* sp. and *B. caudatus*. Based on the appearance of the internal and external features of the cysts, we investigated actin distribution in both predator and prey using a fluorescent actin probe and found that actin localized diffusely over the cell bodies of trophozoites and cysts of both *Colpodella* sp. and *B. caudatus.* Long filaments of actin were not observed. However, in some developmental stages, short rows of filaments were observed on trophozoites. Staining and fluorescence protocols used in combination can provide important insights regarding the life cycle of *Colpodella* species and allow for rapid detection and verification of *Colpodella* sp. in human infections.

591

AN UPDATE ON THE ROLE OF IMAGING IN THE CARE OF PATIENTS WITH SCHISTOSOMIASIS

Celine Lecce[1], Leila Makhani[1], Shveta Bhasker[1], Christian Lecce[1], Jason Kwan[1], Michael Klowak[1], Priyanka Challa[1], Anjola Ogunsina[1], Osaru Omoruna[1], Kimberley Marks-Beaubrun[1], Zachary Corso[1], Rachel Lau[2], Andrea Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario, Toronto, ON, Canada*

Schistosomiasis leads to significant morbidity and mortality worldwide. Infection with *Schistosoma mansoni* and *S. japonicum* can lead to severe hepatic disease including periportal liver fibrosis and portal hypertension. Previous studies recommend the use of abdominal imaging to detect early hepatic changes and improve disease outcome. However, there are no recently published or authoritative resources to guide the use of imaging in the initial diagnosis of schistosomiasis. We searched available literature regarding the role of imaging in the evaluation of patients with schistosomiasis and aim to synthesize clinical recommendations. Eight electronic databases were searched: Ovid Medline, EMBASE, Cochrane Library of Systematic Reviews, Epistemonikos, Global Health, NICE, TRIP and LILACS with the following search terms: [Schistosomiasis OR (Schisto* AND (mansoni OR japonicum))] AND [CT OR (computed AND tomography) OR Ultraso* OR Sonogr* OR MRI OR (Magnetic AND resonance AND Imaging) OR Echo OR Imaging] AND [Liver OR periportal OR peri-portal OR fibrosis OR hepat* OR echogenic* OR (portal AND hypertension)] from database inception to February 28, 2019. A total of 2977 articles were identified: 691 articles on Ovid Medline, 30 Cochrane, 1035 Embase, 10 Epistemonikos, 516 Global Health, 34 NICE, 529 TRIP, and 132 LILACS. A total of 1933 articles remained for title screening after de-duplication. Titles, abstracts and full-texts were systematically double-screened by two reviewers and a tertiary arbitrator. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was employed. Two reviewers performed data extraction and quality of the studies was assessed with the Grading of Recommendations Assessment, Development and Evaluation (GRADE). Data were summarized using qualitative and quantitative measures to evaluate the role of imaging in the clinical management of schistosomiasis. Synthesizing the current literature on abdominal imaging in the evaluation of schistosomiasis can translate into clinical recommendations for improved risk stratification and overall management of schistosomiasis.

592

SCHISTODETECT™: DEVELOPMENT OF A RELIABLE AND SENSITIVE RAPID DIAGNOSTIC TEST FOR *SCHISTOSOMA JAPONICUM* INFECTION IN HUMANS

Jose Ma. M. Angeles[1], Yasuyuki Goto[2], Lydia R. Leonardo[1], Dindo Reyes[3], Kharleezelle J. Moendeg[4], Minh Anh Danh Trinh[4], Elena A. Villacorte[1], Pilarita T. Rivera[1], Masashi Kirinoki[5], Yuichi Chigusa[5], Raymond L. Houghton[3], Shin-ichiro Kawazu[4]

[1]*Department of Parasitology, College of Public Health, University of the Philippines Manila, Manila, Philippines,* [2]*Laboratory of Molecular Immunology, Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [3]*InBios International Inc., Seattle, WA, United States,* [4]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [5]*Department of Tropical Medicine and Parasitology, Dokkyo Medical University School of Medicine, Mibu, Tochigi, Japan*

Zoonotic schistosomiasis continues to be a public health problem in Asian countries including China, the Philippines and Indonesia. Improving the diagnostic tools for surveillance in areas which have reached elimination level will help hasten the possible elimination of this disease. There is a critical need therefore for a rapid, low cost and highly sensitive point-of-care test (POCT) for the detection of specific antibodies in individuals infected with *Schistosoma japonicum*. For the development of the POCT, several schistosome proteins were screened for their antigenicity using different serum panels (negative non-endemic and endemic samples, microscopy/PCR positive samples). ELISA results showed that 5 antigens, thioredoxin peroxidase SjTPx-1, phytochelatin synthase SjPCS, major egg protein fragment Sjp40M tandem repeats Sj7 and Sj11, provided above 70% detection rate. SjTPx-1 and Sj7 showed the highest sensitivity and specificity. Analyses on responses of schistosome-infected individuals to these antigens suggested that SjTPx-1, the best antigen, can be complemented by other antigens for better diagnostic performance. Out of these antigens, 6 fusion proteins were constructed and produced for serological evaluation. ELISA results showed that SjTP7 (SjTPx-1, SjPCS and Sj7 fusion) and SjT47 (SjTPx-1, Sjp40M and Sj7 fusion) have the highest diagnostic potentials among the fusion proteins. However, as compared to the single antigens, SjTPx-1 remained to be the best antigen for the diagnosis of human schistosomiasis. Optimization of different formats of the rapid test kit was done using sera obtained from screening in an endemic municipality in the Philippines. It was concluded that the best working format for the rapid test involves SjTPx-1 antigen. Overall, it was concluded that SjTPx-1 alone is not effective in rendering the rapid test sensitive. Future efforts should focus on generating recombinant fusion proteins with improved yields and robust performance in the rapid test format.

593

DEVELOPMENT OF A SENSITIVE, QUANTITATIVE PCR ASSAY FOR THE DETECTION OF *SCHISTOSOMA MANSONI* TO AUGMENT STOOL SURVEYS FOR STH

Kareen Seignon, Jessica R. Grant, Nils Pilotte, Steven A. Williams

Smith College, Northampton, MA, United States

Schistosomiasis is a neglected tropical disease (NTD) that affects more than 200 million people globally. The causative agents of this infection in humans are the parasitic nematodes *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*. Among other factors, the success of MDA relies on good diagnostic tests to identify endemic regions and to monitor the progress of MDA campaigns. Since we have already developed a series of qPCR tests that are exquisitely sensitive and species-specific for all of the soil-transmitted helminth (STH) parasites using a novel bioinformatics pipeline, it was logical for us to use the same approach to develop a quantitative, real-time PCR diagnostic assay for *S. mansoni* to add to our STH battery of tests for use in the large-scale stool surveys in which we participate. This bioinformatics pipeline uses a novel tool, RepeatExplorer, to identify highly repetitive DNA elements in the genome of any eukaryote. We have already used this approach to successfully develop qPCR assays for *Necator americanus*, *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Trichuris trichiura*, *Ascaris lumbricoides* and others. The same method was applied to both *S. mansoni* and *S. haematobium* to design primers and probes to target these important parasites. Using DNA isolated from *S. mansoni* and *S. haematobium* specimens obtained from the Natural History Museum (London, UK), two new qPCR tests were designed and optimized. In addition to testing the assays on purified DNA, a spiking study was also performed to demonstrate the sensitivity and specificity of the assays on human stool samples with *S.mansoni* and *S. haematobium* eggs added in various concentrations. Next steps include a field study in Uganda to assess the use of these assays in parallel with our existing STH tests.

594

ACCEPTABILITY AND FEASIBILITY OF HOME-BASED GENITAL SELF-SWAB SAMPLING FOR THE DIAGNOSIS OF FEMALE GENITAL SCHISTOSOMIASIS IN ZAMBIA: LESSONS LEARNT FROM THE BILHARZIA AND HIV (BILHIV) STUDY

Comfort Rutty Phiri[1], Amy Sturt[2], Emily Webb[2], Isaiah Hansingo[3], Kwame Shanaube[1], Richard Hayes[2], Helen Ayles[1], Amaya L. Bustinduy[2]

[1]*Zambart, Lusaka, Zambia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Livingstone Central Hospital, Livingstone, Zambia*

The Bilharzia and HIV (BILHIV) study recruited women from two sites in Zambia from the Population Cohort of the HIV prevention trial HPTN071. The aims of the study were to assess the validity, acceptability and feasibility of self-sampling collection methods for the diagnosis of female genital schistosomiasis (FGS). BILHIV Community Workers (BCWs) delivered household based study activities for all sexually–active, non-pregnant women aged 18-31, including home-based education for urine, vaginal and cervical swabs self-collection. Vaginal lavage was performed at the cervical cancer clinic by a midwife and urine microscopy was performed onsite. Egg-patent infections were treated with praziquantel. From January to August 2018, 603 women were enrolled and 527 completed clinic follow up (87.4%). Median age was 24 years (range 22-28). Over half of participants (60.4%, 364/603) completed secondary school education. The majority of participants indicated that self-collection of specimens was "easy" or "very easy" on a 5 point Likert scale: urine 96.2% (580/603), vaginal swab 94.9% (572/603), cervical swab 86.6% (522/603). High proportions of women would be willing to self-collect the following specimens again in future: urine 96.2% (580/603), vaginal swab 96.7% (583/603), cervical swab 96.5% (582/603). The majority reported that vaginal specimen self-collection (93.0%, 561/603) and cervical sampling was not painful (83.6%, 504/603) and would recommend self-sampling to a friend (95.7%, 577/603). Self-sampling at home was preferred over provider-based sampling in the clinic due to: greater privacy (58.5%, 353/603), convenience (46.3%, 279/603) and lack of transport (15.9%, 96/603). Home based self-specimen collection for FGS diagnosis in women ages 18 - 31 years was highly acceptable, with the majority of participants reporting specimen self-collection to be easy/very easy with high willingness to participate in future home-based self-sampling. Results can inform future efforts for community-based diagnosis of FGS.

595

SPECIFIC NUCLEIC ACIDS LIGATION FOR DETECTION OF SCHISTOSOMES: SNAILS

Alexander J. Webb[1], Toby Landeryou[2], Richard Kelwick[1], Fiona Allan[2], Aidan Emery[2], Kirsten Jensen[1], Michael Templeton[1], Paul S. Freemont[1]

[1]*Imperial College London, London, United Kingdom,* [2]*Natural History Museum, London, United Kingdom*

Over 200 million people worldwide are affected by the parasitic infection Schistosomiasis. Fluke worms of the *Schistosoma* genus are the causative agents, with infection only occurring when the cercarial larvae penetrate the hosts' skin barrier. Transmission of the fluke worms occurs via contact with water that is contaminated by infected faeces or urine, where snails, which act as the parasites intermediate host, are also present. To help break the infective cycle of this parasite, point-of-care diagnostics are desirable to help identify infection hot spots as well as monitor and survey sites undergoing control measures. Using a synthetic biology approach, we have systematically designed and developed a new DNA-based biosensor to detect for schistosomes. Our recently developed technology is a DNA-specific molecular probe-based biosensor that can distinguish between schistosome species by targeting specific regions in mitochondrial genomes. Here, we report on the specificity and sensitivity of the biosensors, and show how we envision this biosensor could be used *in situ*.

596

PATHOLOGICAL EFFECT OF URINARY SCHISTOSOMIASIS AMONG SCHOOL CHILDREN IN AN ENDEMIC COMMUNITY OF SOUTHWESTERN NIGERIA

Adeyinka Samuel Adedokun[1], Olusola Ojurongbe[1], Akeem Akindele[1], Segun Akindokun[2], Temitope Bello[1], Victor Oyedepo[2], Johnson Ojo[1]

[1]*Ladoke Akintola University of Technology, Nigeria, Ogbomoso, Nigeria,* [2]*Ladoke Akintola University of Teaching Hospital, Nigeria, Osogbo, Nigeria*

Urinary schistosomiasis continues to thrive in most endemic countries of Africa despite continued drug intervention. Organ pathological changes are rarely investigated during intervention due to the fact that the current and proposed control strategies in endemic communities exclude investigation of pathological effect of the disease. Several association studies have infact linked the disease with debilitating medical conditions. This study therefore determines the prevalence and investigates organ pathological effect of the disease in Nigerian children. Urine specimens were collected from 625 children and examined using the filtration technique. Organ pathological changes were investigated using a portable ultrasound machine, and the World Health Organization method was used for lesions classification scoring. The prevalence of urinary schistosomiasis was 41.8% (261/625), a higher prevalence was recorded in males (52.7%) compared to females (47.3%). Age group 11-15 years recorded the highest prevalence of 48.6%. The pathologic effects recorded in the studied population include, bladder shape distortion (6.7%), bladder wall irregularity (24.3%), bladder wall thickening(41.4%), pseudopolyp of the bladder wall (1.3%) and massing of the bladder wall (2.9%). Moderate dilation of the right and left pelvis of the kidney was recorded in 25 (10.5%) of children. Pathological effects were more commonly seen among males than female, and among children within 11-15yrs old. In conclusion, prevalence of urinary schistosomiasis in this study was high. The pathological changes observed were focal and the affected schistosomiasis positive children were few. Deliberate and targeted intervention must be ensured to limit morbidities/mortalities due to the disease by ensuring continued drug intervention. Ultrasonography should be included as part diagnostic tool in endemic communities.

597

SCHISTOSOMIASIS ASSOCIATED WITH ADVANCED STAGE DUODENAL ADENOCARCINOMA

Daniel A. Welder, Clare McCormick-Baw, Megan Wachsmann, Zhikai Chi, Dominick Cavuoti

UT Southwestern Medical Center, Dallas, TX, United States

Here, we report a case of schistosomiasis associated with stage IV primary duodenal adenocarcinoma. A 41-year-old African American male presented to the ED with complaints of abdominal pain, weight loss, and decreased appetite over one month. He immigrated from Zimbabwe 10 years prior, and most recently visited 4 months ago. His past medical history is non-contributory. He was found to have microcytic anemia (Hgb 9.3, MCV 77.0), pneumonia, and focal small bowel dilation with thickening and inflammatory changes on abdominal CT. Endoscopy revealed a large, villous, infiltrative mass in the third portion of the duodenum. On histologic examination of the biopsy specimen, viable *Schistosoma* eggs were seen within the lamina propria of the duodenal mucosa and associated with adenocarcinoma. *Schistosoma mansoni* eggs were identified on stool ova and parasite exam, and serology was positive for *Schistosoma* IgG antibody. This case is an unusual presentation in the US in that viable ova are typically not seen, and *S. mansoni* is more likely to involve the colon rather than the small bowel. Identifying the parasite has important implications. Although this may be an indolent infection in immunocompetent hosts, in this case immune suppression is impending due to concomitant malignancy. Furthermore, there is evidence that *Schistosoma* infection may down-regulate the immune response by inducing M2 differentiation of macrophages, which has been associated

with a microenvironment favorable to malignancy. It has also been shown that *S. mansoni* is a risk factor for hepatocellular carcinoma and colonic adenocarcinoma, possibly by altering p53 activation.

598

EVALUATION OF OCCUPATIONAL RISK FOR *SCHISTOSOMA MANSONI* AND CHANGING PARASITE POPULATION STRUCTURE IN AGRICULTURAL WORKERS IN SALVADOR, BAHIA

Pedro Santos-Muccillo[1], João R. Cruz[2], Luciano K. Silva[1], Ronald E. Blanton[3], Mitermayer G. Reis[1], Lúcio M. Barbosa[2]

[1]*Oswaldo Cruz Foundation, Salvador, Brazil,* [2]*Bahiana School of Medicine and Public Health, Salvador, Brazil,* [3]*Case Western Reserve University, Cleveland, OH, United States*

In 2011, we described a group of local agricultural workers who showed a higher risk of infection and parasite load than the general population, suggesting an occupational risk. This study aims to evaluate the distribution, prevalence and intensity of Schistosoma mansoni infection in all local agricultural workers. All known urban gardens identified in 2013 were revisited in 2019, and all workers were invited to answer a questionnaire and provide stools. One stool was examined by Kato-Katz test on 2 slides. Participants positive for schistosomiasis were treated with praziquantel and other helminths were treated with albendazole. Of the 43 existing gardens in 2013, 18 remain active in 2019. They are distributed in 4 of 12 sanitary districts and scattered among 6 neighborhoods. Thirty-five agricultural workers were interviewed. S. mansoni infection was found in 22% (4/18) the workers. The majority were male (82.8%) with a mean age of 49.9 ± 15.1 years. Most (77.1%) were born outside of Salvador and lived 58.2 ± 22.9 % of their lives in the city. About 40% indicated a history of schistosomiasis, and of these, only one (7.1%) reported never being treated. Almost 70% were illiterate or did not finished elementary school. All reported having contact with surface water during work. Among workers, 33 were in the parasitological survey, with schistosomiasis prevalence of 18.2% (6/33) and mean parasite load of 224 ±149 eggs per gram of feces. A higher prevalence than the municipal average (2%). Our results indicate an occupational risk for some of the gardens in Salvador. To evaluate the local risk level, these sites will be studied and treated longitudinally every 3 months for 6 months. The parasite´s population structure, pre- and post-treatment, will soon be evaluated to better understand local transmission.

599

INCREASED SERUM CONCENTRATION OF IL-5 AND IL-17 DURING ACUTE FASCIOLIASIS IN CHILDREN LIVING IN HIGHLY ENDEMIC AREA IN CUSCO, PERU

Catalina Aron[1], Martin Montes[1], Maria L. Morales[1], Martha Lopez[1], Miguel Cabada[2]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*University of Texas Medical Branch at Galveston, Galveston, TX, United States*

Fasciolasis is an infection caused by the trematode *Fasciola hepatica* with a worldwide distribution, with high burden in the highlands of Peru. Children under 15 years of age usually represent the population with the highest rate of infection. There is limited information regarding the immune response to *Fasciola hepatica* in children. The purpose of this study is to describe the cytokines involved during fascioliasis infection in children living in endemic areas during different states of the infection. We used stored serum samples from 33 children who lived in communities in Cusco region of Peru. 18 were male and 15 female, with ages ranging from 4 to 15 years old. Children were selected from a previous cross-sectional study of socioeconomic factors associated with *Fasciola hepatica* among children from 26 communities of the Cusco region of Peru. Subjects were classified into three groups, according to stage of disease: acute fasciolasis defined as eosinofilia, positive Fas2 EIA and absence of fasciola eggs in stool; chronic fasciolasis with high egg burden (over 100 eggs per Kato Katz sedimentation test), and a control group with no evidence of disease. We used a cytometric bead array assay to measure TNF-a, IFN-g, IL-2, IL-4, IL-6, IL-10 and IL-17 protein levels. We also determined IL-5 concentrations by EIA method. There was no significant difference in the levels of IL-2, IL-4, IL-6, IL-10, TNF-a and IFN-g between groups ($p > 0.05$, Kruskall-Wallis). However, children with acute infection had higher levels of IL-5 compared with children with chronic infection (median 1.77 vs 0.06 pg/ml, $p < 0.01$, Mann-Whitney). Furthermore, children with acute infection also had higher levels of IL-17 compared to children with chronic infection (median 1.51 vs 0 pg/ml, $p=0.04$, Mann-Whitney). This is the first study of the immune response to fasciolasis in children, comparing acute vs chronic infection. Previos studies in rat model showed mixed cytokine responses during reinfection, or attenuation of Th17 response. Further studies are needed looking for antigen-specific T-cell responses to elucidate the role of Th17 during human fasciolasis.

600

TROPICAL DISEASES RELATED TO WATER AND SANITARY RESILIENCE IN THE MUNICIPALITIES OF ATHIEME AND GRAND-POPO IN SOUTHWEST OF BENIN

Anselme Kpominanon Sede

UAC/LACEEDE, Abomey-Calavi, Benin

The observation in the Athieme-Grand-Popo study area shows the presence of the Mono river, the sea, the marigots and the lowlands, which make these neighboring communities a wetland. Populations often use river water, ponds and backwaters to meet a variety of human concerns. That is not without consequences on their state of health. Therefore, they develop coping strategies (water treatment by elaborate techniques, traditional treatment of diseases) to fight against pathologies related to water. According to health statistics, malaria, schistosomiasis and gastroenteritis are respectively the dominant waterborne diseases in the study area. The objective of this study is to analyze the effectiveness of the control strategies developed by the populations against the waterborne and aquatic diseases, for the reinforcement of the health security measures of the local populations. This makes it possible to understand the level of resilience of population health in relation to the adaptation strategies they develop in their biotope. The climatological (1985-2015), physico-chemical and bacteriological (2019) data obtained before and after the water treatment were used. These data are supplemented by those from the field surveys. The Hazard Analysis Critical Control Point (HACCP) method has identified, assessed the hazards and risks involved in surface water consumption and determined the levels of control through defined strategies. The tools used are observation grids, questionnaires and maintenance guides. The results showed that the techniques used by the populations for the treatment of water do not make it drinkable. Thus, treated water continues to remain a source of disease for human health. It also emerges from this analysis that the traditional treatment of water-related diseases has reliable measurement deficiencies. The availability of drinking water and adequate health centers, the consumption of drinking water, the effective use of health centers in case of illness, the formalization of traditional medicine, are the possible strategies for a better security health.

601

SEASONAL VARIATIONS IN NUTRIENT INTAKE AND DIETARY DIVERSITY AMONG ELECTRONIC WASTE WORKERS, GHANA

Sylvia Akpene Takyi[1], Julius N. Fobil[1], Niladri Basu[2], John Arko-Mensah[1]

[1]*University of Ghana, Accra, Ghana,* [2]*McGill University, Montreal, QC, Canada*

Whereas seasonality on nutrient intake and dietary diversity has been studied in children and female adults, particularly pregnant women; to the best of our knowledge, no dietary assessments have been conducted among electronic waste (e-waste) workers who are widely believed to be exposed to complex mixtures of trace, essential and rare earth elements that are likely to influence nutrient mobilization and their ultimate

metabolism. This study examined the seasonal variations in nutrient intake and dietary diversity among e-waste workers and a control population to inform of their nutritional status. E-waste workers (142) and controls (64) aged between 18 to 55 years were prospectively enrolled. Nutrient intake and diversity were assessed during two distinct seasons: dry and wet seasons, using a 2-day 24-hour recall and dietary diversity questionnaire. Data were analyzed using STATA 15.0, whereas Mann-Whitney and t-tests were used to examine seasonal differences in nutrient intake and diversity. Overall, median energy intake was higher in the e-waste workers (1949(1630-2299) kcal)) than in controls (1823(1507-2259)kcal) (p<0.05). Additionally, there were variations in the amount of nutrients consumed: e-waste workers consumed higher amounts of magnesium (mg), potassium (mg), vitamin A (RE) and B12 (mcg) than controls. However, these did not meet the recommended daily allowance. Furthermore, energy intake, carbohydrates, saturated fats, monounsaturated fats, omega-3 fatty-acids, cholesterol, vitamin C, potassium and phosphorous were higher in the dry compared to the wet season. Although dietary diversity scores were higher in the wet (5.08±1.13), than dry season (4.29±1.00), and higher in e-waste workers(4.93±1.22) than controls (4.67±1.44), they still did not meet the globally accepted requirements. In conclusion, seasonality exists in nutrient intake and dietary diversity of e-waste workers and may affect their nutritional status. Thus, food-based strategies are required to increase nutrient intake and diversity to improve nutritional status of e-waste workers.

602

DRINKING WATER USE BY INFANTS IN A LOW-INCOME COMMUNITY IN THE DOMINICAN REPUBLIC

John D. McLennan[1], Maria Mosquea[2]

[1]*Children's Hospital of Eastern Ontario - Research Institute, Ottawa, ON, Canada,* [2]*Servicio Nacional de Salud, Santo Domingo, Dominican Republic*

Drinking water is not required by infants who are adequately breastfed in the first months of life, however, water may be commonly given to infants, despite ongoing breastfeeding. This practice undermines exclusive breastfeeding and may expose infants to unnecessary health risks. The key objective of this study was to determine the extent of drinking water use, as well as it sources, among infants living in a low-income community in the Dominican Republic. Mothers (or alternative caregivers) of infants attending a well-baby clinic serving the studied community were systematically interviewed with a structured survey about child health practices at each check-up throughout infancy (0-12 months). The survey included questions about the frequency of use of six water sources and preparations pre-identified as the most common in the community. Data were available for 117 infants with 509 surveys (multiple surveys/infant as questions asked at each well-baby check-up). Water use from one or more sources was reported for 74% of one-month old infants and this increased with age, reaching 100% by six months of age. Five gallon bottles, exchanged for refills from local stores, were the most common water source overall and their use increased with infant age. A "special" bottled water marketed for infants was the next most common source overall and its use decreased with increasing infant age. A distant third was boiling water, which dropped as a practice with increasing infant age. Less than 5% used other sources. Greater than 75% of infants were being breastfed at each time point. Childhood diarrhea was reported at 18% of interviews and was significantly related to bottled water use, while controlling for age. Focused initiatives may be needed to encourage less early water use which may increase the extent of exclusive breastfeeding. Examining the quality of bottled water in this setting may also be needed given its extensive use in infancy and possible relationship to diarrhea.

603

FIELD TRIAL OF AN AUTOMATED BATCH CHLORINATOR SYSTEM AT SHARED SHALLOW TUBEWELLS AMONG THE MOST VULNERABLE FORCIBLY DISPLACED MYANMAR NATIONALS (FDMN) IN COX'S BAZAR, BANGLADESH

Nuhu Amin[1], Mahbubur Rahman[1], Anika Tasneem[2], Mahbub Ul Alam[1], Abul Kasham Shoab[1], Tarique Mohammad Nurul Huda[1], Md. Kawsar Alome[3], Maksudul Amin[3], Leanne Unicomb[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*North South University, Dhaka, Bangladesh,* [3]*Action Against Hunger, Dhaka, Bangladesh*

Point-of-use water treatments with chlorine are widely used in emergency responses because of chlorine availability, ease of use, cost-effectiveness, and residual protection in stored water. Despite considerable benefits, chlorination programmes in emergencies remain challenging because of no/lack of standard recommendation for dosing/residual chlorine levels, lack of appropriate chlorine delivery technologies, and limited research in emergencies. We conducted a small-scale field-trial to assess accuracy, and consistency of an automated chlorinator named "Zimba" in a Rohingya camp, Cox's Bazar. From August-September 2018, we selected 2 shallow tubewells, and enrolled 20 households (10 accessing each tubewell) to participate in household surveys. At baseline, field-team tested iron, turbidity, free and total chlorine from tubewell and household stored water using digital field-test kits. The Zimba was installed at the two tubewells dosing with~2.62% NaOCl. During follow-up visits, field-team tested free and total chlorine, turbidity, and iron concentration from Zimba water immediately after chlorination (N=337), household stored water (N=133), and chlorinated stored-water that the field-team placed in households in safe storage containers (N=33). At baseline, mean turbidity was 2.5 NTU, chlorine residual=0.10 ppm, and iron concentration=2.0 mg/L in household stored water. After introducing Zimba, free chlorine >0.2 mg/L was detected in all Zimba water samples (100%) collected immediately after chlorination (mean=2.12 mg/L, SD=1.12), in 24% of household stored water (mean=0.39, SD=0.90), and in 94% household stored safe storage container water (mean=1.4, SD=0.9). Zimba provided water with free chlorine >0.2 mg/L immediately after treatment and when kept in a safe storage container, but not when stored in the household's own containers. Our study suggested that Zimba has potential as a water treatment technology for humanitarian settings. Future research should explore the reasons for low chlorine concentration in the household stored water for effective water treatment, and impact on water contamination.

604

ESTABLISHING AN EFFECTIVE ENVIRONMENTAL SURVEILLANCE FOR POLIOVIRUS IN A DENSELY POPULATED URBAN LOW-INCOME AREA IN DHAKA, BANGLADESH

Md Ohedul Islam[1], Yoann Mira[2], Philippe Veltsos[2], Md Masud Alam[1], Ashraful Islam Khan[1], Sultan Uz Zaman[1], Tahmina Ahmed[1], Md Abdul Karim[1], Tania Ferdousi[1], Tuhinar Arju[1], Rashidul Haque[1], Firdausi Qadri[1], Mami Taniuchi[3]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Novel-T Sàrl, Geneva, Switzerland,* [3]*University of Virginia, Charlottesville, VA, United States*

Environmental surveillance (ES) for polio virus (PV) will play an important role in during the polio endgame as a supplement to acute flaccid paralysis surveillance to detect PV. In low income countries such as Bangladesh, there are many challenges to setting up an effective ES due to frequent migration of people, lack of accurate census and under-developed sewer network. In this study, we established ES in a densely populated urban low-income area of Mirpur (approximately 8.6 sq km), Dhaka to track Sabin 1 and 3 viruses after a supplemental immunization activity with bOPV for children under 5. Access to recent census and sewage/drainage system maps were limited. We developed Open Data Kit (ODK) Collect based forms for smart phones to digitally collect data for blue lines of the sewage/drainage lines and household demographic surveillance system

(HDSS). Close clustering of buildings resulted in interferences and limited line-of-sight for the smart phone GPS to access enough satellites for accurate readings. Subsequently, we replaced digital blue line tracing to physically walking and tracing the sewage/drainage lines throughout our study area using a physical paper map followed by manual digitization using QGIS tool. Forty field workers utilized the HDSS ODK forms to collect GPS location of the household, building and household information, children under 5 years of age and their vaccination status. HDSS data captured a total population of 209,637 of which 19,156 are children under 5 years of age. Novel-T developed an interactive map of the study area using the blue lines and the HDSS data along with other available layers of geographic information such as elevation, roads, schools, hospitals, water lines and buildings. Using this map along with its built-in watershed tool and population selector, we were able to determine candidate ES sites with accurate location and catchment area.

605

KNOWLEDGE, ATTITUDES AND PRACTICES OF MOTHERS REGARDING CHILDREN'S DIARRHEA, UNIVERSITY HOSPITAL OF MIREBALAIS, HAITI, MAY-JUNE 2018

Emmanuel Fabrice Julceus, **Tania Gessie Ramilus**, Raymonde Pinchinat, Emmanuel Mathieu, Ben Bechir Beaubrun, Renault Louis

Zanmi Lasante, Mirebalais, Haiti

Worldwide, diarrhea is the second leading cause of death among children under five years of age. In Haiti, 41% of children under one year of age are affected. WHO believes that improved health behavior could reduce diarrheal disease by 35% and advocates a targeted fight against diarrhea. The objective of this study was to evaluate the knowledge, attitudes and practices of mothers attending the community health department of the University Hospital of Mirebalais (CHDHUM) on prevention and treatment of diarrhea. This study was an exploratory sequential mixed methods. Three focus group were conducted with nurses and community health workers of the CHDHUM about usual attitudes and practices of Mirebalais' mothers. A survey of mothers aged 18 and over, living in Mirebalais area, with at least one child aged two and under, attending the CHDHUM was done from May to June 2018. Questionnaires were made according to five themes from WHO recommendations on diarrhea education (vaccination, hygiene, feeding, treatment, danger signs). Transcripts were analyzed by hand with predetermined codes, quantitative data were analyzed on Epi Info using proportion and chi square test. The results of the qualitative part helped to complete the perceptions (dentition causes diarrhea) and the practices (rice water to treat diarrhea) in the questionnaire. 268 mothers participated in the quantitative part. Knowledge was insufficient or bad for 42.6% of them, worse for those who were illiterate, aged less than 30, living in rural area, or who didn't receive information about diarrhea ($p<0.05$). Attitude was right for 90.7% of the mothers, better for those who attended secondary school or university ($p<0.0001$). Practice was inadequate or harmful for 76.5% of them, worse for those who were illiterate or aged less than 30 ($p<0.05$). A wide part of the mothers had inadequate or harmful practices and insufficient or bad knowledge. Young age, illiteracy, rural area were found as risk factors. These findings can help Haitian public health authorities to better target education initiatives and adapt them to the actual knowledge, attitudes and practices of the mothers.

606

THE USE OF DIGITAL TECHNOLOGY TO IMPROVE AND MONITOR HANDWASHING AMONG CHILDREN 12 YEARS OR YOUNGER IN EDUCATIONAL SETTINGS: A SYSTEMATIC REVIEW

Sylvia K. Ofori[1], Yuen Wai Hung[2], Kamalich Muniz-Rodriguez[1], Reece J. Kakau[1], Sunmisola E. Alade[1], Kadiatou Diallo[1], Kelly L. Sullivan[1], Jessica S. Schwind[1], Benjamin J. Cowling[3], Isaac Chun-Hai Fung[1]

[1]*Georgia Southern University, Statesboro, GA, United States,* [2]*Wilfrid Laurier University, Waterloo, ON, Canada,* [3]*The University of Hong Kong, Hong Kong, China*

Hand hygiene practices are one of the most effective non-pharmaceutical interventions in reducing the incidence, morbidity, and mortality from infectious diseases, especially among children. Digital technologies are used in health-related settings to advance health and wellbeing. Systematic reviews on the use of digital technologies in hand hygiene studies have focused on healthcare workers and community-based applications. The aim of our systematic review was to identify and describe the application of digital technology utilized in hand hygiene research among children in educational settings. We searched PubMed, IEEE Xplore and Web of Science for articles in the English language in January 2019. Titles and abstracts were independently screened for articles that met our inclusion criteria. Eleven articles were found to be eligible. The outcomes measured in the studies included absenteeism, the incidence of diarrhea or respiratory infections, and handwashing frequency. Nine studies used digital technology as an intervention tool and two for monitoring purposes. Three main digital technologies were identified including computer games (n=2), videos (n=7), and video cameras (n=2). Computer games and videos were used as educational tools while video cameras were used for monitoring handwashing behavior. Studies that used computer games focused on improving the self-efficacy of children and for increasing knowledge. Videos were used mostly as a component of a package to teach children about effective hand hygiene to reduce absenteeism or illness. Video cameras were compared with in-person observation in observing students reactivity to being monitored and to observe handwashing after toilet use. The types of digital technologies found in our systematic review proved to be effective in hand hygiene studies, especially when combined with other methods over a short period of time. However, long-term effectiveness remains to be demonstrated. Novel digital technologies adopted in educational environments may aid in helping children develop sustainable handwashing behavior across a multitude of settings.

607

CLEAN WATER ACCESS IN HAITI: IMPACT OF SOURCE TYPE AND MANAGEMENT STRATEGY ON FUNCTIONALITY AND WATER QUALITY

Declan Joseph Devine[1], Neil Van Dine[2], Brian Jensen[2], Mustafa Sikder[1], Daniele Lantagne[1]

[1]*Tufts University, Medfrod, MA, United States,* [2]*Haiti Outreach, Pignon, Haiti*

Access to safe water sources is critical to reducing the rates of waterborne diseases. In 2015, only 64.2% of Haiti had access to improved sources. Additionally, improved sources are often contaminated with *E. coli*. Understanding drivers of functionality and water quality will aid decision makers to identify water source intervention priorities. Between 2016 and 2018, Haiti Outreach collected data at water sources from six departments in Haiti; including type of source, functionality, management type, and presence of *E. coli*. We completed logistic regressions to study how source type and management are associated with functionality and *E. coli*. Data were collected from 12,875 public access water sources and *E. coli* was tested at 7,137 sources. Functionality was higher among unimproved sources (97.9% functional) compared to 65.0% in improved sources. Specifically, 71.2% of protected dug wells were functional, compared to

69.2% of boreholes and 49.4% of public piped systems. Summarizing water quality results, 42.1% of improved sources and 5.5% of unimproved sources met the standard of <1 *E. coli* CFU/100mL. Specifically, over 70% of borehole samples had <1 *E. coli* CFU/100 mL, compared with 25.9% of public piped systems and 23.4% of protected dug wells. Water sources managed by government water and sanitation supply committees (CAEPA) (OR 1.67, 95% CI 1.39-2.01), unsigned community committees (OR 1.48, 95% CI 1.26-1.74), and privately managed (OR 3.52, 95% CI 2.05 - 6.53) were more likely to be functional than those with no management structure. Sources managed by CAEPA (OR 2.05, 95% CI 1.53-2.74) were more likely to have <1 *E. coli* CFU/100 mL than those with no management. More engineered sources delivered safe water but were less likely to be functioning. Sources managed by accountable management structures were more likely to be functioning and produce safe water compared to public and community-based committees. These results inform: the source types and management structures that are most effective in improving access to safe drinking water in Haiti; and, the improvements in source functionality and safety needed to meet the SDGs in Haiti.

608

ZIKA VIRUS RNA PERSISTENCE IN SEWAGE: A NOVEL SURVEILLANCE TOOL

Aaron Muirhead[1], Kevin J. Zhu[2], Joe Brown[2], Margo A. Brinton[1], Federico Costa[3], Matthew J. Hayat[1], Christine E. Stauber[1]

[1]*Georgia State University, Atlanta, GA, United States,* [2]*Georgia Institute of Technology, Atlanta, GA, United States,* [3]*Universidade Federal da Bahia, Salvador, Brazil*

Zika virus (ZIKV) has emerged as one of the most challenging and concerning viral infections in this decade due to its association with congenital brain abnormalities and Guillain-Barré syndrome. An estimated 80% of cases of ZIKV infection are asymptomatic and symptomatic cases are difficult to detect based on symptoms alone as symptoms are shared with many other illnesses. Despite substantial advances in knowledge and understanding about ZIKV, diagnostic challenges remain in resource-limited settings where ZIKV is endemic, highlighting the need for novel and innovative diagnostic tools. The Global Polio Eradication Initiative exemplifies the critical role that environmental surveillance of waters and wastewaters has played in the eradication of a vaccine preventable disease, especially in the absence of clinically detectable cases. Limited research has explored sewage to measure transmission of non-enteric infections of global concern and no one has systematically explored the potential to use sewage to detect and analyze the spread of arboviruses such as ZIKV. To demonstrate the feasibility of ZIKV RNA detection in sewage, we examined ZIKV RNA persistence in sewage from a local wastewater treatment plant. In triplicate, we added ZIKV (MEX 1-44) (10^5/ml) to unpasteurized primary influent and stored the samples at 4°C, 25°C and 35°C for one month. We periodically extracted nucleic acids from the mixture using the QiaAmp Minelute Virus Spin Kit and measured ZIKV RNA as genomic copies via the Taqpath Zika Virus Kit. We found no appreciable decline in ZIKV RNA concentration at 4°C during the month. Based on our analysis, we estimated that 90% degradation or 1-log10 reduction of ZIKA RNA occurred after 14 days at 25°C and after 6 days at 35°C. Our preliminary work suggests that under a range of temperatures and over time, ZIKV RNA will remain stable in sewage. The evidence suggests that detection of ZIKV RNA in sewage may provide a cost-effective, community diagnostic tool that deserves further investigation as a novel surveillance approach.

609

WATER TREATMENT PROCESSES FOR PREVENTING TRANSMISSION OF SCHISTOSOMIASIS

Laura Braun, Lucinda Hazell, Michael R. Templeton

Imperial College London, London, United Kingdom

Schistosomiasis infection occurs through contact with cercaria-infested freshwater. While chemotherapy with praziquantel can have immediate beneficial effects, reinfection can occur rapidly if people are re-exposed to contaminated water. As schistosomiasis control targets become more ambitious and we move towards elimination, interest is increasing in the potentially complementary roles of water, sanitation, and hygiene (WASH) interventions. These may disrupt transmission of the parasite, thereby reducing the likelihood of reinfection following treatment. Water treatment for schistosomiasis control seeks to eliminate viable cercariae from water but the information available to-date about the effectiveness of water treatment processes against cercariae is limited and incomplete. As part of the WISER: Water Infrastructure for Schistosomiasis Endemic Regions project (www.wiserschisto.com), experiments were conducted in the UK, Ethiopia and Tanzania to test the effectiveness of chlorination, sand filtration, and ultraviolet disinfection against schistosome cercariae. *Schistosoma mansoni* cercariae cultured in the Natural History Museum, London were used as well as cercariae and water samples collected from the environment. The effectiveness of each of the treatment processes was examined individually. Guidelines have been produced to enable the design of household and community scale water treatment infrastructure that will help minimise re-exposure to unsafe water by providing safe facilities for water contact activities. This work is very timely considering the recent publication of the World Health Organization's toolkit for WASH and Neglected Tropical Disease (NTDs) programmes, as these two communities come together to find lasting solutions for combating schistosomiasis.

610

ULTRAVIOLET DISINFECTION OF SCHISTOSOME CERCARIAE IN WATER USING ULTRAVIOLET LIGHT EMITTING DIODES

Lucinda Hazell, Michael R. Templeton

Imperial College London, London, United Kingdom

Schistosomiasis is transmitted through skin contact with water containing cercariae, the human infectious stage of the parasitic helminth *Schistosoma spp*. The disease can be treated with a single dose of praziquantel and preventative chemotherapy has been successful at reducing the global health burden. However reinfection is common and hotspots, where prevalence remains high in spite of chemotherapy, persist. Treating water so that it is free from cercariae and can be safely used for contact activities such as laundry and bathing may help to prevent reinfection, but there are no formal guidelines for household or community scale water treatment for inactivating schistosome cercariae. Ultraviolet (UV) light is widely used for water disinfection in Europe, Asia, and the United States, but it is often seen as incompatible with low income settings due to the input energy requirements of conventional mercury source UV lamps. However, the emerging technology of UV light emitting diodes (UV-LEDs), which have considerably lower energy requirements and can be powered by battery or solar cells, may make UV disinfection a realistic option for remote communities in the near future. The literature suggests a relatively low UV fluence of 5-27 mJ/cm^2 is required to achieve a 1-2 log reduction. However, the majority of studies were carried out before the standard protocol for calculating fluence was introduced in 2003, and the data is insufficient to provide robust recommendations for practice. In this research, the UV sensitivity of schistosome cercariae was examined in accordance with the established fluence measurement protocol, using a bench scale UV-LED collimated beam device at three different wavelengths: 255 nm, 265 nm, and 285 nm. Experiments were carried out using *Schistosoma mansoni* cercariae cultured in the Natural History Museum, London along with cercariae and water samples collected from the environment in Mwanza, Tanzania and

the minimum recommended fluence to inactivate cercariae in water was determined. These results are timely, as we look towards inter-sectoral approaches for eliminating schistosomiasis as a public health problem.

611

WATER, SANITATION, AND HYGIENE (WASH) AS AGENTS OF IMPROVED SANITATION IN RURAL LIBERIA ENTREPRENEURS

Alex B. Keimbe[1], Isaac Mwase[1], Marcy Sallor[1], Jannie H. Horace[2]

[1]*Partnership for Advancing Community-Based Services (PACS), Monrovia, Liberia,* [2]*U.S. Agency for International Development (USAID), Monrovia, Liberia*

During the civil war in Liberia, much of the water and sanitation infrastructure was destroyed and has not yet been repaired or replaced. In 2017, about 42% of Liberians practiced open defecation according to the Joint Monitoring Programme. Less than 10% of the population has access to safely managed drinking water and sanitation services. In 2015, the Partnership for Advancing Community based Services (PACS) targeted 1490 rural communities in Bong, Lofa and Nimba Counties with the goal of increasing access to improved sanitation and achieving Open Defecation Free (ODF) status. A group of 280 WASH Entrepreneurs (WEs) were selected and trained to support this initiative. From 2015 to 2018, the WEs built or upgraded 19,460 individual household latrines including hand washing facilities serving an estimated 442,609 people. Additionally, the WEs installed 38,511 dish racks, 48,787 cloth lines, and 4,155 compost fences in the communities. They also constructed 90 hand-dug wells fitted with hand pumps to provide clean water to 22,500 community residents. After four years of PACS support, 82% (1225/1490) of the targeted communities were verified ODF. The ODF community members have access to improved low cost household latrines and handwashing stations. They also contribute money on a monthly basis through Village Savings and Loan (VSL) scheme set up for operation and maintenance of WASH facilities in order to ensure long term sustainability and ownership. PACS support helped rural communities build clean and healthy environments which are expected to reduce the burden of water borne diseases especially among children in these communities. As part of ongoing local capacity development, WEs will receive further training in hand pump installations, fabrication of low cost sanitation improvement products to continue the scale up and promotion of improved sanitation and clean water.

612

SPATIAL PROXIMITY TO WASTEWATER USED FOR IRRIGATION AND CHILDHOOD DIARRHEA IN THE MEZQUITAL VALLEY, MEXICO

Jesse D. Contreras[1], Rob Trangucci[1], Eunice E. Felix-Arellano[2], Sandra Rodríguez-Dozal[2], Horacio Riojas-Rodríguez[2], Rafael Meza[1], Jon Zelner[1], Joseph N.S. Eisenberg[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Instituto Nacional de Salud Pública, Cuernavaca, Mexico*

Wastewater reuse for agriculture is a common practice worldwide with important benefits to climate resilience and water conservation. Associations between local presence of wastewater reuse and negative health outcomes have been found in past research across the globe, but little information exists on the relevant routes of exposure between wastewater reuse and poor health. The Mexico City-Mezquital Valley system is one of the largest wastewater reuse systems in the world and has been the site of key epidemiological studies on wastewater and health. To understand the potential importance of environmental routes of exposure, we assessed the relationship between diarrheal disease in children and spatial proximity to wastewater canals using data from a longitudinal cohort study. We enrolled 581 households from communities in the Mezquital Valley that use wastewater for irrigation. A total of 1,705 surveys were completed during three rounds of interviews. Interviewers collected GPS coordinates of participating households, and local authorities provided digital maps of the wastewater canal system. The shortest distance between a study household and a wastewater canal was calculated in meters (m) using GIS software. The association between distance to a canal and diarrheal disease in children under five was estimated in a hierarchical Bayesian logistic regression model accounting for repeated observations and residual spatial correlations. To account for a non-linear association, household distance was transformed to its natural log. After adjusting for relevant covariates, living farther from a wastewater canal was associated with decreased diarrheal disease in children (100m vs. 10m OR = 0.54, 95% CI 0.37, 0.78; *e*-fold increase OR = 0.76, 95% CI 0.65, 0.90). These results suggest occupational and foodborne exposures are not the only important routes of exposure between wastewater and health, and community exposures may be driving negative health effects. Instead of only targeting specific groups, interventions should focus on whole communities to improve health where wastewater is reused.

613

MODELLING THE IMPACT OF PYRETHROID RESISTANCE ON PERSONAL PROTECTION AND THE MASS COMMUNITY EFFECT OF LONG-LASTING INSECTICIDE TREATED NETS

H. Juliette T. Unwin, Ellie Sherrard-Smith, Thomas S. Churcher, Azra C. Ghani

Imperial College, London, United Kingdom

Long-lasting insecticidal bed nets (LLIN) provide protection against malaria. Direct personal protection, which reduces exposure of users to infectious bites, is conferred by both its physical barrier and the killing and repellency action of the insecticide. Indirect mass protection for the whole community occurs due to the reduction in local mosquito population and the impact that this has on onward transmission. Standard LLIN contain a pyrethroid insecticide, but widespread resistance to this chemistry in mosquitoes may diminish both the direct and indirect impact. Piperonyl butoxide (PBO) synergises with pyrethroid insecticide to restore killing effects and are combined in PBO LLIN. We aimed to quantify the direct versus indirect effects of LLIN, and how these change as mosquito pyrethroid resistance increases. A mathematical model of *Plasmodium falciparum* transmission is used to estimate the reduction of parasite prevalence. Parametrisation of the LLIN model uses data from a systematic review of hut studies. LLIN efficacy wanes as pyrethroid resistance in local mosquitoes, measured using mosquito survival in discriminatory dose bioassays, increases. LLIN users are consistently predicted to have lower levels of malaria prevalence than non-LLIN users irrespective of the level of pyrethroid resistance. In the absence of resistance, in areas of high transmission (pre-intervention EIR of 100, 0-59 month year old parasite prevalence 56%) with 50% LLIN usage, we estimate the absolute reduction in prevalence attributed to the barrier to be 4%, the additional benefit of the insecticide to be 10% and the indirect protection to the user to be 17% (a total reduction of 31%). In non-users, the indirect reduction in prevalence is estimated to be 18%. However, when resistance is 50% the direct protection of the insecticide reduces to 8% and the indirect protection for the users and non-users reduce to 14% and 15% respectively. When PBO nets are simulated, the protection returns to pre-resistance levels. This study shows that pyrethroid resistance may diminish the protection gained from LLIN but PBO LLIN mitigate for this loss of protection.

614

WHOSE ARCHETYPE IS IT, ANYWAY? A MACHINE LEARNING APPROACH FOR CHARACTERIZING MALARIA TRANSMISSION SETTINGS

Amelia Bertozzi-Villa[1], Joshua L. Proctor[1], Jaline Gerardin[2], Caitlin Bever[1], Samir Bhatt[3], Peter Gething[4]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Northwestern University, Chicago, IL, United States,* [3]*Imperial College, London, United Kingdom,* [4]*Oxford University, Oxford, United Kingdom*

Mechanistic malaria models are proving increasingly valuable to policy decision-making and assessment of new tools. Model performance

is often showcased in a range of "archetypal" transmission settings comparing low vs high seasonality, baseline transmission, and indoor biting. These archetypes are used in sensitivity analyses, to demonstrate model versatility, or to highlight varying intervention efficacy. Archetypal sites tend to be selected by convenience or represented by hypothetical locations that possess the desired properties. Each of these heuristic approaches faces a classification challenge when predicting outcomes for a new site: it is difficult to determine which archetype that new site should belong to. We present an alternative method for determining transmission archetypes *a priori* by harnessing the strength of geospatial data. Using the Malaria Atlas Project's covariate library, we assemble a database of malaria-related rasters detailing monthly climate indicators, vector species proportions, and historical ITN, IRS, and ACT coverages, all at 5km^2 pixel resolution for the continent of Africa. We apply singular value decomposition and *k*-means clustering to classify pixels into transmission archetypes. Our method identifies ten data-driven transmission archetypes, capturing a range of vector mixes and seasonality patterns across the continent. Using the agent-based model EMOD, we show that the same intervention can have drastically different effects across archetypes. A three-year intervention of moderate (40%) ITN, IRS, and ACT coverage is sufficient to eliminate in 62.1% of pixels in an archetype with high indoor biting and dual peaked seasonality, whereas that same strategy eliminates in only 48.1% of pixels in a Sahelian setting with low indoor biting and intense seasonality. We also explore novel intervention impact across settings, showing e.g. that ATSBs require a higher kill rate in some archetypes than others to be effective. This methodology is extensible to any mechanistic modeling framework across a range of diseases, and can aid in rapid outcome prediction for novel sites of interest worldwide.

615

MBITES: A MODELLING FRAMEWORK FOR THE STUDY OF MOSQUITO BIONOMICS AND VECTORIAL CAPACITY AS EMERGENT PATTERNS

Sean Wu[1], Héctor M. Sánchez C.[1], Biyonka Liang[1], Daniel T. Citron[2], John Henry[2], David L. Smith[2]

[1]*University of California Berkeley, Berkeley, CA, United States,* [2]*IHME, Seattle, WA, United States*

Mathematical models of malaria transmission by vectors are based on a model for the sporozoite rate first developed by George Macdonald, from which the concept of vectorial capacity (VC) was derived. VC is a parsimonious formula that maps various entomological parameters onto a metric which describes the intensity of transmission. While a useful metric, the mathematical elegance of VC obscures a large degree of uncertainty in how ecology and entomology interact in any specific setting to produce the bionomic parameters that become inputs to the formula for VC. To this end, we developed two models describing mosquito behaviors and behavioral state transitions related to malaria transmission: an individual-based model, and a system of differential equations. By partitioning the complexity of mosquito behavior into atomic units called flight bouts, each of which is taken with a specific behavioral intent (blood feeding, mating, oviposition, etc.), we arrive at a useful mechanistic description of mosquito behavior well-suited to simulation and extension. Using these models, we show how critical parameters, such as mosquito lifespan, feeding rate, and others are not just entomological constants, instead depending upon the local ecology and distribution of resources required for the mosquito life-cycle. In order to facilitate collaboration, critique, and dialogue, MBITES is being developed as an open-source R package to facilitate ease of use and understanding of code, with computationally heavy elements implemented in object-oriented C++.

616

SHAPESHIFTER: A NOVEL MODEL FRAMEWORK FOR SIMULATING INDIVIDUAL-LEVEL MALARIA INFECTION AND IMMUNE DYNAMICS

Jonathan Russell[1], Dan Goes[1], Andre Lin Ouedraogo[1], Chris Lorton[1], Isabel Rodriguez-Barraquer[2], Bryan Greenhouse[2], Edward Wenger[1], Jaline Gerardin[3], Caitlin Bever[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*University of California San Francisco, San Francisco, CA, United States,* [3]*Northwestern University, Chicago, IL, United States*

Targeted interventions used to interrupt malaria transmission in an endemic population depend on an accurate description of the parasite burden: who is at risk for infection, who is infectious, and what is the appropriate response given data from both active and passive surveillance measures. Sampling of parasite densities in malaria-endemic settings gives limited information on the infection and immune status of individuals. Mathematical modeling can help predict what immunological forces underly these sparse measurements. We have developed a novel mathematical model for malaria infection and immune development that functions within the context of EMOD - a highly detailed individual based stochastic model of infectious disease dynamics. Within this model framework, we represent an individual's immune status as a stochastic transition matrix from which infection trajectories are drawn. We then parametrized functions that modify this immune status using a combination of continuous, discrete, and categorical variables that characterize an individual. Next, we calibrated this model to fit both individual-level longitudinal measurements and population-level patterns as measured by cross-sectional surveys. Finally, we tested the robustness of this model in capturing malaria incidence and prevalence metrics as published in studies across a range of geographies and transmission intensities. We demonstrate the utility of this new model in addressing previously intractable epidemiological and programmatic questions in a setting with historically high endemic malaria transmission.

617

MODELING OF HEMATOLOGICAL INDICES DURING SEVERE AND UNCOMPLICATED MALARIA USING ARTIFICIAL NEURAL NETWORKS

Collins Misita Morang'a[1], Thomas D. Otto[2], Saikou Y. Bah[3], Vincent Appiah[1], Gordon A. Awandare[1], Lucas Amenga–Etego[1]

[1]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana,* [2]*Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom,* [3]*Vaccine and Immunity Theme, Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine, Banjul, Gambia*

Hematological indices alone cannot be used to classify severe and uncomplicated malaria, but their accurate interpretation could complement clinical assessments. Our objective was to use hematological indices from 2,691 samples obtained in Ghana, including non-malarial fevers (n=1135), uncomplicated malaria (n=688), and severe malaria (n=868), to identify correlates between clinical malaria outcomes and hematological indices using an artificial neural network (ANN). The data was randomly split (80:20%) to training and test data sets. For training, we used the multi-layer perceptron (a type of ANN used for artificial intelligence on big data) with two hidden layers of rectified linear units. The ANN was compiled and back-propagated using Adam optimizer in 1000 training epochs with cross-validation. Performance on the test data was inspected using accuracy and area under the curve (AUC). The predictions were further investigated by multiple comparisons between groups (P=0.05).We successfully developed three deep learning prediction models. The test accuracy of the first model was 97% (AUC 0.99) and the indices that were predicted to distinguish severe malaria from non-malarial fevers include red blood cell counts (RBC), hematocrit, platelet counts (PLT), monocyte counts, mean cell hemoglobin, and RBC distribution width (RDW) ($P < 0.0001$). Accuracy of the second model was 85% (AUC

0.928) and only PLT and RDW were predicted to distinguish uncomplicated malaria from non-malarial fevers (P < 0.001). The accuracy of the last model was 94% (AUC 0.98) and the levels of lymphocyte counts, white blood cell counts, platelet distribution width, and mean cell hemoglobin concentration (P < 0.001) can be used to distinguish between severe from uncomplicated malaria. We developed an artificial intelligence network that generates accurate classifications of hematological indices between uncomplicated and severe malaria as well as non-malaria fevers. In the future, these deep learning models will be incorporated to a tool/app to support clinical assessment during diagnosis and management of malaria cases.

618

ACCOUNTING FOR HUMAN MOBILITY IN MALARIA ELIMINATION PROGRAMS WITH HETEROGENEOUS TRAVEL DATA

Hsiao-Han Chang[1], Ayesha Mahmud[1], Daniel T. Citron[2], Caroline O. Buckee[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Institute for Health Metrics and Evaluation, Seattle, WA, United States*

Understanding the effect of human movement on malaria transmission is critical for malaria control and elimination. The movement of people between low and high risk regions has consequences for the spread and maintenance of infectious disease transmission, and modifies prospects for disease elimination. In previous work, we used mobile phone calling data, travel survey data, and parasite genetic data to infer parasite flow between populations in Bangladesh and identified the locations with high proportion of imported infections. However, we were unable to consider all the locations in the malaria endemic regions due to the lack of travel data in some areas, hindering our ability to evaluate specific intervention strategies. Here, we build a statistical model based on demographic factors to infer travel for locations that previously had missing data. We use this to parameterize a multi-patch malaria transmission model and infer vectorial capacity by fitting the model to observed incidence data. We highlight the heterogeneity in vectorial capacity across the Chittagong Hill Tract region, which has implications for control strategies. Using our model results, we assess and compare the effectiveness of different malaria elimination strategies, including vector control, mass drug administration, and focal screening and treatment. Our model results allow us to prioritize resource allocation for control efforts and identify the conditions necessary for malaria elimination in the region.

619

IMPLICATION OF SULFADOXINE-PYRIMETHAMINE RESISTANCE-ASSOCIATED MUTATIONS ON THE PROTECTIVE EFFICACY OF SEASONAL MALARIA CHEMOPREVENTION: A PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS

Gina Maria Cuomo-Dannenburg[1], Patrick Walker[1], Robert Verity[1], Matthew Cairns[2], Paul Milligan[2], Lucy Okell[1]

[1]*Imperial College London, London, United Kingdom,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

The Sahel sub-region of Africa has widely utilised seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) since the World Health Organisation recommended its use in 2012. Mutations in the *dhfr* and *dhps* genes are well documented to confer resistance to sulfadoxine-pyrimethamine (SP). In Eastern and Southern Africa, quintuple and sextuple mutations in *dhfr* and *dhps* spread during the use of sulfadoxine-pyrimethamine as a first-line treatment for malaria. Development of these mutations in the Sahel would significantly threaten the efficacy of SMC as an intervention. We are using existing population pharmacokinetic models of SP, amodiaquine and desethylamodiaquine, alongside clinical trial data to determine the relationship between resistance markers and SMC efficacy. We define the protective efficacy to be the probability of SMC preventing an infection when challenged with an infectious bite. This protective efficacy wanes with the drug concentration and we define PC50 to be the concentration achieving 50% protective efficacy. Our initial results show that in a setting with established quadruple mutation, PC50 is 1.365mg/ml and 0.131μg/ml for sulfadoxine and pyrimethamine respectively. This drug concentration occurs at approximately day 35 dependent upon pharmacokinetic factors. The analysis is being extended to include data on resistant parasites which will allow us to estimate how duration of protection will vary with age and level of resistance markers in the population. This model provides for the first time an understanding of SMC efficacy with SP+AQ informed by population pharmacokinetics and a predictive tool for protection provided based upon prevalence of resistance markers. We will determine appropriate thresholds for resistance markers below which SMC retains sufficient protective efficacy with good adherence to the regimen. This work highlights the need for ongoing surveillance of resistance markers and development of a new drug with a similar pharmacokinetic profile to replace SP+AQ as the chosen SMC drug regimen once highly resistant strains become established.

620

THE POWER OF NEXT GENERATION *PLASMODIUM FALCIPARUM* GENETIC CROSSES IN HUMAN LIVER-CHIMERIC MICE

Katrina A. Button-Simons[1], Sudhir Kumar[2], Lisa A. Checkley[1], Meseret Haile[2], Nelly Carmago[2], Catherine Jett[3], Shalini Nair[3], Marina M. White[3], Xue Li[3], François H. Nosten[4], Stefan H. Kappe[2], Timothy J. Anderson[3], Jeanne Romero-Severson[5], Michael T. Ferdig[1], Scott J. Emrich[6], Ashley M. Vaughan[2], Ian H. Cheeseman[3]

[1]*Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States,* [2]*Seattle Children's Research Institute, Seattle, WA, United States,* [3]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [4]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Mae Sot, Thailand,* [5]*Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States,* [6]*University of Tennessee, Knoxville, TN, United States*

Understanding the genetic architecture of complex phenotypes such as drug resistance is critical to developing effective interventions. Resistance to the anti-malarial artemisinin has been primarily associated with mutations in the *Plasmodium falciparum k13* gene and has emerged and spread in Southeast Asia. However, there is growing evidence that additional genetic mutations are involved in artemisinin resistance. Real-time, powerful genetic mapping is needed to understand this complex genetic architecture. Genetic crosses between *P. falciparum* strains are a powerful tool for mapping, but the historical requirement of a splenectomized chimpanzee to complete the *P. falciparum* lifecycle limited the isolation of unique recombinant progeny from crosses of long term culture-adapted malaria parasites. Utilizing the human liver-chimeric FRG huHep mouse infused with human red blood cells has revitalized *P. falciparum* genetic crossing experiments and quantitative genetic analysis. We demonstrate that a genetic cross can now be carried out in less than four months with lab adapted field-derived parasites isolated directly from patients. Over the past year we generated deep sequencing data for the parents and progeny of three novel genetic crosses and developed a bioinformatic pipeline to identify more than 140 unique recombinant progeny. Unlike previous crosses, our field-derived cross shows bi-parental plastid inheritance, contains minimal segregation distortion and exhibits a uniform recombination rate providing consistently high power to detect effects genome-wide. Through detailed simulations we show the increased power and improved mapping resolution that this panel of recombinant progeny will provide. We demonstrate how segregation distortion can decrease power even for qtl with large effect sizes by mapping chloroquine IC_{50} in two independent crosses, one of which includes significant segregation distortion surrounding causal mutations in the *pfcrt* gene. This innovative resource enables rapid dissection of the genetic architecture of emerging anti-malarial drug resistance with unprecedented power and accuracy.

621

UNRAVELING THE EPIGENOME OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*

Chengqi Wang, Samir Jahangiri, Justin Gibbons, Swamy Adapa, Jenna Oberstaller, Xiangyun Liao, Min Zhang, Rays Jiang, John Adams

University of South Florida, Tampa, FL, United States

During malarial blood infections, a hardwired regulatory program secures proper timing of gene transcription and production of functionally relevant proteins, for *Plasmodium* development and pathogenicity. Here, we reveal the critical role of epigenome in malarial development from three aspects in fundamental genome configuration, essential gene regulation and 3D genome architechture. Firstly, we discovered that *P. falciparum* genes are organized in co-expressed linear epigenetic units. We developed a hidden markov model to capture the regulatory cluster formed by adjacent genes during intra-erythrocyte development. We find more genes involved in red blood cell invasion located in same regulatory module with PfAP2-I transcription factor, demonstrating that AP2-I is responsible for regulating RBC invasion. Secondly, we have discovered the underlying epigenetic structures for essential genes in *P. falciparum*. We systematically investigated gene expression, epigenetic markers, and 3D genome configuration of *P. falciparum* essential genes by integrating genomics data with our recently published large-scale mutagenesis data. We found that essential genes form 'hubs' in the regulation network. And we used robust statistical methods to show that the euchromatin is significantly distributed along essential genes, whereas heterochromatin is associated with dispensable genes. Interestingly, we found that the repressive marker H3K36me2 is associated with lowered expression levels in certain sets of essential genes. Finally, we used higher resolution transcriptional data and chromatin conformation capture data to show essential genes tend to be co-regulated and require physical contact in 3D genome space.

622

SELECTION FOR GROUP A *VAR* GENES DOES NOT OCCUR DURING EARLY BLOOD-STAGE INFECTION IN MALARIA-NAÏVE HUMAN VOLUNTEERS

Kathryn Milne[1], Adam Reid[2], Ruth Payne[3], Navin Venkatraman[3], Mandy Sanders[3], Matt Berriman[2], Simon Draper[3], Phil Spence[1], **J. Alexandra Rowe**[1]

[1]*University of Edinburgh, Edinburgh, United Kingdom,* [2]*Wellcome Sanger Institute, Cambridge, United Kingdom,* [3]*University of Oxford, Oxford, United Kingdom*

The *Plasmodium falciparum var* gene family plays a key role in the pathogenesis of severe malaria. Parasites collected from children with severe disease in sub-Saharan Africa show predominant transcription of group A *var* genes, encoding PfEMP1 variants that bind to brain endothelial cells and form rosettes with uninfected erythrocytes. It is hypothesized that parasites expressing group A variants dominate infections in naïve hosts because they promote effective sequestration and confer a growth-advantage compared to parasites expressing group B and C variants. This hypothesis is widely accepted, but is not supported by direct evidence. Previous work using mosquito-infected human volunteers showed expression of a range of mostly group B *var* genes *in vivo*, measured 2-3 cycles after liver egress. Rapid switching or selection in favour of group A *var* genes must then occur if they are a major determinant of severe malaria. We used controlled human malaria infection (CHMI) with blood stage challenge to investigate changes in *var* gene transcription over time *in vivo*. 14 malaria-naïve human volunteers were infected with *P. falciparum* 3D7 by direct intravenous inoculation of infected red blood cells. Volunteers were followed for 7.5-10.5 days until thick smear positivity. Parasite gene expression on the day of diagnosis was assessed by RNA sequencing, and *var* gene transcription was compared to that in the starting inoculum. The inoculum showed transcription of a range of group B *var* genes, with the group A *var* gene Pf3D7_0400400/PFD00020c also detected. Surprisingly, after 4-6 cycles of growth *in vivo*, *var* gene transcription patterns were highly similar to the starting inoculum in all volunteers, with no increase in group A transcripts. The brain endothelial cell-binding group A variant Pf3D7_0400400 detected in the inoculum was not selected for in any of the volunteers, nor was there any evidence for switching towards other group A *var* genes. These data provide direct evidence against the prevailing hypothesis that group A variants dominate infections in naïve individuals because they have a growth advantage *in vivo*.

623

DEVELOPMENT OF AN *EX VIVO* HUMAN BRAIN ORGANOID MODEL TO STUDY SEVERE MALARIA PATHOGENESIS

Adriana Harbuzariu[1], Sidney A. Pitts[1], Andrew P. Shaw[2], Juan C. Cespedes[1], Keri Harp[1], Annette Nti[1], Mingli Liu[1], Jonathan K. Stiles[1]

[1]*Morehouse School of Medicine, Atlanta, GA, United States,* [2]*Georgia Institute of Technology, Atlanta, GA, United States*

Human cerebral malaria (HCM) is a severe neurological manifestation of infection with *Plasmodium* species, with high mortality occurring especially in children. We have previously demonstrated that increased intravascular erythrocyte hemolysis during parasite multiplication releases free heme, leading to blood-brain barrier leakage and neuronal injury in cerebral malaria and placental disfunction during pregnancy. Current experimental models limit translationally relevant studies of malaria pathogenesis at the level of the brain *in utero* or postnatally. Recently, human induced pluripotent stem cells (iPSC) have shown great promise as models for studying neuronal development and diseases in humans. Using iPSC approach, we have generated 3D *ex vivo* human cortical organoids to recreate a developmental heme-induced brain injury model. Our hypothesis is that heme induces human iPSCs and brain cortical organoid injury that can be attenuated by a neuroprotective factor, neuregulin (NRG-1). To test this hypothesis, we employed flow cytometry, western blot, multiplexed immunoassay procedures and immunohistochemistry to assess pluripotency, confirm neuronal and astrocyte markers including NRG-1, its receptor ErbB4, tight junction proteins, matrix metalloproteinases and apoptosis markers at various time points during cortical organoid development. Our results show that iPSCs and cortical organoids express NRG-1 and ErbB4, consistent with observations in the human brain. Heme induced iPSCs apoptosis and differentiation of iPSC at ~30 M concentration and beyond. In addition, heme reduced organoids growth and altered their architecture and structural integrity. NRG-1 attenuated the heme-induced iPSC and cortical organoid injury via ErbB4 mediated pathway. In conclusion, we demonstrate that cortical organoids can be used as a model for studying heme-induced brain injury in CM and can be used to test NRG-1 and other drugs as neuroprotective agents. This innovative model allows for investigation of CM pathogenesis, other brain disorders and hemolytic diseases and will offer new avenues for intervention.

624

A NOVEL *IN VITRO* MODEL OF *PLASMODIUM VIVAX* HYPNOZOITES

Araya Jivapetthai[1], Yongyut Pewkliang[1], Paviga Limudompon[1], Sreekanth Kokkonda[2], Wanlapa Roobsoong[1], Suradej Hongeng[1], Pradipsinh K. Rathod[2], Osamu Kaneko[3], **Rapatbhorn Patrapuvich**[1]

[1]*Mahidol University, Bangkok, Thailand,* [2]*University of Washington, Seattle, WA, United States,* [3]*Nagasaki University, Nagasaki, Japan*

Eradication of *Plasmodium vivax* malaria is difficult because of the ability of the parasites to form dormant liver-stage forms (hypnozoites) that tolerate most drugs and can reactivate after drug pressure dissipates. Research efforts to better understand the biology of *P. vivax* hypnozoites and design relapse prevention strategies have been hampered by the lack of a robust and reliable model for *in vitro* culture of liver-stage parasites. Here, we demonstrate an *in vitro* culture of *P. vivax* hypnozoites in a novel immortalized hepatocyte-like cell line (imHC). We also describe

the establishment of a system to quickly obtain hypnozoites by using DSM265, a chemical compound that efficiently kills only large growing liver stage parasites, not hypnozoites. The enriched hypnozoites exhibit biological characteristics of hypnozoites *in vivo*, including their reactivation from dormancy. Our *in vitro* hypnozoites model offers a significant breakthrough in the characterization of hypnozoite biology, and will facilitate the discovery of anti-relapse interventions.

625

SINGLE-CELL RNA-SEQ REVEALS TIGHTLY REGULATED CHANGES IN GENE EXPRESSION DURING THE INTRAERYTHROCYTIC LIFE CYCLE OF *P. VIVAX* PARASITES

David Serre[1], Matthew V. Cannon[1], Ramon L. Caleon[2], Thomas E. Wellems[2], Juliana M. Sa[2]

[1]*University of Maryland, Baltimore, MD, United States,* [2]*National Institutes of Health, Rockville, MD, United States*

Studies of gene expression in *Plasmodium* are complicated by the continuous developmental changes of the parasites. As a consequence, most studies of blood stage parasites have relied on synchronized *in vitro* cultures of *P. falciparum*. Since *P. vivax* cannot be continuously propagated *in vitro*, this parasite remains poorly studied and our understanding of its biology primarily derives from *P. falciparum,* without accounting for their divergence and differences (e.g., gametocytogenesis, red blood cell (RBC) preference and sequestration). Recently, single-cell RNA-seq has been applied to *P. falciparum* and rodent malaria parasites but viability requirements have precluded such studies in other *Plasmodium* species, including *P. vivax*. Here, we describe the gene expression profiles of 8,172 individual *P. vivax* parasites obtained from infections of 10 splenectomized non-human primates with four *P. vivax* strains. Our data provide, on average, information on 15,251 mRNA molecules from 1,149 genes per individual parasite, allowing evaluations of even weakly expressed genes. Our analyses confirm that multiple stages, including male and female gametocytes, are circulating in each infection, though their relative proportions vary among infections. Our findings highlight the highly specific and tightly regulated expression of most genes during the intraerythrocytic cycle (IEC): many genes are expressed only during a short developmental period and very few genes, if any, are expressed consistently. Transcripts for proteins used in RDTs show strikingly heterogeneous patterns of expression, suggesting that the presence of particular *P. vivax* stages is important for sensitive RDT detection. Stage-specific gene expression regulation is also noticeable for AP2 genes, raising the intriguing possibility that these master controllers of transcription play a key role in regulating the entire IEC. Finally, our analyses revealed that late schizonts expressed a significantly higher proportion of genes without *P. falciparum* orthologs, highlighting the differences in RBC invasion in these species and the need to specifically study *P. vivax*.

626

DEVELOPMENT OF DRUG CANDIDATES FOR CHAGAS DISEASE TARGETING THE *TRYPANOSOMA CRUZI* METHIONYL-TRNA SYNTHETASE

Frederick S. Buckner, John R. Gillespie, Nora R. Molasky, Zhongsheng Zhang, Wenlin Huang, Sayaka Shibata, Yi Liu, Erkang Fan

University of Washington, Seattle, WA, United States

Better drugs are needed to treat Chagas disease, a protozoan infection afflicting 6-8 million people primarily in Latin America. The disease is caused by *Trypanosoma cruzi* which establishes chronic infection lasting decades. The resulting cardiomyopathy (or enteropathy) is fatal in 20-30% of victims. The current antiparasitic drugs are fraught with side effects and have questionable efficacy for treating chronically infected patients. The *T. cruzi* genome contains a single methionyl-tRNA synthetase (MetRS) that is essential for protein synthesis. This enzyme is being targeted with novel inhibitors to develop leads for anti-*T. cruzi* drug development. The MetRS inhibitors were developed against the *Trypanosoma brucei* MetRS assisted by crystal structure of that enzyme bound to inhibitors. Approximately 400 compounds from a library of MetRS inhibitors have been screened against mammalian-stage *T. cruzi* cultures establishing structure activity relationships. Twenty-five compounds have been identified with EC_{50} values between 1-10 nM and another 54 in the range of 11-100 nM. These compounds are highly selective with cytotoxicity (CC_{50}) values on mammalian cells of greater than 10,000 nM in the large majority. An exemplary compound was tested in an *in vitro* washout assay (16-day exposure at 25X the EC50) and shown to have trypanocidal activity (with no outgrowth in the 60 day observation period), comparable to the clinical drug, benznidazole. The active compounds represent several distinct scaffolds within the MetRS inhibitor series and are associated with different physicochemical (e.g. LogP, solubility) and pharmacological properties. A subset of compounds has been tested in the murine acute *T. cruzi* infection model, demonstrating strong suppression of *T. cruzi* growth and 100% survival of the mice. New compounds have been synthesized with low plasma clearance and high volume of distribution (feature that are believed to be favorable for treating this tissue parasite) and will be tested in the *in vivo* efficacy model. The MetRS inhibitors represent a promising class of compounds to be developed as potential novel drugs for Chagas disease.

627

XENODIAGNOSIS OF DOGS VERTICALLY INFECTED WITH *LEISHMANIA INFANTUM* REVEALS SKIN PARASITE BURDEN AS STRONGEST CORRELATE OF CANINE INFECTIOUSNESS TO SAND FLY VECTOR

Breanna M. Scorza[1], Kurayi Mahachi[1], Erin C. Cox[1], Angela J. Toepp[1], Adam Lima[1], Anurag Kushwaha[2], Patrick Kelly[1], Claudio Meneses[3], Katherine N. Gibson-Corley[1], Lyric Bartholomay[4], Shaden Kamhawi[3], Christine A. Petersen[1]

[1]*University of Iowa, Iowa City, IA, United States,* [2]*Banaras Hindu University, Varanasi, India,* [3]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [4]*University of Wisconsin, Madison, WI, United States*

Dogs are the primary reservoir for human visceral leishmaniasis due to *Leishmania infantum*. Phlebotomine sand flies maintain the zoonotic disease by transmitting parasites among dogs and to humans. A subset of dogs is infected during gestation, but the extent to which they contribute to the infected sand fly pool is unknown. We examined infectiousness of dogs naturally infected with *L. infantum* via vertical transmission (n=16) to the vector *Lutzomyia longipalpis* using xenodiagnosis. We also examined transmission correlates including dog age, clinical status, serostatus, and parasite burden in skin, spleen, and blood. Sand flies fed on two sites/animal for 30 min. 48 hrs post feeding, blood fed female flies were separated, DNA extracted and subject to Real Time-qPCR for detection of *Leishmania* DNA. A standard curve allowed quantification of equivalents taken up by individual sand flies. Clinical status was staged according to LeishVet guidelines based on physical exam and serum chemistry panel. We found vertically infected dogs are infectious to sand flies. Further, sand fly parasite uptake did not correlate with clinical status where dogs with moderate disease (stage 2-3) showed significantly higher transmission to the vector than dogs with mild (stage 1) or severe (stage 4) disease. Interestingly, we documented a substantial parasite burden in the skin of vertically infected dogs by RT-qPCR, despite never receiving intradermal parasites via sand flies. There was a significant correlation between skin parasite burden at the feeding site and sand fly parasite uptake ($p<0.01$), while positive xenodiagnosis did not correlate with parasitemia ($p=0.23$). This suggests dogs with high skin parasite burden contribute more to the infected sand fly pool. Although skin parasite load and parasitemia correlated with one another ($p<0.01$), the average parasite number detected in skin was higher compared to blood in all dogs tested. Thus, dogs without detectable parasitemia may already be infectious to the vector. Together, our data implicate skin parasite burden as a strong indicator of outward transmission potential.

628

TESTING FOR CHAGAS CARDIAC DISEASE AT A LARGE SAFETY-NET HOSPITAL IN NEW ENGLAND

Alyse Wheelock[1], Sukhmeet Sandhu[1], Davidson Hamer[2], Rachel Marcus[3], Deepa Gopal[4], Natasha Hochberg[5]

[1]*Internal Medicine Residency Program, Department of Medicine, Boston Medical Center, Boston, MA, United States,* [2]*Department of Global Health, Boston University School of Public Health; Section of Infectious Disease, Department of Medicine, Boston University School of Medicine, Boston, MA, United States,* [3]*MedStar Heart and Vascular Institute, Medstar Union Memorial Hospital, Baltimore, MD, United States,* [4]*Department of Medicine, Cardiovascular Division, Boston University Medical Center, Boston, MA, United States,* [5]*Section of Infectious Disease, Department of Medicine, Boston University School of Medicine, Boston, MA, United States*

Prior research suggests that Chagas disease, caused by the parasite *Trypanosoma cruzi*, is an important etiology of non-ischemic cardiomyopathy and cardiac arrhythmias among people who originate from Mexico, Central America, and South America. Less than 1% of the estimated 300,000 people living with Chagas disease in the United States have been diagnosed and treated, however. This study aims to determine the frequency of Chagas disease testing among individuals from endemic regions who display signs consistent with Chagas heart disease. Retrospective chart review was performed of electronic medical records from 2012-2017 at an academic safety net hospital in Boston. Inclusion criteria were patients born in Mexico, Central America, and South America with non-ischemic cardiomyopathy, conduction abnormalities (including bundle branch blocks or atrioventricular blocks), or arrhythmias (including sinus bradycardia, frequent premature ventricular complexes, or ventricular tachycardias). Data on demographics, electrocardiograms (EKG), echocardiography, Chagas disease diagnostics, and treatment were collected. Descriptive statistics were performed. 3099 patients met the inclusion criteria during the study period, including 1494 women and 1605 men. The most frequent countries of origin were El Salvador (n=755), Colombia (n=546) and Brazil (n=494). Only 42/3099 (1.4%) patients had Chagas serologies ordered. Of these patients, 8/42 (19.0%) tested positive for Chagas disease, 33/42 (78.6%) tested negative, and 1/42 (2.4%) had indeterminate results. Rates of Chagas disease testing were very low among this cohort of patients from endemic countries with EKG and echocardiographic findings consistent with Chagas cardiac complications. Among those patients tested, a significant proportion tested positive. Further analyses will be targeted towards determining factors associated with Chagas disease test ordering to explore opportunities for expanded testing. The initial findings of our study show a need to expand diagnosis and treatment of Chagas cardiac disease among Latin American immigrants in North America.

629

COMPARISON OF CHAGAS DISEASE SEROLOGY TEST PERFORMANCE IN THE UNITED STATES

Jeffrey D. Whitman[1], Christina A. Bulman[1], Emma L. Gunderson[1], Rebecca L. Townsend[2], Susan L. Stramer[2], Judy A. Sakanari[1], Caryn Bern[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*American Red Cross, Gaithersburg, MD, United States*

Chagas disease affects an estimated 300,000 individuals in the US. Diagnosis in the chronic phase requires positive results by two IgG serological tests. Three ELISAs (Hemagen, Ortho, Wiener Recombinante 3.0) and one lateral flow assay (InBios ChagasDetectPlus) are FDA-cleared. Comparative data in US populations are sparse. We evaluated plasma samples from 800 blood donors tested by the American Red Cross, 500 classified as seropositive by >2 blood bank tests (Ortho, RIPA, Abbott PRISM, Abbott ESA) and 300 classified as seronegative (by Ortho or PRISM). Country of birth was known for 255 seropositive specimens: 94 Mexico, 88 Central America and 73 South America. Seronegative samples were selected randomly, frequency-matched with seropositive samples by region of donation. Specimens were tested by the four FDA-cleared tests. We compared test performance to two references, blood bank classification (BB) and positive results by >2 FDA-cleared tests (consensus). A latent class analysis is planned. Compared to BB, sensitivity/specificity were 88.0%/100% for Hemagen, 92.4%/100% for Ortho, 94.0%/99.3% for Wiener and 97.4%/91.7% for InBios. Compared to consensus, sensitivity/specificity were Hemagen 90.7%/99.7%, Ortho 95.3%/99.7%, Wiener 96.3%/98.1% and InBios 99.2%/89.9%. Compared to BB status, sensitivity was lowest for those born in Mexico (83.0%, 85.1%, 91.5% and 97.9%) and highest for South America (93.2%, 97.3%, 98.6% and 98.6% for Hemagen, Ortho, Wiener and InBios). Some specimens positive by BB testing were negative or discordant in this evaluation, presumably due to antibody degradation. In conclusion, no test used alone had optimal performance. InBios was the most sensitive FDA-cleared test, detecting 97% of BB positives, but with specificity of 90-91%. Hemagen showed lower sensitivity in both analyses (88-91%) but high specificity. Ortho and Wiener had intermediate sensitivity and high specificity. Improved availability of the FDA-cleared tests and their combined use would facilitate laboratory diagnosis of Chagas disease in the US.

630

ADDRESSING EARLY DIAGNOSIS OF CONGENITAL CHAGAS DISEASE IN THE TIME OF THE ELIMINATION GOAL OF MOTHER-TO-CHILD TRANSMISSION IN THE AMERICAS

Yagahira E. Castro[1], Freddy Tinajeros[1], Caryn Bern[2], Gerson Galdos-Cardenas[1], Edith S. Malaga[3], Edward Valencia Ayala[3], Syamal Raychaudhuri[4], Kathryn Hjerrild[4], Steven J. Clipman[1], Andrés G. Lescano[3], Tabitha Bayangos[1], Walter Castillo[5], María Carmen Menduiña[6], Kawsar R. Talaat[1], Robert H. Gilman[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*University of California, San Francisco, CA, United States,* [3]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [4]*InBios International, Inc., Seattle, WA, United States,* [5]*Asociación Benéfica PRISMA, Lima, Peru,* [6]*Percy Boland Maternity Hospital, Santa Cruz, Plurinational State of Bolivia*

Early diagnosis of congenital Chagas disease (CChD) represents a good opportunity for early treatment. We evaluated factors of maternal adherence to the 6- or 9-month follow-up program (FP) and the ability IgM Shed Acute Phase Antigen (SAPA) ELISA in the early diagnosis of CChaD). Methods: Two prospective studies (consisting of a training and a validation cohort) were conducted in three hospitals in Santa Cruz, Bolivia. Pregnant women were screen for Chagas disease, and infants from seropositive mothers were examined to determine vertical transmission at birth and 1-month by microscopy, qPCR, and IgM TESA-blot (trypomastigote excreted-secreted antigens), and at 6- or 9-months for anti-*Trypanosoma cruzi* IgG antibodies. An IgM SAPA ELISA was optimized using a recombinant SAPA and camelid antibodies for IgM detection. Findings: A total of 5318 women were evaluated, overall maternal seroprevalence was 25.49% (95% CI: 24.33%-26.69%) and adherence to FP was 37.24% (95% CI: 34.66%-39.88%), respectively. Lower maternal education (adjusted OR: 0.75, p=0.049), owning a computer (adjOR: 1.97, p=0.002), and living in a house with more than three persons per room (adjOR: 0.73, p=0.022) impacted the odds of maternal adherence. When only one infant sample obtained at birth was evaluated in the validation cohort, the qPCR and the IgM SAPA ELISA have similar accuracy and were both higher than microscopy (sensitivity: 86.49%, 32/37; 81.58%, 31/38; 57.58%, 19/33, respectively, and specificity: 99.53%, 212/213; 96.15%, 209/217; 99.47%, 189/190, respectively). Interpretation: Socioeconomic factors were mainly determinants of maternal adherence. None of the early diagnostic tests achieved sensitivities higher than 90%, likely because of the biology of the infection. The IgM SAPA ELISA has the potential to be implemented in the short-term as an early diagnostic tool.

631

EVALUATING COMMUNITY-BASED SCREENING CAMPAIGNS FOR SEROLOGICAL DIAGNOSIS OF *TRYPANOSOMA CRUZI* AND *STRONGYLOIDES STERCORALIS* IN MADRID, SPAIN

Maria Delmans Flores-Chavez[1], Olvido Bocos[2], Francisca Vivas[3], Briggitte Jordan[4], Javier Nieto[5], Emilia Garcia[5], Belen Garcia[4], Ignacio Peña[6], Cristina Arcas[6], Carmen LLanos Aguilar[2], Ana Orellana[2], Jose Saugar[5], Juan Jose De Los Santos[4]

[1]*Fundación Mundo Sano / Centro Nacional de Microbiología, Madrid, Spain,* [2]*Centro Municipal de Salud Comunitaria de Usera, Madrid, Spain,* [3]*Ayuntamiento de Madrid, Madrid, Spain,* [4]*Fundación Mundo Sano, Madrid, Spain,* [5]*Centro Nacional de Microbiología-ISCIII, Madrid, Spain,* [6]*Salud Entre Culturas, Madrid, Spain*

Chagas disease (CD) and Strongyloidiasis (SS) are two parasitic infections which have poor visibility due to the absence or scarce presence of symptoms. The causative agents of these pathologies are the protozoan *Trypanosoma cruzi* and the nematode *Strongyloides stercoralis*, respectively. Both infections are diagnosed several years after having acquired them, in fact, most of those affected are unaware of their infection and, therefore, they did not receive a specific treatment at the appropriate time. In order to access to the diagnosis CD and SS, three strategies of community-based screening campaigns were evaluated: two Thursday afternoons each month (Thursday's screening), the first Saturday of each month (Saturday's screening) and one Sunday per semester (Sunday's screening). The population was included by the active action of health workers prior to the day of sampling in the Thursday's screening and Sunday's screening, while on Saturday's screening people were included at the same day of the activity when they were conducting an administrative process at the Bolivia Consulate. The management of samples and results was performed through the Municipal Community Health Centre of Usera. Subsequently all positive cases were referred to their reference hospitals according to the preference of each participant. CD was detected by three serological tests and SS by IgG detection of anti-*Strongyloides* antibodies. From April to December 2018, 522 samples were analyzed. The overall prevalence of CD and SS were 20% and 11% respectively, 99% of CD cases came from Bolivia, whereas SS was detected in people from different countries of Latin America. The combined search of both parasites facilitates the appropriate use of resources. Considering the economic costs of each strategy, Saturday screening is the most profitable system. Community-based screening campaigns allowed the detection of CD and SS outside the primary health care system, at time intervals according to the time availability of the population at-risk. Knowledge of the needs of the affected population will improve the efficiency of intervention strategies.

632

IMPROVED BIOMARKERS AND IMAGE ANALYSIS FOR CHARACTERIZING PROGRESSIVE CARDIAC FIBROSIS IN A MOUSE MODEL OF CHRONIC CHAGASIC CARDIOMYOPATHY

Kristyn Hoffman, Peter Hotez, Maria Bottazzi, Kathryn Jones

Baylor College of Medicine, Houston, TX, United States

Chronic Chagasic cardiomyopathy (CCC), caused by *Trypanosoma cruzi* infection, is an important public health problem due to progressive cardiomyopathy in patients, for which there is no curative treatment. CCC is characterized by myocarditis and cardiac fibrosis, which leads to life-threatening arrhythmogenic and circulatory abnormalities. This study aimed to identify non-invasive markers of cardiac fibrosis to monitor disease progression in a mouse model of CCC. Adult Balb/c cardiac cells were infected with *T. cruzi* H1 trypomastigotes (Tc1) or exposed to parasite lysate and concentrations of TGF-β, CTGF, ET-1 and PDGF-D were significantly elevated in the infected cell group supernatants. Female Balb/c mice were chronically infected with *T. cruzi* H1 and compared to an uninfected control group. Cardiac fibrosis, inflammation, mRNA expression of pro-fibrotic genes, and serum levels of pro-fibrotic biomarkers were measured. TGF-β genes, and other TGF-β superfamily genes, CTGF, ET-1 and PDGF were upregulated in the infected mouse heart. Serum concentrations of TGF-β, CTGF and PDGF-D, were significantly higher in infected mice and significantly correlated to cardiac fibrosis progression. Strain analysis was performed on MR images at 111 and 140 days post infection (DPI) and echocardiography images at 212 DPI. Chronic infection induced significantly elevated LV strain and concomitantly significantly decreased cardiac output at each time point. LV strain was significantly correlated to decline in cardiac output and increase in cardiac fibrosis. TGF-β, CTGF and PDGF-D can be used as biomarkers of fibrosis in CCC, as they positively correlated with levels of fibrosis measured on histopathology. Strain analysis of both MRI and echocardiography is a sensitive method to acquire longitudinal measurements in the same animal, which is not possible with the traditional method of measuring fibrosis on histopathology. Fibrosis biomarkers and strain analysis can be applied as longitudinal, non-invasive tools to study the effects of interventions and/or therapeutics on cardiac fibrosis development when using a mouse model of CCC.

633

CHILD MOUTHING OF CONTAMINATED FOMITES AND ANIMAL CONTACT IS ASSOCIATED WITH DIARRHEA AND STUNTING (REDUCE PROGRAM)

Christine Marie George[1], Ronald Saxton[1], Jennifer Kuhl[1], Jamie Perin[1], Nicole Coglianese[2], Elizabeth Thomas[1], Sarah Bauler[2], Anthony Koomson[2], Phil Moses[2], Geoffrey A. Nyakuni[3], Amagana Togo[3], Ruthly Francois[1], Patrick Mirindi[3], Lucien Bisimwa[3]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Food for the Hungry, Phoenix, AZ, United States,* [3]*Food for the Hungry, Bukavu, Democratic Republic of the Congo*

Exploratory play behavior is important for motor and cognitive development in young children. However this behavior also presents an exposure route to fecal pathogens for susceptible pediatric populations. In our prospective cohort study of 515 children under 5 years of age in South Kivu, Democratic Republic of the Congo, we are investigating the association between child play behaviors, diarrheal disease, and child growth. Child mouthing of soil and objects is assessed by five hour structured observation, and contact with domestic animals is assessed by caregiver reports. Spot checks of signs of open defecation or flying toilets on the household compound are also performed. In this abstract we report our baseline findings. Fifty percent of children were observed putting soil in their mouth, and 90% of children were observed mouthing a visibly dirty object during structured observation. Caregivers reported that 42% of children touched chickens, 36% touched guinea pigs, and 20% touched rabbits in the last week. Children observed putting soil in their mouth had a significantly higher odds of stunting (Odds Ratio (OR): 1.72 (95% Confidence Interval (CI): 1.06, 2.82)). Children observed putting a visibly dirty object in their mouth had a significantly higher odds of diarrhea (OR: 4.97 (95% CI: 1.17, 21.04)). Children that were reported to have touched chickens had a significantly higher odds of diarrhea (OR: 1.76 (95% CI: 1.06, 2.92)). In addition, children in households with signs of open defecation or flying toilets were at significantly higher odds of being wasted (OR: 2.87 (95% CI: 1.02, 8.04). Child mouthing of soil and visibly dirty objects and contact with chickens was associated with stunting and diarrhea in rural Democratic Republic of the Congo. These findings are being used to design an evidence-based Baby water, sanitation, and hygiene intervention to target these identified risk factors in the upcoming REDUCE trial. Interventions that focus on clean child play spaces are urgently needed to reduce pediatric exposure to fecal pathogens. This study is funded by the USAID Office of Food for Peace.

634

HAND CONTAMINATION WITH PATHOGENIC, ZOONOTIC AND ANTIMICROBIAL RESISTANT BACTERIA AMONG CAREGIVERS RESIDING WITH DOMESTIC ANIMALS IN INDIA

Marlene K. Wolfe[1], Karin Gallandat[2], Daniele Lantagne[1], Amy Pickering[1]

[1]*Tufts University, Medford, MA, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Exposure to zoonotic pathogens and antibiotic resistant bacteria is an increasing concern in low-income domestic settings. Antibiotics in animal feed is expected to increase by 82% in India by 2030, likely intensifying environmental transmission of antimicrobial resistant bacteria. This study's goal was to identify whether hands are an exposure pathway for pathogenic, zoonotic, and antimicrobial resistant bacteria among caregivers living in close proximity to animals in India. We conducted a survey on animal care and collected hand rinse samples from 108 caregivers of young children in India. We cultured *E. coli* and total coliform from hand rinsate and extracted and analyzed DNA from cultured biomass to identify *E. coli* virulence genes (ECVG) by multiplex PCR and bacterial pathogens and antimicrobial resistance genes (ARG) by untargeted long-read genomic sequencing. We used quantitative PCR to identify a host-associated genetic marker (BacCow) specific to animal feces. Ruminants were present in 67% of compounds and poultry in 44%. *E. coli*, ECVG, and BacCow were identified in 78%, 29%, and 97% of samples respectively. Providing animal care was positively associated with cultured *E. coli* and presence of any ECVG, animal presence was positively associated with BacCow, and number of animals was negatively associated with ECVG. We identified 28 species of pathogenic bacteria (including zoonotic species such as enterotoxigenic *E. coli* and *Campylobacter spp.*), and 106 distinct ARGs, the majority conferring ß-lactam resistance. Animal fecal contamination on hands was highly prevalent. Caregivers providing animal care had increased ECVG on hands, suggesting a link between animal contact and diarrheagenic *E. coli* on hands. Results suggest hands are carriers of important pathogens and ARGs in this setting where animals are in close contact with humans. To our knowledge, this is the first study to conduct long-read sequencing of viable bacteria from caregiver hand samples in a low-income context. This untargeted, flexible approach is capable of identifying hundreds of pathogens at once and could be a valuable addition to models of risk assessment.

635

A PROSPECTIVE COHORT STUDY INVESTIGATING THE RELATIONSHIP BETWEEN THE GUT MICROBIOTA, ENVIRONMENTAL ENTEROPATHY AND IMPAIRED GROWTH IN RURAL BANGLADESH

Jamie Perin[1], Mathieu Almeida[2], Vanessa Burrowes[1], Shahnawaz Ahmed[3], Rashidul Haque[3], Tahmina Parvin[3], Shwapon Biswas[3], Ishrat J. Azmi[3], Md. Sazzadul Islam Bhuyian[3], Kaiser A. Talukder[3], Abu G. Faruque[3], O. Colin Stine[2], Christine Marie George[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*University of Maryland, Baltimore, MD, United States,* [3]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh*

There is a growing body of literature demonstrating an association between environmental enteropathy (EE) and stunting in young children. This condition is defined by reduced intestinal barrier function, and increased inflammation resulting in low nutrient absorption. Microbial communities in the child gut are thought to play an important role in this condition, although the relationship is poorly understood. In this study we investigated the associations between gut microbial communities, EE, and prospective child growth among children in rural Mirzapur, Bangladesh. We examined four fecal markers of EE (alpha-1-antitrypsin, calprotectin, myeloperoxidase, and neopterin) and anthropometric measures from a cohort of 68 children. We sequenced the 16S rRNA gene of bacterial DNA from stool, and used the resulting sequences to estimate amplicon sequence variants (ASV). We then age-matched children with poor growth to children with normal growth within one month based on the WHO child growth standards, and compared the change in abundance and diversity of ASV over time. Children had a mean baseline age of 17 months and there was a stool sample from each child at baseline and the 18 month follow-up. Using tertiles of standardized anthropometry and the four fecal markers of EE, we compiled child pairs to compare ASV identity and diversity by child growth. We observed increases in *Streptococcus* and *Escherichia/Shigella* among children with elevated alpha-1-antitrypsin compared to controls (3% increase versus 12% decrease, p=0.008 and 4% increase versus no change, p=0.042, respectively). There was higher *Proteobacteria* among children with poor growth (bottom tertile of height for age z-scores) than among controls, (31% versus 12%, p=0.015). We observed increased *Proteobacteria* presence among calprotectin defined EE cases than controls (15% increase versus 13% decrease, p=0.007). Diversity in ASV was lower among children with poor growth at study enrollment. Further research is necessary to determine the attributes of gut microbial communities most likely to promote or inhibit growth among young children in low- and middle-income countries.

636

PTM202, A BOVINE COLOSTRUM BASED NUTRITIONAL SUPPLEMENT, DECREASES THE ENTERIC INFLAMMATION OF ENVIRONMENTAL ENTERIC DYSFUNCTION IN BANGLADESHI INFANTS

Jeffrey Donowitz[1], Masud Alam[2], Mamun Kabir[2], Tahsin Ferdous[2], Aysha Zerin[2], Uma Nayak[3], Rashidul Haque[2], William A. Petri[3]

[1]*Virginia Commonwealth University, Richmond, VA, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*University of Virginia, Charlottesville, VA, United States*

PTM202 is a nutritional supplement composed of a combination of bovine colostrum and whole egg. Both the cows and hens are specifically vaccinated to induce antibodies in colostrum and eggs which provide passive immunity against human enteric pathogens. PTM202 has been shown to decrease the duration of diarrhea in Guatemalan and Filipino children. We conducted a randomized placebo-controlled trial to determine the effect of PTM202 on environmental enteric dysfunction (EED). Healthy 6 to 9-month-old Bangladeshi infants were randomized to receive either 7g of PTM202 plus micronutrient sprinkles (intervention arm [IA]) or micronutrient sprinkles alone (control arm [CA]), twice a day for 30 days via directly observed therapy. Biomarkers of EED (fecal myeloperoxidase [MPO], fecal Reg 1B, serum C-reactive protein [CRP], serum sCD14, and lactulose:mannitol ratio [L:M]) were measured on days 0 and 30. Compliance was 96.5% (193/200 took their respective supplement for 30 days). No significant differences in enrollment characteristics was found between the intervention and control arms. None of the EED biomarkers differed significantly between groups on day 0 other than CRP (IA 7.5 vs CA 16.5 mg/L, p = 0.05). At 30 days, children in the intervention arm had significantly lower MPO (IA 5972.1 vs. CA 7849.2 ng/ml, p = 0.05) and Reg 1B (IA 121.8 vs CA 145.3 ug/ml, p= 0.04) compared to children in the control arm. No difference was noted for CRP, sCD14, or L:M between arms at 30 days. In conclusion, 7g of PTM202 given twice a day decreases EED associated enteric inflammation and epithelial cell damage in Bangladeshi infants.

637

MODIFYING TOILETS TO MAKE THEM CHILD FRIENDLY IN RURAL BANGLADESH

Tarique Mohammad Huda[1], Ruhul Amin[1], Abdullah Al Masud[1], Elli Leontsini[2], Mahbubur Rahman[1], Tania Jahir[1], Jyoti Bhushan Das[1], Farzana Yeasmin[1], Fosiul Alam Nizame[1], Abul Kasham Shoab[1], Laura Kwong[3], Stephen P. Luby[3], Peter J. Winch[2]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Stanford University, Stanford, CA, United States*

In low income countries, child feces are often not collected nor disposed safely. Most toilets have dimensions and features most appropriate for adults. We aimed to understand how to modify existing latrines to make them child friendly. Our objectives were a) to identify barriers to young children using existing latrines and b) develop enabling technologies and behavioral recommendations to increase toilet use by 3 to 7 years old children. The study was part of a pilot trial to improve child development in rural Bangladesh. We conducted 4 Focus Groups with mothers of children 3 to 7 years old to explore the barriers of toilet use. The barriers to children using toilets included fear of darkness, long distance from the main house, discomfort to sit given large size of pan, and fear of falling due to slippery floor. We enrolled 20 households to try out the following enabling technologies: ring attached with rope for child to hold onto while squatting, wooden board with smaller hole placed on pan, transparent fiberglass tile on the roof, picture of a cartoon character with handwashing messages, and wooden step stool at the toilet entrance. Two to three weeks after installing the enabling technologies, we conducted 19 in-depth interviews with the caregivers to explore the acceptance and feasibility of the installed technologies. The holding ring was well liked by the children. Caregivers considered it affordable and available. The wooden board with hole reduced the fear of falling and the discomfort of sitting on pan. However, it was inconvenient for mothers to handle and maintain due to its heavy weight. Mothers felt disgust when the time came to clean it. The transparent fiberglass roof tile, an affordable and available technology, reduced the darkness during the day time, and kept the latrine floor dry as sunshine could come through it. The picture in the toilet was interesting to the children but only for the first few days. In conclusion, the fiberglass roof tile holding ring and step stool can make the toilets more child friendly in rural Bangladesh. Further studies could provide insight on the prospect for sustained use and willingness of beneficiaries to purchase.

638

EVALUATING A COMPLEMENTARY FOOD HYGIENE BEHAVIOR CHANGE INTERVENTION IN RURAL MALAWI

Kondwani R. Chidziwisano[1], Save Kumwenda[1], Jurgita Slekiene[2], Joachim H. Mosler[2], Tracy Morse[3]

[1]*University of Malawi, Blantyre, Malawi,* [2]*Eawag aquatic research, Zurich, Switzerland,* [3]*University of Strathclyde, Glasgow, United Kingdom*

Globally, diarrheal disease accounted for over 90% of foodborne illness in 2010, with over 70% of this burden in Sub-Saharan Africa. However, traditional diarrheal prevention interventions focused on water, sanitation, and handwashing (WASH), with little integration of food hygiene. We designed and implemented a theory-based complementary food hygiene and WASH intervention in rural Malawi and evaluated its impact on food hygiene behaviors. Formative research and intervention development was grounded in the RANAS (Risk, Attitude, Norms, Ability and Self – regulation) Model and targeted five key behaviors: 1) cleaning of feeding and cooking utensils, 2) safe utensil storage, 3) reheating of left-over food, 4) child self feeding, and 5) handwashing with soap at critical times. The intervention was delivered for 9 months through village meetings and household visits. Formative research indicated that norms, ability and self-regulation factors were the primary determinants of selected behaviors. Intervention activities were linked to Behavior Change Techniques of the RANAS model. We randomly assigned villages to a control or intervention group and targeted caregivers of children aged six months to two years. Food hygiene behaviors were measured through randomized before after study in the intervention and control villages and changes assessed through difference in the group level proportions of caregivers observed practicing the key behaviors. At endline, four behaviors showed a significant difference: cleaning utensils (95% vs 70%, *P*=0.008); keeping utensils in an elevated place (98% vs 16%, *P*=0.000), reheating of food (24% vs 4%, *P*=0.024) and handwashing with soap (46% vs 6%, *P*=0.000). Significant changes in perceived norms and perceived ability (*P*=0.000) related to the four behaviors among the intervention participants indicates an effective influence of the intervention on targeted behavioral determinants. The study suggests that theory driven behavior change initiatives using contextual and psychosocial factors effectively improved food hygiene behaviors in rural Malawi.

639

DOES A SCHOOL BASED INTERVENTION TO ENGAGE PARENTS CHANGE OPPORTUNITY FOR HANDWASHING WITH SOAP AT HOME? PRACTICAL EXPERIENCE FROM AN ON-GOING RANDOMIZED TRIAL IN NORTHWEST TANZANIA

Elialilia S. Okello[1], Heiner Grosskurth[2], Kenneth Makata[1], Onike Mcharo[1], Safari Kinungh'i[3], Saidi Kapiga[1], Belen Torondel[2], Robert Dreibelbis[2]

[1]*Mwanza Intervention Trials Unit, Mwanza, United Republic of Tanzania,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*National Institute for Medical Research, Mwanza Centre, United Republic of Tanzania*

School-based deworming campaigns are a common approach to control of ascariasis and trichuriasis infections. However, preventing reinfection requires an integrative approach that combines de-worming with sustainable hygiene behaviour change at schools and homes. There is limited data on how school-based water, sanitation, and hygiene interventions can influence behaviour in the home. This sub-study examined the influence of the parental engagement sessions on access to handwashing materials and clean latrines at home. This longitudinal qualitative study included three rounds of household spot checks and interviews with 20 parents of children in 4 schools in northwest Tanzania participating in the Mikono Safi trial. At each school, parental engagement sessions were completed where parents were provided information on their own child's infection levels and information on STH prevention followed by a group discussion. Baseline data were collected before parental engagement sessions were implemented. Subsequent data collection occurred at 4 and 8 months following the intervention. At baseline, 19 out of 20 households had toilets but most of them were in poor condition and unsafe for children and no household had handwashing facilities near the toilet. All participating households participated in the parental engagement activities. Discussions regarding transmission of STHs evoked feeling of disgust and motivated parents to improve sanitation and access to handwashing at home. At the 4 months follow-up, 13 out of 20 households had made some repairs on the existing toilets or built new toilets and 12 built handwashing facilities next to the toilet. However, only 4 included soap at the handwashing facility. At 8 months, 16 households had installed handwashing facilities but only 8 had water and soap at handwashing facilities. Our strategy for engaging parents was effective in generating the intended emotional drivers of household behaviours. This resulted in substantial sustained structural hygiene. However, success was limited with regards to the provision of water and soap.

640

OPTIMIZING THE NUMBER OF CHILD DEATHS AVERTED WITH MASS AZITHROMYCIN DISTRIBUTION

Catherine Oldenburg[1], Ahmed Arzika[2], Ramatou Maliki[2], Ying Lin[1], Kieran O'Brien[1], Jeremy Keenan[1], Thomas Lietman[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*The Carter Center Niger, Niamey, Niger*

The MORDOR trial demonstrated a 14% reduction in all-cause child mortality with biannual mass azithromycin distribution compared to placebo. The largest observed reduction in mortality was in children aged 1-5 months. With the greatest absolute risk of mortality, these children may have the most to gain from interventions that reduce child mortality. Some implementation strategies may target younger ages, such as during infancy. We estimated the number of deaths averted by targeting different age groups versus 1-59 month-old children in the Niger site of MORDOR. Communities were randomized to 24 months of biannual mass azithromycin or matching placebo distribution to all children aged 1-59 months in the community. Mortality was measured via door-to-door census. Analyses were restricted to the Niger site only as any implementation of azithromycin for reduction of child mortality may be limited to settings with higher under-5 mortality. We calculated incidence rate differences (IRDs) for all-cause mortality in communities randomized to biannual azithromycin versus biannual placebo in children aged 1-5, 1-11, and 1-59 months at the time of treatment to estimate the absolute number of deaths averted per person-year based on the person-time observed in MORDOR-Niger. In 594 communities in Boboye and Loga, Niger, 3,615 deaths were observed over 145,694 person-years. Mortality in the placebo arm decreased with increasing age. IRDs for azithromycin versus placebo were -5.0 (95% confidence interval, CI, -6.6 to -3.4), -11.5 (95% CI -16.0 to -5.4), and -11.7 (95% CI -19.4 to -3.9) deaths per 1,000 person-years in the 1-59 month, 1-11 month, and 1-5 month age groups. This corresponded to 729 (95% CI 492 to 966), 297 (95% CI 168 to 427), and 126 (95% CI 43 to 209) deaths averted in each age group over the course of the trial. In conclusion, Although targeting higher risk groups is tempting, this may constitute a "paradox of prevention"—a far greater number of deaths may be averted by treating all preschool children than by targeting specific high-risk individuals or subgroups, thus maximizing the number of deaths averted.

641

PROJECTED IMPACT AND COST-EFFECTIVENESS OF HIGH-RISK TARGETED VERSUS COMMUNITY-BASED ADMINISTRATION OF AZITHROMYCIN FOR REDUCING CHILD MORTALITY IN SUB-SAHARAN AFRICA

Rebecca Brander[1], Marcia Weaver[1], Benson Singa[2], Grace John-Stewart[1], Patricia Pavlinac[1], Judd Walson[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Kenya Medical Research Institute, Nairobi, Kenya*

Mass drug administration (MDA) of azithromycin (AZM) is being considered to reduce child mortality in Sub-Saharan Africa (SSA). Targeted AZM administered to high-risk children is also being tested. To illustrate the health impact and resource implications of targeted vs population-based strategies, we modeled the cost effectiveness (CE) of MDA vs AZM administered at hospital discharge, an accessible population at high risk of death. CE was modeled from the payer's perspective with a 6-month time horizon. The model included AZM-related adverse events, growth faltering, and mortality as health effects, and the cost of AZM delivery and healthcare utilization. Model parameters were sourced from published literature when possible. In estimates with a base-case 1.65% mortality risk and 1.9% hospitalization risk in the population and 4.5% mortality risk and 17.7% re-hospitalization risk post-discharge, and a mortality reduction of 13.5% based on recent trial results, we found post-discharge AZM averted ~2800 deaths and ~170000 DALYs and would be cost-saving, whereas MDA would avert ~15000 deaths and ~860000 DALYs at a cost of $147/DALY averted. In sensitivity analyses, results were most affected by parameters for hospitalization and mortality rates, effects of AZM on hospitalization rates, and the costs of drug delivery in MDA. Targeted AZM was cost-saving in any scenario in which AZM reduced hospitalization rates, and was more cost-effective than MDA when mortality was >2% higher or hospitalization was >10% higher in the targeted vs general population, or when post-discharge delivery costs were <500% of MDA costs. Both post-discharge AZM and MDA costs per DALY doubled with each 50% increase in macrolide resistance prevalence. Targeting AZM to children at highest risk of death may be an antibiotic-sparing and cost-effective, or even cost-saving, strategy to reduce child mortality if trials of targeted AZM demonstrate a similar effect as MDA trials. However, targeted AZM averts fewer absolute deaths and DALYs and may not reach all children who would benefit. Any AZM administration decision must consider implications for antibiotic resistance.

642

"HE'S CONSIDERING HIS INVESTMENT, NOT HIS HEALTH": ECONOMIC DETERMINANTS OF RISK, PREVENTION AND RESPONSE BEHAVIORS RELATED TO THE FIVE PRIORITY DISEASE GROUPS IN COTE D'IVOIRE

Danielle Naugle[1], Natalie Tibbels[1], Abdul Dosso[2], William Benié[2], Walter Kra[3], Corinne Fordham[1], Mieko McKay[2], Valère Konan[4], Jeanne Brou[5], Jocelyne Nebre[5], Adaman Kouadio[4], Zandra Andre[6], Diarra Kamara[2], Stella Babalola[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Johns Hopkins University, Abidjan, Côte D'Ivoire,* [3]*Alassane Ouattara University, Bouaké, Côte D'Ivoire,* [4]*Department of Veterinarian Services Ministry of Animal Resources and Fisheries, Abidjan, Côte D'Ivoire,* [5]*National Institute of Public Hygiene, Abidjan, Côte D'Ivoire,* [6]*U.S. Agency for International Development, Abidjan, Côte D'Ivoire*

After the 2014-2016 Ebola outbreak in West Africa, countries are mobilizing to strengthen preparedness for future threats. To support that effort, this qualitative study explored individual, social, and cultural determinants of risk, prevention, and response behaviors related to the five priority zoonotic disease groups in Côte d'Ivoire (rabies, mycobacterium, bacterial and parasitic diseases, viral hemorrhagic fevers, and respiratory zoonotic diseases). Data were collected through 32 focus group discussions, 33 interviews, 20 observations, and 20 community maps engaging 234 adult men and women across four urban sites. Participants were purposively sampled from populations that interact regularly with animals like farmers, hunters, and butchers. The interviews were recorded, transcribed, coded and analyzed. The findings highlight the role of economic factors in shaping behavior. First, many risk, prevention and response behaviors around zoonotic diseases are inextricably linked to the actors' primary means of subsistence; as a result, decisions are made based on careful calculations to maximize financial gain and minimize loss. Second, an underlying economic precariousness favors close co-habitation with animals, curative rather than preventative approaches to health, self-medication of both animals and people, and the consumption and sale of sick and dead animals. Third, echoes of past experiences and fear of financial loss generate reluctance to inform authorities of potential outbreaks. Without overlooking other important behavioral determinants (including structural factors, lack of information, low perceived risk, and cultural/religious beliefs and practices), this presentation seeks to underscore the pervasiveness of economic determinants of key risk, prevention, and response behaviors in Côte d'Ivoire. Social and behavior change communication programs seeking to reduce the public health burden of zoonotic diseases might benefit from aligning prevention and response behaviors with the expressed priorities of the actors: maximizing financial gains and minimizing financial losses.

643

AN INVESTMENT CASE FOR MATERNAL NEONATAL TETANUSELIMINATION

Sarah K. Laing[1], Ulla Griffiths[2], Sophia Bessias[1], Sachiko Ozawa[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*UNICEF, New York City, NY, United States*

Maternal neonatal tetanus elimination (MNTE) has been a global public health goal since 1989. To date, 14 countries have yet to reach elimination targets of less than one case per 1000 live births per district. Many of the remaining districts are hard to reach, facing implementation challenges that require novel implementation strategies. The Strategic Advisory Group of Experts at the World Health Organization (WHO) proposed five strategies to achieve MNTE by 2020: 1) vaccination campaigns using standard tetanus-toxoid containing vaccines with regular syringes as well as single-dose, pre-filled, auto-disposable syringes (TT-Uniject^{TM}), 2) increased routine immunization of pregnant women, 3) promotion of clean delivery and umbilical cord care, 4) neonatal tetanus surveillance, and 5) conducting elimination validation activities. We estimated the costs, averted deaths, and cost-effectiveness of achieving MNTE between 2018 and 2020. We used data from the WHO-Maternal Child Epidemiology Estimation Group, the United Nations Development Program, and UNICEF for our analysis. We estimated that it will cost US $199.6 million to eliminate maternal neonatal tetanus by 2020. These costs include $162.3 million for vaccination campaigns, $6.2 million for routine immunization of pregnant women at antenatal care clinics, $23.8 million for promotion of clean delivery, $4.2 for surveillance, and $3.1 for elimination validation activities. Achieving and sustaining elimination through 2030 will avert over 74,000 neonatal deaths and 4.6 million life years. MNTE in the remaining 14 countries will cost $2,696 per death averted and $43 per life year gained. Maternal neonatal tetanus can be eliminated with significant investment and high prioritization. While reaching hard-to-reach populations require substantial costs, MNTE can bring about broader benefits through health systems strengthening and improved access to care. Achieving MNTE by the current target of 2020 will require financial commitment and strong political will.

644

DIRECT AND INDIRECT COSTS TO THE DOD FOR MILITARY BENEFICIARIES PROVIDED CARE IN THE MILITARY HEALTH SYSTEM FOR DENGUE FEVER, 2012 - 2017

Bria Graham-Glover[1], Lanna Forrest[1], Calli Rooney[2], Stephen Barnes[1], Stefan Fernandez[3], Emily Cisney[4], Jacob Ball[5], John Ambrose[1]

[1]*DHA US Army Satellite, Aberdeen Proving Ground, MD, United States,* [2]*U.S. Army Medical Materiel Development Activity (USAMMDA), Ft. Detrick, MD, United States,* [3]*Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand,* [4]*DoD JPEO CBRND, Ft. Detrick, MD, United States,* [5]*Army Public Health Center, Department of Defense, Aberdeen Proving Ground, MD, United States*

Dengue virus (DENV) is the mosquito-borne pathogen, causative of dengue fever (DF) and endemic in most of the world's tropical and sub-tropical regions. DF is a threat to troops deployed in these areas. While DF is known or suspected to have cause disease among US troops as early as the 1898 Spanish-American War, to-date the burden of dengue infections among U.S. Service Members (SMs) is largely unknown. No specific treatment or vaccine is licensed in the US and treatment is limited to supportive care. Further, there is little consensus of the true cost of DF among DoD service personnel and other MHS beneficiaries. To facilitate DoD funding prioritization and support decisions around dengue vaccines and therapeutics development, this study provided DoD estimates of dengue direct medical and lost productivity (as available) costs. Military Health System (MHS) beneficiaries (e.g., Active duty (AD) SMs and non-AD military beneficiaries) from all components and Services, meeting the case definition for DF between 2012 and 2017 were included (n=1,346). Dengue cases were identified by querying DoD paid hospitalizations and outpatient medical encounters for dengue associated diagnosis codes; and from reviews of reportable medical events of confirmed dengue cases. Each individual was included in the analysis only once per calendar year. The cost of an encounter was determined separately for outpatient and inpatient care and reported in 2018 dollars. Lost productivity costs were calculated for AD SMs only and included (1) lost duty time where a SM is not able to perform his duties (e.g., number of inpatient hospital days); and (2) limited duty time where a SM performs his duty following dengue care, but is considered to be at diminished capacity (e.g., working while sick). Treatment of DF in the MHS is costly to the DoD, totaling almost $2 million over a five year period in direct medical costs. Additionally, the DoD experienced approximately $1 million in lost productivity costs for SMs. These findings, in light of the global health agenda, suggest that the dengue vaccine should remain a priority.

645

EVALUATION OF A SAVINGS LED FAMILY-BASED ECONOMIC EMPOWERMENT INTERVENTION FOR AIDS-AFFECTED ADOLESCENTS IN UGANDA WITH HEALTH ECONOMIC EVALUATION: A FOUR-YEAR FOLLOW-UP

Yesim Tozan[1], Ariadna Capasso[1], Sicong Sun[2], Julia Sh Wang[3], Ozge S. Bahar[2], Christopher Damulira[2], Fred M. Ssewamala[2]

[1]*College of Global Public Health, New York University, New York, NY, United States,* [2]*International Center for Child Health and Development, Brown School of Social Work, Washington University, St. Louis, MO, United States,* [3]*Department of Social Work and Social Administration, The University of Hong Kong, Hong Kong, Hong Kong*

In Uganda, 660,000 children have lost a parent to HIV/AIDS. Family economic empowerment interventions can improve health and developmental outcomes among children in communities impacted by AIDS and mitigate the risks they face. In resource-limited settings, it is important to identify cost-effective, scalable and sustainable interventions to inform allocation of resources given competing health priorities. Yet, evidence is limited on the cost-effectiveness (CE) of interventions aimed at improving adolescents' physical and mental health. We present a CE analysis of *Bridges*, a NIH-funded savings-led economic empowerment intervention among children and adolescents impacted by AIDS in Uganda. We use longitudinal data corrected at 4 time-points: 12, 24, 36 and 48 month-follow-up (2012-2016). Intent-to-treat analyses using multi-level difference-in-differences models compared the effects on self-rated health, mental health functioning, and sexual health of the two treatment arms: 1:1 (*Bridges) and 2:1 (BridgesPLUS)* incentivized savings match to usual care over the 48 months. Per-participant costs for each arm were calculated using the treatment-on-the-treated sample. Intervention effects and per-participant costs were used to calculate the incremental CE ratios (ICERs). At 48-months, *BridgesPLUS* significantly improved self-rated health, (0.25, 95% CI 0.06–0.43), HIV knowledge (0.21, 95% CI 0.01–0.41), self-concept (0.26, 95% CI 0.09–0.44) and self-efficacy (0.26, 95% CI 0.09–0.43) and lowered hopelessness scores (-0.28, 95% CI -0.43–0.125); whereas Bridges improved self-rated health (0.26, 95% CI 0.08–0.43) and HIV knowledge (0.22, 95% CI 0.05–0.39) compared to usual care. ICERs ranged from $224 for hopelessness to $298 for HIV knowledge. Intervention effects were measurable two years post-intervention, with higher savings match (2:1) positively impacting more outcomes than lower savings match (1:1). Our findings support the incorporation of economic empowerment interventions for poor AIDS-impacted children and communities within national social protection frameworks in low- and middle-income countries.

645A

EVALUATION OF THE IMPACT OF THE CERTIFIED OPHTHALMIC PARAMEDIC PROGRAM AT A LARGE CHARITY EYE HOSPITAL IN DELHI, INDIA

Vimal Konduri[1], Ishaana Sood[2], Shalinder Sabherwal[2], Sunita Arora[2], Parul Datta[2], Kyle McDaniel[1], Suresh R. Chandra[1], Cat N. Burkat[1]

[1]*University of Wisconsin School of Medicine and Public Health, Madison, WI, United States,* [2]*Dr. Shroff's Charity Eye Hospital, Delhi, India*

Vision loss is a major healthcare issue in India, driven by lack of access to care. In 2014, the Certified Ophthalmic Paramedic (COP) Program based at Dr. Shroff's Charity Eye Hospital (SCEH) in Delhi, supported by Combat Blindness International, began training young women from Delhi and rural North India to become paramedics (COPs) through a free 2-year program. Afterwards, COPs work at SCEH facilities in their communities. The program aims to improve SCEH's reach and quality of care and advance COPs' socioeconomic mobility and gender equity. This mixed-methods study examined the program's impact on graduates, their families, and delivery of care. Program graduates from Delhi and 5 SCEH secondary centers (located in outlying rural areas) who completed training at least 1 year prior were considered. All answered "before" and "after" questionnaires with over 200 total questions, reflecting previous and current socioeconomic status (SES), gender equity, healthcare choices, and confidence. Qualitative interviews were administered to some subjects and their family members. The program's impact on care was assessed using hospital data on procedures and screenings. 53 of 69 eligible graduates were included in the study. 18 family members of 16 COP subjects were interviewed. Questionnaire responses showed that COP program participation was associated with a significant ($p=1.037x10^{-5}$) increase in participants' SES, measured by principal component analysis of household asset ownership, with a greater increase at secondary centers ($p=7.508x10^{-5}$) than in Delhi (p=0.03094). Program graduates also reported a significant ($p=2.152x10^{-5}$) increase in their contribution to family economic decision-making. Interviewed graduates and their families had positive opinions of the program, also offering some suggestions for improvement. At secondary centers, surgery volume increased by 164% and outpatient visits increased by 109% between 2013 and 2017. Overall surgery volume at SCEH increased by 62% and number of patients screened increased by 64% over same period, indicating a substantial increase in patient volumes since the COP program began.

646

ASSOCIATION OF PRE-CONTROL INFECTION PREVALENCE OF LYMPHATIC FILARIASIS WITH CLINICAL MORBIDITY

Natalie V. Vinkeles Melchers[1], Wilma A. Stolk[1], Belén Pedrique[2], Joost W. Vanhommerig[1], Luc E. Coffeng[1], Sake J. de Vlas[1]

[1]*Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands,* [2]*Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland*

Lymphatic filariasis (LF), commonly known as elephantiasis, is a neglected tropical disease targeted for elimination through mass drug administration (MDA). Affected persons can experience painful and severely disfiguring clinical manifestations, which can lead to permanent disability and economic loss. We assess the statistical association between the prevalence of LF infection and morbidity before the initiation of MDA. A systematic review was performed by two independent researchers to collect population-based data on the pre-control prevalence of microfilariae (mf) and morbidity (lymphoedema/elephantiasis and hydrocele). Diagnostic techniques for the detection of mf prevalence were standardised by calculating a transformation factor, such that mf prevalence was standardised to thick blood smear (mf/20 μl). We quantified the association between pre-control prevalence of infection and morbidity using a logistic regression curve, with stratifications by geographical region, LF parasite species, age and sex. Model parameters were estimated using a maximum likelihood. The pre-control prevalence of mf was non-linearly associated with the prevalence of lymphoedema/elephantiasis. Pre-control prevalence of lymphoedema/elephantiasis was higher in South-East Asia (SEA) than in Africa, given similar pre-control mf prevalence in areas with low pre-control mf prevalence. The association between the prevalence of community infection and hydrocele was approximately linearly related, with higher hydrocele prevalence rates in Africa than in SEA or the Western-Pacific region. The associations were then applied to published data on pixel-level pre-control mf prevalence in Africa to estimate the prevalence of lymphoedema/elephantiasis and hydrocele at baseline. These pre-control associations can assist policy-makers, programme managers, drug developers, and mathematical modellers to better predict the baseline occurrence of disease in specific geographical areas with specific population distributions. In addition, these associations can be used to re-calculate the Global Burden of Disease due to LF.

647

THE EFFICACY OF TRIPLE DRUG THERAPY IN MASS DRUG ADMINISTRATION TO REDUCE LYMPHATIC FILARIASIS IN HAITI

Marisa A. Hast[1], Christine L. Dubray[1], Anita D. Sircar[1], Madsen Beau De Rochars[2], Joshua Bogus[3], Abdel N. Direny[4], Jean Romuald Ernest[5], Carl Fayette[5], Katiuscia O'Brian[3], Charles W. Goss[3], Daniel Frantz Sabin[5], Ryan Wiegand[1], Jean Frantz Lemoine[6]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*University of Florida, Gainesville, FL, United States,* [3]*Washington University in St. Louis, St. Louis, MO, United States,* [4]*RTI International, Washington, DC, United States,* [5]*IMA World Health, Port-au-Prince, Haiti,* [6]*Ministere de la Sante et de la Population, Port-au-Prince, Haiti*

In Haiti, transmission of *Wucheria bancrofti* lymphatic filariasis (LF) persists in several regions of the country despite a decade of annual mass drug administration (MDA). Recently, studies have shown that annual "IDA" triple drug MDA using ivermectin , diethylcarbamazine (DEC), and albendazole is more effective in eliminating microfilaria (Mf) than "DA" double drug MDA using DEC and albendazole alone. To further determine the efficacy of IDA MDA in Haiti, a longitudinal study was conducted within a community-randomized trial in Quartier Morin commune from November 2016 to January 2018. As part of the community trial, ten localities were randomized to receive either IDA or DA MDA. Participants were tested at baseline for LF antigenemia using filariasis test strips (FTS), and FTS positive participants were also tested for Mf positivity and Mf count per milliliter. FTS positivity at baseline was 8.0% (239/3004) in the IDA arm and 11.5% (344/2994) in the DA arm. Mf positivity was 1.4% (42/3004) and 2.4% (72/2994), respectively. Male sex, older age, locality, and presence of LF complications were significantly associated with both FTS and Mf positivity before treatment. Participants who were FTS positive were followed-up 12 months later for repeat FTS and Mf testing, and 84% successfully completed a second visit. Among FTS positive participants retained at follow-up, 20.5% became FTS negative in the IDA arm compared with 25.6% in the DA arm (P=0.3). Among retained participants who were Mf positive at baseline, 94.4% were free of Mf at 12 months in the IDA arm compared with 75.9% in the DA arm (P=0.02). Conversion to Mf or FTS negativity was not modified by age or sex in the IDA arm. In the DA arm, participants aged 5-9 years were more likely to become FTS negative compared with older age groups (P=0.001). Among FTS positives at 12 months, FTS score was significantly lower than at baseline in both arms, and median Mf count per ml was lower in the IDA arm (P=0.04). Overall, MDA using IDA was more effective than DA in reducing microfilaremia in Haiti. Adopting a three-drug regimen for yearly MDA may accelerate the elimination of LF as a public health problem in this region.

648

COMMUNITY-LEVEL EFFECTIVENESS OF MASS DRUG ADMINISTRATION OF IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE VERSUS DIETHYLCARBAMAZINE AND ALBENDAZOLE FOR ELIMINATION OF LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

Moses Laman[1], Livingstone Tavul[1], Stephan Karl[1], Bethuel Kotty[1], Zebedee Kerry[1], Steven Kumai[2], Anna Samuel[1], Lina Lorry[1], Lincoln Timinao[1], Samuel C. Howard[3], James Wangi[4], Leo Makita[5], Lucy John[6], Sibauk Bieb[6], Charles W. Goss[7], Katiuscia O'Brian[7], Gary J. Weil[7], James W. Kazura[3], Daniel J. Tisch[3], Catherine Bjerum[3], Christopher L. King[3], Leanne J. Robinson[8]

[1]*PNG Institute of Medical Research, Madang, Papua New Guinea,* [2]*Bogia District Health Office, Madang, Papua New Guinea,* [3]*Case Western Reserve University, Cleveland, OH, United States,* [4]*World Health Organisation PNG, Port Moresby, Papua New Guinea,* [5]*VBD Control Program, PNG National Department of Health, Port Moresby, Papua New Guinea,* [6]*PNG National Department of Health, Port Moresby, Papua New Guinea,* [7]*Washington University, St Louis, MO, United States,* [8]*Burnet Institute, Melbourne, Australia*

Evidence from Papua New Guinea (PNG) and elsewhere, has shown that a co-administered dose of Ivermectin, Diethylcarbamazine (DEC), and Albendazole (IDA) is more effective for clearing *W. bancrofti* microfilaremia (Mf) than treatment with the standard therapy of DEC plus ALB (DA). However, the number of rounds of IDA required to interrupt LF transmission is unknown. A large open-label, parallel group, cluster randomized trial conducted in PNG from 2016-2019 confirmed IDA has an acceptable safety profile in a moderate transmission region and superior individual-level efficacy (96% IDA versus 83% DA; p=0.038). To investigate the effectiveness of one round of IDA versus DA on the community-level prevalence of microfilaremia and antigenemia, all consenting community members were re-tested one year after MDA. Compared to baseline, communities that received a single dose of IDA showed a greater reduction in Mf prevalence with IDA (4.4%, N=2388 to 0.4%, N=2299) compared to communities that received DA (4.2%, N=2189 to 1.5%, N=1945). After controlling for MDA coverage and average bednet usage, the odds of Mf infection were 3.8 times greater in DA vs IDA villages 1 year after MDA (p=0.0005). MDA coverage was a significant predictor of village Mf prevalence decrease (p=0.032), but bednet usage was not (p=0.509). Filarial Strip Test positivity significantly decreased slightly after MDA (p<0.001) but to a similar degree in IDA villages (from 22% to 16%) and DA villages (23% to 18%; p=0.506). Xenomonitoring for LF in four villages was conducted at baseline and four to six weeks following MDA and 6 villages 12 months later, divided equally among the two treatment arms. Four to six weeks following MDA there was a >10-fold reduction in LF infected mosquitoes, equivalent between treatment arms. The 1-year data are pending. Data showing the effect of a second year of MDA with the same regimens will be available for presentation at the meeting. IDA has a greater impact than DA on community Mf prevalence after a single round of MDA and has the potential to significantly accelerate LF elimination in settings such as PNG by requiring fewer rounds of MDA to interrupt transmission.

649

EFFICACY AND EFFECTIVENESS OF A 3 DRUG REGIMEN AGAINST A STANDARD 2 DRUG REGIMEN FOR LYMPHATIC FILARIASIS: RESULTS OF AN OPEN-LABELLED CLUSTER RANDOMIZED TRIAL

Jambulingam Purushothaman[1], Subramanian Swaminathan[1], Gary J. Weil[2], Vijesh K. Sreedhar[1], Srividya Adinarayanan[1], Krishnamoorthy Kalianna Gounder[1]

[1]*Vector Control Research Centre (ICMR), Puducherry, India,* [2]*Washington University School of Medicine, St. Louis, MO, United States*

An open labelled cluster randomized trial was carried out to assess the efficacy and effectiveness of a 3-drugs (ivermectin, DEC, albendazole IDA) vs standard 2-drugs (DEC, albendazole, DA) for lymphatic filariasis (LF). Filarial infections were detected with the FTS test for filarial antigenemia. Participants with positive antigen tests were tested for microfilaremia (Mf) with 60 μl thick night blood smears. Clusters (villages) were block randomized by population and infection prevalence to receive either IDA or DA. Participants were treated with a single oral dose of 3-drug (ivermectin, 200 μg/kg; diethylcarbamazine, 6 mg/kg; albendazole, a fixed dose of 400 mg) or with DA alone. Treatment was offered to residents who were >5 years of age and not pregnant. Approximately 70% of the population consumed the drugs. Separate surveys were conducted 12 mo after treatment to compare the efficacy and effectiveness of the two drug regimens. For the efficacy study, a cohort of individuals with filarial infections at baseline were followed. 80% of 591 persons with Mf at baseline were re-examined for Mf at 12 mo. Mf prevalence after DA and IDA were 38% and 16%, respectively. Reductions in Mf counts were 71.1% and 86.5% after DA and IDA. The reductions were significantly better after IDA than after DA (P<0.05). Repeat cross-sectional surveys were performed to assess the overall effectiveness of a single dose of the two treatments at the community level. 4272 and 3549 persons were tested during pre- and post-treatment surveys in the 2-drug treatment villages. The corresponding figures in the 3-drug arm were 4782 and 3584. Mf prevalence decreased from 6.2 to 5.4% (by 13%) after DA and from 6.8 to 3.5% (by 48%) after IDA. Mf density was reduced by 18.8% after DA and by 52.6% after IDA. A single dose of DA or IDA were not effective for clearing CFA. Results from this study suggest that IDA is superior to DA regarding efficacy for treatment of individuals and regarding effectiveness at the community level for reducing the Mf prevalence and intensity in endemic communities. IDA has the potential to accelerate LF elimination if it is used for MDA in areas that currently receive DA.

650

PERCEPTIONS AND REPORTED SEVERITY OF ADVERSE EVENTS FOLLOWING TREATMENT FOR LYMPHATIC FILARIASIS: RESULTS OF A MULTICENTER COMMUNITY BASED STUDY

Alison Krentel[1], Shruti Mallya[1], Charles W. Goss[2], Charles Thickstun[3], Daniel Dilliott[1], Nandha Basker[4], Purushothaman Jambulingam[4], Valery Madsen Beau De Rochars[5], Abdel N. Direny[6], Jean Frantz Lemoine[7], Adriani Lomi Ga[8], Taniawati Supali[9], Joshua Bogus[2], Cade Howard[10], Zebedee Kerry[11], Leanne J. Robinson[11], Myra Hardy[12], Andrew C. Steer[13], Josaia Samuela[14], Ken B. Schechtman[2], Peter U. Fischer[2], Christopher L. King[10], Gary J. Weil[2]

[1]*Bruyere Research Institute, Ottawa, ON, Canada,* [2]*Washington University, St. Louis, MO, United States,* [3]*University of Ottawa, Ottawa, ON, Canada,* [4]*Vector Control Research Centre, Pondicherry, India,* [5]*University of Florida, Gainesville, FL, United States,* [6]*RTI Envision, Washington, DC, United States,* [7]*Ministère de la Santé Publique et de la Population (MSPP), Port au Prince, Haiti,* [8]*Planning Department in East Nusa Tenggara Provincial Government, Kupang, Indonesia,* [9]*Universitas Indonesia, Jakarta, Indonesia,* [10]*Case Western Reserve University, Cleveland, OH, United States,* [11]*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea,* [12]*University of Melbourne, Melbourne, Australia,* [13]*Department of General Medicine, Royal Children's Hospital, Melbourne, Australia,* [14]*Ministry of Health and Medical Services Fiji, Suva, Fiji*

A multicenter community-based safety trial compared a standard two-drug treatment (DEC and albendazole, DA) with a three-drug treatment (ivermectin and DA, IDA) for lymphatic filariasis (LF). Treatment acceptability was measured using mixed methods. In each country, acceptability was assessed with a survey of 400 randomly selected participants (≥14 yrs) within four months of treatment. Focus group discussions were conducted in each country in communities where IDA was administered. 1919 participants were surveyed across 5 countries. 27 focus group discussions were analyzed for emergent themes. Survey data was linked to clinical data on adverse events (AE) collected by the safety trial teams. 17.4% (325/1870) of the sample had clinically assessed AE during the 7 day post-treatment monitoring period. When

surveyed, 43.7% (142/325) of these participants reported that they did not experience any AE. Survey data showed women reported AE more than men (71.7% vs 64.4%; p=0.01), including more frequent nausea (p=0.0015) and headache (p=0.0001). Men felt better more often after treatment than women did (10.1% vs 5.1%; p<0.0001). Participants reporting no AE in the day following treatment varied across sites: Fiji (68.9%), Haiti (55.2%), India (88.7%), Indonesia (52.9%) and PNG (72.6%) (p<0.0001). Indonesian participants reported a higher rate of passing intestinal worms following treatment (22.4%) as compared to the other sites (p<0.001). Qualitative data confirmed that participants were sometimes hesitant to take medication because of possible AE. Access to telephone numbers, presence of health staff, free treatment for AE and seeing family members whose AE cleared reassured community members about AE. Results showed that perceptions of AE varied between countries and between genders. Active AE management has the potential to reduce the perceived severity of AE as people are reassured about where to seek care following treatment. These results indicate that AE management must be an integral component of mass drug administration beforehand to reassure community and afterwards to care for AE. Gender-specific approaches to AE may be needed.

651

USE OF REAL-TIME DAILY REPORTING TO IMPROVE MASS DRUG ADMINISTRATION IN AN URBAN SETTING

Abdel N. Direny[1], Alain Javel[2], Jean F. Lemoine[3], Franck Monestime[2], Wenser Estime[2], Eurica Denis[2], Carl R. Fayette[2], Scott Torres[1], Ellen Knowles[1], Nancy Stroupe[1], Caitlin Worrell[4], Tara Brant[4]

[1]*IMA World Health, Washington, DC, United States,* [2]*IMA World Health, Port au Prince, Haiti,* [3]*Ministere de la Sante Publique et de la Population, Port au Prince, Haiti,* [4]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Haiti is approaching elimination of lymphatic filariasis, with 84% of the 140 endemic communes under post-treatment surveillance in 2019. Achieving effective mass drug administration (MDA) in five communes of the Port-au-Prince metropolitan area remains a challenge, with MDA coverage steadily declining from 2012 to 2017 (79% to 41%). In 2017, the Ministry of Health (MOH) and partners adopted a new strategy to improve MDA coverage in urban settings. The investigators hypothesized that the use of mobile phones could improve MDA coverage through real-time daily reporting and staff feedback. A network of 750 community promoters (CPs) were trained to collect and submit data daily through an interactive voice response (IVR) using simple mobile phones. Data were cleaned, and daily reports generated with projected epidemiological coverage. Daily update reports were presented during next day morning briefings with the MOH and its partners, allowing reallocation of resources and focused efforts to strengthen areas of low performance. A total of 9,147 (81%) distribution post reports were received, including 7,830 (69%) received before the close of the MDA day. Reports were most commonly missing due to delay of submission of register forms from all posts and poor network connections. Based on analysis of the collected data, CPs provided feedback to community drug distributors to make improvements in the MDA process. Before using the mobile data collection system, it took months for the MOH and partners to obtain MDA performance data; with the new system, the program could estimate expected coverage before concluding MDA and make adjustments as needed. This strategy contributed to substantially improved data quality and MDA coverage (from 41% in 2017 to 80% in 2018) in the Port-au-Prince area at relatively low program cost ($USD 6000 for over 2 million people treated). For 2019 MDA, improvements will be made to resolve the issue of poor network connections by moving from an IVR platform to a short message service (SMS) platform.

652

PERSISTENT LYMPHATIC FILARIASIS TRANSMISSION IN HOTSPOT COASTAL COMMUNITIES DESPITE 10 OR MORE ROUNDS OF IVERMECTIN + ALBENDAZOLE MASS DRUG ADMINISTRATION. HOW CAN PROGRAMS ACCELERATE ELIMINATION TOWARDS THE 2020 TARGET?

Andreas Nshala[1], Abdel N. Direny[2], Kerry Dobies[2], Nancy Stroupe[2], Katie Crowley[3], Upendo J. Mwingira[4]

[1]*IMA World Health, Dar es Salaam, United Republic of Tanzania,* [2]*IMA World Health, Washington, DC, United States,* [3]*RTI International, Washington, DC, United States,* [4]*NTD Control Program and National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania*

Fifty-five million Tanzanians are at risk of lymphatic filariasis (LF) infection. Two communities along the eastern coast of the Indian Ocean in Tanzania, Mafia and Lindi districts, had a baseline antigen prevalence of 46% and 53% respectively. Annual district-wide mass drug administration (MDA) started in 2001 in Mafia and 2003 in Lindi using Ivermectin (IVM) + Albendazole (ALB). After over 10 rounds of MDA in which epidemiological coverage varied from 44% to 87% in Mafia (12/13 rounds were effective) and from 29% to 90% in Lindi (7/10 were effective), with a steady trend of increased coverage through 2018, these coastal communities have not yet met the WHO criteria for stopping MDA. Sentinel and spot check sites assessments conducted in 2003, 2006, 2013, and 2016 revealed an average LF antigen of 28%, 27%, 4%, and 4% in Mafia, and 5%, and 7% in Lindi in 2013 and 2016. These results provided evidence that active transmission was ongoing in both districts. The program target is to reach below 2% LF antigen before conducting the transmission assessment survey (TAS) and eventually stopping MDA. In 2018, instead of once per year MDA as done in previous rounds, the program provided IVM+ALB twice per year targeting the eligible population, and reached 81% and 75% epidemiological coverage respectively in both MDA rounds. Sentinel and spot check sites reassessed in December 2018 after one round of MDA in 2017 and two rounds of MDA in 2018 revealed a remarkable reduction of LF antigenemia levels per site ranging from 100% (i.e. 0% prevalence) to 38% (i.e. over one thirds of infections reduced). It is demonstrated that LF antigenemia in hotspots could be reduced at an even faster rate than previously thought. Twice a year MDA with effective coverage for LF is feasible and acceptable, despite the challenge of perceived MDA fatigue from volunteers and the population at large. This approach may be key to reducing LF transmission in hotspots to achieve the elimination goal.

653

ANTIBODIES TO PEPTIDES REPRESENTING *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN REFLECT ACQUISITION OF NATURALLY ACQUIRED IMMUNITY IN MALIAN ADULTS AND CHILDREN

DeAnna J. Friedman-Klabanoff[1], Mark A. Travassos[1], Sonia Agrawal[1], Amed Ouattara[1], Andrew Pike[2], Jason A. Bailey[3], Matthew Adams[1], Drissa Coulibaly[4], Kirsten E. Lyke[1], Matthew B. Laurens[1], Shannon Takala-Harrison[1], Bourema Kouriba[4], Abdoulaye K. Kone[4], Ogobara K. Doumbo[4], Jigar J. Patel[5], Mahamadou A. Thera[4], Philip L. Felgner[6], John C. Tan[5], Christopher V. Plowe[7], Andrea A. Berry[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*U.S. Food and Drug Administration, Silver Spring, MD, United States,* [3]*Emmes Corporation, Rockville, MD, United States,* [4]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali,* [5]*Roche Sequencing Solutions, Madison, WI, United States,* [6]*Vaccine Research and Development Center, Department of Physiology and Biophysics, School of Medicine, University of California Irvine, Irvine, CA, United States,* [7]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Plasmodium falciparum circumsporozoite protein (CSP) is the most studied malaria subunit vaccine antigen. CSP epitopes include the conserved

junction between the N-terminal and central repeat region (CRR), the immunodominant NANP CRR, and C-terminal polymorphic T and B cell epitopes. To investigate acquired CSP immunity, we probed sera from ten adults and ten children from Bandiagara, Mali, on a peptide microarray. 73 CSP variants from reference genomes and field-derived genomic data were represented as 16 amino acid (aa) peptides with 12 aa overlap. This resulted in 98 peptide positions with 1-23 variants at each position, totaling 251 peptide sequences. Log_2 transformed fluorescence intensities (FI) and serorecognition, defined as individual FI >2.5 standard deviations above FI for five U.S. malaria naïve controls, were compared. Adult sera had higher FI to 160 CSP peptides than children. Adult sera recognized more variant peptides than children (median adult 207.5 (range 157-241) and median children 86.5 (range 58-196), p=0.0001, Mann Whitney test). 60 CSP peptides were serorecognized by more adults than children and were located throughout the CSP ectodomain. In a longitudinal analysis of sera from children over three time points during the malaria season, overall FI was higher post- than pre-season (p<0.0001, Wilcoxon Signed Rank (WSR) test), and lower post- than mid-season (p< 0.0001, WSR test). Sera from children recognized a median (range) of 86.5 (58-196), 148 (75-84), and 115.5 (76-179) peptide variants pre, mid, and post season (pre to mid comparison p = 0.02, and mid to post comparison p = 0.08, WSR test). FI and serorecognition comparisons suggest that children develop antibody responses to diverse CSP epitopes during a malaria season, but responses wane. Overall, we described responses to CSP epitopes and their variants, and observed differential responses to the immunodominant NANP region, and other regions previously identified as potential markers of protective immunity. Next steps will be to differentiate immunodominant from protective responses in a larger study with longitudinal infection surveillance data.

654

IDENTIFYING ANTIBODY AND MONOCYTE RESPONSES ASSOCIATED WITH PROTECTION FROM MALARIA IN PREGNANT WOMEN

Amaya Ortega[1], Elizabeth Aitken[1], Wina Hasang[1], Holger Unger[2], Maria Ome-Kaius[3], Amy Chung[1], Stephen Rogerson[1]

[1]*The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia,* [2]*Victoria Hospital Kirkcaldy, Kirkcaldy, United Kingdom,* [3]*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea*

Antibodies to VAR2CSA, member of the PfEMP1 family, have been associated with protection from placental malaria. Most studies have focused on the neutralising antibody that blocks placental sequestration, by inhibiting the adhesion of infected erythrocytes to chondroitin sulphate A, mediated by VAR2CSA. Placental malaria is frequently accompanied by accumulation of monocytes, and studies using promonocytic THP-1 cells demonstrate the importance of antibodies that promote phagocytosis of infected erythrocytes. To produce a more physiological model of how functional antibody can clear IgG-opsonised infected erythrocytes, we developed a novel phagocytosis assay using purified human primary monocytes. Results from intra-donor assays were highly correlated (r=0.70), inter-donor assays were less so (r=0.31). To investigate the role of antibody which promotes phagocytosis, monocytes isolated from Australian donors (n=3) and plasma samples from Papua New Guinean women were used. Women were categorized as non-infected with *Pf* (n = 50), infected with *Pf* placental malaria (n = 50) or infected with *Pf* but no placental malaria (n = 27). Phagocytosis assays measured antibody in sera to infected erythrocytes expressing VAR2CSA. Compared to women with placental malaria, pregnant women infected with *Pf* with no placental malaria were more commonly positive for (Chi-square test, p = 0.02) and had higher levels of (Mann-Whitney U test, p = 0.001) opsonising antibodies which promote monocyte phagocytosis infected erythrocytes. The assay has been further optimised to use beads coated with VAR2CSA domains, to examine domain specific responses, and we are characterising monocyte populations involved in phagocytosis (CD14, CD16, CD32 and CD64 markers). Primary human monocytes can reliably be used to measure opsonising antibody to parasite proteins on infected erythrocytes or coated on fluorescent beads, and opsonizing antibodies measured using these cells are associated with protection from placental malaria. Characterisation of functional immunity to VAR2CSA is an important complement to assays of adhesion blocking activity of antibody.

655

REPEATED MALARIA EXPOSURES SKEW MONOCYTES/ MACROPHAGES TOWARDS A REGULATORY PHENOTYPE

Rajan Guha[1], Anna Mathioudaki[2], Gunjan Arora[1], Shangping Li[1], Shafiuddin Siddiqui[3], Jeff Skinner[1], Didier Doumtabe[4], Safiatou Doumbo[4], Kassoum Kayentao[4], Aissata Ongoiba[4], Boubacar Traore[4], Judith Zaugg[2], Peter Crompton[1]

[1]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States,* [2]*EMBL, Heidelberg, Germany,* [3]*NCI/National Institutes of Health, Bethesda, MD, United States,* [4]*Mali International Center of Excellence in Research, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali*

In malaria-naïve individuals, *Plasmodium falciparum* (*Pf*) infection results in high levels of *Pf*-infected red blood cells (iRBCs) that trigger systemic inflammation and fever. Conversely, individuals in endemic areas who are repeatedly infected are often asymptomatic and have low levels of iRBCs, even children who have yet to acquire fully protective antibodies. The molecular mechanisms underlying these clinical observations remain unclear. We previously showed that PBMCs collected from healthy Malian children before the malaria season responded to iRBCs by producing pyrogenic, pro-inflammatory mediators such as IL-1β, IL-6 and TNF. However, following febrile malaria there was a marked shift in the response to iRBCs with the same children's PBMCs producing lower levels of pro-inflammatory cytokines and higher levels of anti-inflammatory cytokines (IL-10, TGF-β). Moreover, genome-wide expression analysis showed that molecules involved in phagocytosis and intracellular killing were upregulated in PBMCs after malaria as compared to before. Together, these data suggested that malaria-induced epigenetic reprogramming of innate immune cells might play a role in immunity to malaria. In follow-up studies, age-stratified analysis of monocytes collected before the malaria season showed an inverse relationship between age and pro-inflammatory cytokine production capacity. Accordingly, monocytes of Malian adults expressed higher levels of CD163, CD206 and arginase 1, known molecules associated with a regulatory phenotype. These observations were recapitulated with an *in vitro* system of monocyte-macrophage differentiation whereby re-exposure to iRBCs was associated with diminished expression of pro-inflammatory mediators and a corresponding decrease in epigenetic markers of active gene transcription (i.e. H3K4me3) at the TSS/promoter regions of the same pro-inflammatory mediators. Together these data support the hypothesis that epigenetic reprogramming of monocytes/macrophages toward a regulatory phenotype contributes to the acquisition of clinical immunity to malaria.

656

CLEARANCE OF *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS BY NK CELLS AND MONOCYTES

Padmapriya Sekar, Gunjan Arora, Eric O. Long

National Institutes of Health, Rockville, MD, United States

Antibodies naturally acquired during repeated exposure to the parasite *Plasmodium falciparum* are essential for control of blood-stage malaria. NK cells are innate immune cells with an important role in containing viral infections and tumor growth, mainly by direct cytotoxicity and inflammatory cytokine production. Recently, we reported that primary human NK cells lyse *P. falciparum*-infected red blood cells (iRBCs) via antibody-dependent cellular cytotoxicity (ADCC) in presence of IgG isolated from adults living in a malaria-endemic region. This NK-mediated lysis results in inhibition of parasite growth *in vitro*. Live imaging showed release of parasitophorous vacuoles (PV) from iRBCs after incubation with NK cells. Many of these PVs gave propidium iodide-positive signals, suggesting loss of viability of *P. falciparum* inside the PVs after NK-mediated iRBC lysis. We are interested to know whether and how PVs

are eliminated from circulation and postulate that monocytes play a role in clearance of PVs, thereby preventing inflammatory responses by the innate immune system. Preliminary live imaging experiments suggest that monocytes phagocytose PVs that have been released from iRBCs after incubation with NK cells. We are testing plasma samples of individuals from malaria-endemic regions for antibodies reactive to *P. falciparum* antigens at the surface of PVs, and the contribution of Fc receptors on monocytes to phagocytosis of PVs.

657

MALARIA SPECIFIC GENE EXPRESSION SIGNATURE IN B CELLS FROM PAPUA NEW GUINEAN CHILDREN

Arlene E. Dent[1], Grace Weber[1], Bruce Rosa[2], Adam Pelletier[1], Paula Embury[1], Daisy Mantila[3], Moses Laman[3], Benishar Kombut[3], Maria Ome-Kaius[3], Christopher L. King[1], Leanne Robinson[4], Rafick-Pierre Sekaly[1], Makedonka Mitreva[2], James Kazura[1]

[1]*Case University, Cleveland, OH, United States,* [2]*Washington University, St. Louis, MO, United States,* [3]*PNG IMR, Madang, Papua New Guinea,* [4]*Burnet Institute, Melbourne, Australia*

Naturally acquired immunity to malaria develops slowly with age and is largely mediated by antibodies that protect against blood stage infection and malaria illness. Long-lived humoral immunity depends on the activation of T follicular help (Tfh) cells that support the differentiation of naïve B cells into long-lived plasma cells and memory B cells (MBC) in the germinal center reaction. The objective of this study was to advance understanding of the molecular pathways regulated in B cells and T cells of children with acute malaria and their specificity with respect to other acute febrile non-malaria illnesses. Papua New Guinean children aged 1-10 yrs old presenting with fever were enrolled in a prospective cohort study in which venous blood samples were collected at presentation and ~9 weeks later upon recovery. Children with fever were diagnosed with acute *Plasmodium falciparum* (Pf) malaria or acute febrile non malaria (Pf PCR negative) illness, e.g., pneumonia. We characterized B and T cell subsets by flow cytometry and performed RNA-Seq. Children with acute malaria had increased frequencies of classical, activated, and non-class switched MBC compared to children with acute febrile non malaria illness. Additionally, children with acute malaria had increased frequencies of quiescent Tfh subsets whereas children with acute febrile non malaria illness had increased frequencies of activated Tfh subsets. Using RNA-Seq, we found that total B cells from children with acute malaria had 272 upregulated genes compared to their recovery B cells and acute febrile non malaria B cells. These upregulated genes unique to malaria were significantly over-represented among 5 KEGG pathways: Protein processing endoplasmic reticulum, Parkinson's disease (mitochondrial function), Base excision repair, B cell receptor signaling, and Oxidative phosphorylation. We conclude that acute malaria B cells may have a unique immunometabolic signature that may reveal specific pathways useful for adjunct therapies. We are currently validating these findings with alternative methods and acute malaria B cells from other geographically distinct populations.

658

ENHANCING ATTENUATED SPOROZOITE VACCINES AGAINST MALARIA WITH A GLYCOLIPID ADJUVANT

Sumana Chakravarty[1], Charles Anderson[2], Moriya Tsuji[3], Andrew Ishizuka[4], Robert A. Seder[4], Stephen L. Hoffman[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*Aaron Diamond AIDS Research Center, New York, NY, United States,* [4]*Laboratory of Cellular Immunology, VRC, National Institutes of Health, Rockville, MD, United States*

A highly effective malaria vaccine is urgently needed. In clinical trials, Sanaria® PfSPZ-based vaccines, composed of aseptic, purified, cryopreserved, radiation attenuated PfSPZ Vaccine, or non-attenuated PfSPZ with chloroquine (PfSPZ-CVac) were safe and have been highly protective. Because PfSPZ Vaccine is based on eliciting long-lived tissue resident responses in the liver, novel approaches to improving its immunogenicity should be evaluated. Accordingly, we have been working on the effects of a novel glycolipid, 7DW8-5 that binds CD1d, and stimulates iNKT cells, as a rare immune stimulant in a mouse malaria model involving irradiated (irr) *P. yoelii* (Py) sporozoites (irrPySPZ). 7DW8-5 enabled reduction in number of doses from 3 to 1 for 75% protection with irrPySPZ by direct venous inoculation (DVI) and from 4 to 2 doses intradermally (ID), facilitated a contracted 1-week dosage regimen by DVI; and enhanced splenic CD8+ and CD4+ T cells responses with PfSPZ Vaccine in NHPs. In informal safety assessments, Balb/C and CD-1 mice injected with 10 μg (dose used in NHPs) of 7DW8-5 by DVI developed no signs of illness and doses of up to 100 μg 7DW8-5 administered intramuscularly (IM) with a recombinant adenovirus have been shown to be well tolerated in NHPs. These findings provided strong rationale for clinical development of 7DW8-5, and we are manufacturing 7DW8-5 under cGMPs, while refining preclinical regimens. Mice were durably protected at 16 weeks when immunized (DVI) with either 4 doses of 2×10^3 irrPySPZ (days 1, 3, 5, 8) or 2 doses of 2.5×10^4 (days 1,8) mixed with adjuvant. Durable protection declined when 7DW8-5 was omitted. Despite early studies showing efficacy by ID routes, irrPySPZ administered by non-DVI SC and ID routes resulted in 70-80% protection only with four doses of 1.5×10^5 irrPySPZ at two weeks after the last dose. Protection declined to 50% by 16 weeks, and two doses were not effective even at early challenge time points. Details of GMP 7DW8-5 testing *in vitro*, and immunogenicity in NHPs will be reported. Future use of this adjuvant is expected to vastly improve commercial, clinical, and public health benefits of PfSPZ vaccines.

659

CELLULAR IMMUNOLOGICAL ANALYSIS OF NAÏVE EUROPEAN AND PRE-EXPOSED AFRICAN VOLUNTEERS INFECTED WITH *PLASMODIUM FALCIPARUM* SPOROZOITES

Mikhael Dito Manurung[1], Sanne de Jong[1], Koen A. Stam[1], Meta Roestenberg[1], Stephen L. Hoffman[2], Peter G. Kremsner[3], Benjamin Mordmüller[3], Bertrand Lell[3], Maria Yazdanbakhsh[1]

[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Sanaria Inc., Rockville, MD, United States,* [3]*Universität Tübingen, Tübingen, Germany*

Malaria still causes significant morbidity worldwide. However, a safe and effective vaccine remains elusive. Vaccines that showed promising results in the early phase of trials, which was usually conducted in malaria-naive areas, eventually proved suboptimal when tested in the endemic areas. Coinfections and pre-exposure to malaria are known to dysregulate immune system considerably, which might contribute to the differences in the efficacy of vaccines tested. To address this, we recruited five malaria-naïve Europeans and 20 malaria-preexposed Gabonese for a controlled human malaria infection (CHMI) with *Plasmodium falciparum* sporozoites (PfSPZ) Challenge (SanariaTM) via direct venous inoculation (DVI). Following the CHMI, all Europeans developed parasitaemia, whereas 8/20 (40%) African volunteers did not for the whole study period. This outcome enables the comparison of groups with a varying degree of antimalarial immunity. We analysed PBMCs from baseline, five and eleven days after DVI and stimulated them with *P. falciparum*-infected red blood cells (PfRBC) and controls to analyse antigen-specific cytokine response by flow cytometry. We demonstrate that the Gabonese who were able to control their parasitaemia (TBS-) had a higher percentage of IFN-g-producing CD4 T cells compared to TBS- Africans, who in turn had higher frequencies of these cells compared to the malaria-naïve Europeans. Additionally, we observed higher baseline frequency of PfRBC specific CM CD4 T cells in TBS- Africans, whereas TBS+ Africans have a higher frequency of TNF-producing CD8 NKT cells. Distinct patterns of immune responses over time associated with parasite control were also observed, which when supplemented with targeted gene set enrichment analyses, suggests migration of cells into the peripheral tissues to exert antimalarial responses. Our data provide information on the geographic differences in the immune response against malaria as well as the underlying mechanism of naturally-acquired immunity that lead to parasite control.

659A

IN VITRO AND *IN VIVO* ASSAYS TO ESTABLISH THE INFECTIVITY OF *IN VITRO*-PRODUCED *PLASMODIUM FALCIPARUM* SPOROZOITES

Abraham G. Eappen[1], Hashani Hettiarachchi[1], Tao Li[1], Sumana Chakravarty[1], Christiane Urgena[1], Benjamin U. Hoffman[2], McWilliams Ian[1], Patricia De La Vega[1], Ayyappan Rathakrishnan[1], Lixin Gao[3], MingLin Li[3], Peter F. Billingsley[1], B. Kim Lee Sim[1], Stephen L. Hoffman[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Columbia University Irving Medical Center, New York, NY, United States,* [3]*Protein Potential LLC, Rockville, MD, United States*

We are developing *in-vitro*-produced *Plasmodium falciparum* (Pf) sporozoites (SPZ) (iPfSPZ) to substitute for mosquito-produced PfSPZ (mPfSPZ) in controlled human malaria infections, vaccines, and biology studies. During the past 2 years we produced >10^8 iPfSPZ, and systematically studied iPfSPZ *in vitro* in HC-04 cells (hepatocyte line) and cryopreserved primary human hepatocytes (PHH), and *in vivo* in immunocompromised and fumarylacetoacetate hydrolase-deficient mouse (Fah-/-, Rag2-/-, Il2rg-/-, termed the FRG mouse) engrafted with human hepatocytes (FRG huHep) that has been shown to be an excellent model for *Plasmodium falciparum* (Pf) liver stage development. In HC-04 cells and PHH we added 5x10^4 iPfSPZ or mPfSPZ and after 6 days in culture assessed numbers of parasites > 10 μM expressing PfMSP1, PfLSA1, or PfHSP70 per well. In multiple experiments there were more parasites after adding iPfSPZ than mPfSPZ. Results of one set of assays comparing parasites/well expressing PfMSP1 after adding iPfSPZ vs PfSPZ were respectively 44±18 vs 36±8 in HC-04 cells and 116±35 vs 43±8 in PHH. We injected 0.8-2x10^6 iPfSPZ into FRG mice then 6 and 7 days later we infused the mice with human erythrocytes, and 6 hours after last infusion sacrificed the mice, processed the livers for immunohistochemistry (IHC) and cultured the blood to identify Pf. In two consecutive experiments, we successfully infected FRG mice with iPfSPZ and mPfSPZ producing asexual erythrocytic stage infection. In livers from FRG mice, by IHC there were qualitatively similar numbers of mature liver stage parasites 7 days after infection with iPfSPZ or mPfSPZ. In contrast to the *in vitro* assays and IHC of mice livers, the levels of asexual erythrocytic stage infection in the mice injected with iPfSPZ were substantially lower than in mice injected with mPfSPZ. Development of iPfSPZ and mPfSPZ to late liver stage parasites (*in vitro* and *in vivo*) were morphologically and quantitatively similar. We are now working to increase the efficiency of conversion from late liver stage to highly infectious erythrocytic stage parasites.

660

CHARACTERIZATION OF SUBSTANCE P DURING WEST NILE VIRUS INFECTION

Shannon E. Ronca, Sarah M. Gunter, Rebecca B. Kairis, R. Elias Alvarado, Allison Lino, Rodion Gorchakov, Kristy O. Murray

Baylor College of Medicine, Houston, TX, United States

In the United States, West Nile virus (WNV) has infected more than 7 million individuals since its introduction in 1999. Up to 40% of patients with symptomatic WNV infections will develop long-term, severe neurological complications. We must identify viable therapeutic options to prevent death and poor clinical outcomes, as no preventive vaccines have yet been licensed. A key player in neuroinflammation, substance P (SP), and its receptor neurokinin-1(NK1R) may play a valuable role in the progression of WNV infection. We previously conducted a study in the wild-type BL6 mouse model of WNV neuroinvasive disease using a highly selective NK1R antagonist and observed increased survival and modulation of SP that correlated with survival outcomes. Treatment successfully lowered levels of SP RNA in brains of treated mice, but did not alter levels of WNV RNA, suggesting a role for SP in modulating WNV infection. We aimed to characterize the levels of SP in the same mouse model during infection in the absence of treatment. When compared to uninfected controls, increased levels of SP are obvious within 1 day post infection (DPI) in whole blood and 5DPI in brains and levels correlate to survival. Additionally, we used flow cytometry to characterize the immune cell types present in brain and spleen during infection to identify which are key players to the exacerbating role of SP to WNV infection, including astrocytes and microglia, which have been previously identified to play roles in WNV infection and neuroinflammation of other diseases. This study provides evidence that SP is, at minimum, a valuable marker to detecting the severity of WNV infection, and further supports that treatment with an NK1R antagonist has potential as a treatment for WNV during acute infection.

661

THE RE-EMERGENCE OF YELLOW FEVER EPIDEMICS IN NIGERIA MAY CONTINUE DESPITE THE INCLUSION OF ITS VACCINE IN NATIONAL PROGRAM ON IMMUNIZATION. THE LOW POPULATION IMMUNITY SPEAKS

Marycelin M. Baba[1], Bamidele Soji Oderinde[1], Erick Mora Cardenas[2], Alessandro Marcello[2]

[1]*University of Maiduguri, Maiduguri, Nigeria,* [2]*International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*

Globally, YF is a re-emerging disease with approximately 200,000 cases and 30,000 deaths occurring annually, with 90% of them in Africa. In 2016, 32 African countries with a total population of 610 million people were at the risk of YF. Nigeria is one of 37 countries in Africa with high risks of YF epidemics. YF epidemics in Nigeria were presumptively diagnosed in 1946, 1951, 1952, 1953 1957 but was confirmed in 1969 and it reoccurred in 1970. The estimated and laboratory confirmed cases from these epidemics (1946-1970) ranged from several thousands and 124 respectively. Among estimated cases, death claimed more than 1000 lives and 118 from confirmed cases. After 21 years of silence, YF epidemic hit Kwara State in September 2017 and extended to six other states and twelve Local Government Areas (LGAs) in January, 2018. These epidemics involved 358 suspected, 33 (51.6%) confirmed cases with 45 deaths (CFR :21.1% and 28.1% among suspected and confirmed respectively). Within this period, sporadic YF epidemics were reported in 16 states and 62 LGAs. In November 2018, another YF epidemics were reported in six states and FCT involving 11,140 confirmed cases. To assess the possibility of the persistence of these epidemics in the country, the population immunity studies were conducted in three states in northeastern Nigeria using micro-neutralization (MN), IgM/IgG ELISA and plaque reduction neutralization tests (PRNT). While other tests (ELISA and PRNT) are on ongoing, the MN which denotes the ability of the protective antibody to inhibit the infectivity of the virus shows that only 68 (9.2%) of 738 patients tested, had YF neutralizing antibody with a titre of 8 and above. Thirty –six of the 68 patients (52.9%) had antibody titre of 1:8 while 670 (90.8%) had no neutralizing antibody to this virus. The huge population immunity gap is a pointer to the possibility that YF epidemics will persist in Nigeria. It also implies that inclusion of YF vaccination in NPI is not sufficient to yield the desired herd immunity and the occurrence of these epidemics in Nigeria poses serious global threats.

662

SYNDECAN-1 AS A BIOMARKER OF SEVERITY IN ACUTE YELLOW FEVER

Francielle Tramontini Gomes de Sousa[1], Erika R. Manuli[2], Luiz G. Zanella[3], Yeh-Li Ho[3], Lucas Chaves Netto[3], Mariana P. Marmorato[3], Juliana Z. Dias[3], Mateus V. Thomazella[3], Carolina A. Correia[3], Cássia G. Silveira[3], Priscilla R. Costa[3], Geovana M. Pereira[3], Midiã S. Ferreira[3], Camila M. Romano[2], Esper G. Kallas[3], Eva Harris[1], Ester C. Sabino[4]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California-Berkeley, Berkeley, CA, United States,* [2]*Laboratory of Medical Investigation, Hospital das Clínicas HCFMUSP, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil,* [3]*Hospital das Clínicas*

HCFMUSP, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil, [4]Department of Infectious and Parasitic Diseases, Institute of Tropical Medicine, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil

Symptomatic infections by yellow fever virus (YFV) are characterized by intense vasculopathy and organ impairment, especially liver, kidneys, lungs, intestine, and brain, leading to high mortality rates (~30%). The aim of this study was to evaluate clinical and laboratorial factors related to endothelial damage, such as syndecan-1 serum levels and Transendothelial Electric Resistance (TEER), in individuals with acute yellow fever (YF). Blood samples of patients with suspected YF were collected after informed consent was obtained, when individuals were admitted to Hospital das Clínicas, University of São Paulo, Brazil. YF cases were confirmed by detection of YFV in blood and/or autopsy tissues by RT-PCR. Cases were classified in three groups as follows: i) non-severe (N=11): individuals who recovered and presented neutrophil count <4000/mL, indirect bilirubin (IB) <0.6 mg/dL, and aspartate aminotransferase (AST) <1500 U/L; ii) severe (N=37): individuals who presented one or more of the following criteria: neutrophil >4000/mL, IB >0.6 mg/dL, AST <1500, and/or death; iii) control (N=11): healthy individuals. Serum levels of syndecan-1 (a transmembrane protein that is shed during vascular disruption) were significantly (p<0.001) higher in the severe group (mean 170.5 ng/ml) when compared to the non-severe (mean 50.6 ng/ml) or control groups (mean 2.3 ng/ml). Moreover, syndecan-1 serum levels strongly correlated with AST (Pearson R=0.469, p=0.001) and creatinine levels (Pearson R=0.512, p<0.001), as well as with death (Pearson R=0.469, p=0.001). Syndecan-1 serum levels were also significantly (p=0.0048) higher in YFV-infected individuals who died (mean 215.9 ng/ml) than in patients who recovered (mean 106.7 ng/ml). Notably, TEER values of human umbilical vein endothelial cells (HUVECs) treated with serum from the three different groups correlated with circulating levels of syndecan-1 (Pearson R=-0.493, p=0.044). In summary, these results indicate that syndecan-1 is associated with vascular impairment and yellow fever severity and therefore may be used as a biomarker of disease outcome.

663

AVIAN SUSCEPTIBILITY TO AFRICAN AND EUROPEAN USUTU VIRUS STRAINS

Sarah Kuchinsky[1], Francesca Frere[1], Eric Mossel[2], **Nisha Duggal**[1]

[1]*Virginia Tech, Blacksburg, VA, United States,* [2]*Centers for Disease Control and Prevention, Fort Collins, CO, United States*

Usutu virus (USUV; *Flaviviridae*) is an emerging mosquito-borne virus closely related to West Nile virus (WNV). USUV is likely maintained in an enzootic cycle between birds and *Culex* spp. mosquitoes and can cause severe neuroinvasive disease in humans. USUV was initially discovered in South Africa in 1959, with detection in additional African countries in subsequent years. In the past two decades, USUV has been introduced into Europe multiple times, with increasing geographic spread across the continent. Large-scale bird die-offs have accompanied the spread of USUV in Europe, particularly in Eurasian blackbirds (*Turdus merula*). However, no avian models have been developed for USUV, and whether the virus has become more transmissible or pathogenic during emergence is unknown. To determine whether USUV has evolved during emergence in Europe, we sequenced several African isolates and compared sequences to circulating European strains. We found evidence of positive selection in the USUV NS3 gene, with derived mutations occurring in European isolates. To test whether USUV may have become more pathogenic in birds during emergence, we selected two low-passage European and two low-passage African USUV strains to evaluate (Netherlands 2016, Spain 2009, Uganda 2010, and South Africa 1959). All strains replicated to high titers in chicken, duck, goose, and quail cell lines. Next, locally hatched two-day-old chickens were inoculated subcutaneously with the USUV strains. All chicks developed viremia between days 1 and 4 post-inoculation, with no morbidity or mortality in any group and a trend towards higher viremia in birds inoculated with European strains compared to African strains. In an *ex vivo* model using chicken PBMCs, the European isolates reached significantly higher titers than the Uganda 2010 isolate. Further comparisons will be performed in a susceptible mouse model and in wild birds to determine whether differences in viremia are species-specific.

664

NS2B/NS3 MUTATIONS ENHANCE THE INFECTIVITY OF GENOTYPE I JAPANESE ENCEPHALITIS VIRUS IN AMPLIFYING HOSTS

Yi-Chin Fan[1], Jian-Jong Liang[2], Jo-Mei Chen[3], Jen-Wei Lin[3], Yi-Ying Chen[3], Kuan-Hsuan Su[4], Chang-Chi Lin[5], Wu-Chun Tu[3], Ming-Tang Chiou[4], Shan-Chia Ou[3], Gwong-Jen J. Chang[6], Yi-Ling Lin[2], Shyan-Song Chiou[3]

[1]*National Taiwan University, Taipei, Taiwan,* [2]*Academia Sinica, Taipei, Taiwan,* [3]*National Chung Hsing University, Taichung, Taiwan,* [4]*National Pingtung University of Science and Technology, Pingtung, Taiwan,* [5]*National Defense Medical Center, New Taipei City, Taiwan,* [6]*Centers for Disease Control and Prevention, Fort Collins, CO, United States*

Genotype I (GI) virus has replaced genotype III (GIII) virus as the dominant Japanese encephalitis virus (JEV) in the epidemic area of Asia. The mechanism underlying the genotype replacement has remained unclear. We focus our current study to investigate the role of mosquito vector and amplifying host(s) in JEV genotype replacement by comparing the replication ability of GI to GIII viruses *in vitro* and *in vivo*. GI and GIII viruses had similar infection rate and replicated the similar viral titers by blood meal feeding in *Culex tritaeniorhynchus*. But GI virus yielded a higher viral titer in amplifying host-derived cells, especially at the elevated temperature, and produced an earlier and higher viremia in experimentally inoculated amplifying hosts (pigs, ducklings, and young chicken). The amplifying advantage of viral genetic determinants of GI viruses was subsequently identified by utilizing chimeric and recombinant JEVs (rJEVs). As comparing to the recombinant GIII virus (rGIII virus), we observed a higher replication ability of the recombinant GI virus as well as the chimeric rJEVs encoding GI virus-derived NS1-3 genes in the amplifying hosts. The replication advantage of the chimeric rJEV abolished after the introduction of a single substitution of GIII viral mutation (NS2B-L99V, NS3-S78A, or NS3-D177E). In addition, the gain-of function assay further supported that rGIII virus encoding GI virus NS2B-V99L/NS3-A78S/E177E substitutions re-gained the enhanced replication ability. Thus, we conclude that the replication advantage of GI virus in the pigs and poultries is the result of three critical NS2B/NS3 substitutions. This may lead to more efficient transmission of GI virus than GIII virus in the amplifying hosts-mosquitoes cycle.

665

PROTECTIVE EFFICACY OF JAPANESE ENCEPHALITIS VIRUS MONOCLONAL ANTIBODIES DERIVED FROM VACCINATION IN A MINIATURE SWINE MODEL

Christian L. Cook[1], Victoria B. Ayers[1], Amy C. Lyons[1], So Lee Park[1], Ashley N. Doerfler[1], Susan M. Hettenbach[2], Ashley M. Zelenka[1], Konner R. Cool[1], Gregory J. Peterson[3], Stephen Higgs[2], Dana L. Vanlandingham[1], Yan-Jang S. Huang[1]

[1]*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States,* [2]*Biosecurity Research Institute, Kansas State University, Manhattan, KS, United States,* [3]*University Research Compliance Office Kansas State University, Manhattan, KS, United States*

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus which causes neurotropic disease in humans and is maintained in swine and avian species which serve as amplification hosts. Current vaccines derived from a genotype III (GIII) isolates have significantly reduced the number of pediatric encephalitis cases. However, there has been increasing concern of potential vaccine failure and immune breakthrough due to the rapid emergence of genotype I (GI) throughout the Asia Pacific region. In this study, heterologous protective efficacy of two mouse anti-JEV monoclonal antibodies (mAbs), derived from immunization with GIII isolates, was

evaluated through the passive immunization and experimental challenge of pigs with a representative strain of the emerging GI. Prophylactic treatment of JEV-31 and JEV-169 mAbs, which target domain III and domain I of the envelope (E) protein, respectively, conferred protection against challenge of GI JE-91 strain based on the delayed onset of fever and viremia. Overall, our results demonstrated that anti-E mAbs derived from vaccination are capable of protecting against the infection with heterologous JEV genotypes and provide the basis for our understanding of the breadth of humoral immune responses induced by JEV vaccines in susceptible vertebrate hosts.

666

DISEASE SURVEILLANCE AND VIROME ANALYSIS STUDY OF JAPANESE ENCEPHALITIS VECTOR, *CULEX TRITAENIORHYNCHUS*, COLLECTED FROM THREE PREFECTURES IN JAPAN

Astri Nur Faizah[1], Daisuke Kobayashi[2], Michael Amoa-Bosompem[3], Haruhiko Isawa[2], Kyeong Soon Kim[4], Mamoru Watanabe[2], Kozue Miura[1], Kazuhiro Hirayama[1], Kyoko Sawabe[2]

[1]*The University of Tokyo, Tokyo, Japan,* [2]*National Institute of Infectious Diseases, Tokyo, Japan,* [3]*Tokyo Medical and Dental University, Tokyo, Japan,* [4]*Tottori University, Tottori, Japan*

Japanese encephalitis (JE) is the leading cause of viral encephalitis with over 60,000 cases annually and is endemic in many Asian countries, including Japan. Japanese encephalitis virus (JEV) is mainly transmitted by *Culex tritaeniorhynchus*. Knowledge on the paddy-breeding mosquitoes' nature is vital in understanding the transmission cycle of JEV. A lot of effort has been put into virome determination of virus-transmitted mosquitoes. Mosquito viruses, based on host range, are divided into arbovirus (which infect vertebrates) and insect-specific virus (ISV). Recent studies have indicated the ability of ISVs in affecting vector competence to transmit arboviruses. Using an extensive approach of high-throughput RNA-sequencing, JE surveillance together with virome analysis has become practicable. Therefore the aim of this study was to combine JE surveillance and determination of the JE vector's virome. In this study, we collected *Cx. tritaeniorhynchus* mosquitoes in three prefectures (Ishikawa, Tottori, and Nagasaki) in 2017. RNA viromes were processed using metagenomic sequencing. Subsequently, the results were verified by PCR and followed by phylogenetic analysis. We successfully identified eight novel viruses out of eighteen viral genomes, including one JEV detected in only Ishikawa prefecture. Novel viruses belonged to divergent families, notably *Flaviviridae* and Iflaviridae. The JEV identified belonged to Genotype I based on its envelope (E) gene sequences, and was closely related to JEV isolated from cattle in Miyazaki prefecture in 2009. We are currently comparing virome of mosquitoes from different prefectures and determining the correlation with JEV, if any. This study not only adds up the knowledge pool of ISVs in Culex mosquitoes, but it also reveals continuous amplification and active transmission cycle of JEV and highlights the need for constant national surveillance and vaccination. Japan is one of the countries in Asia to implement regular JE vaccination since 1930, however, the JEV circulation shows no sign of a halt, thus alerting other endemic countries where vaccination has never been applied.

667

EXPLANTS OF HUMAN AND RUMINANT PLACENTAS ARE TARGETED BY MEMBERS OF THE *BUNYAVIRALES* ORDER DIFFERENTLY: COMPARING CELLULAR AND MOLECULAR MECHANISMS OF INFECTION TO UNDERSTAND DISPARATE RATES OF MISCARRIAGE BETWEEN SPECIES

Cynthia M. McMillen, Devin A. Boyles, Joseph R. Albe, Amy L. Hartman

University of Pittsburgh, Pittsburgh, PA, United States

Rift Valley fever virus (RVFV) is an arbovirus that causes disease in livestock and humans in Africa and the Middle East. Ruminants are highly susceptible to RVFV infection resulting in synchronous miscarriages, or "abortion storms". Abortogenic rates reach as high as 100% within a given outbreak. In pregnant women, there have only been two confirmed cases of vertical transmission and infection increases the risk of late-term miscarriage. To understand the disparate ability of RVFV to infect placentas from ruminant livestock and women, we compared tissue permissivity and tropism of RVFV infected ruminant and human placentas. Secondly, because other phylogenetically similar viruses may have conserved mechanisms of infection, tropism, and pathogenicity, we sought to understand whether other *Bunyavirales* target reproductive tissues similar to RVFV. The decidua, chorioallantoic membrane, and villi from sheep, cattle, or human mid- or term-gestation placentas were infected with arboviruses from the *Bunyavirales* order (Heartland, Jamestown Canyon, La Crosse) or Zika virus for comparison. RVFV cell-tropism was visualized by confocal fluorescent microscopy and viral titer was quantitated by qRT-PCR and plaque assay. RVFV replicated in sheep and cow placenta; infection was more efficient in the chorioallontoic membrane compared to separate cultures of the chorion and allontoic membranes. Highest viral yields occurred in the chrioallontoic membrane of ruminants, whereas highest replication occurred in the chorionic villus of humans. Heartland, Jamestown Canyon, and La crosse viruses also replicated in ruminant tissue. These results incidate a previously unrecognized potential for Heartland, Jamestown Canyon and La crosse viruses to target placental tissue. This is the first example of *Bunyavirales* such as Heartland and La crosse viruses to infect tissues from species other than their natural hosts. Further comparison of the permissive nature of *Bunyavirales* to structurally different placenta structures will help pinpoint the viral and cellular components needed for viral entry into reproductive organs and induction abortions.

668

INVESTIGATION OF AIM2 LOSS IN BATS REVEALS FUNCTIONAL DAMPENING OF THE INFLAMMASOME PATHWAY

Geraldine X. Goh, Matae Ahn, Aaron T. Irving, Zhu Feng, Lin-Fa Wang

Duke-NUS Medical School, Singapore, Singapore

in the co-evolution between hosts and their commensal infectious agents, bats have emerged as mammals which appear to harbor a range of highly lethal zoonotic viruses such as filoviruses, lyssaviruses, henipaviruses, and coronaviruses. We previously reported a complete loss of the PYHIN gene family, including the mammalian AIM2 gene, a cytosolic dsDNa sensor capable of activating the inflammasome. AIM2 recruits its adaptor ASC and triggers formation of the multi-protein inflammasome complex, activating caspase-1 to cleave key cytokines such as IL-1β for secretion, and mediating a pro-inflammatory cell death program called pyroptosis. While AIM2 mediates an essential role in sensing and alerting mammalian cells to invasion by foreign pathogens, over-activation can trigger detrimental cell death and tissue injury in the host. We observed that wild-type bat immortalized kidney and primary bone marrow derived macrophages (BMDMs) indeed lack AIM2 inflammasome signaling and ASC-speck formation in response to cytosolic dsDNA. AIM2 reconstitution restored adaptor recruitment of ASC *in vitro*, with dose-wise increase of ASC-speck formation in response to treatment with cytosolic dsDNA.

Intriguingly, cytokine release and cell death remained relatively dampened in spite of AIM2 inflammasome reconstitution. in comparison to human caspase-1, bat caspase-1 exhibited decreased activity and was identified as responsible for the lack of IL-1β secretion. Taken together, our data indicates that loss of AIM2 in bats results in diminished ability to respond to cytosolic dsDNA, augmented by further dampening of the downstream inflammasome pathway. Thus bats may have evolved to protect against inflammasome hyperactivation during metabolic or oxidative stress, in part conferring them the ability to harbor zoonotic agents without immune-mediated pathogenesis.

669

PATHOGENIC *E. COLI* IN DRINKING WATER: ARE THEY HUMAN OR ANIMAL IN ORIGIN?

Jannatul Ferdous[1], Ridwan Bin Rashid[1], Rebeca Sultana[2], Sabera Saima[1], Musharrat Jahan[1], Anowara Begum[1], Peter Kjær Jensen[2]

[1]*University of Dhaka, Dhaka, Bangladesh,* [2]*University of Copenhagen, Copenhagen, Denmark*

The occurrence of pathogenic bacteria in drinking water is a global health concern. The aim of this study was to investigate the presence of diverse pathotypes of *E. coli* isolates in piped-to-plot 'improved' communal source water and in point-of-consumption of drinking water (point-of-drinking) and, to identify their origin of fecal contamination. The study was conducted from September 2014 to October 2015 in a low-income urban community of Bangladesh. PCR was performed for characterization of pathogenic *E. coli* and phylogenetic grouping. a total of 229 *E. coli* isolates were randomly collected where 125 isolates were from 108 point-of-drinking and 104 were from 76 communal source water samples. Diverse pathotypes were identified where ETEC was the most prevalent pathotype found in point-of-drinking water (37%, 46/125) and communal source water (46%, 48/104). Phylogenetic grouping showed substantial presence of subgroup B1 (most prevalent in animals feces) in both of point-of-drinking (50%, 91/181) and source water (50%, 90/181) isolates followed by the presence of B2-3 (most prevalent human feces) in (65%, 13/20) point-of-drinking and (35%, 7/20) source water. Our findings suggest that both communal sources and point-of-drinking water of the study area were mostly contaminated by the feces from animals (181/229) and to a lesser extent by human feces (20/229). The non-human mammals and birds played a vital role in fecal contamination of the water and require priority attention in future intervention effort of water quality improvement. Our results indicate that addressing human sanitation without consideration of fecal contamination from livestock sources may not be enough to prevent drinking water contamination and thus will persist as a greater contributor of diarrheal pathogens in low-income urban communities.

670

ANTIBODIES AGAINST *TREPONEMA PALLIDUM* SHOW THAT YAWS IS ENDEMIC IN NONHUMAN PRIMATES IN KENYA

Emily H. Hardgrove[1], Dawn M. Zimmerman[2], Michael E. von Fricken[3], Graham A. Matulisb[3], Joseph Kamau[4], Daniel Chai[4], Samson Mutura[4], Velma Kivali[5], Fatima Hussein[4], Peris Ambala[4], Andrea Surmat[6], Sascha Knauf[7]

[1]*Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, United States,* [2]*Global Health Program, Smithsonian Conservation Biology Institute, Washington, DC, United States,* [3]*George Mason University, Fairfax, VA, United States,* [4]*Institute of Primate Research, Karen-Nairobi, Kenya,* [5]*International Livestock Research Institute, Nairobi, Kenya,* [6]*Mpala Research Centre and Wildlife Foundation, Laikipia, Kenya,* [7]*German Primate Center, Goettingen, Germany*

Yaws is a disease caused by the bacterium *Treponema pallidum* subsp. *pertenue* (*TPE*), believed to be a solely human pathogen. *TPE* has recently been identified in African nonhuman primates (NHP) raising concerns about a possible zoonotic reservoir. Kenya is one of 76 countries the World Health Organization (WHO) categorizes as previously endemic for yaws, but there are no current data on presence or absence. Sustainable yaws eradication will, however, rely on information about transmission dynamics and potential links between human and NHP *TPE* strains. in October of 2016, 65 olive baboons (*Papio anubis*) and two vervet monkeys (*Chlorocebus pygerythrus*) were sampled in Laikipia County, Kenya. As no skin lesions were observed, a preliminary serological screening was performed using the immunochromatographic Dual Path Platform (DPP) HIV-Syphilis Assay (Chembio Diagnostic Systems, Inc.) and the *T. pallidum* Particle Agglutination Assay (SERODIa ® TP-PA). The DPP test found antibodies in one of the two vervet monkeys and in 73.8% (48/65) of the baboons. Of the 37 baboon samples with definitive *TP-Pa* results, 31 were positive, including four that tested negative with the DPP assay, yielding a combined seropositivity of 54% (34/63). Based on the DPP assay, males (84.4%, 38/45) had a relative risk of 1.77 (p=.003, 95% [1.205, 3.015]) compared to females (47.62%, 10/21). Adult male baboons exhibited 100% (20/20) seropositivity compared to adult females, at 81.8% seropositivity (9/11). Infants exhibited the lowest seroprevalence rates (25%, 1/4), followed by juveniles (50%, 11/22), subadults (85.7%, 6/7), and adults (88.23%, 30/34). The propensity towards sexually mature animals is suggestive for a sexual transmission mode. Our data contribute evidence that in East Africa, *T. pallidum* infection is endemic in NHPs and multiple NHP taxa contain antibodies indicating latent infection. Providing reliable information on the epidemiology of treponematoses in both humans and NHPs, especially in regions where both co-exist, has important programmatic implications for yaws eradication.

671

HIGH INCIDENCE OF HUMAN BRUCELLOSIS IN A RURAL PASTORALIST COMMUNITY IN KENYA, 2016

Peninah M. Munyua[1], Eric Osoro[2], Elizabeth Hunsperger[1], Mathew Muturi[3], Athman Mwatondo[4], Doris Marwanga[5], Philip Ngere[6], Rebekkah Tiller[7], Clayton Onyango[1], Kariuki Njenga[2], Marc-Alain Widdowson[1]

[1]*US Centers for Disease Control and Prevention - Kenya, Nairobi, Kenya,* [2]*Washington State University Global Health Program, Nairobi, Kenya,* [3]*Kenya Ministry of Agriculture Livestock and Fisheries, Zoonotic Disease Unit, Nairobi, Kenya,* [4]*Kenya Ministry of Health, Zoonotic Disease Unit, Nairobi, Kenya,* [5]*Center for Global Health Research, Kenya Medical Research Institute, Nairobi, Kenya,* [6]*Kajiado county Department of Health, Nairobi, Kenya,* [7]*Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention Atlanta, Nairobi, Kenya*

Brucellosis is a zoonotic infection of ruminants. Humans are generally infected through unpasteurized milk or contact with infected animals. Globally, incidence is higher in the Middle East and Asia, but data in sub-Saharan Africa are scarce. We estimated the incidence of human brucellosis in a pastoralist community with high brucellosis sero-prevalence from a previous study. We enrolled all household members of randomly selected households in Kajiado County. Between February 2015 and January 2016, any household member who fell ill was asked to visit any of three study health facilities. Those who met the clinical case definition for suspect brucellosis (fever and at least one of night sweats, joint or muscle pains, headache, fatigue and anorexia) provided a blood sample. Samples were tested by Rose Bengal test (RBT) for agglutination antibodies and TaqMan Array Card (TAC) for *Brucella* DNA. Positive tests by TAC or RBT constituted the laboratory-confirmed brucellosis cases. Data on demographics, clinical presentation and risk factors were collected using a standardized questionnaire. Estimated crude cumulative incidence, by age and sex, was the number of brucellosis cases in one year per total population in enrolled households. Of 4,746 enrolled persons in 804 households, 52% were males and median age was 18 (range, 1-99) years. We enrolled 236 suspect brucellosis cases at the health facilities; 64% were females and median age was 41 (range, 1-97 years). Of the 236 suspect cases, four (2%) tested positive by RBT and of 217 tested, 23 (11%) positive by TAC. Laboratory-confirmed acute brucellosis cases were 24 (10%), yielding a crude cumulative annual incidence of 506/100,000 persons (95% confidence interval; 441 - 580). Incidence among females was two times higher than males (662 vs 362) and two to eight times

higher in the 41-60 age compared to other age groups. Education on risk factors for *Brucella* transmission and animal vaccination would help reduce the high incidence of brucellosis in this pastoralist community.

672

SEASONALITY OF AGRICULTURAL EXPOSURE MORE IMPORTANT THAN SEASONALITY OF CLIMATE FOR PREDICTING YELLOW FEVER TRANSMISSION IN BRAZIL

Arran Hamlet[1], Daniel G. Ramos[2], Katy Gaythorpe[1], Alessandro P. Romano[2], Tini Garske[1], Neil Ferguson[1]

[1]*MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom,* [2]*Secretariat for Health Surveillance, Brazilian Ministry of Health, Brasilia, Brazil*

Yellow fever virus (YFV) is a zoonotic arbovirus affecting both humans and non-human primates (NHP's) in Africa and South America. In South America since 1942 almost all cases have arisen from sylvatic spillover. Previous descriptions of YF's highly seasonal nature have relied on climatic explanations, with the role of the seasonality in exposure neglected - despite the high proportion of cases occurring in those involved in agriculture. This study investigates the role of seasonality in agriculture, as well as climate, in YF reporting for humans and non-human primates (NHP's). We fit a series of random forest models to yellow fever (YF) occurrence in both human and NHP's (2003-2018) at the 2nd administrative division against covariates accounting for the seasonality of climate, the seasonality of agriculture (planting, harvesting), agricultural output and host dynamics. An exhaustive combination of covariate groupings to produce 15 models which were assessed for accuracy and predictability. Models fit to human reports performed better than NHP with an average area under the curve (AUC) value of 0.924 (95% CI: 0.914 - 0.933) vs 0.893 (0.882 - 0.905). Models fit to covariates including the seasonality of agriculture, but not the seasonality of climate, fit better than the inverse, in humans 0.948 (0.941 - 0.955) vs 0.903 (0.891 - 0.916) and NHP's, 0.908 (0.897 - 0.918) vs 0.859 (0.845 - 0.873). Models also highlighted the influence of planting and harvesting corn, beans and soya as predictors of human YF reporting. These findings illustrate the importance of the seasonality of exposure and that it is not necessarily just an increased viral transmission in zoonotic reservoirs which leads to spillover, but also an increased interaction with the sylvatic cycle. Direct application of these findings in a public health setting is possible, with 45% of YF cases in Brazil occurring in those involved in agriculture. By highlighting both the crop types involved, and the temporal window, vaccination activities and public health outreach could effectively, and pro-actively, educate and vaccinate those at greatest risk of YF.

673

FARMERS AND FECES: A ONE HEALTH APPROACH TO EMERGING SWINE ZOONOSES

Emily Bailey[1], Vida Ahyong[2], Cristina Tato[2], Maria Phelps[2], Norma Jeff[2], Michelle Tan[2], Rene Sit[2], Joseph DeRisi[3], Gregory Gray[1]

[1]*Duke University, Durham, NC, United States,* [2]*Chan-Zuckerberg Biohub, San Francisco, CA, United States,* [3]*University of California at San Franscisco, San Franscisco, CA, United States*

North Carolina is the second largest producer of pork in the United States. Disease outbreaks, such as those from porcine epidemic diarrheal virus (PEDV), highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus 2 (PCV2), can readily threaten swine production operations sometimes resulting in large economic losses. Pathogen surveillance within swine farms can be an effective approach for the early identification of new disease threats and the mitigation of transmission before broad dissemination among a herd occurs. However, standard surveillance practices might not always be feasible as they may disrupt normal production operations. Additionally, the cost of conducting surveillance, particularly in large-scale settings, may also be a barrier to its routine implementation. The focus of this study was to describe a non-invasive swine slurry sampling method as an alternative approach for conducting routine surveillance in farms, providing an additional tool for farmers to protect their animals and themselves from new disease threats. Our preliminary molecular analysis of viruses detected in fecal slurry indicates that non-swine origin viruses are present these samples. These findings were then further validated using a metagenomic sequencing approach in partnership with the Chan-Zuckerberg Biohub and the Chan-Zuckerberg Initiative and their new bioinformatic pipeline, IDseq. The results of our preliminary analysis likely reflect the effects of virus recombination as well as close interaction between livestock and wildlife. This work also indicates the need for additional work further understand the impact of zoonotic viral pathogens on swine exposed workers particularly in areas where zoonotic disease transmission is known to occur, such as on large scale swine farms.

674

MALARIA MORBIDITY IN VENEZUELA 1995-2018: AN OBSERVATIONAL ANALYSIS OVER 1.7 MILLION CASES

Leopoldo Villegas[1], Leonor Pocaterra[2], Luis F. Chavez[3], Jorge Moreno[4], Elia Sanchez[5], Melfran Herrera[5], Angela Martinez[6], Gustavo Bretas[7], Anderson Martinez[7], Maria M. Villegas[7], Mary Ann Torres[8], Maria E. Guevara[7], Jose Oletta[9]

[1]*ASOCIS, Tumeremo, Bolivar, Bolivarian Republic of Venezuela,* [2]*Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela,* [3]*INCIENSA, San Jose, Costa Rica,* [4]*Centro de Investogacion de Campo Dr. Francesco Vitanza, Tumeremo, Bolivar, Bolivarian Republic of Venezuela,* [5]*Fundasalud Sucre, Cumana, Bolivarian Republic of Venezuela,* [6]*Instituto de Salud Publica, Ciudad Bolivar, Bolivarian Republic of Venezuela,* [7]*Global Development One, Silver Spring, MD, United States,* [8]*International Council Of Aids Service Organizations (ICASO), Montreal, ON, Canada,* [9]*Alianza Venezolana por la Salud, Caracas, Bolivarian Republic of Venezuela*

Venezuela is facing a complex humanitarian emergency with the worst malaria resurgence in the Americas. The national malaria information system (MIS) collects weekly malaria cases since 1955. This is an observational study based on national surveillance data as part of long-term (25 y) epidemiological studies on the dynamics of malaria transmission. Individual-level data on all malaria cases between 1995-2006 (nationwide) and 2007-2018 (84% of the national caseload) were obtained from the national/state information systems. Malaria cases (reported and estimated) were triangulated with publicly available datasets. Case notification forms contained basic information on demographics (age, sex), case classification (indigenous, imported), and the reporting location (municipality, State). From 2008 onwards new variables were added: key dates (symptom onset, diagnosis, and treatment), and reporting unit location (health facility, parish) and occupation, residence and, ethnicity. Between 1995-2018, data from 1.74 M (out of 2.1 M cases reported) were consolidated. Annual malaria cases showed marked heterogeneity in time and space. *Plasmodium vivax* was the most important parasite involved in the transmission nationwide (>70%). Over the study period, *P. falciparum* and *P.malariae* infections were clustered in hotspots in Bolivar and Amazonas States while most cases in Sucre state, are due to *P.vivax* (99%). Consistent with the increase in transmission over the years, local transmission occurred in 23/24 states (2018); mixed infections rose significantly (7 times). Key populations affected by malaria include miners, plantation workers, indigenous groups, pregnant women, adolescents, and young adults. Malaria resurgence has been driven by internal migration to mining areas, stock out of antimalarial drugs and limited control activities. Venezuela offers a unique opportunity to better understand the epidemiological characteristics of malaria resurgence in a humanitarian emergency context. Data-driven approaches to target interventions are required to curtail the negative effects on this malaria epidemic in Venezuela.

675

ESTIMATING CONTRIBUTIONS TO MALARIA TRANSMISSION BY MEASURING INDIVIDUAL HUMAN-TO-MOSQUITO *PLASMODIUM FALCIPARUM* TRANSMISSION EVENTS IN A NATURAL SETTING USING PARASITE GENOTYPING AND LONGITUDINAL HOST SAMPLING

Kelsey M. Sumner[1], Elizabeth Freedman[2], Lucy Abel[3], Andrew Obala[3], Steven Meshnick[1], Steven Taylor[2], Wendy Prudhomme-O'Meara[2]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Duke University, Durham, NC, United States,* [3]*Moi University, Eldoret, Kenya*

Despite increased malaria reduction efforts, *Plasmodium falciparum* prevalence has remained high in Western Kenya where parasites are found in up to 80% of residents. This malaria persistence highlights the need to identify the region's malaria reservoirs. Asymptomatic malaria could be a reservoir, but the amount asymptomatically-infected humans transmit malaria to mosquitoes remains unresolved. We estimated the relative contributions of asymptomatic and symptomatic *P. falciparum* human infections to successful mosquito transmission events in a high transmission area. We hypothesized that asymptomatic compared to symptomatic humans contributed more to mosquito transmission. For a 14-month longitudinal cohort of 243 participants across 3 villages in Webuye, Western Kenya, we collected blood samples from human participants asymptomatic (monthly) and symptomatic (as suspected) for malaria as well as mosquitoes from participant households (weekly). Using these human and mosquito samples, we sequenced 2 *P. falciparum* gene targets: (1) circumsporozoite protein and (2) apical membrane antigen 1. For every *P. falciparum*-infected mosquito, we counted the number of shared parasite haplotypes between a human infection and mosquito abdomen collected in the participant's household within 7 days following infection detection in humans. a shared parasite haplotype indicated a successful human-to-mosquito transmission event. Using a multi-level Poisson regression model, we compared asymptomatic vs. symptomatic human infections to the number of parasite haplotypes shared between the infected humans and mosquito abdomens. Across humans, we identified 895 asymptomatic and 153 symptomatic infections. Across mosquitoes, we identified 199 abdomens infected with *P. falciparum*. We found asymptomatic infections in humans were transmitted to mosquitoes more often than symptomatic ones, establishing that asymptomatic malaria highly contributes to mosquito infection and onward transmission. These findings inform the utility of targeted active test and treat strategies for finding asymptomatic malaria in endemic regions.

676

PERFORMANCE OF A CASE-BASED MALARIA SURVEILLANCE SYSTEM TO SUPPORT MALARIA ELIMINATION IN HAITI

Wilmar Belizaire[1], Reginald Joseph[2], Samson Marseille[3], Kenold Rendel[3], Jean Frantz Lemoine[4], Alyssa J. Young[1]

[1]*Clinton Health Access Initiative, Boston, MA, United States,* [2]*Ministère de la santé publique et de la population, Jeremie, Haiti,* [3]*Direction d'Epidémiologie des Laboratoires et de la Recherche, Port-au-prince, Haiti,* [4]*Ministère de la santé publique et de la population, Port-au-prince, Haiti*

Haiti has committed to eliminate malaria by 2025. Achieving this target requires a robust surveillance system that facilitates the timely reporting of high-quality individual case data. in December 2017 the Direction d'Epidémiologie des Laboratoires et de la Recherche (DELR) in collaboration with the Clinton Health Access Initiative (CHAI), and the National Malaria Program (PNCM) piloted a DHIS2 case-base surveillance system in 13 health facilities in the department of Grand-Anse, the region which reported 33.26% (n=2937) of confirmed cases in 2018. Benefits of using DHIS2 case-based reporting include an opportunity to conduct more in-depth analysis, leading to a better understanding of individual drivers of transmission. Demographic, diagnostic, geolocation and travel history data were collected for each confirmed malaria case by designated health facility personnel using DHIS2 through a mobile-based application. The performance of the case-base surveillance system evaluation process included monitoring four indicators a) proportion of forms with key indicator data fields completed, b) proportion of confirmed cases for which a paper notification form is completed, c) proportion of confirmed cases with a paper notification form completed that are also reported to DHIS2, and d) proportion of cases reported on a paper notification form that are reported in DHIS2 within one week of confirmation. Results from the 13-month DHIS2 case-based reporting pilot showed that form completeness, concordance, and electronic reporting rates remained high throughout the duration of the pilot, at 78.3%, 99.7%, and 93.9%, respectively. a large part of the success of the pilot was a result of weekly site supervision and data validation visits. Specific challenges to implementation included rapid diagnostic test stock-outs at health facilities, limited internet connectivity causing subsequent delays in data-server synchronization, and a lack of human and financial resources required to conduct weekly on-site data validation. These obstacles should be addressed before expansion to all health facilities and communities in Grand-Anse.

677

USE OF HEALTH FACILITY-BASED SEROLOGICAL SURVEILLANCE TO INVESTIGATE *PLASMODIUM FALCIPARUM* AND *P. VIVAX* TRANSMISSION DYNAMICS IN A PRE-ELIMINATION SETTING, INDONESIA

Henry Surendra[1], Supargiyono Supargiyono[2], Riris A. Ahmad[3], Rizqiani A. Kusumasari[2], Theodola B. Rahayujati[4], Siska Damayanti[4], Jackie Cook[5], Chris Drakeley[1]

[1]*Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Department of Parasitology, Faculty of Medicine-Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia,* [3]*Department of Biostatistics, Epidemiology and Population Health, Faculty of Medicine-Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia,* [4]*District Health Office of Kulon Progo, Wates, Indonesia,* [5]*MRC Tropical Epidemiology Group, Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom*

Measuring malaria burden in low transmission settings requires more sensitive tools, and more efficient sampling strategies. Here, we use serology and repeated health facility-based cross-sectional surveys including all clinical attendees and accompanying people to investigate *Plasmodium falciparum* and *P. vivax* transmission dynamics in a pre-elimination setting in Indonesia. The rolling surveys were conducted in 8 health facilities in Kulon Progo Regency, Indonesia, May 2017 to April 2018. Demographic data were collected from all participants and household coordinates were collected using participatory mapping methods. In addition to standard microscopy test, bead-based serological assays were performed on finger-prick bloodspot samples from 9453 people. Seroconversion rates (SCR) were estimated by fitting a simple reversible catalytic model to seroprevalence data. Mixed effect logistic regression was used to investigate factors associated with exposure and spatial analysis was performed to identify the clustering of higher values of antibody responses. Standard microscopy tests identified a parasite prevalence of 0.06% (95% CI: 0.03-0.14, n=6) and 0 for *P. vivax* and *P. falciparum,* respectively. The estimated seroconversion rate in the population was very low, SCR 0.003 (95% CI: 0.002-0.006) and 0.003 (95% CI: 0.002-0.004) for *P. falciparum* and *P. vivax*, respectively. Multivariate analysis revealed that adults and forest goers were more likely to have higher risk of exposure compared to their counterparts. Spatial analysis of antibody responses identified potential high-risk areas where a subsequent outbreak occurred. These findings suggest the use of health facility-based serological surveillance to supplement the existing surveillance systems to better understand malaria transmission dynamics in a pre-elimination setting. Findings from this study could be used by surveillance and control programmes to better target interventions. Further implementation research is needed to enable integration of these methods with existing surveillance systems.

678

MALARIA MORBIDITY AND MORTALITY FOLLOWING INTRODUCTION OF A UNIVERSAL POLICY OF ARTEMISININ-BASED TREATMENT FOR MALARIA IN PAPUA, INDONESIA: A RISING BURDEN OF *PLASMODIUM VIVAX* MALARIA

Jeanne Rini Poespoprodjo[1], Enny Kenangalem[2], Nicholas Douglas[3], Lenny Burdam[2], Ketut Gdeumana[4], Ferry Chalfien[2], Pak Prayoga[2], Fransciscus Thio[2], Angela Devine[3], Jutta Marfurt[3], Govert Waramori[4], Shunmay Yeung[5], Rintis Noviyanti[6], Pasi Penttinen[4], Michael J. Bangs[4], Paulus Sugiarto[7], Julie A. Simpson[8], Yati Soenarto[1], Nicholas M. Anstey[3], Ric N. Price[3]

[1]*Universitas Gadjah Mada, Yogyakarta, Indonesia,* [2]*Papuan Health and Community Development Foundation, Timika, Indonesia,* [3]*Menzies School of Health Research, Darwin, Australia,* [4]*PT Freeport Indonesia/International SOS, Timika, Indonesia,* [5]*The London School of Hygiene & Tropical Medicine, London, United Kingdom,* [6]*Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [7]*Rumah Sakit Mitra Masyarakat, Timika, Indonesia,* [8]*University of Melbourne, Melbourne, Australia*

Malaria control activities can have a disproportionately greater impact on *Plasmodium falciparum* than *P. vivax* in areas where both species are coendemic. We present the temporal trends in malaria-related morbidity and mortality in Papua, Indonesia before and after introduction of ACT for all *Plasmodium* species. a prospective, district-wide malariometric surveillance system was established in April 2004 to record all cases of malaria at community clinics and the regional hospital and maintained until December 2013. in March 2006, antimalarial treatment policy was changed to artemisinin combination therapy for uncomplicated malaria and intravenous artesunate for severe malaria due to any *Plasmodium* species. Over the study period a total of 418,238 patients presented to the surveillance facilities with malaria. The proportion of patients with malaria requiring admission to hospital fell from 26.9% (7,745/28,789) in the pre-policy change period (April 2004 to March 2006) to 14.0% (4,786/34,117) in the late transition period (April 2008 to December 2009). There was a significant fall in the mortality of patients presenting to the hospital with *P. falciparum* malaria (0.53% (100/18,965) versus 0.32% (57/17,691)), but this was less apparent for patients with *P. vivax* malaria (0.28% (21/7,545) versus 0.23% (28/12,397)). Between the same periods, the overall proportion of malaria due to *P. vivax* rose from 44.1% (30,444/69,098) to 53.3% (29,934/56,125) in the community clinics, and from 32.4% (9,325/28,789) to 44.1% (15,035/34,117) at the hospital. After controlling for population growth and changes in treatment seeking behaviour, the incidence rate ratio for the reduction of *P. falciparum* malaria was 0.40 (95%CI 0.40,0.41) and that *P. vivax* malaria was 0.60 (95%CI 0.59,0.60)). a universal policy of artemisinin-based blood schizontocidal treatments in Papua, Indonesia was associated with a greater decrease in the burden of *P. falciparum* compared to *P. vivax.* Widespread access of patients to safe and highly effective radical cure of *P. vivax* will be critical to malaria elimination efforts in co-endemic regions.

679

THE EPIDEMIOLOGY OF *PLASMODIUM VIVAX* AMONG ADULTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Nicholas F. Brazeau[1], Cedar Mitchell[2], Molly Deutsch-Feldman[2], OJ Watson[3], Andrew Morgan[1], Cory Keeler[4], Kyaw Thwai[2], Melchior Mwandagalirwa[5], Antoinette Tshefu[5], Joris Likwela[6], Robert Verity[3], Steven Meshnick[2], Jonathan Juliano[1]

[1]*University of North Carolina School of Medicine, Chapel Hill, NC, United States,* [2]*Gillings School of Global Public Health, Chapel Hill, NC, United States,* [3]*Imperial College London, London, United Kingdom,* [4]*Department of Geography, University of North Carolina, Chapel Hill, NC, United States,* [5]*Programme National de la Lutte contre le Paludisme, Kinshasa, Democratic Republic of the Congo,* [6]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo*

Although *Plasmodium vivax* has long been considered absent from Sub-Saharan Africa, recent reports of *P. vivax* infections among Duffy-negative hosts in Sub-Saharan Africa continue to accumulate. However, to date, no studies have performed a nationally representative survey of *P. vivax* in a Sub-Saharan African country. To overcome this critical gap, we used data collected from the 2013-2014 Standard Demographic Health Survey in the Democratic Republic of the Congo (*Study ID: DRC DHS-II*) to screen over 17,000 adults for *P. vivax*. Overall, we detected 472 cases of *P. vivax* (weighted prevalence: 2.96%, 95% CI: 2.28%, 3.65%). Among the 489 clusters that were sampled in the DRC DHS-II, the number of *P. vivax* cases ranged from 0 - 9 (weighted prevalence range: 0 - 46.15%). Among the 472 *P. vivax* cases, three infected hosts were Duffy-positive (0.64%) and 175 were co-infected with *P. falciparum* (31.70%). To identify the epidemiological and spatial risk factors associated with *P. vivax* infection, we evaluated over 30 covariates with generalized estimating equations. Among these covariates, individual-level wealth, access to health insurance, and precipitation were identified as significant predictors of *P. vivax* infection. However, *P. vivax* infections did not appear to cluster spatially (Moran's I: 0.02, $p > 0.05$), possibly due to interference by *P. falciparum* ($p < 0.05$). To evaluate the effect of scalable antimalarial interventions on *P. vivax* versus *P. falciparum*, we calculated the relative risks of bed-net use, housing materials, and health-insurance using inverse-probability weights with an ensemble supervised machine learning approach. Among these three interventions, only housing materials demonstrated a reduction in *P. vivax* cases [RR: 1.95 (1.11, 3.42)], while all three interventions reduced *P. falciparum* cases. Future work will include *P. vivax* phylogenetics and creating a Bayesian hierarchical spatial prediction model. The return of *P. vivax* to Sub-Saharan Africa is a renewed threat that complicates malaria elimination efforts. The results of this study will aid the DRC government in its efforts to halt the spread of *P. vivax.*

680

TRENDS IN OUTPATIENT MALARIA CASES AND THE EFFECTS OF MALARIA CONTROL IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Filippo Lechthaler[1], **Barbara Matthys**[2], Giulia Lechthaler-Felber[2], Joris Losimba Likwela[3], Hypolite Muhindo Mavoko[4], Junior Matangila Rika[4], Meschac Mutombo Mutombo[2], Laura Ruckstuhl[2], Joanna Barczyk[2], Estifanos Shargie[5], Helen Prytherch[2], Christian Lengeler[2]

[1]*Bern University of Applied Sciences, Bern, Switzerland,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Soins de Santé en Milieu Rural (non-profit organization SANRU), Kinshasa, Democratic Republic of the Congo,* [4]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [5]*The Globa Fund to Fight AIDS, Tuberculosis and Malaria, Geneva, Switzerland*

The Democratic Republic of the Congo (DRC) has the second highest case load from malaria in the world after Nigeria. Malaria control interventions have been strengthened in line with the Millenium Development Goals. We analyzed the effects of these interventions on malaria at health facility level using a retrospective trend analysis of malaria cases between 2005 and 2014, with data collected from outpatient and laboratory registers based on a representative sample of 175 health facilities across the country. We applied a time series analysis to assess trends of suspected and confirmed malaria cases by health province and for different age groups. a linear panel regression model controlled for non-malaria outpatient cases, rainfall, light intensity, health province and time fixed effects, to examine the relationship between the interventions and malaria case occurrences and positivity rates. Overall, recorded suspected and confirmed malaria cases in the DRC have increased. a sharp increase in confirmed cases from 2010 coincides with the introduction of the new treatment policy and the resulting scale-up of diagnostic testing. Controlling for confounding factors, the introduction of rapid diagnostic tests (RDTs) was significantly associated with the number of tested and confirmed cases. The evolution of the positivity rate does not indicate a downward trend in morbidity due to malaria. The sharp increase in confirmed malaria cases from 2010 is associated with improved diagnostic availability, mainly the introduction of RDTs. Before that, a great part of malaria cases were treated based on clinical suspicion. This finding points

to a better detection of cases that potentially contributed to improved case management. Furthermore, the expansion of diagnostic testing along with the increase in confirmed cases implies that before 2010, cases were underreported, and that the accuracy of routine data to describe malaria incidence has improved.

681

INTEGRATING GENETIC AND ENVIRONMENTAL DATA TO MODEL TRANSMISSION PARAMETERS (MOVEMENT AND HABITAT USE) IN THE MAJOR INSECT VECTOR OF SLEEPING SICKNESS IN UGANDA (*GLOSSINA FUSCIPES*)

Norah Saarman, Evlyn Pless, Anusha Bishop, Giuseppe Amatulli, Adalgisa Caccone

Yale University, New Haven, CT, United States

Tsetse flies (genus *Glossina*) are the obligate vectors of the trypanosome parasite that cause animal and human African trypanosomiasis (also known as nagana and sleeping sickness, respectively). One of the most effective strategies in controlling these dangerous and costly diseases is through vector control of tsetse flies. Establishing feasible programs that reduce on-the-ground disease risk require knowledge of vector movement and habitat use. Spatial modeling of these parameters using genetic and ecological data can fill this knowledge gap. However, integrating these two data types remains a challenge in spatial ecology. In this study, we build upon maximum likelihood methods developed by Bouyer et al (2015 in PLoS NTD) to predict movement and habitat use in the insect vector *Glossina fuscipes fuscipes* in Uganda. We use a novel strategy that applies a machine learning algorithm (random forest regression) to model both genetic and ecological parameters (gene flow and fly density, respectively) based on remotely-sensed environmental data (temperature, precipitation, isothermality, altitude, etc.). The final output integrates the two models into a single bivariate map that can identify areas (i) with the highest disease risk and greatest need for medical infrastructure, (ii) with marginal habitat that can be controlled at low cost but also require dedicated monitoring to prevent re-colonization, and (iii) likely to harbor isolated populations that can be effectively eradicated and/or use in the development of novel vector control strategies. To our knowledge, this is the first application of machine learning to integrate genetic and ecological data to predict these important disease transmission parameters (vector gene flow and habitat use). We also apply our approach to forecast future patterns of gene flow and habitat use under alternative global warming and solar geoengineered scenarios. Future forecasting will help to predict changes in parasite transmission dynamics, and can improve strategic planning to reduce human and animal African trypanosomiasis in a changing climate.

682

MUTUALISTIC BACTERIA-PROVISIONED RESOURCES IMPACT VECTOR COMPETENCY

Brian L. Weiss[1], Rita Rio[2]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*Department of Biology, West Virginia University, Morgantown, WV, United States*

Many symbionts supplement their host's diet with essential nutrients. However, whether these nutrients also enhance parasitism is unknown. In this study, we investigated whether folate (B9) production by the tsetse fly (*Glossina* spp.) essential mutualist, *Wigglesworthia,* aids auxotrophic African trypanosomes in completing their lifecycle within this obligate vector. We show that the expression of *Wigglesworthia* folate biosynthesis genes changes with the progression of trypanosome infection within tsetse. The disruption of *Wigglesworthia* folate production caused a reduction in the percentage of flies that housed midgut (MG) trypanosome infections. However, decreased folate did not prevent MG trypanosomes from migrating to and establishing an infection in the fly's salivary glands, thus suggesting that nutrient requirements vary throughout the trypanosome life cycle. We further substantiated that trypanosomes rely on symbiont-generated folate by feeding this vitamin to *G. brevipalpis*, which exhibits a low trypanosome vector competency and houses *Wigglesworthia* incapable of producing folate. Folate supplemented *G. brevipalpis* were significantly more susceptible to trypanosome infection, further demonstrating that this vitamin facilitates parasite infection establishment. Our cumulative results provide evidence that *Wigglesworthia* provides a key metabolite (folate) that is 'hijacked' by trypanosomes to enhance their infectivity, thus indirectly impacting tsetse species vector competency. Parasite dependence on symbiont-derived micronutrients, which likely also occurs in other arthropod vectors, represents a relationship that may be exploited to reduce disease transmission.

683

PARATRANSGENIC MANIPULATION OF MICRORNA275 IN THE TSETSE FLY AND ITS DOWNSTREAM EFFECT ON TRYPANOSOME INFECTIONS

Liu Yang, Brian Weiss, Serap Aksoy

Yale University, New Haven, CT, United States

Tsetse flies (*Diptera Glossina*) are prominent vectors of African trypanosomes, which are the causative agents of "sleeping sickness" in humans and "Nagana" in domesticated animals throughout sub-Saharan Africa. As effective vaccines and affordable treatments for these diseases are still lacking, we investigated alternative methods, such as interfering with trypanosome infection progression in tsetse, as strategies to reduce disease. We discovered previously that trypanosomes manipulate the expression of a host microRNa 275 (*miR275*), which in turn regulates the structural integrity of tsetse's gut associated peritrophic matrix (PM). This outcome promotes the establishment of trypanosome infections in the fly's gut. To investigate the role of *miR275*in tsetse vector competence, we developed a novel system to constitutively manipulate the expression of tsetse *miR275*. We engineered a tsetse commensal endosymbiont, *Sodalis glossinidius,* to express antagomir-275, which binds to *miR275* thus reducing its expression. We subsequently recolonized flies with recombinant (rec)*Sodalis* and evaluated the downstream effects on tsetse midgut physiology as well as trypanosome infection traits. We found that flies colonized with rec*Sodalis*display impaired digestion phenotypes and are highly susceptible to infection with trypanosomes. Our study is a proof of concept that paratransgenic expression is an effective method to regulate microRNa expression levels for downstream functional studies.

684

TSETSE CONTROL IN G-HAT *FOCI*: FOR HOW LONG AND HOW TO STOP?

Jean Baptiste Rayaisse[1], Inaki Tirados[2], Dramane Kaba[3], Mahamat Hissène Mahamat[4], Moise Kagbadouno[5], Albert Mugenyi[6], Mallaye Peka[7], Fabrice Courtin[8], Mamadou Camara[5], Philippe Solano[9]

[1]*CIRDES, Bobo - Dioulasso, Burkina Faso,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*IPR, Bouaké, Côte D'Ivoire,* [4]*IRED, Ndjaména, Chad,* [5]*PNLTHA, Conakry, Guinea,* [6]*COCTU, Kampala, Uganda,* [7]*PNLTHA, Moundou, Chad,* [8]*IRD, Bouaké, Côte D'Ivoire,* [9]*IRD, Montpellier, France*

With the development and the use of tiny targets, implementation of tsetse control in complement to the "screen and treat" strategy has significantly impacted gambiense Human African Trypanosomiasis (g-HAT) incidence in Guinea, Chad, Côte d'Ivoire and Uganda. This combination of methods, in addition to saving lives and reducing/eliminating transmission, is easily accepted by populations that are not anymore disturbed by tsetse bites and that are more protected against g-HAT. However, with the reduction of the tsetse densities together with decrease in disease incidence, and decrease in funding when the number of cases has gone down, the question of sustainability of vector control operations emerges. Tsetse eradication can be contemplated in potentially isolated areas (e.g. like the Mandoul in Chad), but in many HAT *foci* tsetse populations are not isolated, e.g. in areas like the mangrove in Guinea. In these areas, the

question becomes: until when should the vector control be maintained, and by who? If it has to be stopped, what are the conditions to be fulfilled in before stopping? We propose here an algorithm aiming at helping decision on tsetse control in g-HAT *foci* according to the incidence of HAT cases in the focus, which consists in: 1) maintaining vector control as long as new cases are still found, 2) decreasing vector control when number of new cases reaches zero, but keeping a capacity of reaction to be able to implement vector control in the vicinity of new cases, should they be found (« reactive vector control »), and 3) stopping vector control when no cases have been found during 5 consecutive years with similar medical effort. Parallel to these decisions, other tools (xenomonitoring in tsetse, monitoring in animals and tsetse densities surveillance) should be used to help for early response if necessary, using prediction models. Such an approach is useful and will give some common guidelines for decision making, depending on the context, provided stakeholders have the same understanding on the description/definition of the different situations.

685

AMBLYOMMA VARIEGATUM, VECTOR OF AFRICAN TICK-BITE FEVER, CONTAINS AN INTEGRATED *RICKETTSIA AFRICAE* CHROMOSOME IN ITS NUCLEAR GENOME

Alistair C. Darby[1], Alaa M. Al-Khafaji[1], Mark Whitehead[1], Catherine S. Hartley[1], Glen Robinson[1], Stuart D. Armstrong[1], Aleksandra Y. Beliavskaia[1], Germanus S. Bah[2], Naftaly Githaka[3], Lesley Bell-Sakyi[1], **Ben Makepeace**[1]

[1]*University of Liverpool, Liverpool, United Kingdom,* [2]*Institut de Recherche Agricole pour le Développement, Ngaoundéré, Cameroon,* [3]*International Livestock Research Institute, Nairobi, Kenya*

Amblyomma variegatum, the tropical bont tick, is one of the most important ticks involved in pathogen transmission to humans and livestock in sub-Saharan Africa and the Caribbean, and also has direct impacts on ruminant productivity. It is the primary vector of two bacteria in the Rickettisiales: *Ehrlichia ruminantium*, the aetiological agent of heartwater disease in ruminants, and *Rickettsia africae*, which causes African tick-bite fever in humans. Unusually for a pathogenic *Rickettsia* sp., the prevalence of *R. africae* in *A. variegatum* has been reported to be close to fixation. We confirmed this finding using specimens collected from the Adamawa Region of Cameroon, where 95.3% of ticks (*n* = 192) removed from cattle were positive by qPCR. However, the normalised density of rickettsial to tick single-copy genes was often low (rickettsia:tick ratio of ~0.5 - 5). The Tick Cell Biobank maintains two *A. variegatum* cell lines (AVL/CTVM13 and AVL/CTVM17) that were found to be positive by PCR for several rickettsial genes; Sanger sequencing confirmed that these genes were of *R. africae* origin. Unexpectedly, no microscopic or proteomic evidence of a bacterial infection in these cell lines was evident, and tetracycline treatment of cultures over two months, which was sufficient to eliminate *Rickettsia raoultii* from parallel tick cell cultures, had no significant effect on *R. africae* DNa signal in the *A. variegatum* lines. We extracted high molecular-weight DNa from AVL/CTVM17 cells and a single adult *A. variegatum* male from a colony maintained at the International Livestock Research Institute in Nairobi. Using Chromium 10x libraries, we sequenced these genomes on an Illumina NovaSeq and obtained assemblies of ~6 Gb, the first from an *Amblyomma* sp. The cell line and the tick genomes contained an integrated *R. africae* chromosome, although a large deletion representing ~10% of the rickettsial genome was present in both assemblies. This finding has significant implications for the epidemiology of *R. africae* and suggests that other tick genomes may contain integrated rickettsial DNA.

686

A NOVEL GROUP OF SCABIES MITE INACTIVE CYSTEINE PROTEASES WITH PRO-COAGULATORY FUNCTIONS

Deepani D. Fernando[1], Simone Renolds[2], Gunter Hartel[2], Bernard Cribier[3], Nicolas Ortonne[4], Katja Fischer[2]

[1]*Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka,* [2]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [3]*Universite de Strasbourg Faculte de Medecine, Strasbourg, France,* [4]*CHU Henri Mondor, Service d'Anatomo-Pathologie, Paris, France*

Scabies is an infectious skin disease caused by the burrowing mite *Sarcoptes scabiei* affecting an estimated 100-300 million people worldwide and various companion, farm and wild animals. There is no vaccine and the few available broad-spectrum anti-parasitic drugs fail to control the disease. Scabies is not simply an itch; its mechanical damage to the skin allows the entrance of *Staphylococcus aureus* and group a *Streptococcus* leading to serious secondary complications and mite essential secretory / excretory proteins contribute to this bacterial establishment. Scabies mite express cysteine proteases homologous to the group 1 allergen of house dust mites. However, in contrast to their free-living relatives scabies mites express multiple cysteine proteases comprised of 5 proteolytically active (Sars1a-e) and 5 inactive (SMIPP-Ca-e) members. Recombinant, soluble SMIPP-C proteins were successfully expressed and purified from *Escherichia coli*. Localisation studies using immune-histology demonstrated that SMIPP-Cs are present in the mite gut and excreted within the faeces. Gene expression analysis found that SMIPP-Cs are highly expressed in the adult female mite, and less in other life stages. Initial functional investigations showed that SMIPP-Cs bind to calcium ions and the skin protein Dermatopontin, a fibrin formation accelerator. Two SMIPP-Cs were found to accelerate blood coagulation, accelerate fibrin formation during fibrinogen polymerisation and delay plasmin induced fibrinolysis, hence maintaining the fibrin clot for a longer time. Scanning electron micrographs of SMIPP-C induced fibrin clots revealed a complex structure compared to normal fibrin clot structures, suggesting that SMIPP-C proteins are responsible for an aberrant fibrin formation. Immuno-histological localisation of SMIPP-C proteins in the microthrombi within skin biopsies from human scabies lesions further consolidate the SMIPP-C role in the host-pathogen interplay. We propose that scabies mite SMIPP-Cs cause the pathophysiology of the micro-thrombi formation commonly found in scabies infected skin histology.

687

RHODNIUS ECUADORIENSIS POPULATION GENOMICS IN SOUTHERN ECUADOR FOR GUIDING VECTOR CONTROL PROGRAMS

Luis E. Hernandez Castro[1], Anita G. Villacís[2], Björn Andersson[3], Jaime A. Costales[2], Sofía Ocaña-Mayorga[2], Erin L. Landguth[4], Cesar A. Yumiseva[2], Mario J. Grijalva[2], Martin S. Llewellyn[1]

[1]*University of Glasgow, Glasgow, United Kingdom,* [2]*Pontifical Catholic University in Ecuador, Quito, Ecuador,* [3]*Karolinska Institutet, Stockholm, Sweden,* [4]*University of Montana, Missoula, MT, United States*

Understanding triatomine population dynamics at a region scale is key for an effective vector control program. Here we aimed to investigate province-wide population structure and gene flow in the main Chagas disease vector in southern Ecuador, *Rhodnius ecuadoriensis*, and provide guidelines for vector control. To achieve this, we genotyped 2,552 SNP markers of 282 *R. ecuadoriensis* samples from 25 communities in Loja, Ecuador from 2004 to 2018. a strong signal of structuring (F_{ST} = 0.225, P-value = 0.001) was detected by our hierarchical analysis of molecular variance (AMOVA), which attributed most of the variation to regions (17.21 %) and individuals within populations (77.50%) as compared to populations within regions (domicile vs sylvatic populations – 5.29%). Interestingly, populations pairwise F_{ST} comparisons showed similar patterns of structure. After that, we identified 13 genomic clusters among samples using discriminant analysis of principal components (DAPC). Additionally, we explored phylogenetic relationship using a neighbour-joining tree of pairwise genetic distance from samples allele counts which clustered them by region, similarly to pairwise F_{ST} comparisons and DAPC results. Once genomic differentiation pattern was stablished, we tested isolation-by-distance (IBD) as null hypothesis using mantel test and correlograms which drew a significant correlation (mantel r = 0.55, P-value = 0.001). Finally, we tested the usability of our 2,552 SNPs to detect *loci* more differentiated than the expected of neutral *loci* and found 157 candidate F_{ST}-outlier

locus which can be the target of local adaptation. Our findings suggested little differentiation between domicile and sylvatic vector population exists and that vector migration among communities within a region is high. Although spatial correlation was strong, other processes may be shaping Loja vector populations as indicated by correlograms. Vector control design should account for the high vector population gene flow within in a region for successful eradication, although sylvatic population still represent a risk for recolonization.

688

THE GENETIC BASIS OF PRAZIQUANTEL RESISTANCE IN *SCHISTOSOMA MANSONI*

Winka Le Clec'h[1], Frederic D. Chevalier[1], Marina McDew-White[1], Robbie Diaz[1], Amanda Strickland[1], Meghan Guzman[2], Ana Carolina de Mattos[2], Philip T. LoVerde[2], **Tim Anderson**[1]

[1]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [2]*University of Texas Health, San Antonio, TX, United States*

Praziquantel (PZQ) monotherapy is the basis for schistosome control. Mass treatment campaigns (2016-2021) aim to distribute 250 million PZQ tablets per year, constituting a 10-fold increase in tablet distribution, and intensifying selection for PZQ resistance. PZQ-resistant (PZQ-R) parasites have been reported from the field and can be selected in the laboratory, but the genetic basis of PZQ-R is unclear. We measured the *in vitro* dose-response of male worms from the laboratory selected SmLE-PZQ-R resistant population and SmLE PZQ-sensitive (PZQ-S) population and observed a 14-fold difference in IC_{50} (12.75 ± 4.49 (SE) μg/mL vs. 0.86 ± 0.14 (SE) μg/mL). We also demonstrated that SmLE-PZQ-R population contains individuals that are impervious to high dose PZQ. To determine the genetic basis of PZQ resistance in this SmLE-PZQ-R population, we used a pooled association approach. We phenotyped individual male worms 72h after PZQ treatment (24.3 μg/mL) using a quantitative metabolism-based assay (lactate production), which provides an excellent proxy for worm recovery. We then pooled male worms showing the lowest and highest lactate production, and illumina sequenced these pools. Comparison of allele frequencies between the two pools unambiguously ($p=10^{-20}$) identifies a single ~3 Mb region on chromosome 3 containing 72 genes, including several very promising candidate *loci*. To enrich PZQ-R or PZQ-S alleles, we genotyped larval parasites to identify those carrying SNPs in the QTL region associated with PZQ-R or PZQ-S and established infections with these two populations in hamsters. This "marker-assisted" selection approach resulted in two parasite populations differing by >368 fold in PZQ response. We conclude that PZQ resistance in this laboratory population has a single gene, recessive inheritance and results in a dramatic change in resistance. We are now using RNAi to knock down genes within the QTL region to identify the gene involved in PZQ-R. Our central aim is to identify the genetic basis of PZQ-R in order to understand the mechanism of action and to develop molecular markers for monitoring PZQ-R in schistosome control programs.

689

CAN CIRCULATING ANTIGENS BE PREDICTORS FOR FEMALE GENITAL SCHISTOSOMIASIS AS DIAGNOSED BY EXPERT REVIEW AND COMPUTER AUTOMATED IMAGE ANALYSIS

Sigve Holmen[1], Eyrun Kjetland[2], Bellington Vwalika[3], Isaiah Hansingo[4], Comfort Rutty Phiri[5], Maina Mudenda[6], Joyce Mapandi[6], Govert Van Dam[7], Paul Corstjens[7], Claudia de Dood[7], Emily Webb[8], Amy Sturt[8], **Amaya Lopez Bustinduy**[8]

[1]*Holmen Innovative Solutions, Oslo, Norway,* [2]*University of Oslo, Oslo, Norway,* [3]*University of Zambia, School of Medicine, Lusaka, Zambia,* [4]*Livingstone Central Hospital, Livingstone, Zambia,* [5]*Zambart, Lusaka, Zambia,* [6]*Livingstone Central Hospital, Lusaka, Zambia,* [7]*Leiden University Medical Center, Leiden, Netherlands,* [8]*London School of Hygiene &Tropical Medicine, London, United Kingdom*

Urogenital schistosomiasis (UGS) caused by *S. haematobium* is estimated to affect more that 200 million people, with the main burden of disease in Africa. Female Genital Schistosomiasis (FGS) presents with sandy patches (SP) and abnormal blood vessels (ABV) in the lower female genital tract. These lesions are associated with post-coital bleeding, dyspareunia, infertility and prevalent HIV. in a study of 603 females aged 18 – 31 years in Zambia, we investigated whether urine Circulating Anodic Antigen (CAA), can be used as a diagnostic indicator of FGS. CAa is regurgitated into the bloodstream by *Schistosoma* worms, and after rapid renal clearance it can be detected in urine. CAa detection was performed at Leiden University Medical Center in the Netherlands. FGS (SP and ABV) was diagnosed by expert review of digital colposcopic images and by computer automated image analysis (CAIA), using previously published algorithms detecting areas of the cervical mucosa that appear more yellow than the surrounding tissue. The reviewer was blinded to the clinician's original diagnoses and CAIA. ABVs were identified by circular template matching and mean distance between blood vessels. The prevalence of live *Schistosoma* worms, as determined by a positive CAA, was 15.1 % (91 / 601). FGS prevalence was 36.9 % (179 / 485) by expert review and 49.5 % (240 / 485) by CAIA, with a Cohen's Kappa of 0.25 ($p < 0.001$). FGS prevalence was 25.6 % (124 / 485) when the analysis was restricted to images positive for FGS by both expert review and CAIA. FGS positive images were associated with a positive CAa ($p < 0.001$). CAa had an FGS detection rate of 5.8 % and a positive predictive value of 35.1 %, indicating that CAa results alone fail to detect most cases of FGS in adults in a setting with a relatively low prevalence of current infection. This supports previous findings in which SP and ABV may appear in the absence of current infection with *S. haematobium.* The lesions in FGS are most likely late-stage, chronic manifestations of the disease, reinforcing the urgent need for visual diagnostics and early treatment. Further research is needed to explore if live worms influence FGS symptoms and HIV risk.

690

TREATMENT OUTCOMES OF *FASCIOLA HEPATICA* INFECTION IN PRE-SCHOOL AND SCHOOL AGE CHILDREN IN CUSCO, PERU

Melinda B. Tanabe[1], Camille M. Webb[1], Maria L. Morales[2], Marta Lopez[3], Miguel M. Cabada[1]

[1]*University of Texas Medical Branch, Galveston, TX, United States,* [2]*IMT - Universidad Peruana Cayetano Heredia, Cusco, Peru,* [3]*IMT - Universidad Peruana Cayetano Heredia, Galveston, TX, United States*

Fasciola hepatica is endemic in the Andes highlands with an infection prevalence reaching almost 70% among children in some communities. Triclabendazole (TCBZ) is the only recommended treatment for humans. Resistance to TCBZ is an emerging problem. Our aim was to evaluate treatment outcomes of TCBZ in children with chronic fascioliasis in the highlands of Cusco, Peru. a community-based study evaluated the impact of Fasciola among pre-school and school-age children in 26 communities in Cusco. We identified children with chronic fascioliasis defined as the presence of eggs in stool. Children were treated with one or two doses of TCBZ 10mg/kg given with a fatty meal. We evaluated stool samples for Fasciola eggs at least one month after treatment to assess response. Three stool samples per subject were tested with Kato Katz and rapid sedimentation each time. Of 2958 children included in the study, 166 had Fasciola eggs in stool (prevalence of 5.6%). The mean age of those infected was 10.6 years and 50.6% were male. The mean egg count was 56 eggs/gram of stool. 29 (17%) children had eosinophilia, 34 (20%) had anemia, and 52 (31%) had stunting at baseline. in total 162 children were treated, 157 with one dose and 5 with 2 doses of TCBZ. Follow up was within 90 days in half the cases (median 90 days, IQR 56 - 118). Of 86 children that followed up within 90 days, 33 (37.5%) had positive eggs in stool and 32 of these received a second course of treatment. Twenty (62.5%) continued to pass eggs in stool. Overall effectiveness was 62.5% with one dose and 77% after two doses of TCBZ. in the highlands of Cusco in Peru, a single dose TCBZ had a low effectiveness and almost one in four subjects failed to respond to a second dose of treatment. Research for alternative safe effective drugs for fasciolasis is urgently needed as the emergence of resistance threatens control programs.

691

POC-CCA PERFORMANCE FOR MAPPING LOW AND MODERATE ENDEMICITY AREAS FOR SCHISTOSOMIASIS MANSONI AND THERAPEUTIC EFFICACY EVALUATION FOLLOWING SCHOOL-BASED PRAZIQUANTEL ADMINISTRATION (60 MG/KG) IN BRAZIL

Agostinho Gonçalves Viana[1], Pedro Henrique Gazzinelli-Guimarães[2], Vanessa Normandio de Castro[1], Yvanna Louise Oliveira[3], Lílian Lacerda Bueno[1], Stefan Michael Geiger[1], Sílvio Santana Dolabella[3], Anna Phillips[4], Ricardo Toshio Fujiwara[1]

[1]*Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,* [2]*Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, EUA, Bethesda, MD, United States,* [3]*Universidade Federal de Sergipe, Aracaju, Brazil,* [4]*Imperial College London, London, United Kingdom*

Current diagnostic methods for intestinal schistosomiasis (detection of eggs in stool by Kato-Katz (KK) are limited and may be particularly unreliable at low levels of infection. in order to overcome some of the pitfalls of the KK method, there has been interest in developing more sensitive tests for the diagnosis of schistosomiasis. The Point-Of-Care (POC) Circulating Cathodic Antigen (CCA) urine assay (POC-CCA) has been reported to be a sensitive and specific alternative to KK in different endemic settings. Specific data comparing the performance of microscopy and antigen detection tests in low Schistosomiasis prevalence settings and mainly, a good method for cure control, however, is currently not available. in this context, the aim of this work was to compare the performance of POC-CCa and KK in the urine and stool samples of school-aged children (SAC) (5-16 years-old) from low endemic area of Minas Gerais State, and in a moderate endemic area of Sergipe State Brazil as well as to test the performance of POC-CCa as a tool for therapeutic efficacy evaluation in areas under MDa programs. For the KK assay, three stool samples were collected, and 2 KK slides were prepared per sample. Up to the present moment, of the 1,285 SAC surveyed in the state of Minas Gerais, 32 (2.5%) of the samples were positive for KK (intensity mean, 20 eggs mg/stool). However, when evaluated the results of the POC-CCa test, 245 (19%) samples were positive in Minas Gerais state. Moreover, out of 542 SAC surveyed until the moment in Sergipe state, 130 (23.9%) of the samples were for KK (intensity mean, 43 eggs mg/stool), 183 (33.7%) samples were positive by the POC-CCa test, considering trace as positive. The results of the analysis 30 days after treatment (praziquantel 60mg/Kg) showed that out of the 210 and 197 POC-CCa positive and treated patients in both areas respectively, 31.5 (84%) and 29.5 (85%) turned out to negative by POC-CCA. Only 15.2% of the positive individuals in both areas remained positive 30 days after treatment by the rapid test. Taken together our preliminary results POC-CCa appears to be a good test to evaluate and monitor infection cure control after treatment.

692

VALIDATION OF HOME-BASED GENITAL SELF-SWABS FOR THE DIAGNOSIS OF FEMALE GENITAL SCHISTOSOMIASIS IN ZAMBIAN WOMEN FROM AN HIV-1 PREVENTION TRIAL

Amy Sturt[1], Comfort Rutty Phiri[2], Emily Webb[1], Isaiah Hansingo[3], Lisette Van Lieshout[4], Paul Corstjens[4], Govert Van Dam[4], Claudia de Dood[4], J. Russell Stothard[5], Richard Hayes[6], Helen Ayles[6], Amaya L. Bustinduy[6]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Zambart, Lusaka, Zambia,* [3]*Livingstone Central Hospital, Livingstone, Zambia,* [4]*Leiden University Medical Center, Leiden, Netherlands,* [5]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [6]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

The diagnosis of Female Genital Schistosomiasis (FGS) is challenging as it relies on resources rarely available in low income settings. The BILHIV (bilharzia and HIV) study assessed the performance of two home-based self-collection methods (cervical and vaginal swabs) compared to cervicovaginal lavage (CVL) for the detection of *S. haematobium* DNa by real-time polymerase chain reaction (PCR). Between January and August 2018, participants from the Population Cohort of HPTN071 (PopART), an HIV prevention trial, were recruited if they were non-pregnant, sexually active, 18-31 years old and residents of two communities (Maramba and Dambwa) in Livingstone, Zambia. Genital self-swabs and a urine specimen were collected and a questionnaire administered at home visits. a midwife obtained a CVL at the cervical cancer clinic. 603 women were enrolled, 319 (53%) from Maramba and 284 (47%) from Dambwa, and 87.3% (527/603) completed CVL. The median age was 24 years (IQR 22 - 26) and 60.4% (364/603) had completed some secondary school. Overall prevalence of *Schistosoma* infection was 5.5% (33/603) based on urine microscopy and 15.1% (91/601) based on urine Circulating Anodic Antigen (CAA). Maramba had higher prevalence of microscopy 8.2% (26/319) and CAa positivity 20.5% (65/317) compared to Dambwa, 2.5% (7/284) and 9.2% (26/284) respectively. *Schistosoma* PCR was positive in: 3.1% (14/451) vaginal self-swabs (3.7% in Maramba and 2.8% in Dambwa), 3.5% (9/257) cervical self-swabs (4.8% in Maramba and 2.6% in Dambwa), and 2.7% (14/523) CVL (3.8% in Maramba and 1.2% in Dambwa). Sample processing is ongoing (CVL (523/527), vaginal swabs (452/603) and cervical swabs (257/603)). Preliminary analysis suggests that home-based genital self-sampling for the detection of *Schistosoma* DNa is comparable to clinic-based collection. Sensitivity and specificity analysis will be performed at completion of specimen processing. Genital self-sampling may provide a future option for FGS community diagnosis at scale.

693

POC-LAMP FOR HUMAN SCHISTOSOMES COMPARATIVE COST AND TIME ANALYSIS FOR VARIABLE ARRANGEMENTS

Brittany Pulkkila[1], Chummy S. Sikasunge[2], James Mwansa[2], **Nilanjan Lodh**[1]

[1]*Marquette University, Milwaukee, WI, United States,* [2]*University of Zambia, Lusaka, Zambia*

Schistosomiasis is one of the major Neglected Tropical Diseases (NTDs), with more than 200 million people infected and close to 800 million at risk, mostly in sub-Saharan African countries. It is caused by two major species, *Schistosoma mansoni* and *S. haematobium*. These often share the same location, raising the problem of accurate and specific diagnosis and can impact any control strategies based on targeted mass drug administration (MDA). World Health Organization has drawn attention to the need for field applicable tests with high specificity and improved sensitivity. To address the above-mentioned issues, we have evaluated the amplification of both *S. mansoni* and *S. haematobium* by loop-mediated isothermal amplification (LAMP), which can be used as a point-of-care (POC) test, from filtered urine samples collected from school children after treatment in Zambia by four different extraction techniques: Qiagen, LAMP-PURE (LP), Chelex and heating. The feasibility of POC-LAMP is also evaluated by determining comparative cost analysis and person-time involvement for each approach. DNa extraction by LP is the fastest (average 20 minutes) compared to the other three methods, although it is the most expensive including amplification ($9.35 compared to $4.90 for heating extraction and amplification). Chelex extraction is slower and simpler than LP and also detected 20% more positive infection than heating. Extraction by heating is also very fast, inexpensive and probably the simplest to perform. However, LAMP performed on heating-extracted samples resulted into many false-negative results, possibly indicating the presence of inhibitor(s). Qiagen and LP extraction both detected 100% of positive infections, but Qiagen extraction is more cost effective than LP. We have demonstrated the sensitivity, cost-effectiveness and time requirement of the LAMP method to detect both schistosome parasites from field collected urine samples. LAMP can be used as a POC test for surveillance and assessing success of control intervention in Zambia as part of their ongoing local schistosomiasis control program.

694

THE DIAGNOSTIC POTENTIAL OF GLYCAN SPECIFIC ANTIBODIES IN SCHISTOSOMIASIS ASSESSED BY GLYCAN MICROARRAYS

Anna O. Kildemoes[1], Angela van Diepen[1], Tom Veldhuizen[1], Linh Nguyen[1], Mio Tanaka[2], Govert J. van Dam[1], Meta Roestenberg[1], Shinjiro Hamano[2], Cornelis H. Hokke[1]

[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Institute of Tropical Medicine (NEKKEN), Nagasaki, Japan*

During *Schistosoma* infections, antibodies specific for numerous antigens are induced by parasite larvae, adult worms and eggs. a large proportion of these antibodies are directed against antigenic glycans, which are part of the parasite's glycoprotein and glycolipid repertoire. It remains poorly understood whether these anti-glycan antibodies play a role in immunity to schistosomiasis or contribute to parasite immune evasion. Irrespective of the function of antibodies to defined schistosome glycans in infection, they may constitute a valuable sofar untapped potential in a diagnostic context. As animal experimental work has shown that these anti-glycan antibody responses are highly dynamic, we aim to identify specific glycan targets of the antibody response in schistosome infection. After glycomics analyses of *Schistosoma mansoni* life stages and other helminths, we have isolated and identified hundreds of glycans of which several are unique to blood flukes. These native *S. mansoni* glycans combined with synthetic glycans relevant to schistosomes and other helminths were used to construct a glycan microarray. As such the antigenic repertoire on the microarray represent targets uniquely present in schistosomes as well as targets cross-reactive with other organisms: helminths, other invertebrates, food stuff of plant origin or the mammalian host. We have used this microarray to screen sera from a controlled human *S. mansoni* infection model and from schistosomiasis cohorts from both low and high transmission endemic areas, pre- and post-treatment, in order to identify targets with diagnostic potential. We focus on elucidating anti-glycan antibody responses, which are not only specific to schistosomes but also provide grounds for distinction between current infection and prior exposure/infection. Additionally, we aim to identify differences between responses to *S. mansoni* and *S. haematobium* infection. Development of an antibody based diagnostic tool with such properties would be a useful addition to the diagnostic toolbox available for monitoring treatment efficacy and in schistosomiasis control/elimination programs.

695

PERSISTENCE OF AFEBRILE SUBMICROSCOPIC *PLASMODIUM* SPP. INFECTIONS IN AN ENDEMIC AREA FOR MALARIA IN COLOMBIA

Jehidys E. Montiel Ramos[1], Luisa F. Carbal Reyes[1], Lina M. Zuluaga Idarraga[1], Ana M. Vasquez Cardona[1], Daniel C. Aguirre Acevedo[1], Berlin Londoño Renteria[2], Alberto Tobon Castaño[1]

[1]*Universidad de Antioquia, Medellín, Colombia,* [2]*Kansas State University, Manhattan, KS, United States*

in malaria endemic regions, many of *Plasmodium* infections are below the level of detection by microscopy (submicroscopic) which is the standard test for malaria diagnosis. Submicroscopic infections (SI) persist undetectable and could contribute to malaria transmission. Understanding of dynamic and duration of SI is indispensable to develop control strategies. Afebrile and SI detected in an endemic Colombian population were longitudinally followed for 60 days. The clearance time of SI and associated factors were estimated. Inhabitants of 4 endemic villages in Tumaco (Pacific Coast) were enrolled between August/2017-March/2018. SI were defined as those detected by LAMP (Loop-mediated isothermal amplification) but no by microscopy with an axillary temperature lower than 37,5°C. An ELISa test was performed to evaluate Antiplasmodial IgG Antibody levels at cero day. SI people were followed up for two months by LAMP and microscopy in at least 6 measurements to estimate the parasite positivity in time. a survival analysis was performed to calculate the survival function and a log rank test to compare the survival distributions regarding malaria history. a cox regression model was done to identify risk factor associated to the persistence. SI in the community were detected in 64/958 individuals (6,7%), 67,2% were *P. falciparum* and 32,8% were *Plasmodium* spp. The median persistence was 53 days and 47,5% had at least three negative consecutive LAMP results with a clearance rate of 0,013/100 SI/day. The median survival was higher in individuals with history of malaria (58 days) compared to individuals without history of malaria (31 days) but there were not statistically significant differences (p 0,205). On the other hand, an increased in the antibody levels against Pf-msp was associated with a decrease in the clearance *Plasmodium* risk (HR 0,14; CI 95%: 0,043- 0,475). There is a high proportion of SI which can persists over time and this persist is related with antibody levels Pf-msp. It is suggested that a cumulative exposure to *Plasmodium* antigens is associated with protection from future clinical malaria episodes and therefore with SI.

696

FALCIPARUM BUT NOT VIVAX MALARIA DURING EARLY GESTATION IS ASSOCIATED WITH INCREASED RISK OF SUBSEQUENT HYPERTENSIVE DISORDERS OF PREGNANCY

Whitney E. Harrington[1], Aung Myat Min[2], Mary Ellen Gilder[2], Nay Win Tun[2], Kerryn Moore[3], Moo Kho Paw[2], Jacher Wiladphaingern[2], Kesinee Chotivanich[4], Nicholas J. White[5], Francois Nosten[2], Rose McGready[2]

[1]*Seattle Children's / University of Washington, Seattle, WA, United States,* [2]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand,* [3]*Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia,* [4]*Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [5]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Old Road Campus, Oxford, United Kingdom*

Malaria and hypertensive disorders of pregnancy (HDoP) continue to affect millions of pregnancies world-wide, particularly those of young, first-time mothers. Prior research suggests a link between the two conditions, but to date, no large, prospective analysis has considered the effect of peripheral falciparum or vivax malaria on risk of HDoP, by gestational age of infection. Using data from the Thai-Myanmar border collected between 1986 and 2016, we evaluated the relationship between (1) any falciparum or vivax malaria and (2) gestational age of first falciparum or vivax infection and the risk of gestational hypertension (GH), pre-eclampsia (PRE-EC), and eclampsia (EC). 23,262 singleton, live-born pregnancies were considered, in which women were enrolled during first trimester and followed fortnightly with data recorded on blood smear positivity, blood pressure, and proteinuria. Logistic regression models were adjusted for maternal age, first trimester weight, weight gain, chronic hypertension, number of consultations, place of delivery, refugee status, and year of delivery. Among all women, any falciparum infection was associated with risk of any HDoP (AOR 1.63, 95%CI: 1.13-2.37), with strongest effect for GH (AOR 1.84, 95%CI: 1.18-2.87). When stratified by gravidity, falciparum malaria predicted risk of PRE-EC among primigravidae (AOR 4.11, 95%CI: 1.35-12.49) and risk of GH among multigravidae (AOR 2.16, 95%CI: 1.33-3.51). Falciparum infection between 0-11 weeks gestation (AOR 2.51, 95%CI: 1.40-4.50) or 12-21 weeks gestation (AOR 1.75, 95% CI:1.00-3.05) was associated with risk of HDoP. There was no association between vivax malaria and HDoP. In this large, prospectively followed cohort we observed a strong association between early peripheral falciparum malaria and HDoP. The data suggest that falciparum but not vivax malaria during a key period of placental development may result in chronic placental hypoxia, eventually progressing to clinical GH or PRE-EC. These data highlight the critical need to prevent malaria early in gestation, during a window when many women may not yet be aware of the pregnancy.

697

THE EFFECT OF DELAYED TREATMENT ON PROGRESSION TO SEVERE *PLASMODIUM FALCIPARUM* MALARIA: A POOLED MULTICENTRE INDIVIDUAL-PATIENT ANALYSIS

Andria Mousa[1], Joseph D. Challenger[1], Aubrey J. Cunnington[2], Abdullah Al-Taiar[3], Nicholas M. Anstey[4], Cyril Badaut[5], Bridget E. Barber[6], Dibyadyuti Datta[7], Chris Drakeley[8], Jamie T. Griffin[1], Matthew J. Grigg[9], Chandy C. John[7], Florence Migot-Nabias[10], Hugh Reyburn[11], Eleanor M. Riley[12], Colin J. Sutherland[11], Firmine Viwami[13], Christopher J. Whitty[14], Timothy William[15], Azra C. Ghani[1], Lucy C. Okell[1]

[1]*MRC Centre for Global Infectious Disease Analysis, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom,* [2]*Section of Paediatrics, Department of Medicine, Imperial College London, Imperial College London, London, United Kingdom,* [3]*Faculty of Medicine, Kuwait University, Kuwait City, Kuwait,* [4]*Global Health Division, Menzies School of Health Research, Darwin, Australia,* [5]*Unité de Biothérapie Infectieuse et Immunité, Institut de Recherche Biomédicale des Armées, Brétigny sur Orge, France,* [6]*Menzies School of Health Research, Darwin, Australia,* [7]*Ryan White Center for Pediatric Infectious Disease and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States,* [8]*Department of Infection Biology, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [9]*Global and Tropical Health Division, Menzies School of Health Research, Darwin, Australia,* [10]*MERIT, Institut de Recherche pour le Développement (IRD), Université Paris Descartes, Sorbonne Paris Cité, Paris, France,* [11]*Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [12]*Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [13]*Centre d'Etude et de Recherche pour le Paludisme associé à la Grossesse et à l'Enfance, Faculté des Sciences de Santé, Université d'Abomey-Calavi, Cotonou, Benin,* [14]*Clinical Research Department, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [15]*Infectious Diseases Society Sabah-Menzies School of Health Research Clinical Research Unit, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia*

Delay in receiving treatment for uncomplicated malaria (UM) is often reported to increase the risk of developing severe disease, but findings are not consistent across all studies. Understanding which factors underpin progression to severe disease is important in quantifying the potential impact of improving access to treatment and identifying high-risk groups. We searched Ovid MEDLINE and Embase to identify studies on severe P.falciparum malaria with any information on treatment delay, such as duration of symptoms or fever, and contacted authors to obtain individual-patient data. To date, we have pooled data from seven studies in Uganda, The Gambia, Benin, Yemen and Malaysia of 1,377 patients with severe malaria and 2,273 UM controls. Definitions of severity phenotypes were standardised across the studies to compare treatment delay in UM patients with different severe disease manifestations using mixed-effects logistic regressions adjusted for age. An illness duration of ≥ 24 hours prior to arriving at the health facility in children was associated with increased odds of severe malarial anaemia (SMA) compared to UM controls; OR=4.50 (2.06-9.84). Arriving at the facility within 4 days of symptom onset is estimated to prevent 49% of SMa cases, whilst treatment within 24 hours could prevent 76% of SMa cases. Duration of illness was significantly shorter in those with cerebral malaria (CM), hyperlactatemia and hyperparasitaemia compared to controls, whilst no difference was observed for respiratory distress syndrome (p=0.23) or hypoglycaemia (p=0.12). Our results suggest improving rapid access to treatment would probably be highly effective at preventing SMA. CM, hyperlactatemia and hyperparasitaemia have a faster onset and trigger treatment seeking earlier, thus access to treatment would be required within a shorter time-frame to prevent these faster-developing phenotypes. We are continuing to gather additional datasets and explore the pathway to severe disease as well as the effects of accessibility, failure and source of initial treatment prior to the study, transmission intensity, seasonality and socioeconomic determinants.

698

MALARIA ATTRIBUTABLE FEVER IN LOW AND HIGH TRANSMISSION SETTINGS OF ZAMBIA: DIFFERENCES BETWEEN ACTIVE AND PASSIVE CASE DETECTION

Japhet M. Matoba[1], Philip Thuma[1], Jennifer C. Stevenson[2], Julia Pringle[3], Caison Sing'anga[1], Mukuma Lubinda[1], Amy Wesolowski[4], Tamaki Kobayashi[4], Douglas Norris[5], William J. Moss[6]

[1]*Macha Research Trust, Choma, Zambia,* [2]*Macha Research Trust & Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Choma, Zambia,* [3]*Johns Hopkins Bloomberg School of Public Health, Department of Molecular Microbiology and Immunology, Baltimore, MD, United States,* [4]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [5]*Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [6]*Department of Epidemiology, Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Fever is a common symptom of malaria. It is also one of the main symptoms of many other illnesses such as diarrhea and pneumonia. For these diseases, diagnosis with a fever is usually the first point of entry into the health surveillance system. The Zambian government uses active and passive sampling strategies like the malaria indicator survey and rapid surveillance reporting systems for monitoring febrile illnesses and assessing their burden in the community. Understanding the characteristics of febrile illness obtained through these methods can help in adapting treatment guidelines suited to various malaria transmission settings, thereby helping to mitigate threats due to changes in the epidemiological profile as countries strive for malaria elimination. Towards this goal, we examined the correlation of fever and malaria between a cross-sectional analysis of 7,875 actively detected individuals and 513, 307 passively detected health seeking individuals from a health center rapid reporting surveillance system over the period of 2012 to 2015. The participants were drawn from low and high transmission areas in Choma and Nchelenge Districts in Southern and Luapula Provinces of Zambia. Choma and Nchelenge Districts have a malaria prevalence of 1% and 30% respectively. Fever was classified as having a body temperature above 37.5 ^{0} C, while malaria was assessed by a point-of-care rapid diagnostic test (RDT) for *Plasmodium falciparum*. From Choma District and the actively detected data, none of the febrile individuals yielded a positive RDT while 0.5% were RDT positive but afebrile. In Nchelenge District, 80% of the actively detected fevers were attributable to malaria. Among the passively sampled individuals, only 5% of those febrile were RDT positive in Choma District while 56% of fevers were attributable to malaria in Nchelenge. All fever cases were once considered as malaria but as transmission declines, there is need for more information on what is causing fever and how to address it. Our results suggest the need for rigorous integrated management of febrile illness and introduction of point of care diagnostics for non-malaria fever.

699

EPIDEMIOLOGY OF SUBPATENT *PLASMODIUM FALCIPARUM* INFECTIONS IDENTIFIED BY HIGH-SENSITIVITY REAL-TIME PCR DETECTION DURING COMMUNITY-BASED PROACTIVE AND REACTIVE CASE DETECTION IN WESTERN KENYA

Steve M. Taylor[1], Kelsey M. Sumner[2], Betsy Freedman[1], Judith Mangeni[3], Andrew A. Obala[3], Wendy P. O'Meara[1]

[1]*Duke University, Durham, NC, United States,* [2]*UNC Gillings School of Global Public Health, Chapel Hill, NC, United States,* [3]*Moi University, Eldoret, Kenya*

Low-level, asymptomatic *Plasmodium falciparum* infections are common in high-transmission settings, and the availability of conventional malaria

rapid diagnostic tests (cRDTs) for case detection enables community-based screening for these infections. These screening approaches include reactive case detection (RACD), wherein index cases of malaria trigger ring testing, and proactive case detection (PACD), wherein high-risk groups are tested in targeted fashion. We sought to measure the suitability and efficiency of a common cRDT that detects *P falciparum* HRP2 for these purposes by performing sensitive PCR-based parasite detection on specimens collected in 2013-14 in a high-transmission setting in Western Kenya. Over 3,500 participants were enrolled: index children were RDT-positive (cases) or matched RDT-negative controls, and they and all household members were tested with cRDT at enrollment and blood spots were archived. We designed, validated, and applied a TaqMan real-time PCR assay targeting a multi-copy motif *pfr364* in the *P. falciparum* genome that detects down to 0.1 parasite/uL of whole blood. Overall, the parasite prevalence was 22.9% by cRDT and 61.5% by PCR; compared to members of control households, Prevalence Ratios were higher for case household members by both cRDT (PR: 2.21; 95% CI 1.82 - 2.7) and PCR (PR: 1.74; 95% CI 1.58 - 1.92), indicating significant household infection clustering. The overall sensitivity of cRDT compared to PCR was 36%. Sensitivity was highest in infections > 100,000 p/uL (98.2%) and lowest in infections < 1p/uL (8.5%); using a conventional density cutoff of 100p/uL, the sensitivity above the limit was 84% and below was 21%. Based on cRDT prevalence in subgroups, we estimated that the number of household members needed to screen with a cRDT to identify one additional infection was 9.8 in a PACD program and 4.4 in an RACD program. In our site in Western Kenya, *P. falciparum* infections detected by cRDT or PCR were clustered within households of children with malaria; compared to a PACD approach, the RACD approach using cRDT to screen asymptomatic members of the community is twice as efficient at case detection.

700

ONGOING ASSESSMENT OF *PLASMODIUM FALCIPARUM* PARASITE PREVALENCE IN SOUTHERN PROVINCE ZAMBIA: RESULTS FROM A 2019 PARASITE SURVEY THREE YEARS AFTER A MASS DRUG ADMINISTRATION TRIAL

Brooke Mancuso[1], Travis Porter[1], Maya Fraser[2], Kafula Silumbe[2], Busiku Hamainza[3], Hawela Moonga[3], Adam Bennett[4], Josh Yukich[1], Caterina Guinovart[2], Kammerle Schneider[2], John M. Miller[2], Thomas P. Eisele[1]

[1]*Tulane University School of Public Health, New Orleans, LA, United States,* [2]*PATH, Seattle, WA, United States,* [3]*National Malaria Control Center, Lusaka, Zambia,* [4]*University of California San Francisco, San Francisco, CA, United States*

Between 2014-2016 10 districts along Lake Kariba in Southern Province, Zambia were the site of a community randomized controlled trial investigating the impact of mass drug administration (MDA) strategies. The study area received a continuous effort to maintain a fully scaled intervention package (SIP) of proven malaria interventions, including universal coverage of long-lasting insecticidal nets (LLINs), up to 50% targeted coverage of indoor residual spraying (IRS) with the highly effective Actellic-300cs, improved access to malaria diagnosis and treatment through community case management, improved supply chain and program management, high quality malaria surveillance using DHIS2, and targeted programmatic MDa in higher transmission areas after the initial MDa trial ended. Yearly parasite surveys in the study area during the peak transmission season (April-May) from 2014 - 2016 showed *Plasmodium falciparum* parasite prevalence (*Pf*PR) in children <5 years old to have declined by 87%, irrespective of trial arm, from a baseline of 31.3% in 2014 to 4.0% immediately following the MDa trial in 2016. Since the trial, the Zambian National Malaria Elimination Centre (NMEC) has maintained the full SIP across Southern Province. The decline in *Pf*PR was maintained (*Pf*PR = 3.7%) in the former trial area in 2017, 15 months after the MDa trial. Another parasite survey is currently underway (April-May 2019); we will report if the SIP has successfully maintained the large declines in malaria 3 years after the MDa trial. We will also report SIP components and other factors associated with *Pf*PR in 2019.

701

TARGETED SURVEILLANCE FOR FOREST-BASED MALARIA TRANSMISSION: RESULTS OF A CLUSTER RANDOMIZED CONTROLLED TRIAL IN SOUTHERN LAO PDR

Adam Bennett[1], Emily Dantzer[1], Bouasy Hongvanthong[2], Francois Rerolle[1], Sophia Hocini[1], Jennifer Smith[1], Jimee Hwang[3], Roland Gosling[1], Joshua Yukich[4], Bryan Greenhouse[1], Rattanaxay Phetsouvanh[5], Andrew A. Lover[6]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Center for Malariology, Parasitology, and Entomology, Vientiane, Lao People's Democratic Republic,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [5]*Department of Communicable Disease Control, Vientiane, Lao People's Democratic Republic,* [6]*University of Massachusetts-Amherst, Amherst, MA, United States*

Lao PDR is targeting elimination of *Plasmodium falciparum* by 2025, and all species of malaria by 2030, but transmission due to forest exposure remains a challenge to these goals. We conducted a cluster randomized controlled trial in Champasak Province, Lao PDR to evaluate the role of village-level mass test and treat (MTAT) and forest worker peer navigator-led focal test and treat (FTAT) for reducing *Pf* prevalence and incidence. Testing included the use of ultra-sensitive rapid diagnostic tests (uRDTs). Using a split-plot design, 56 villages and 14 health facility catchment areas were randomized to receive either the MTAT or FTAT intervention. Two MTAT rounds were conducted in the intervention villages June-Sep 2018, and continuous FTAT implemented Mar-Nov 2018. Of 24,912 tests conducted during MTAT, only 8 were positive by uRDT/RDT for *Pf* (0.03%) and 9 for *P. vivax* (0.04%). PCR detected slightly higher *Pf* positivity (0.07%) and far higher *Pv* positivity (1.58%) than RDTs. During FTAT, 2,906 forest workers were tested at or near the forest fringe, and 67 positive for *Pf* by uRDT/RDT (2.31%). PCR detected 51 *Pf* infections (1.75%) and 116 *Pv* infections (4.04%). Across interventions, diagnostic sensitivity was similar for uRDT (70%) and RDT (68%) compared to PCR for *Pf,* but very low for *Pv* by RDT (8%). Mean monthly *Pf* incidence decreased in the FTAT areas from 0.16/1000 in 2017 to 0.12/1000 in 2018, and was constant in control areas, but this difference did not reach significance in a random effects negative binomial model (Incidence rate ratio (IRR): 0.62, 95% CI 0.23 - 1.64). End-line cross-sectional survey results including PCR will also be presented. The far higher positivity results in forest-worker based FTAT compared to village-based MTAT highlights the importance of targeting forest transmission through active surveillance, and the low sensitivity of RDTs for *Pv* highlights the need for more sensitive diagnostics to detect this reservoir. Engaging the forest worker community through peer navigators is a potentially effective and feasible approach and should be considered as part of a malaria elimination strategy in areas with continued forest transmission.

702

GENOME SEQUENCES OF THE FILARIAL PARASITES *MANSONELLA PERSTANS* AND *MANSONELLA OZZARDI*

Amit Sinha[1], Catherine B. Poole[1], Richard D. Morgan[1], Zhiru Li[1], Laurence Ettwiller[1], Nathalia F. Lima[2], Marcelo U. Ferreira[2], Samuel Wanji[3], Clotilde K. Carlow[1]

[1]*New England Biolabs, Ipswich, MA, United States,* [2]*University of Sao Paulo, Sao Paulo, Brazil,* [3]*University of Buea, Buea, Cameroon*

Mansonella perstans and *M. ozzardi* are filarial parasites that cause human mansonelliasis. *M. perstans* is highly prevalent in areas of sub-Saharan Africa and South America, whereas, *M. ozzardi* is found exclusively in the Americas and Caribbean islands. *Mansonella* parasites are severely understudied and little is known about their biology, epidemiology, or pathogenesis. Clinical symptoms following infection can be absent or non-distinct, and diagnostic as well as treatment options are lacking. We have obtained the first draft sequence of the genomes of *M. perstans* and *M. ozzardi*. Multiple isolates of both the species were sequenced,

using multiple sequencing platforms including Illumina, PacBio and 10X Genomics. These data were assembled to yield genome sizes of ~80Mb in ~1000 to ~5,000 contigs, encoding ~10,000 proteins in each genome. The completeness of these genomes was evaluated using BUSCO, a software that measures the fraction of Single Copy Orthologs expected to be conserved within a taxa. The BUSCO score for both *M. perstans* and *M. ozzardi* is > 85%, similar to the scores of other filaria with complete genome sequences, indicating the high quality of these *Mansonella* genomes. We performed orthology analysis and comparisons to other filarial parasites to identify shared as well as species-specific proteins. The availability of genomes sequences from *M. perstans* and *M. ozzardi* will provide further insight into the evolution and phylogenetic relationships between the *Mansonella* species from different continents. The genomes will also serve as an important resource for further biochemical, molecular and genetic studies, as well facilitate the development of new diagnostic biomarkers and therapeutic tools.

703

FILARIAL POPULATION GENOMICS AND ITS ROLE IN ELIMINATION PROGRAMS

Warwick Grant[1], Michel Boussinesq[2], Katie Crawford[1], Patricia Graves[3], Shannon Hedtke[1], Annette Kuesel[4], Colleen Lau[5]

[1]*La Trobe University, Bundoora, Australia,* [2]*IRD UMI* [233]*-INSERM U*[1175]*-Montpellier University, Montpellier, France,* [3]*James Cook University, Cairns, Australia,* [4]*WHO/TDR, Geneva, Switzerland,* [5]*Australian National University, Canberra, Australia*

Lymphatic filariasis and onchocerciasis have been targeted for elimination, primarily using mass drug administration at the community level, with global elimination as a public health problem the endpoint for lymphatic filariasis and elimination of transmission in 80% of affected sub-Saharan countries by 2025 as the current onchocerciasis target. Where duration, treatment coverage, and compliance are sufficiently high, it has been demonstrated that elimination is achievable for both parasites within defined geographic areas. However, transmission has re-emerged after apparent elimination in some areas, and in others has continued despite years of drug treatment. a critical question is whether this observed re-emergence and/or persistence of transmission is due to local parasites—i.e., the result of inadequate duration, drug coverage, poor response of the parasite to drugs, or inadequate methods of assessment and/or cutoffs for determining when to stop treatment—or due to parasites introduced to the area via human or vector movement from another endemic area. We review population genomics in *Onchocerca volvulus*, the filarial nematode that causes onchocerciasis, and *Wuchereria bancrofti*, the major pathogen for lymphatic filariasis. We focus in particular on the combination of genetic epidemiology and genome-wide associations to define transmission zones and distinguish between local and introduced parasites as the source of resurgence and to identify genetic markers associated with parasite response to chemotherapy. Our ultimate goal is to assist elimination efforts by developing easy-to-use tools that incorporate genetic information about transmission and drug response for more effective mass drug distribution and surveillance strategies.

704

ONCHOCERCA VOLVULUS SECRETOMES: A SOURCE OF POTENTIAL TARGETS FOR DETECTING VIABLE PARASITES

Sasisekhar Bennuru[1], Sara Lustigman[2], Thomas Nutman[1]

[1]*National Institutes of Health, Bethesda, MD, United States,* [2]*New York Blood Center, New York, NY, United States*

As efforts shift from control to elimination of *Onchocerca volvulus (Ov)*, additional tools to identify those contributing to ongoing Ov transmission in low transmission settings will be needed to reliably detect viable adult female. Building on our previously identified targets for antigen detection, we did comparative proteomic analyses of the secretomes of adult male (AMES) and adult female (AFES) with previously published somatic proteomes of adult female (OvAF), adult male (OvAM), microfilariae (MF), embryonic stages (EMB), L3 and L4 larval stages. Compared to the AFES (650 proteins), the AMES had 7 times as many proteins (~4600) detected. Principal component analyses indicated a high degree of similarity between the OVAFES with the somatic proteomes of MF and embryonic stages. Multivariate analyses resulted in the identification of clusters of proteins that were commonly detected in the AFES, OvAF, MF and/or EMB. Functional analyses of the proteins suggest that the AMES were enriched for proteins involved in nuclear export, post-translational modifications, and peptidase activity. In contrast AFES was enriched for unspecified secreted class of proteins and those that had endopeptidase inhibitor activity. To evaluate if any of the proteins identified commonly between OvAF-AFES or OvAF-AFES-EMB/MF, could be detected in body fluids of infected individuals, we also performed proteomic profiles of serum, exosomes and urine, and compared these with uninfected control fluids. The intersection of these data show that there only a relatively few proteins commonly found in each of these developmental stages and/or anatomical compartments. Among these, however, are a number of potential targets that can be exploited for biomarker assessment in onchocerciasis.

705

THE ANTHELMINTIC PRAZIQUANTEL ACTIVATES A SCHISTOSOME TRANSIENT RECEPTOR POTENTIAL CHANNEL

Jonathan S. Marchant, Sang-Kyu Park

Medical College of Wisconsin, Milwaukee, WI, United States

Schistosomiasis (Bilharzia) is a parasitic worm infection that infects over 200 million people worldwide. No effective vaccine currently exists and the drug praziquantel (PZQ), discovered around 40 years ago, is the key clinical therapy. The clinical formulation of PZQ is a racemate (±PZQ) composed of the enantiomers (*R*)-PZQ and (*S*)-PZQ. (*R*)-PZQ causes Ca^{2+} influx and spastic paralysis of adult worms, with (*S*)-PZQ acting as a less active distomer. From a treatment perspective, it is problematic that despite decades of clinical use, as well as demonstration of PZQ resistance in both lab and field, the target of PZQ remains unknown. This lack of knowledge has proved a longstanding roadblock in schistosomiasis chemotherapy. Resolution of the target of PZQ action in schistosomes would facilitate discovery of new anthelmintics and novel vulnerabilities of parasitic flatworms to chemotherapy. Here, we demonstrate (*R*)-PZQ activates a Ca^{2+}-permeable schistosome transient receptor potential (TRP) channel in *Schistosoma mansoni*, christened *Sm*.$TRPM_{PZQ}$. *Sm*.$TRPM_{PZQ}$ was activated by ±PZQ with an EC_{50} of 1.08±0.14μM in a Ca^{2+} imaging assay and this activation was stereoselective, with the (*R*)-PZQ evoking Ca^{2+} signals over a considerably lower concentration range (EC_{50} of 597±10nM) than (*S*)-PZQ (EC_{50} of 27.9±3.1μM). At 37°C, (*R*)-PZQ activated *Sm*.$TRPM_{PZQ}$ over an even lower concentration range (EC_{50}=154±33nM, Figure 2E), corresponding to the concentration-dependency of (*R*)-PZQ evoked contractions of schistosome worms *in vitro*. Analysis of *Sm*.$TRPM_{PZQ}$ in transcriptomic datasets evidences expression across various schistosome life cycle stages, and analysis of flatworm genomes revealed expression of $TRPM_{PZQ}$ in both free-living and parasitic flatworms that exhibit sensitivity to PZQ. These data provide the first report of a schistosome target activated by PZQ, are consistent with *Sm*.$TRPM_{PZQ}$ being a target of this clinically important therapeutic

706

EVALUATION OF A PROTOTYPE DUAL ANTIGEN RAPID TEST TO DETECT EXPOSURE TO *ONCHOCERCA VOLVULUS*

Vitaliano A. Cama[1], Guilherme Maerschner Ogawa[1], Alison Golden[2], Austin Newsam[1], Sara Lustigman[3], Paul T. Cantey[1], Thomas B. Nutman[4], Sasisekhar Bennuru[4]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*PATH, Seattle, WA, United States,* [3]*New York Blood Center, New York, NY, United States,* [4]*National Institutes of Health, Bethesda, MD, United States*

Serological tools for onchocerciasis are currently limited to the OV-16 ELISa and rapid diagnostic test, with reported high specificity (>98%) but

sensitivities between 45-80%. Therefore, there is a need for improved assays to support elimination mapping. The novel recombinant antigen OVOC3261 was identified from >400 *O. volvulus* protein candidates spotted on a protein array and screened for IgG4 reactivity with sera samples of infected individuals. The performance of OVOC3261 (~82% sensitivity) alone and in conjunction with OV-16 to detect IgG4 antibodies in skin snip positive patients was assessed and validated by Enzyme Linked Immunosorbent Assay (ELISA), a multiplex bead assay (Luminex™) and luciferase immunoprecipitation systems (LIPS). a prototype dual antigen rapid test (DART) was developed; reactivity to either (or both) antigens was considered positive. Two well-characterized panels of serum (n=279) and dried blood spots (DBS, n=440) were assembled to evaluate the diagnostic performance of DART. Each test was read by 3 different people and scored band intensities relative to a semi-quantitative color intensity scale. DART had a combined sensitivity of 93.9% (OVOC3261 87.9%, OV-16 85.5%) and a specificity of 83.1% (OVOC3261 89.2%, OV-16 92.3%) when used with serum, and a 90% sensitivity (OVOC3261 81.4%, OV-16 73.3%) and 94.6% specificity (OVOC3261 95.3%, OV-16 99.2%) when tested with DBS. Data from 231 individuals tested in both panels showed lower band intensities with DBS when compared to serum ($p<0.01$, paired t-test). Overall, OVOC3261 added to the sensitivity of OV-16, although with some reduction in specificity. DART is simple to use and could support the ongoing efforts to eliminate the transmission of onchocerciasis.

707

IN SILICO IDENTIFICATION OF NEW BIOMARKERS AND DEVELOPMENT OF RAPID DIAGNOSTIC TESTS FOR THE FILARIAL PARASITES *MANSONELLA PERSTANS* AND *MANSONELLA OZZARDI*

Catherine B. Poole[1], Amit Sinha[1], Laurence Ettwiller[1], Lynne Apone[1], Karen McKay[1], Vaishnavi Panchapakesa[1], Nathália F. Lima[2], Marcelo U. Ferreira[2], Samuel Wanji[3], Clotilde K. Carlow[1]

[1]*New England Biolabs, Ipswich, MA, United States,* [2]*University of São Paulo, São Paulo, Brazil,* [3]*University of Buea, Buea, Cameroon*

Mansonelliasis is a widespread yet neglected tropical infection of humans caused by any of three species of filarial nematodes, *Mansonella perstans*, *M. ozzardi* and *M. streptocerca*. *M. perstans* is found throughout Saharan Africa and in South America, whereas *M. ozzardi* and *M. streptocerca* are localized to certain areas of Latin America or Africa, respectively. Clinical symptoms are nondistinct and diagnosis mainly relies on the detection of microfilariae in skin or blood. Species-specific DNA repeat sequences have been used as highly sensitive biomarkers for filarial nematodes such as *Brugia malayi*, *Loa loa* and *Onchocerca volvulus*. We have developed a bioinformatic pipeline to mine Illumina reads obtained from sequencing *M. perstans* and *M. ozzardi* genomic DNA for new biomarker candidates. This approach led to the discovery of species-specific, repeat-based biomarkers and the successful development of loop-mediated isothermal amplification (LAMP) diagnostic tests with remarkable levels of specificity and sensitivity for each *Mansonella* infection. The assays performed successfully on clinically defined human samples and experimentally infected *Culicoides milnei*. These new tools are field deployable and suitable for the reliable detection and differentiation of *M. perstans* and *M. ozzardi* from other co-endemic filarial species in human hosts and insect vectors.

708

LOA LOA: DETECTION OF CIRCULATING CELL-FREE DNA IN BODY FLUIDS

Frimpong Kodua[1], Sasisekhar Bennuru[1], Papa Drame[2], Eric Dahlstrom[3], Thomas Nutman[1]

[1]*National Institutes of Health, Bethesda, MD, United States,* [2]*Duke Global Health Institute, Duke University, Durham, NC, United States,* [3]*National Institutes of Health, Hamilton, MT, United States*

We have previously described the utility of qPCR-based detection of *Loa loa (Ll)*microfilariae (mf) that detected a single copy gene LLMF72. Using pipelines based on raw reads from next generation sequencing data and RepeatExplorer, we identified 15 high copy tandem repetitive elements in the *Ll*genome (with coverage across the genome ranging from 1000-20000x). Using custom primer-probe sets designed to amplify each of these 15 repeats we assessed their ability to amplify genomic DNA of *Loa*loa and not amplify the often co-endemic filarial species*Onchocerca volvulus*and *Wuchereria bancrofti*. Among all of the repeats, one LL2643 was found to be the most sensitive, though each primer probe set had 100% specificity. When compared to LLMF72, LL2643 was found to be the 256-fold more sensitive by qPCR with a limit of detection reliably shown to be 1fg/ul. Given the increased sensitivity of the assay to detect gDNA equivalent to $1/10^{th}$of a mf, we tested the utility of LL-2643 in detecting circulating cell-free DNA (ccfDNA) in Ll-infected individuals. Analyses of the ccfDNA extracted from serum/plasma of 32 mf+ Ll-infected individuals indicated a significant correlation between Ct values and mf counts ($r=-0.68$, $p<0.0001$). More importantly, in patients that were followed longitudinally following treatment, almost all of the patients' ccf DNA was undetectable. Interestingly, the kinetics of the disappearance of ccfDNA showed a marked reduction of Ll DNA at 1 month following diethylcarmbazine therapy ($p<0.001$) that continued to fall over to undetectable over time. Currently, the assay is being tested to evaluate if the ccfDNA can be detected in urine of infected individuals so that a non-invasive method of diagnosis and treatment can be developed.

709

CTL4 GENE-KNOCKOUT TO BLOCK *PLASMODIUM* INFECTION IN THE VECTOR MOSQUITO

Maria L Simoes, Yuemei Dong, Godfree Mlambo, George Dimopoulos

Johns Hopkins University, Baltimore, MD, United States

The development of genetically modified (GM) mosquitoes for malaria control has gained strength through the recent advances in gene-drive technology and an increased understanding of vector-parasite interactions. While most work aiming at the development of GM malaria resistant mosquitoes, suitable for population replacement, has focused on the over-expression of anti-parasitic genes, here we have explored a strategy relying on CRISPR/Cas9-based inactivation of mosquito encoded *Plasmodium* agonists. During its sexual cycle inside the mosquito vector, *Plasmodium* engages in intimate interactions and relies in numerous *Anopheles*-derived host factors, which act as facilitators of infection. The C-type lectin CTL4 has been identified as a *Plasmodium* agonist (Osta *et al*., 2004). in our recent work we showed that the C-type lectin-mediated protection against parasite melanization in the African vector *A. gambiae* is dependent on the intensity of infection, rather than the mosquito-parasite combination (Simões *et al*., 2017). RNa interference (RNAi)-based silencing of CTL4 resulted in melanization and reduction of live parasites, albeit RNAi results in only partial gene silencing. We hypothesized that the knockout (KO) of CTL4 would result in complete melanization of the parasites, and consequently a complete halt of *P. falciparum*'s development inside the mosquito. We are currently using CRISPR/Cas9 technology for targeted CTL4-KO in *A. gambiae*. We generated gRNa overexpressing *A. gambiae* transgenic lines that were crossed with the Vasa::Cas9 strain, to generate CTL4-KO *A. gambiae* mutants. These CTL4-KO GM mosquitoes are being evaluated for parasite blocking of total *Plasmodium*-agonist disruption. The effects of CTL4-KO on bacterial and fungal development inside the mosquito are also being screened.

710

INSECT STEROID HORMONE SIGNALING REGULATES NON-COMPETITIVE *PLASMODIUM FALCIPARUM* DEVELOPMENT IN *ANOPHELES GAMBIAE* MOSQUITOES

Kristine Werling[1], W. Robert Shaw[1], Maurice A. Itoe[1], Kathleen A. Westervelt[1], Perrine Marcenac[1], Douglas G. Paton[1], Duo Peng[1], Naresh Singh[1], Andrea L. Smidler[1], Adam South[1], Amy A. Deik[2], Liliana Mancio-Silva[3], Allison R. Demas[3], Sandra March[3], Eric Calvo[4], Serge Rakiswendé Yerbanga[5], Thierry Lefèvre[5], Abdoulaye Diabaté[5], Roch K. Dabiré[5], Sangeeta N. Bhatia[3], Clary B. Clish[2], Flaminia Catteruccia[1]

[1]*Harvard TH Chan School of Public Health, Boston, MA, United States,* [2]*Broad Institute, Cambridge, MA, United States,* [3]*Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, United States,* [4]*Laboratory of Malaria and Vector Research, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [5]*Institut de Recherche en Sciences de la Santé/Centre Muraz, Bobo-Dioulasso, Burkina Faso*

Anopheles gambiae mosquitoes are the major African vectors of *Plasmodium falciparum*, the deadliest human malaria parasite. Transmission of this parasite occurs when a female mosquito takes a blood meal to produce eggs, but whether parasite development interacts with oogenesis in the initial phases after blood feeding remains largely unknown. Here we show that in the naturally occurring *An. gambiae-P. falciparum* association, parasite development is non-competitively linked to biological processes leading to egg development. We reveal that *An. gambiae* experience no reproductive costs of *P. falciparum* infection, such that there is a positive correlation between the number of eggs a female develops and the number of oocysts in her midgut. This interaction is regulated by the insect steroid hormone 20-hydroxyecdysone (20E) produced by the female post-blood meal, as impairing 20E signaling breaks the positive egg-oocyst correlation and leads to fewer oocysts—suggesting that 20E may regulate relative female investments in reproduction and immunity. Strikingly, females that invest less in reproduction support faster parasite development, via the accumulation of lipids carried by transporters involved in oogenesis. Faster growth in turn leads to earlier sporozoite infectivity, increasing the chances of parasite transmission. We observe similar phenomena in field infections with *P. falciparum* parasites from West Africa, where we additionally reveal that parasite genetic components affect the interaction of parasite growth with 20E-regulated mosquito metabolism. Overall, our results suggest that *Plasmodium* has adopted a developmental strategy in *Anopheles* that limits costs to the mosquito while also promoting its transmission. Moreover, by highlighting the ability of the parasite to rapidly adapt in response to changes in mosquito physiology, our findings have profound implications for currently proposed vector control tools targeting mosquito reproduction.

711

KNOCKOUT OF *ANOPHELES STEPHENSI* LRIM1 USING CRISPR-CAS9 REVEALS ITS CRUCIAL ROLE IN VECTOR COMPETENCE

Ehud Inbar[1], Abraham Eappen[1], Robert Alford[2], William Reid[1], Tao Li[1], Robert Harrel[2], Sumana Chakaravarty[1], Donald F. Ward[1], Kim Lee Sim[1], Stephen L. Hoffman[1], Peter F. Billingsley[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Insect Transformation Facility, Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, United States*

Sanaria® PfSPZ Vaccine and PfSPZ-CVac confer high protective efficacy against *Plasmodium falciparum* (Pf) infections. These vaccines are composed of aseptic, purified, cryopreserved Pf sporozoites (SPZ), and are manufactured using aseptically grown *Anopheles stephensi*. *Anopheles* immune genes such as Leucin-Rich Immune (LRIM) protein and Thioester-Containing Protein 1 (TEP1) inhibit *Plasmodium* sporogony by melanization and phagocytosis of the oocysts. Knocking out these genes should theoretically increase PfSPZ infection intensities and improve manufacturing efficiency of PfSPZ products. We generated an *A. stephensi* LRIM1 knockout line, Δ*aslrim1*, using CRISPR-Cas9. LRIM1 protein was undetectable in homozygous Δ*aslrim1* by western blot with anti-AsLRIM1 antibody. Knocking out LRIM1 profoundly affected the mosquito. The bacterial load increased overwhelmingly and the microbiome composition was altered. These alterations probably accounted for the increased adult mosquito mortality. There was also a reduction in the number of eggs oviposited, and the fertility of males decreased. Contrary to expectation, Δ*aslrim1* non-aseptic mosquitoes were unable to support growth and development of PfSPZ. Pf infection intensities were partially restored when adult mosquitoes were maintained on 10% sugar solution containing antibiotics, and fully restored when Δ*aslrim1* mosquitoes were produced aseptically. In summary, LRIM1 is crucial to *A. stephensi*, being essential for survival of mosquitoes (probably because of their reduced capacity to survive bacterial insult), affecting reproductive capacity. Reductions in Pf infections are probably due also to bacterial overload as the ability to support growth and development of Pf in aseptic mosquitoes is unaffected.

712

BACTERIAL SUPPRESSION OF MALARIA TRANSMISSION BY MOSQUITOES

Wei Huang[1], Janneth Rodrigue[2], Alfonso Mendoza-Losana[2], Marcelo Jacobs-Lorena[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*GlaxoSmithKline plc, Tres Cantos, Spain*

The intolerable burden of malaria demands the urgent development of novel approaches to fight this deadly disease. Previously, we have shown that engineered mosquito symbiotic bacteria can render mosquitoes resistant to the parasite. However, translation of these findings to the field faces major regulatory barriers. Here we describe two non-modified symbiotic bacteria - *Delftia* sp. and *Pseudomonas* sp. These bacteria were originally isolated from mosquitoes that had lost the ability to sustain the development of *Plasmodium falciparum* parasites. While *Pseudomonas* easily colonizes the mosquito and is transmitted vertically, it is a poor inhibitor of *Plasmodium* development. *Delftia* on the other hand, is a potent inhibitor of *Plasmodium* development in mosquitoes. This bacterium does not impose a fitness load: it does not affect mosquito survival, blood feeding behavior, fertility or fecundity. *Delftia* has promise for use in the control of malaria.

713

NUCLEASES IN THE MOSQUITO GUT REDUCE EFFICIENCY OF RNA INTERFERENCE

David J. Giesbrecht[1], David Boguski[2], Ian Wiens[1], Lisa Zhan[1], Daniel Heschuk[1], Steve Whyard[1]

[1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*Fisheries and Oceans Canada, Winnipeg, MB, Canada*

Sterile insect technique (SIT) against mosquitoes suffers from high costs of sex-sorting and sterilization. RNa interference (RNAi) against transcripts essential for sex-determination and testis development has been proposed as a cost-effective means of producing sterile males for SIT. For this goal to be realized, optimization of dsRNa delivery is needed. Because endogenous nucleases can impede dsRNa delivery in some insects, we investigated the impacts of nucleases in mosquito guts. Ten putative nucleases were identified in the *Aedes aegypti* genome, with two highly expressed in the guts of larvae. Using an *ex-vivo* assay, we observed that dsRNa is rapidly degraded within the mosquito larva's gut. Double-stranded RNa targeting these two nucleases, when fed to the larvae, effectively reduced both the nuclease transcripts and gut nuclease activity. When these nuclease-specific dsRNAs were co-delivered with dsRNa targeting a reporter gene, improved knockdown of the reporter gene's transcripts was observed.

These results suggest that inhibiting nuclease activity could bring RNAi-based SIT within reach. Finally, we discuss several approaches to package dsRNa in mosquito food to avoid nuclease degradation.

714

INTERACTION BETWEEN PLASMODIUMAL PHATUBULIN AND *ANOPHELES* FREP1 ASSISTS MALARIA TRANSMISSION

Genwei Zhang[1], Guodong Niu[2], Manpreet Kaur[2], Luara Perez[2], Xiaohong Wang[2], **Jun Li**[2]

[1]*University of Oklahoma, Norman, OK, United States,* [2]*Florida International University, Miami, FL, United States*

Passage of *Plasmodium* through a mosquito is essential for malaria transmission. FREP1, a mosquito midgut peritrophic matrix protein, binds to the parasite and mediates *Plasmodium* infection in *Anopheles*. Notably, this FREP1-mediated *Plasmodium* invasion pathway is highly conserved across multiple species of *Plasmodium* and *Anopheles*. Previous experiments show that FREP1 binds to *P. falciparum*. FREP1 also binds to rodent malaria pathogen *P. berghei*. Furthermore, nine *P. berghei* proteins were co-precipitated with FREP1-conjugated beads. After cloning these nine genes and expressing them in insect cells, six of them were confirmed to interact with FREP1. Among them, α-tubulin and heat shock protein 70 (Hsp70) are highly conserved in *Plasmodium* species with >95% identity. Thus, antiserum against α-tubulin and Hsp70 were produced in mice to determine their effects on *Plasmodium* transmission. Our results show that anti-α-tubulin serum significantly inhibits *P. falciparum* transmission to *An. gambiae*, while anti-Hsp70 serum does not. In addition, purified rabbit polyclonal antibody against human α-tubulin blocks *P. falciparum* transmission to *An. gambiae*. Although α-tubulin is normally inside a cell, fluorescence microscope assays show that anti-α-tubulin Ab can access and bind to *Plasmodium* ookinete apical invasive apparatus. Therefore, we propose that the interaction between FREP1 and α-tubulin may direct the ookinete invasive apparatus towards midgut PM for efficient parasite invasion.

715

IMMUNITY AND MEMORY AGAINST MALARIA: AN ATLAS OF THE MOSQUITO IMMUNE SYSTEM AT SINGLE-CELL RESOLUTION

Gianmarco Raddi

University of Cambridge/Wellcome Sanger Institute, Cambridge, United Kingdom

Malaria is yearly responsible for 219 million cases and over 400,000 deaths. *Anopheles gambiae* mosquitoes are the main African vectors for the most virulent malaria parasite: *Plasmodium falciparum*. Mosquitos are not mere bystanders however, and rely on both humoral and cellular innate immune responses to defeat invading pathogens, including malaria. These efforts are coordinated by hemocytes, the insect equivalent to vertebrate's white blood cells. Yet, hemocyte biology is largely unknown, mainly due to their fragility and low numbers. in order to identify unknown cell types, their gene signatures, and their spatial-temporal localization in the mosquito we isolated *Anopheles* hemocytes and characterized them by single-cell RNa sequencing. a total of 5,218 individual *Anopheles* hemocytes were profiled 1,3 and 7 days after sugar-feeding, blood-feeding, or infection with *Plasmodium berghei*. Ten cell sub-types were identified, including novel effector, inhibitory, phagocytic, and secretor cells. Bulk RNAseq of *Anopheles* hemocytes, guts, and carcasses was also performed to identify selective marker genes. The putative cell types were validated with fluorescence in situ hybridization (RNA-FISH) in mosquito sections, whole guts and carcasses, and isolated hemocytes, showing an increase in active granulocytes and novel effector cells with malaria infection. After validation, challenged hemocytes' transcriptomic changes with time were investigated to understand hemocyte lineage and development. Both a rapidly dividing hemocyte progenitor pool and a more general trajectory of cell activation were identified, showing a progressive increase in immunity, signal transduction, spliceosome, and cell cycle genes from day 1 to 2 and 3, before returning to baseline at day 7.Finally, *Plasmodium* infection leads to a dramatic increase of the novel secretor cell type, as well as active granulocytes. Our results are the first comprehensive transcriptomic study of a whole invertebrate organism's immune system, demonstrating hemocytes' complexity far exceeds what is currently described in the literature

716

IMPLEMENTING MALARIA DIAGNOSTIC COMPETENCY ASSESSMENT COURSES IN NON-ENGLISH SPEAKING COUNTRIES OF AFRICA

Mamadou Alpha Diallo[1], Mame Cheikh Seck[1], Ibrahima Diallo[2], Khadim Diongue[1], Aida Sadikh Badiane[1], Mouhamadou Ndiaye[1], Daouda Ndiaye[1]

[1]*Cheikh Anta Diop University, Dakar, Senegal,* [2]*National Malaria Control Program, Dakar, Senegal*

Accurate malaria diagnosis is important for rational use of antimalarials and prevent drug resistance. Hence, a need to implement a comprehensive and regular malaria microscopy training and competency assessment program. Since 2016, Cheikh Anta Diop University (UCAD) is conducting External Competency Assessment in malaria microscopy (ECAMM) courses for non-English speaking countries in Africa. Here, we assess the outcomes of the first three years of this program. The audience was limited to 12 individuals per course. Each course was conducted over five consecutive days. On Day 1, a pre-assessment of participants in malaria theoretical knowledge and malaria microscopy was done. Days 2 through 4 comprised both learning units and assessment modules for malaria microscopy. Participants were assessed on parasite detection, species identification, and parasite quantitation. The slides sets were from the WHO Slide Bank. Only Level 1 and Level 2 microscopists were certified as WHO experts. Since 2011, twelve ECAMM courses have been conducted with 138 participants from 22 countries. Out of the 138, 96 (69.6%) were certified as experts either as level 1 or level 2. Eighteen countries had at least one microscopist certified. Out of the 138, 116 participants (84.1%) had both their sensitivity and specificity higher than 90%. The assessment results showed that accuracy in parasite detection ranged from 68% to 100%, with 60 out of 138 participants achieving 100%. Accurate results for species identification were variable, ranging from only 39% to 100%, with 55 out of 138 achieving more than 90%. The results for parasite counting were also variable, ranging from 14% to 100%, with 81 out of 138 achieving 50% or more of their counts within 25% of the true count. Significant improvements were reported in malaria microscopists after attending the program. Although all participants gained knowledge and awareness about the benefits of ECAMM courses, its implementation should be extended to more participants. We recommend that all higher officials and policymakers in the field of malaria to pay attention to it and allocate adequate budget on a continuous basis.

717

ENGAGING COMMUNITIES TO SUPPORT CONSENTING FOR MINIMALLY INVASIVE TISSUE SAMPLING (MITS) PROCEDURE: LESSONS LEARNED FROM BANGLADESH

Faruqe Hussain[1], Emily Gurley[2], Md. Saiful Islam[1], John Blevins[3], Ahoua Kone[3], Abdush Suban Mulla[1], Abu Uzayer[1], Afroz Zahan[1], Aziz Ahmed[1], Shikha Datta Gupta[1], Suruj Ali[1], Abdullah Al Masud[1], Mamunur Rashid[1], Ahmed Shahriar[1], Shams El Arifeen[1], Shahana Parveen[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka, Bangladesh,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Rollins School of Public Health, Emory University, Atlanta, GA, United States*

The Child Health and Mortality Prevention Surveillance (CHAMPS) Network is using community engagement platform to involve the community into the implementation of post-mortem minimally invasive tissue sampling (MITS) using needle, to identify the aetiology of death among <5 children

in Baliakandi sub district, Bangladesh. Communities could confuse MITS with full autopsy leading to refusal. We aimed to establish and sustain trust and partnership engaging community leaders and residents to facilitate decision making on MITS consent. We first conducted 50 institutional level consultations with community and religious leaders and then held meetings in the courtyards and tea stalls with general adult residents between May 2017 to March 2018, before and after inception of MITS in 3 different health facilities. We informed and educated ~28% adult populations (out of 146,601) through 810 meetings in 261 villages. Community meetings provided a useful platform responding to community queries and concerns related to MITS procedure. Almost all of the community and religious leaders consulted showed positive responses to determine cause of child death through MITS implementation. Cascading sharing information and consultation from community leaders to general community residents built trust and partnership. Communities appreciated the respect CHAMPS team showed to families of deceased children and team participation in funeral activities facilitated trust. Clarifying from the beginning the extent of what the program will offer effectively minimized community expectations to financial or medical care support beyond the program scope and reduced further queries and arguments. Greater than half (53%) of parents and relatives of deceased children who consented for MITS procedure were exposed to engagement activities. Engaging the residents who live remotely requires additional time and effort. It is important to actively engage community leaders and residents to develop and strengthen trust, increase acceptance and minimize expectations when implementing socio-culturally sensitive community-based public health programs.

718

HEALTH AND SAFETY OF UCLA INTERNAL MEDICINE RESIDENTS PARTICIPATING IN SHORT-TERM INTERNATIONAL CLINICAL ELECTIVES

Jesse E. Ross, Noah Kojima, Chris Tymchuk

University of California Los Angeles, Los Angeles, CA, United States

International clinical electives continue to be very popular among resident physicians; however, very little is known about their health and safety while on these electives. The UCLa Department of Medicine Global Health Pathway offers short-term international clinical electives to 25 residents each year to rotate at partner sites in Malawi, Peru and Thailand. All residents undergo a mandatory pre-departure orientation, which consists of lectures with Infectious Disease faculty and a pre-travel visit with a travel medicine physician. Residents are also directly supervised by a UCLa faculty member on site for the majority of their elective. We surveyed past participants of the electives to determine if there were any significant health or safety issues that they faced or were diagnosed with during or after their electives. We distributed an anonymous, online survey to 142 former participants and received 63 responses for a response rate of 44%. The majority of respondents rotated at our partner site in Lilongwe, Malawi (87%) and the remainder at partner sites in Iquitos, Peru (11%) and Bangkok, Thailand (2%). The results of our survey revealed the most common adverse health events were: diarrhea (45%), fever (17%), and vomiting or flu like symptoms (7%). None of the reported adverse health events led to hospitalization or early termination of the elective. Four participants (6%) reported a bodily fluid exposure, with two exposures necessitating the initiation of HIV post exposure prophylaxis. There were no reported HIV seroconversions among the respondents. While 19% of participants reported a concerning tuberculosis exposure, there were no reported PPD or IGRa conversions. Three participants reported concern for personal safety and one reported being a victim of robbery. While this study is limited by its retrospective nature and a relatively low response rate, the results suggest that short-term international clinical electives are safe if appropriate steps are taken to mitigate any potential health or safety risks of performing clinical work in international settings.

719

BUSINESS MODEL INITIATIVES TO IMPROVE ACCESS TO ESSENTIAL MEDICINES IN LIMITED RESOURCES COUNTRIES. A PHARMACEUTICAL COMPANY'S APPROACH

Harald Nusser, Tayyab Salimullah, Viviam Canon, Nadine Shecker, Rachel Hinder, Rebecca Stevens

Novartis Social Business, Basel, Switzerland

According to WHO ~2 billion people have no access to essential medicines. Pharmaceutical companies are requested to contribute to improving access for patients in limited resources settings. Novartis Social Business (NSB) is a unit based on a patient-centric model aiming to support under-resourced healthcare systems, placing equal weight on financial and social returns. NSB brings together different access to medicine programs; in addition to the well established Malaria initiative that by 2018 has delivered without profit more than 880m antimalarials, NSB includes "Healthy Family", "SMS for life" and "Novartis Access". Healthy Family has built local, sustainable capabilities for healthcare in India, Kenya and Vietnam, where local health educators teach their communities, host educational meetings and guide for seeking diagnosis and treatment from a qualified doctor. Only in 2018, ~7.8 million people attended health educational meetings; there were 15.123 health camps in place, providing medical care to more than 700.000 people. In addition, a pilot was conducted in Jigani, India to incorporate digital tools and test online doctor's support to trained nurses when they are with patients in the field. "SMS for life" was implemented initially in 2009 in Tanzania, and more recently in Zambia as a tool for surveilling availability of essential medicines and diagnostic tests for Malaria, HIV and TB in remote rural areas. "Novartis Access" includes a portfolio available to governments, NGOs and other public sector providers in low-income countries at a price of UDS1 per treatment per month, and covers treatments for key diseases including malaria, pneumonia, diabetes, hypertension, asthma, dyslipidemia and some types of cancer. The number of monthly treatment delivered went from 84.448 in 2016 to 2.274.700 in 2018, reaching more than 1.5 m patients the last year. The first year of Novartis Access in Kenya has been recently evaluated and suggests an initial positive impact on the availability of drugs for diabetes and hypertension at the healthcare facility level.

720

BURNOUT AND WELLBEING IN GLOBAL HEALTH: OBSERVATIONS FROM RECIPIENTS OF THE CONRAD N. HILTON HUMANITARIAN PRIZE

David G. Addiss[1], Leslie Leonard[2], Deirdre Guthrie[3]

[1]*Task Force for Global Health, Atlanta, GA, United States,* [2]*Emory University, Atlanta, GA, United States,* [3]*Spore Studios, Three Oaks, MI, United States*

Global health and humanitarian organizations attract highly motivated people who care deeply about social justice and alleviating suffering. They often work in conditions of deprivation, conflict, and chronic stress, which can contribute to burnout. Burnout leads to high staff turnover, decreased performance, and sub-optimal organizational effectiveness. To understand work-related burnout and the capacity of humanitarian organizations to recognize, manage, and prevent it, we invited recipients of the Conrad N. Hilton Humanitarian Prize - among the most influential humanitarian and global health organizations - to complete a survey and participate in interviews. Fourteen human resource (HR) directors completed surveys and 39 semi-structured interviews were completed with CEOs (10), HR directors (10), and up to three staff per organization (19). Factors identified by more than 50% of HR directors as contributing to burnout included: poor relationships with supervisors or other leaders (86%); long, unpredictable working hours (71%), ambiguous job expectations (64%), communication challenges (64%), and barriers to professional and personal growth (57%). HR directors identified significant deficiencies in the quality and effectiveness of resources available to manage employee stress. Perceived barriers to wellbeing included lack of time (86%), funding (64%) and expertise (57%). Interviews revealed four types of stressors:

structural dynamics, including internal (e.g., workload, supervision) and external (e.g., contracts, pressure to perform) factors; and safety, both physical (e.g. threat of violence) and psychological (e.g., lack of civility). Types of stressors differ by gender and setting (e.g., headquarters or "field"), and residency status ("national" or "expatriate"). Staff who over-identify with work find it difficult attend to their own wellbeing. These findings suggest that among elite humanitarian and global health organizations, stress is ubiquitous, burnout is not uncommon, self-care is often sub-optimal, and resources for wellbeing are inadequate.

721

RISK FACTORS FOR INFECTIOUS DISEASES IN URBAN ENVIRONMENTS IN SUB-SAHARAN AFRICA: A REVIEW

Matthew R. Boyce, Rebecca Katz, Claire J. Standley

Georgetown University, Washington, DC, United States

Our world is rapidly urbanizing. According to the United Nations, between 1990 and 2015, the percent of the world's population living in urban areas grew from 43% to 54%. Estimates suggest that this trend will continue and that over 68% of the world's population will call cities home by 2050, with the majority of urbanization occurring in African countries. This urbanization will have a profound effect on global health and could significantly impact the epidemiology of infectious diseases owing to the trade, travel, and migration inherent to cities. a better understanding of infectious disease risk factors specific to urban settings is needed to plan for and mitigate against these impacts. We conducted a systematic literature review of the Web of Science and PubMed databases to assesses the risk factors for infectious diseases in urban environments in sub-Saharan Africa. a search combining keywords associated with cities, migration, infectious disease, and risk were used to identify relevant studies. Original research and meta-analyses published between 2004 and 2019 investigating geographical and behavioral risk factors, changing disease distributions, or control programs were included in the study. The search yielded 414 papers, of which 37 met the criteria for inclusion in the analysis. Papers were categorized according to risk factor, geographic area, and study type. The papers covered 27 countries in sub-Saharan Africa with West Africa the most represented sub-region. Malaria and enteric diseases were the most frequent focus of the studies. The results of this work can inform public health policy as it relates to capacity building and health systems strengthening in rapidly urbanizing countries, as well as highlight knowledge gaps that warrant additional research.

722

COSTING ANALYSIS OF SEROLOGICAL SURVEILLANCE FOR MEASLES AND RUBELLA IMMUNITY IN ZAMBIA

Andrea Carcelen[1], Bryan Patenaude[1], William J. Moss[1], Phil Thuma[2], Simon Mutembo[3], Kyla Hayford[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Macha Research Trust, Choma, Zambia,* [3]*Ministry of Health, Choma, Zambia*

Despite reportedly high vaccination coverage, measles and rubella outbreaks remain common. This is a product of the fact that vaccination coverage is only a proxy measure of population immunity. However, estimating immunity requires biological markers and laboratory testing through serological surveillance. a better understanding of the costs of serosurveys and the tradeoff of cost per information gained is needed to inform how serological surveillance can be optimally used to support immunization programs. There is limited systematically collected data on the actual cost of serosurveys. This study aims to assess the tradeoff between cost and precision of seroprevalence estimates obtained by collecting serological specimens during a household survey to estimate population immunity levels for measles and rubella using empiric data and simulation models. Costs were calculated with data from the Zambian post-campaign coverage evaluation survey using an ingredients-based approach from the government-funded healthcare perspective and were linked with seroprevalence results. To frame the collection of serosurvey data, a costing framework for integrated disease surveillance was adapted to capture the categories of implementation inputs across serosurvey functions. We estimated the total serosurvey cost in Southern province was US $88,382 to collect dried blood spots (DBS) from 658 participants in 16 clusters. Personnel was the largest contributing input (52%) followed by transportation (16%). Compared to a traditional vaccination coverage survey, collecting DBS for serological surveillance added $27,253 to the total survey costs. Cost and precision tradeoffs will be further investigated by simulating various sampling strategies for serological surveys and varying survey parameters. As more countries add specimen collection to their surveys, it is important to understand the costs and cost per information gained. This cost-precision model will allow policy makers to determine what the added cost of collecting specimens and argue the case for using serosurveys to identify potential pockets of susceptible individuals.

723

DEVELOPING GLOBAL HEALTH PRACTITIONERS: A NOVEL INTERNATIONAL HEALTH EXPERIENCE FOR US MEDICAL STUDENTS

Sara U. Schwanke Khilji, Justin Denny

Oregon Health & Science University, Portland, OR, United States

Student interest in global health is burgeoning (Khan 2013). As the number of students seeking international health experiences (IHE) increases, training programs are challenged to accommodate this demand with opportunities that prepare students for work in global health while providing professionally and culturally relevant learning experiences, embedded within an appropriate ethical framework (Melby 2016). The *OHSU Global Southeast Asia Clinical Field Experience* is a novel elective IHE offered through the Oregon Health & Science University (OHSU) School of Medicine. The four-week elective is structured as a hybrid immersion experience, integrating traditional clinical observership opportunities at an OHSU Global partner site in Thailand with interactive didactics led onsite by OHSU faculty, introducing learners to fundamental concepts in global health such as globalization of healthcare, global burden of disease, and ethics (Jogerst 2015). Broadly, the course aims to develop students› ability to conceptualize health as the result of macro-level influences on both individuals and populations - including historical, political, economic, and cultural - and to apply these concepts in a non-US setting. The course was developed in accordance with core OHSU Global programmatic principles, including long-term, bidirectional, and mutually beneficial relationships with partnering institutions in Southeast Asia. It is complemented by a reciprocal four-week observership experience at OHSU for Thai medical students from the OHSU Global partner site in Bangkok. Student narrative feedback has been highly positive, with the majority of students citing the beneficial impact of the experience on their perceptions of what constitutes global health; knowledge and ability to analyze the main drivers of disease globally; and recognition of the importance of cultural humility. Ethical approval for a post-course survey is under development to more fully analyze the impact of the elective on learner knowledge, attitudes, perceptions, and career decisions.

724

INEQUITIES IN THE BURDEN OF FEVER, DIARRHEA AND ACUTE RESPIRATORY INFECTION IN CHILDREN UNDER FIVE IN LOW- AND MIDDLE-INCOME COUNTRIES AND THE ROLE OF INTEGRATED COMMUNITY CASE MANAGEMENT IN TARGETING THOSE MOST AT RISK

Peter Winskill, Ben Lambert, Alexandra B. Hogan, Patrick G. Walker

Imperial College London, London, United Kingdom

Upon surviving the neonatal period, the biggest killers of children aged under five years in low- and middle-income settings are malaria, pneumonia and diarrhoea. The burden of all three diseases can be greatly reduced with prompt access to the effective treatments that are available. However, inequities in society and access to treatment affect both the

risk of acquiring one or more of these conditions as well as being able to access healthcare for appropriate treatment if infected. In an effort to address inequities, the WHO recommends integrated Community Case Management (iCCM) as an "equity-focused strategy that complements and extends the reach of public health services by providing timely and effective treatment of malaria, pneumonia and diarrhoea to populations with limited access to facility-based health care providers, and especially to children under 5". Here we present a multi-country, multivariate, multilevel, Bayesian logistic regression analysis of Demographic and Health Surveys (DHS) data quantifying the inequitable burden of fever, diarrhoea and acute respiratory infection (ARI) in children aged under five years and the relationship between burden and measures of inequity such as wealth, rurality and remoteness. The analysis evaluates international, national and subnational trends in over half a million children in twenty-six countries. We also assess the potential for iCCM to address these imbalances by targeting treatment to those most in need.

725

HEALTH SERVICES IN LONG-TERM REFUGEE CAMPS: IMPLICATIONS FOR INTEGRATION WITH NATIONAL SURVEILLANCE SYSTEMS

Aurelia Attal-Juncqua, Aashna Reddy, Erin M. Sorrell, Claire J. Standley

Georgetown University, Washington, DC, United States

The number of displaced persons today is estimated at over 65 million, the highest ever in history. Of these, 25 million are classified as refugees (over half of which are children), and 10 million are considered stateless. Millions more have been placed at risk of displacement or homelessness over the past decade due to natural disasters and other non-conflict emergencies. Displaced people therefore represent a major population in jeopardy of not receiving essential health services, sometimes over long durations of time. Establishing formal shelters or camps for displaced persons is the traditional approach to addressing their basic needs. However, there is a known risk of emergence and transmission of infectious diseases in crowded camp settings. Moreover, the countries who host displaced persons and refugees are usually low and middle income themselves, and vulnerable to added strains on their health systems, therefore large numbers of displaced persons can threaten national or even global health security. In prolonged resettlement, where camps persist for months, years, or even transition towards establishment as permanent settlements, there is no clear process for maintaining health services long-term or to whom that responsibility falls. In our study we selected three major refugee camps—Zaatari in Jordan, Dadaab in Kenya, and Cox's Bazaar in Bangladesh—as case studies to explore the extent to which health service provision is integrated with the host country's national health system, particularly with respect to surveillance for infectious diseases. Through examination of the literature, as well as key informant interviews, we note the challenges, opportunities, and lessons learned for supporting long-term health systems strengthening during the creation and management of displaced persons camps.

726

INFECTIOUS DISEASE SURVEILLANCE WITH MLAB - AN APP FOR AUTOMATED RAPID DIAGNOSTIC TEST ANALYSIS

Thomas F. Scherr[1], Carson Moore[1], Caison Sing'anga[2], Japhet Matoba[2], Ben Katowa[2], Philip Thuma[2], David Wright[1]

[1]*Vanderbilt University, Nashville, TN, United States,* [2]*Macha Research Trust, Macha, Zambia*

There is no doubt that malaria control strategies have made progress, noted by an estimated 274 million fewer cases and 1.1 million fewer malaria-related deaths reported in the last decade. Significant hurdles remain, however, including case identification, surveillance, and resource management, that must be addressed prior to countries embarking on elimination campaigns. It is implausible that a one-size-fits-all solution exists for malaria elimination, and an endemic nation's decision is based on available resources, geography, population, and current level of disease prevalence. a reactive case detection strategy used in and around Macha, Zambia has shown great success. in this approach, an index malaria case is identified at a health center and a response team travels to the home of the index case to screen and treat nearby individuals. While "test-and-treat" has been successful in localized areas of low transmission, concerns over scalability and sustainability keep the approach from being more widely accepted. Regardless of strategy, the international community agrees that all elimination strategies would benefit from improved surveillance, better information workflow, and data aggregation. There remains a need for a scalable surveillance solution that can be readily implemented by elimination-ready countries. To meet this need, we present mLab, a mobile application for analyzing point-of-care rapid diagnostic tests, as part of a mobile Health and Treat (mHAT) strategy. Together, these address the challenges that elimination campaigns currently face. mLab utilizes image-processing of diagnostic tests to provide an automated, semi-quantitative, rapid malaria diagnosis. This data serves as the gateway to improved surveillance, as the mHAT web platform will more quickly mobilize a reactive-case response team, provide near real-time RDT results reporting, and in-depth analytics. Ultimately, the mHAT approach will enable resource monitoring for improved supply chain management, data-driven decisions, and more targeted interventions for malaria elimination campaigns.

727

A NATIONAL SURVEY OF EMERGENCY MEDICINE PROVIDERS' KNOWLEDGE AND MANAGEMENT OF TROPICAL DISEASES IN THE RETURNING TRAVELER

Nelly Gonzalez-Lepage[1], Ashley Iannantone[1], Megan Rech[2], **Theresa Nguyen**[2]

[1]*Loyola University Medical Center - Stritch School of Medicine, Maywood, IL, United States,* [2]*Loyola University Medical Center - Department of Emergency Medicine, Maywood, IL, United States*

Due to a combination of socioeconomic, climate, and geographical factors, there has been an increased prevalence of tropical diseases within the United States (U.S.). An estimated 87 million U.S. travelers visit international destinations per year, and many of them may present to the Emergency Department (ED) for travel-related illnesses. The objective of this survey study was to assess the level of EM physicians' training and confidence in the diagnosis and management of tropical diseases. We developed a 30 question online survey to explore self-reported gaps in knowledge regarding tropical diseases and to determine the availability of institutional resources for assisting EM physicians in the clinical management of such diseases. This survey was developed, validated, and administered to 834 EM physicians nationwide via the Emergency Medicine Practice Research Network. Descriptive statistics were used to present survey results. a total of 204 responses (24% response rate) were received. Overall, 91.7% of physicians had received prior training specific to treating and/or managing tropical diseases (in medical school, residency, fellowship, or at their current institution). Despite this, 40.7% of EM physicians reported gaps in knowledge regarding which diagnostic tests were appropriate to order for different tropical diseases and 21% reported the absence of an established screening process for returning travelers at their home institution. Of seven tropical diseases (Dengue, Zika, Cholera, Typhoid, Chagas, Malaria and Schistosomiasis), the highest perceived knowledge gap was for Dengue, with 71.1% of physicians reporting a need for Continuing Medical Education (CME) training focused on diagnosis and management. This is the first national survey to date assessing general EM providers' knowledge and management of tropical diseases in the returning adult traveler. Our needs assessment has identified specific gaps in knowledge which can be filled by development of CME resources and guidelines for standardized screening processes within the ED.

728

DEVELOPMENT OF GLOBAL HEALTH CURRICULUM, TRAINING AND PARTNERSHIPS IN AN ACCREDITED MPH PROGRAM IN CLEVELAND, OHIO

Daniel J. Tisch, Ronald E. Blanton, Charles H. King, Peter A. Zimmerman

Case Western Reserve University, Cleveland, OH, United States

The Case Western Reserve University (CWRU) Master of Public Health (MPH) Program developed the Concentration in Global Health in 2008. This coincided with a University-wide NIH-funded framework to link global health training across 5 Schools and 9 departments. This CWRU Framework for Global Health provided the administrative and training environment to identify and develop integrated curricula, workshops, a certificate program, and mentored field experiences. These incorporated MPH students, faculty, and MPH-dual degree programs and partnerships. The MPH Global Health Concentration has recently undergone a systematic revision of global health competencies in order to address revised accreditation standards through the Council on Education for Public Health (CEPH). The program implemented a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis utilizing surveys and feedback from meetings with students, faculty, internal partners, and external partners as part of a three year self study. The Global Health Concentration competencies were re-defined according to discipline standards, academic/training strength and opportunities, and the missions of the MPH program and University. The five new Global Health competencies were linked with curricula, training experiences, assessments, professional needs, outcomes, and partnerships. Alignment of these competencies to courses and learning experiences, as well as our institutional, regional, and global partnerships will be described. The lessons learned from our global health research and training history, self-study, and revised competencies/curriculum will be presented.

729

A QUALITATIVE ASSESSMENT OF VICO (VIGILANCIA INTEGRADA COLABORATIVA) IN GUATEMALA

Mariangeli Freitas Ning[1], Jahn Jaramillo[2], Michael Park[3], Terrence Q. Lo[3], Loren Cadena[4], Olga L. Henao[3], Andres Espinosa-Bode[4]

[1]*TEPHINET/Centers for Disease Control and Prevention, Guatemala City, Guatemala,* [2]*Public Health Institute/Centers for Disease Control and Prevention, Guatemala City, Guatemala,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Centers for Disease Control and Prevention, Guatemala City, Guatemala*

The collaborative integrated surveillance system (VICo) was implemented in 2007 to better understand and characterize the burden of diarrheal, respiratory and febrile illnesses in Guatemala. VICo is a collaboration of the Ministry of Health (MSPAS) of Guatemala, the Universidad del Valle de Guatemala (UVG), and the Centers for Disease Control and Prevention (CDC). To evaluate the usefulness of VICo and inform both the current redesign of the system and new surveillance activities in the Central American region, CDC personnel in Guatemala conducted semi-structured interviews with 39 key stakeholders responsible for the development and operationalization of VICo. Participants included local, regional, and national level staff from MSPAS, UVG, and CDC and were selected using purposive sampling methods. We used grounded theory to explore stakeholder perceptions of VICo and generate recommendations for improvement. Thematic analysis was conducted to identify and summarize emergent themes. Preliminary analysis identified five areas relevant to the usefulness of VICo: inter- and intra- agency communication and collaboration, policy and strategy, use and timeliness of data, simplicity of the surveillance system, and prioritization of activities. It also identified needed improvements in capacity building and workforce development. Further analysis of the usefulness groupings will develop specific themes that underscore the strengths and weaknesses of VICo from the perspectives of the stakeholders and the organizations they represent. The lessons learned will inform decision makers on needed improvements to activities in Guatemala and provide insights for new surveillance activities in the region. Finally, the qualitative assessment approach serves as a model that can be adapted for evaluation and assessment of impact of other surveillance activities. The ongoing evaluation of surveillance is essential to ensure activities are meeting the needs of countries and generating the data needed for the planning and implementation of effective public health policies and strategies.

730

AFRICAN CENTERS OF EXCELLENCE IN BIOINFORMATICS: AN EVIDENCED-BASED APPROACH TO BIOMEDICAL RESEARCH COLLABORATION IN AFRICA

Darrell E. Hurt[1], Christopher Whalen[1], Mamadou Wele[2], Daudi Jjingo[3], Michael Tartakovsky[1]

[1]*National Institute of Allergy and Infectious Diseases, North Potomac, MD, United States,* [2]*University of Sciences, Techniques, and Technologies of Bamako, Bamako, Mali,* [3]*Makerere University, Kampala, Uganda*

The African Centers of Excellence in Bioinformatics, or ACE, is a program established to increase bioinformatics research collaborations for US-sponsored research in a region deeply impacted by emerging and re-emerging pathogens. ACE attempts to overcome some of the fundamental challenges faced by computational researchers in Africa: the lack of access to the tools and cyberinfrastructure that keep skills and understanding current with global research efforts. The Centers are established and nurtured through a public-private partnership facilitated by the Foundation for the NIH between the NIH's National Institute of Allergy and Infectious Diseases and a consortium of research and educational institutions in Africa, their governments, and private sector companies. Their collaboration delivers in-kind contributions of high-performance computing infrastructure, bioinformatics tools, training, and mentoring to students and faculty. The first ACE Center opened its doors in April of 2015 in collaboration with the Republic of Mali and the University of Sciences, Techniques, and Technology of Bamako. The second ACE Center opened in March 2019 at the Infectious Diseases Institute on the Makerere University campus in Kampala, Uganda. This Center, the most advanced to date, includes a telelearning-capable classroom, a visualization lab featuring virtual reality-based training, and significant compute, storage, and network infrastructure, including special provisions for power and efficiency.

731

AN EXPLORATORY STUDY OF THE MIGRATORY PATTERNS OF NOMADIC FULANI OF NORTHEASTERN FOR HEALTH CARE DELIVERY

Oladele B. Akogun

The Health Programme, Common Heritage Foundation, Yola, Nigeria

In Africa, many minority groups such as the nomadic Fulani are often denied access to health because of their itinerant lifestyle. Few migrant communities benefited from the community-directed intervention strategy for increasing access to health service. An understanding of the drivers of migration is necessary for developing universal health coverage that will include the nomads. An 11-month exploratory study of the nomadic Fulani communities around the river Benue-Gongola confluence, Northeastern Nigeria was carried out using qualitative techniques. Three migratory routes were identified. During harmattan season (October-January), the nomads migrate occupy the Benue-Gongola river confluence while their livestock eats from an abundance of dry pasture, during the dry-hot season (February-April) they congregate in the valley taking advantage of the perennial river. The dry-hot season is the best season for implementing health interventions. We trained their volunteers to recognize, diagnose and distinguish between fevers using the rapid diagnostic test kit for malaria. The nomads desert the valley at the onset of the rain (May-June). During July-October when the rain becomes intense and the lowland is flooded large nomadic communities break into manageable units and disperse further uplands where the land is more visible. It is at this time

that nomads are farthest from health facilities. It is the time to apply the health education and skills as well as use the health supplies that they had obtained in previous locations. We identified the types of health activities in which the nomads could participate during each of the periods. While two clans completed a migratory cycle in approximately twelve months, another did so in 24 months and the fourth clan completed a cycle in about 32 months. The commonest diseases follow similar seasons and commodities can be delivered to fit the season's needs. Nomads have a highly effective communication system, revered leadership and organization structure which could be explored for extending the universal health coverage to them.

733

WORKING WITH THE COMMUNITY TO ESTABLISH A HEALTH PROMOTION PLAN IN THE KINDERGARTENS IN THE CONTEXT OF A CHILD MORTALITY SURVEILLANCE IN MANHIÇA, MOZAMBIQUE

Saquina Cossa[1], Maria Maixenchs[2], Felismina Tamele[1], Zubaida Manhenge[1], John Blevins[3], Inacio Mandomando[1], Quique Bassat[2], Khatia Munguambe[1]

[1]*Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique,* [2]*ISGlobal, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain,* [3]*Emory Global Health Institute, CHAMPS Program Office, Emory University, Atlanta, GA, United States*

The ultimate goal of the Child Health and Mortality Prevention Surveillance Program (CHAMPS) is to implement activities focused on maternal and child health with input from the community in collaboration with existing, trusted community organizations and key leaders. Through a community engagement (CE) strategy and with the collaboration of the District's Health, Women and Social Welfare Services, kindergartens from Manhiça District, in Southern Mozambique, were identified as key stakeholders and approached to explore their views on the most prevalent diseases affecting pre-school children and currently prevention measures in order to jointly design a plan to improve children´s health in general. Kindergarten's educators and parents and caregivers of children attending kindergartens were involved in a series of participant meetings, which took place from April to July 2018 in the kindergartens. Reports and field notes were organized in a matrix for data analysis. Twenty-seven meetings were held (19 with parents and caregivers and 8 with kindergartens educators) involving a total of 456 participants. Malaria, fevers, cough and diarrhea were the most prevalent perceived diseases on pre-school children. The main factors affecting child mortality, according to participants, were the fact that the hospital is distant and poorly supplied, the cost of medicines in private pharmacies, the preference for the use of traditional medicine due to lack of access to health care, the poor condition of the kindergartens 'latrines and the fact that kindergartens do not provide lunch to children. Participants proposed health education campaigns on specific diseases, nutrition and hygiene; a mobile health care team to attend to those in distant areas, emergency kits available at the kindergartens; and training health workers to express empathy when providing assistance. a plan has been designed to provide education in nutrition, hygiene and first aid to parents, caregivers and educators and use them as disseminators of information to the wider community. Working with the people involved when designing strategies to improve their own health is crucial.

734

BUILDING TRUST, RESPECT, AND EMPATHY IN PUBLIC HEALTH INTERVENTIONS: AN ETHIOPIAN CASE STUDY

Caroline Ackley[1], Berhanu Damise[2], Ketema Degefa[2]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Haramaya University, Harar, Ethiopia*

In this paper we suggest approaches to community engagement activities that facilitate clinical public health interventions. In Eastern Hararghe, Ethiopia, children under-5 are dying from clinically unknown causes of death, and often buried with an inadequate or non-existent medical history. One reason for uncertainty into causes of death is that 55-60% of child deaths in the study sites are considered community-based according to Kersa Demographic and Health Surveillance.[KA1] The Child Health and Mortality Prevention Surveillance (CHAMPS) study aims to identify leading and underlying causes of death in children under-5 through multiple techniques, including minimally-invasive tissue sampling (MITS). We performed a 13-month qualitative study intended to elicit perspectives related to the community engagement approaches and strategies for conducting MITS, including 8 in-depth interviews, 2 semi-structured interviews, 2 focus groups discussions, and 5 instances of participant observation. Additionally, we analysed reports from 12 community advisory board meetings. We found that trust, respect, and empathy were the most important factors for the community in successful public health interventions. Prior to CHAMPS activities in Ethiopia, communities felt they knew what was causing under-5 child death; namely malnutrition, and poor sanitation and hygiene - each linked to the will of God. Given the lack of clinical data into causes of death, MITS presented an opportunity for the community to gain certainty in their convictions. However, MITS is highly contentious in the study site and the community demanded engagement activities to align study objectives with community priorities, while highlighting the themes of trust, respect and empathy. Based on the findings, we suggest that community engagement be a component in public health interventions to facilitate alignment between biomedical investigation and community perception. For interventions to be successful, socio-cultural and religious understandings of respect, trust, and empathy must first be qualitatively explored and then acted upon in study implementation.

735

MEASURING THE "KNOW-CAN GAP": DIFFERENCE IN KNOWLEDGE AND SKILLS FOR ASSESSING CHILDHOOD DIARRHEA AND PNEUMONIA AMONG PUBLIC AND PRIVATE FRONTLINE WORKERS IN UTTAR PRADESH, INDIA

Lopamudra Ray Saraswati, Ashutosh Mishra, Prince Bhandari, Animesh Rai, Ambrish Chandan

RTI International India, New Delhi, India

In India, frontline workers (FLWs) - public accredited social health activists (ASHAs) and private rural medical providers (RMPs) - play a pivotal role in the early detection and treatment of childhood diarrhea and pneumonia. This study measures *knowledge* and *skills*, and the gap between the two ('*know-can*' gap), related to assessment of childhood diarrhea with dehydration and pneumonia among public and private FLWs, and explores factors associated with them. We surveyed 473 ASHAs and 447 RMPs in six districts of Uttar Pradesh (UP) in India. Knowledge was assessed using face-to-face interviews and skills using video vignettes. While 'sunken eyes' and 'loss of skin turgor' were considered key signs of dehydration, 'fast breathing' and 'chest in-drawing' were considered for pneumonia. The '*know-can*' gap was defined as the lack of skills among those who had knowledge. We used logistic regression (separately for ASHAs and RMPs) to identify the correlates of knowledge (among all) and of skills (in a subset of FLWs that had correct knowledge). FLWs' knowledge related to signs of dehydration ranged from 23% to 48%, and that of pneumonia ranged from 27% to 37%. Their skills ranged from 3% to 42% for dehydration and 3% to 18% for pneumonia. There was a significant '*know-can'* gap in all the signs, except 'sunken eyes'. Regression analyses showed a strong association of training and supervisory support with better knowledge and skills related to diarrhea with dehydration, but only better knowledge related to pneumonia. Mass-media exposure, interpersonal communication with a health worker, and seeing a patient in the last week were other factors associated with correct knowledge and skills. This finding suggests that knowledge-focused trainings are ineffective. Programs should, instead, focus on hands-on trainings followed by refresher trainings. Given the important contribution of FLWs in the Indian health system, the quality of their services will be central to India's continued progress against under-five deaths, and the gap between their knowledge and skills warrants immediate attention.

736

A QUALITATIVE ASSESSMENT OF ACCEPTABILITY DETERMINANTS TO FACILITATE MINIMALLY INVASIVE TISSUE SAMPLING (MITS) IMPLEMENTATION IN QUELIMANE, MOZAMBIQUE

Amilcar Magaco[1], Edu Namarogolo[1], Saquina Cossa[1], Rui Anselmo[1], Dianna Blau[2], Mischka Garel[2], Robert Breiman[2], Jesuel Cassimo[3], Quique Bassat[4], Maria Maixenchs[4], Inácio Mandomando[1], Khátia Munguambe[1]

[1]*Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique,* [2]*Emory Global Health Institute, Atlanta, GA, United States,* [3]*Direção Provincial de Saúde da Zambézia, Quelimane, Mozambique,* [4]*Centro de Investigação em Saúde de Manhiça, Manhica, Mozambique*

The Countrywide Mortality Surveillance for Action (COMSA) aims to implement a child mortality surveillance program through strengthening vital registration event reporting (pregnancy, birth and death) and investigating causes of death (CoD) in sample Mozambique based in verbal autopsies. in Quelimane district (central Mozambique), Minimally Invasive Tissue Sampling (MITS) will be added to fine-tune the CoD determination approaches. Before MITS´ implementation, an evaluation of the feasibility (i.e. acceptability, practicality and implementation) and ethical considerations of child mortality surveillance was considered fundamental. a socio-anthropological study is being conducted at community level. a total of 26 semi-structured interviews (SSI) with health care providers and *nharrube (traditional authorities who washes the bodies before the funeral)* and 14 focus group discussions (FGD) with community leaders and midwives were completed to understand the local attitudes and perceptions related to the death of children. Audio materials were transcribed, systematically coded and analyzed using NVIVO12®. Participants desire to know the CoD influenced the initial discourse in favor of acceptability. However, some participants were skeptical about the procedure. Poor community mobilization, disagreement with Islamic religious practices, and with local traditional beliefs and practices were identified as potential barriers to MITS implementation. in contrast, desire to know the causes of child deaths, intention to clear the elders from accusations of witchcraft, involvement of leaders in the process of disseminating project information, and the provision of transport for bodies back to the community constitute potential facilitators for the implementation of MITS. Although MITS was considered an innovation to determine the CoD in children, community members remain skeptical about the procedure in lifeless bodies due to tensions with religion and tradition. However, this initiative in Quelimane should emphasize the importance of the high involvement of a variety of influential community and religious leaders.

737

BUILDING AN ACCESSIBLE EVIDENCE BASE FOR MEDICAL COUNTERMEASURE USE IN BIOEMERGENCIES - THE SPECIAL PATHOGEN RESEARCH NETWORK INITIATIVE

Lauren M. Sauer[1], Mark J. Kortepeter[2], Nahid Bhadelia[3], Theodore Cieslak[2], Richard Davey[4], Kerry Dierberg[5], Jared D. Evans[6], Maria G. Frank[7], Jonathan Grein[8], Colleen S. Kraft[9], Christopher J. kratochvil[2], Susan McLellan[10], Gregory T. Measer[11], Aneesh K. Mehta[9], Vanessa Raabe[9], George Risi[12], Erica Shenoy[13], Timothy M. Uyeki[14]

[1]*Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*University of Nebraska Medical Center, Omaha, NE, United States,* [3]*Boston University School of Medicine, Boston, MA, United States,* [4]*National Institute of Allergy and Infectious Diseases (NIH), Bethesda, MD, United States,* [5]*Bellevue Hospital, New York, NY, United States,* [6]*Johns Hopkins University Applied Physics Laboratory, Laurel, MD, United States,* [7]*Denver Health Medical Center, Denver, CO, United States,* [8]*Cedars Sinai Health System, Los Angeles, CA, United States,* [9]*Emory University, Atlanta, GA, United States,* [10]*University of Texas Medical Branch, Galveston, TX, United States,* [11]*United States Food and Drug Administration (FDA), White Oak, MD, United States,* [12]*Biomedical Advanced Research and Development Authority, Washington, DC, United States,* [13]*Massachusetts General Hospital, Boston, MA, United States,* [14]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

During the 2014-2016 Ebola virus disease (EVD) outbreak, clinicians lacked a readily available resource cataloguing available medical countermeasures (MCMs) to treat EVD patients in West Africa and other regions. Some patients received investigational interventions under uncontrolled compassionate use protocols. Therefore, the Special Pathogens Research Network (SPRN) of the National Ebola Training and Education Center (NETEC) formed the MCMs Working Group (WG) with members from 10 Regional Ebola and other Special Pathogen Treatment Centers (RESPTC) and NETEC partners. The goal of the MCMs WG is to develop an evidence-informed, easily accessible, practical assessment of potential countermeasures for clinicians managing patients with selected highly hazardous communicable diseases. Pathogens were selected based on 1) ability to cause severe disease; 2) potential to cause large outbreaks; 3) paucity of currently available cleared MCMs; 4) occurrence of nosocomial spread to health care providers; and 5) transmissibility requiring specialized care in a biocontainment unit. After a standardized literature review is conducted, an assessment of potentially available countermeasures is performed by 2-4 subject matter experts, followed by peer review. Pathogens selected to date include Marburg, Lassa, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Crimean Congo Hemorrhagic Fever (CCHF), Nipah, Variola (and Monkeypox) and South American Hemorrhagic Fever (Junin, Machupo, etc.) viruses. The list overlaps with the World Health Organization's list of "Blueprint" priority diseases. Reviews on seven pathogens are in progress. In conclusion, the NETEC SPRN MCM WG was created to provide a preemptive compendium of systematic and clinically relevant summaries of available MCMs for selected pathogens based on pre-specified criteria. Challenges will include updating current documents as new scientific evidence becomes available. Plans are in place for regular review and updates, with the most up-to-date materials available on the NETEC website for open-source access.

738

INTEGRATING TRADITIONAL HEALERS INTO THE HEALTH CARE SYSTEM TO IMPROVE EARLY DIAGNOSIS OF EPIDEMIC PRONE DISEASES IN WEST NILE REGION, UGANDA, 2010-2019

Titus Apangu[1], Gordian Candini[1], Janet Abaru[1], Bosco Candia[1], Felix J. Okoth[1], Linda A. Atiku[1], Harriet Apio[1], Amy Schwartz[2], Paul Mead[2], Kiersten Kugeler[2]

[1]*Uganda Virus Research Institute, Entebbe, Uganda,* [2]*United States Centers for Disease Control and Prevention, Fort Collins, CO, United States*

In rural Uganda most patients consult traditional healers during times of illness before visiting allopathic health facilities. Traditional healers provide access to supportive care for common illnesses but their use can delay diagnosis and treatment for severe conditions and hinder early detection of epidemic-prone diseases. Because early antimicrobial treatment saves lives and stops plague outbreaks, the Uganda Plague Program has engaged traditional healers to improve recognition and referral in the plague-endemic West Nile region since 2010. Participating healers receive general health education, plague specific training, referral cards and record books. Healers refer patients they feel might have plague or other severe illnesses to nearby health facilities with a designated referral card. Presentation of this card at participating facilities allows the sick individual to bypass any queue. Plague Program personnel meet monthly with all participating healers to discuss facility referrals, verify arrival in facility registers and provide feedback on the discharge diagnoses and outcomes. During 2010-2019, 43 healers made 748 referrals to 21 facilities in the West Nile region. One plague case was identified as part of this program. Common discharge diagnoses among those referred to facilities included malaria (n=262), pneumonia and respiratory tract infections (n=182), gastrointestinal illness (n=41), parasitic infections (n=33), sexually transmitted infections (n=30) and peptic ulcer disease (28). Most patients

(724; 97%) improved after receiving care at a health clinic. This program was instituted during a time when the episodic nature of plague was on a decline. Nevertheless, patients with wide-ranging life-threatening conditions such as malaria and pneumonia received more appropriate therapy for their suspected condition than had they remained in the care of a healer alone. Traditional healers serve as a first line of detection for unusual illnesses, and improving trust and communication between traditional and allopathic health care systems may improve early detection of epidemic-prone diseases in rural Uganda.

739

HUMAN RESOURCE FOR HEALTH CHALLENGES IN SIERRA LEONE

Haja Abie Manasaray, Beah Joe Lebby, Ivan Macauley-Black, Julian Mattia

Njala University/Ministry of Health, Bo, Sierra Leone

Sierra Leone has some of the worst health indicators in the world. It was one of the three West African countries seriously heat by the deadly outbreak of Ebola in 2014 which left a total of 3,589 confirmed death according to the World Health Organization (WHO). The outbreak affected an already crippled health system where health care workers accounted for 307 cases with 221 deaths. Several efforts have been made in post-Ebola to improve the health workforce in the country. The current study conducted in July 2017, looks at health worker density in rural communities in Sierra Leone and the challenges they face in their work environment. a total of 84 peripheral health units (PHUs) were targeted in 7 health districts. Questionnaires were administered to the head of each PHU to track the human resource capacity at the facility level. Key questions included; the number of staff available, their cadre, whether they had received in-service training and number of villages covered by the facility. Overall, 70 PHUs were assessed in 7 health districts. These comprised 39 males and 31 females with an average age of 42 years. About 90% confirmed haven received at least 1 in-service training. The average number of staff at each facility was 5.4 with an average 3.7 trained professional staff. Furthermore, only 46% of the total staff are on payroll. The average number of population per PHU was 6,960 people. The density of trained health professional was 0.53 staff per 1,000 population. Each PHU administer health care to an average 19 villages with the nearest village being within an average 11 kilometers. Also, 37% and 26% of the PHU staff confirmed that they do outreach on a twice monthly and monthly basis respectively. The 0.53 trained health professionals per 1000 population is below the WHO recommended threshold of 4.5 skilled health professionals per 1000 population. This is an improvement to the 2013 ratio of 0.19 skilled health professional per 1000 population. These results shows that the health sector especially at the primary level is overburdened with limited motivation. It however shows an improvement but there is still very huge challenge in reaching the WHO threshold.

740

STILLBIRTH BURDEN AND CHALLENGES FOR REPORTING: INITIAL RESULTS FROM THE CHAMPS MAKENI SITE, SIERRA LEONE

Mary Claire Worrell[1], Carri Jo Cain[2], Solomon Samura[2], Erick Kaluma[3], Baindu Kosia[4], Michelle Dynes[1], Kevin Clarke[1], Navit Salzberg[5], Sorie I. Kamara[6], Foday Sesay[7], Mohammed Sheku[7], Amara Jambai[8], Robert F. Breiman[5], Reinhard Kaiser[9]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*World Hope International, Makeni, Sierra Leone,* [3]*Crown Agents, Makeni, Sierra Leone,* [4]*FOCUS* [1000]*, Makeni, Sierra Leone,* [5]*Emory Global Health Institute, Atlanta, GA, United States,* [6]*ICAP, Makeni, Sierra Leone,* [7]*Ministry of Health and Sanitation, Makeni, Sierra Leone,* [8]*Ministry of Health and Sanitation, Freetown, Sierra Leone,* [9]*Centers for Disease Control and Prevention, Freetown, Sierra Leone*

Sierra Leone has a stillbirth rate of 24.4 per 1000 total births [2015]. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network site in Makeni, Sierra Leone aims to improve understanding of stillbirth etiology through prospective surveillance and collection of comprehensive cause of death data, including advanced laboratory and histopathology diagnostics. Data will allow decision makers to develop interventions and national policies. We present initial results on stillbirth enumeration and assess misclassification. We used the World Health Organization definition for stillbirth: a baby born with no signs of life either ≥ 28 weeks of gestation or with birth weight ≥ 1000 grams. We assessed misclassification by reviewing the stillbirth status designation at eligibility screening and stillbirth criteria for each case and assessed concordance between data from clinical records and verbal autopsies. We consider clinical data to be the most reliable source of information. From July 2018 to February 2019, CHAMPS detected 79 stillbirths. Of these, 97% of caregivers provided consent for enrollment for clinical data collection and verbal autopsy. Seventy-four (96%) of the reported stillbirths were delivered at a health facility; six (8%) cases were misclassified when comparing eligibility stillbirth status to clinical records, including one miscarriage misclassified as stillbirth, three neonatal deaths misclassified as stillbirths, and two stillbirths misclassified as neonatal deaths. Clinical and verbal autopsy data yielded discordant status on 10 cases (14%). As nearly all stillbirths identified occurred in a health facility, we very likely missed community stillbirths, highlighting a need to improve community surveillance. We found evidence of stillbirth misclassification suggest the need to improve education of healthcare staff. Stillbirth status discordance between clinical and verbal autopsy data needs further investigation particularly to determine adherence to case definitions and validity of verbal autopsy data for classifying stillbirths in this context.

741

TWO STEPS FORWARD, ONE STEP BACK: THE IMPACT OF REDUCING PRIVATE SECTOR CO-PAYMENT MECHANISM SUBSIDIES ON THE AVAILABILITY, USAGE, AND PRICE OF QUALITY-ASSURED ACTS IN KENYA, NIGERIA, AND UGANDA

Tayo Olaleye[1], Luke Baertlein[1], Patricia Njiri[2], Alex Ogwal[3], Theodoor Visser[4], Abby Ward[4], Leslie Wentworth[4], Aaron Woolsey[4]

[1]*Clinton Health Access Initiative, Abuja, Nigeria,* [2]*Clinton Health Access Initiative, Nairobi, Kenya,* [3]*Clinton Health Access Initiative, Kampala, Uganda,* [4]*Clinton Health Access Initiative, Boston, MA, United States*

In 2010, the Global Fund launched the Affordable Medicines Facility for malaria (AMFm) to increase accessibility of WHO-quality-assured artemisinin combination therapies (QAACTs) through a manufacturer-level subsidy. Access to QAACTs in participant countries increased, on average with a four-fold expansion in the percent of private sector facilities stocking QAACTs. AMFm continued as the private sector Co-Payment Mechanism (CPM); however, in 2018, it was terminated in Nigeria (NG), and reduced in Kenya (KE) and Uganda (UG). Population representative samples of private medicine retailers were surveyed across these countries to assess the subsidy reduction impact on availability, price and market share of antimalarials at two time periods: during the CPM (2014-2016, n=2760) and after its reduction (2018-2019, n=1146). Comparing retail prices of antimalarials before and after the reduction of the CPM, the average retail price of QAACTs increased from $0.48 to $1.31 in NG and $0.99 to $1.23 in UG, but decreased from $1.11 to $0.80 in KE. The average price of non-quality assured ACTs remained stable at $1.45 in NG, but decreased from $3.64 to $2.05 in UG, and $4.25 to $0.86 in KE. Concurrently, the proportion of outlets with QAACTs in stock dropped from 72% to 49% in NG, 71% to 64% in UG, and 72% to 35 % in KE. Availability of non-quality assured ACTs increased from 13% to 56% in NG, 6% to 40% in UG, and 42% to 68% in KE. Non-ACT antimalarial availability increased in NG but decreased in UG and KE. The QAACT market share declined in each country relative to other ACTs, but non-ACT antimalarial market share remained constant or declined. Results suggest the retail price of QAACTs increased in 2 of 3 countries after the CPM reduction, coinciding with a decline in availability and market share of QAACTs. However, availability and market share of non-quality assured ACTs increased during this time, resulting in no net decline in ACT availability or market share. There was no indication that artemisinin

monotherapies gained market share following the subsidy reduction. The public health impact depends on the uncertain quality of ACTs that are not WHO-quality-assured.

742

SPIDER ENVENOMATIONS THERAPEUTICS AND ANTIVENOM ACCESSIBILITY: A SYSTEMATIC REVIEW

Christian Lecce, Avinash N. Mukkala, Aisha Khatib, Michael A. Klowak, Pryanka Challa, Eric Shao, Jason Kwan, Tianna Chong-Kit, Jamie Sookhoo, Emma Hagopian, Dylan Kain, Mofe Adeosun, Andrea K. Boggild

Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada

Spiders are a group of arthropods in the order *Araneae* and class *Arachnida* which have eight legs and fangs. Modern advancements in transportation allow increased human travel to areas which are endemic to spiders, increasing the possibility of envenomation. Physicians could select the optimal envenomation treatment using a clinical resource that compares efficacy statistics of antivenom versus other therapeutics. Our goal is to compile existing prevention and treatment data in the literature in order to synthesize this clinical resource. PubMed (NCBI), MEDLINE (OVID), EMBASE (OVID), Cochrane Database of Systematic Reviews (CIDR) and TOXLINE (TOXNET) were searched from inception to June 2018 using combinations of the search terms "spider," and "envenomation*. Iterative inclusion and exclusion of search terms was employed to maximize extraction. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. Evidence will be summarized using descriptive measures for each intervention type, as well as a qualitative synthesis. Meta-analysis will be planned if sufficient efficacy measures exist. 961 MEDLINE articles, 1053 PubMed, 1486 EMBASE, 0 CIDR and 149 TOXLINE records were retrieved for title and abstract screening; after a multi-step de-duplication pipeline, 1928 remained. Following abstract screening, 282 full-text records were eligible for inclusion. Upon initial review of these records, *Latrodectus hasseltii*, *Latrodectus mactans, Loxosceles reclusa,* and *Phoneutria spp.* were the most medically relevant. Data will be grouped and summarized by prevention, therapeutic strategies, geographic location and species. The recommended mode of treatment and management will be provided on an evidence-based, per-species basis. Increased transcontinental movement of people and tropical produce has facilitated importation of arachnids to non-endemic regions where clinicians lack familiarity with envenomation syndromes and appropriate therapeutics. Synthesizing the current evidence around therapeutic strategies can inform the development of treatment and prevention protocols.

743

A SYSTEMATIC REVIEW OF SCORPION ENVENOMATION THERAPEUTICS AND ANTIVENOM ACCESSIBILITY

Avinash N. Mukkala, Christian Lecce, Aisha Khatib, Michael A. Klowak, Priyanka Challa, Eric Shao, Jason Kwan, Tianna Chong-Kit, Jamie Sookhoo, Emma Hagopian, Dylan Kain, Mofe Adeosun, Andrea K. Boggild

Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada

Scorpions (Scorpiones) are eight-legged arthropods of the class Arachnida. With increased human migration and transcontinental shipment of produce from the tropics, the incidence of scorpion envenomations may increase in non-endemic areas. We aim to synthesize existing evidence around prevention and treatment of scorpion envenomations into a clinical resource, including provision of information on access to, and indications for, antivenom usage. PubMed (NCBI), MEDLINE (OVID), EMBASE (OVID), Cochrane Database of Systematic Reviews (CIDR) and TOXLINE (TOXNET) were searched from inception to June 2018 using combinations of the search terms "scorpion" and "envenomation". Iterative inclusion and exclusion of search terms was employed to maximize article extraction. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. Evidence will be summarized using descriptive measures for each intervention type, as well as a qualitative synthesis. Meta-analysis will be planned if sufficient efficacy measures exist. 961 MEDLINE articles, 1053 PubMed, 1486 EMBASE, 0 CIDR and 149 TOXLINE records were retrieved for title and abstract screening; after a multi-step deduplication pipeline, 1928 remained. After title and abstract screening, 422 studies were eligible for inclusion. Some of the main medically important species include: Mesobuthus tamulus, Androctonus australis, Hemiscorpius lepturus, Tityus serrulatus, and Centruroides sculpturatus. Data will be grouped and summarized for ease of clinician use by prevention, therapeutic strategies, geographic location and species. The recommended mode of treatment and management will be provided on an evidence-based, per-species basis. Increased transcontinental movement of people and tropical produce has facilitated importation of scorpions to non-endemic regions where clinicians lack familiarity with envenomation syndromes and appropriate therapeutics. Synthesizing the current evidence around therapeutic strategies for scorpion envenomations can inform the development of appropriate treatment and prevention protocols.

744

GEOGRAPHIC DISTRIBUTION UPDATE OF ARGENTINIAN TRIATOMINE SPECIES AS VECTORS OF CHAGAS DISEASE FRAMED IN A CITIZEN SCIENCE PROJECT

Soledad Ceccarelli[1], Agustin Balsalobre[1], Maria Eugenia Cano[1], Delmi Canale[2], Patricia Lobbia[2], Joaquín Cochero[3], Raúl Stariolo[2], María del Pilar Fernandez[4], Jorge E. Rabinovich[1], Gerardo A. Marti[1]

[1]*Centro de Estudios Parasitológicos y de Vectores, La Plata, Argentina,* [2]*Centro de Referencia de Vectores, Coordinación Nacional de Vectores, Ministerio de Salud de la Nación, Santa María de Punilla, Argentina,* [3]*Instituto de Limnología "Dr. Raúl A. Ringuelet", La Plata, Argentina,* [4]*Laboratory of Eco-epidemiology, Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY, United States*

The Wallacean Shortfall phenomenon indicates that there is very little knowledge of the geographic distribution for the vast majority of the species described today, especially for invertebrates. in the case of insect vectors species and associated vector-borne pathogens, there are current initiatives that compile occurrence data, providing geographic information that enables policymakers to make evidence-based decisions. Other vector species are often sparsely recorded and there are few globally comprehensive sets of primary data compiled. Such is the case of triatomine species (Reduviidae: Triatominae), vector of *Trypanosoma cruzi* - Chagas disease etiological agent. Currently, there are about 150 species described worldwide for the Triatominae subfamily, 137 species distributed in the Americas, and 17 species cited for Argentina. Although all species are considered as potential vectors, around 70 species have been found naturally infected with this parasite. Beyond the ‹Atlas of the Tratominae› published by Carcavallo et al. (1998), no work carried out a full integration of the existing geographic information of Argentinian triatomine species, as some successful efforts completed in other Latin American countries. Recently, an updated and integrated occurrence database of 135 American triatomine species called 'DataTri' was published. Additionally, for the last 15 years, the National Vector Reference Center of Argentina (CeReVe) has been compiling occurrence data during their own fieldwork, which has remained unpublished. Finally, a citizen science project called 'GeoVin' was developed to gather occurrence data of Argentinian triatomine species through citizen participation using a mobile app. Here we report a multi-source database of distributional records for all Argentinian triatomine species. a total of 9593 occurrence data were collected between 1918-2019. We hope that this study helps and encourage colleagues and citizen to keep this information updated that can be used as basic information by public health agencies to guide surveillance actions and control of Chagas disease.

745

MOLECULAR DETECTION OF TICK-BORNE BACTERIA AND ARBOVIRUSES AT LIVESTOCK MARKETS AND SLAUGHTERHOUSES FROM WESTERN KENYA

Tatenda Chiuya[1], Daniel K. Masiga[1], Jandouwe Villinger[1], Eric M. Fevre[2], Amanda Bastos[3]

[1]*International Centre of Insect Physiology and Ecology, Nairobi, Kenya,* [2]*International Livestock Research Institute, Nairobi, Kenya,* [3]*University of Pretoria, Pretoria, South Africa*

Ticks are vectors of viral, bacterial, and protozoal pathogens of public health importance, including *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Theileria*, and Crimean-Congo hemorrhagic fever (CCHF) virus, which can cause severe systemic illness and mortality. Active surveillance of tick-borne bacteria and viruses is necessary for intervention planning, and to detect novel pathogens or novel vector-pathogen associations. We collected 462 ticks from cattle, sheep, and goats at livestock markets and slaughterhouses in three neighboring counties in western Kenya over a period of 13 months. Livestock farming and trade in this region is characterized by peri-urban livestock markets and close association with humans in areas of high population density. We identified the ticks using both morphological and molecular techniques targeting the cytochrome oxidase 1 (COI), 16S rRNA, and internal transcribed spacer (ITS2) genes and pooled them (334 pools of 1-3 ticks) according to sex, species, and host of origin. We then screened the pools for zoonotic and livestock pathogens by PCR coupled with high-resolution melting (HRM) analysis using primers targeting bacterial 16S rRNA, 18S rRNA, and *omp*B genes and arboviral non-structural protein, RNa dependent RNa polymerase, and nucleoprotein genes. Amplicons with unique melting profiles were sequenced. The sampled ticks identified were from the genera *Amblyomma*, *Rhipicephalus*, and *Haemaphysalis*. *Rickettsia africae* was detected in 82 pools of *A. variegatum*, while 18 pools of *Rhipicephalus decoloratus* were positive for *Anaplasma platys.* Two pools of *R. decoloratus* were positive for CCHFV, while sindbis virus was detected in *A. variegatum* (1 pool) and *R. decoloratus* (1 pool). in addition, 201 pools of ticks were positive for *Coxiella* endosymbionts. Our findings demonstrate the presence of infected ticks on animals being traded, and translocated across the counties, which thus may disseminate these pathogens into disease free areas.

746

RESISTANCE MECHANISMS OF *PHLEBOTOMUS ARGENTIPES* THE VECTOR OF LEISHMANIASIS IN SRI LANKA

Ruwanika K. Pathirage[1], Karunaweera N. Karunaweera[1], Karunaratne S. Karunaratne[2]

[1]*Department of Parasitology, Faculty of Medicine, Colombo, Sri Lanka,* [2]*Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka*

Phlebotomus argentipes is the known vector of *Leishmania donovani,* the causative organism of leishmaniasis. a major challenge of an effective vector control program is the development of insecticide resistance in the vector. in order to develop an effective vector control programme it is important to identify the vector susceptibility to insecticides. Thalawa and Pannala and Mamadala and Mirigama sites were selected for sand fly collection. F1 progeny were exposed to different concentrations of DDT, malathion, deltamethrin and propoxur. Results were validated with the control mortalities using Abbott's formula. For biochemical analysis, esterase assay, glutathione S-transferase assay, oxidase assay, protein assay and acetylcholinesterase assay were performed with individually homogenized sand flies. DNa was isolated from individual sand flies using a method by Livak, 1984 followed by PCR to determine the insensitivity of the pyrethroid target site sodium channel regulatory proteins to identification of possible mutations of the gene. According to the susceptibility test, the colony population was susceptible to concentrations of >0.6% DDT and >0.7% Malathion, >0.007% Deltamethrin and >0.015% Propoxur. Biochemically proved that, the most of the flies had protein activity <0.3μmol min^{-1} mg^{-1}, esterase activity <1.00 μmol min^{-1} mg^{-1} and GST activity <0.4μmol min^{-1} mg^{-1}. All protein, esterase and GST activity were < 0.35 equivalent units of monooxygenase amounts. Over half of this population had <30% residual AChE activity in the presence of propoxur. However, 6.5% had >60% residual activity. Hence, the majority of the population may be considered susceptible to the insecticides used in this study. Furthermore, the kdr mutations were not seen hitherto in wild *P. argentipes* populations in this study. However, further studies are being conducted to ascertain the genetic variations related to the susceptibility patterns of Sri Lankan *P. argentipes* to synthetic insecticides.

747

UNUSUAL REACTIONS TO ARTHROPOD BITES: HORSE FLY, BED BUG, BROWN RECLUSE SPIDER AND TICK BITES

Don W. Kannangara, Dhyanesh Pandya

St Luke's University Health Network, Phillipsburg, NJ, United States

Arthropod bites affect human and animal health by envenomation, allergic reactions and disease transmission by acting as biological or mechanical vectors. Hematophagous arthropods also inject vasodilators, anticoagulants, platelet inhibitors, anti-itch and analgesic compounds that assist in blood feeding. Death may result from 1. potent toxins injected such as the paralytic toxin of *Ixodes holocyclus* 2. bacterial, viral and parasitic infections transmitted or 3. by allergic reactions resulting in anaphylactic shock. The compounds in arthropod saliva also may cause local reactions and skin necrosis. Arthropod salivary compounds play a key role in pathogen transmission and their establishment in a host by inhibition of host defenses. We present case examples of a horse fly bite acting as a mechanical vector resulting in a severe local reaction caused by a bacterial infection, eschar formation following bed bug bites and severe skin necrosis following "brown recluse spider bites". Brown recluse spiders are not native to New Jersey or Pennsylvania, but could be transported from endemic areas. We report one case each from these two states, highly likely to be brown-recluse bites with skin and deep tissue necrosis requiring debridement. We also present different examples of skin reactions after tick bites that include blistering, "vesiculo-pustular poison-ivy like rashes", eschar associated rashes and involvement of large areas of skin.

748

CDC'S INTERNATIONAL RESPONSE TO THE ZIKA VIRUS OUTBREAK: STRENGTHENING REGIONAL PUBLIC HEALTH ENTOMOLOGY NETWORKS

Rebecca Levine[1], Norma Padilla[2], Rajesh Ragoo[3], Daniel Impoinvil[1], Samuel Dadzie[4], Mamadou Coulibaly[5], Audrey Lenhart[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Universidad del Valle, Guatemala City, Guatemala,* [3]*Caribbean Public Health Agency, Port of Spain, Trinidad and Tobago,* [4]*Noguchi Memorial Institute for Medical Research, Legon, Ghana,* [5]*University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali*

The 2016 Zika virus outbreak highlighted capacity gaps in vector surveillance and control programs, particularly in smaller, resource-constrained countries. in addition, there were limited opportunities for countries to share expertise in public health entomology and few formal mechanisms for countries to work together to bring vector borne disease outbreaks under control. CDC supported a regional network-based strategy to improve the ability of countries to conduct vector surveillance and leverage resources for vector control. Regional networks were developed or strengthened to create frameworks for vector personnel from neighboring countries to share surveillance data, collaborate on vector control strategies, build capacity, and improve information exchange. Three entomological networks focusing on *Aedes* surveillance were supported in Central America, the Caribbean, and West Africa, improving capacity for preparedness and response to *Aedes*-borne diseases. As an example, by the end of 2017, a unified strategy was formalized for the island of Hispaniola, whereby both Haiti and the Dominican Republic

conducted joint cross-border *Aedes* surveillance activities, agreed to share entomological data, and adopted the same entomological surveillance platform. The networks were also used to provide trainings to personnel from over 40 countries on *Aedes* trapping methods, integrated vector management, insecticide resistance surveillance, mapping and use of GIS, digital surveillance platforms, and data management and analysis. a formal evaluation of the networks is being conducted in 2019, to assess their role in improving *Aedes*-borne disease surveillance, preparedness, and response capacity among the member countries. Network-based approaches in resource-constrained settings can empower countries to leverage resources in vector surveillance and control, which contributes to sustainable improvements in entomological capacity and outbreak response.

749

VENEREAL TRANSMISSION OF VESICULAR STOMATITIS VIRUS BY *CULICOIDES SONORENSIS* MIDGES

Paula Rozo-Lopez[1], Yoonseong Park[1], Berlin Londono[1], Barbara Drolet[2]

[1]*Kansas State University, Department of Entomology, Manhattan, KS, United States,* [2]*United States Department of Agriculture, Agricultural Research Service, Arthropod-Borne Animal Diseases Research Unit, Manhattan, KS, United States*

Culicoides sonorensis midges are well-known arbovirus vectors that play an important role in the spread of diseases such as vesicular stomatitis virus (VSV). VSV epidemiology has many complex variables to consider, including a broad range of vertebrate hosts, multiple routes of transmission, and extensive diversity of suspected vector species. Understanding non-conventional routes for VSV vector transmission might help explain viral maintenance during inter-epidemic periods and times of adverse conditions for horizontal (bite) transmission. To determine whether infected females, though intrathoracic and oral inoculation, can transmit VSV to uninfected naïve males. Our results showed the presence of viral RNa in 11.4% of males mated with injected females and 11.9% of the males mated with orally infected females during the third gonotrophic cycle. Additionally, we evaluated whether intrathoracically inoculated male midges can venereally transmit VSV to uninfected naïve females. a total of 41.7% of the females tested positive for the presence of viral RNa 7 days after copula. Lastly, we conducted ultrastructural studies with immunohistochemistry on reproductive organs of males and females and established relevant anatomical sites for virus location that results in transmission through copulation. Our research indicates that *Culicoides* midges are capable of efficient venereal transmission and may help explain ecological, temporal, and spatial aspects of VSV epidemiology.

750

SUBLETHAL ACOUSTIC TRACHEAL RUPTURE PROVIDES NOVEL INSIGHTS OF LARVAL MOSQUITO RESPIRATION, CONTRADICTING THE CLASSICAL THEORY OF OBLIGATE METABOLIC GAS EXCHANGE VIA THE SIPHON

Herbert Joseph Nyberg

New Mountain Innovations, Inc., Old Lyme, CT, United States

Acoustic larvicide occurs by exposing mosquito larvae to acoustic energy rupturing the dorsal tracheal trunks (DTTs) by the expulsion of gas bubbles into the body. In studying this we identified undescribed anatomical and physiological respiratory features. The classical theory of respiration is that the siphon and DTTs play an obligate role in respiration. In contrast, the results of the present study contradict the widely accepted theory that culicine larvae respire via atmospheric gas exchange. Gas exchange necessary for survival occurs in the absence of a tracheal connection to the atmosphere through the siphon. We identified an undescribed tracheal occlusion necessary for the acoustic larvicide rupture of the DTTs, this constriction prevents the escape of energized gas from the siphon as well as precluding the exchange of metabolic gas with the atmosphere. This contradiction of the classical theory of respiration will impact future efforts in controlling mosquito populations and the associated diseases they transmit.

751

CHARACTERIZATION OF THE REPRODUCTIVE BEHAVIOR OF THE NATURAL POPULATION OF *ANOPHELES COLUZZII* AND *ANOPHELES GAMBIAE* IN SENEGAL

Oumou K. Gueye[1], El Hadj A. Niang[1], Mouhamed B. Faye[1], Amblat A. Ahmad[1], Abdoulaye K. Dia[1], Frederic Tripet[2], Abdoulaye Diabate[3], Charles Wondj[4], Ibrahima Dia[5], Lassana Konate[1], Ousmane Faye[1], Oumar Gaye[1]

[1]*Universite Cheikh Anta Diop, Dakar, Senegal,* [2]*Keele University, Newcastle-under-Lyme, United Kingdom,* [3]*Institut de Recherche en Science de la Santé/Centre Muraz, Bobo-Dioulasso, Burkina Faso,* [4]*Centre for Research in Infectious diseases (CRID) and International Institute of Tropical Agriculture (IITA), Yaounde, Cameroon,* [5]*Unité d'Entomologie Médicale, Institut Pasteur de Dakar, Dakar, Senegal*

Anopheles gambiae s.s., the major malaria vector in Africa, has long been known to exist in nature as two distinct and sympatric populations. Initially described as M and S molecular forms, now formally named as *Anopheles coluzzii* Coetzee & Wilkerson while the S form retains the nominotypical species name, *Anopheles gambiae*. In most of their sympatric areas, the reproductive isolation between the incipient species is thought to be the main barrier to hybridization. However, in Senegal, the barrier to the gene flow seems to be porous in some areas with relatively high hybridization rates. Here, we studied the swarming behavior of these two species to investigate its impact on the hybridization.The study was carried out in the south and center of Senegal during the 2018 raining season. Swarms were surveyed at sunset towards the lightest part of the sky, about 0.5 - 4m above the ground. Once located, swarm were collected using a net. Indoor resting populations was also collected during the same period from each sentinel village by pyrethrum spray catch earlier the morning to estimate the frequency of the two species and their hybrids. All specimens collected were identified morphologically then by PCR. Results show that *An. gambiae* swarms mainly over bare ground whereas *An. coluzzii* swarms over various objects forming a dark-light contrast with the ground. The height of swarms varies between 0.5 to 2.5 meters and the swarming duration was about 10 minutes. Swarming was correlated with sunset and no mixed swarm were found in the sympatric area despite the high level of hybridization rate. Swarming behavior shows a pre-mating reproductive barrier between *An. coluzzii* and *An. gambiae* in Senegal. No link was found between swarming behavior and hybridization, but the lack of mixed swarm may be the result of low number of sample obtained in the sympatric area.

752

PREDICTION OF MICROCLIMATES USING MACHINE LEARNING

Rachel Sippy[1], Diego Herrera[2], David Gaus[2], Ronald Gangnon[3], Jonathan Patz[3], Jorge Osorio[3]

[1]*SUNY-Upstate Medical University, Syracuse, NY, United States,* [2]*Salud y Desarollo Andino, Pedro Vicente Maldonado, Ecuador,* [3]*University of Wisconsin - Madison, Madison, WI, United States*

Microclimates are an important component of our ecosystem and can impact human health through heat illnesses or by impacts on vector habitats. *Aedes aegypti*, the vector of dengue, chikungunya, and Zika, is a highly localized species and its abundance is impacted by microclimate conditions. Research on microclimates can be difficult, as precise on-the-ground measurements are required; our understanding microclimate stability is limited. Although we know microclimates affect human health, there have been no attempts to predict microclimates. HOBO Temperature/Relative Humidity Data Loggers were deployed in four sites per month for 24 to 576 continuous hours (1—24 days) each month from September 2016 to August 2017 in a community in rural Ecuador. Data

were summarized for each 24-hour period. We assessed the variability of summary microclimate variables across time and urban environments. We combined remotely-sensed and climate station weather data with urbanicity, elevation, and spatial components to predict summary microclimate variables across the entire community using machine learning approaches. Two-hundred and eighty-seven log-days of data were collected. We found that some microclimate variables were temporally stable, urban sites had warmer temperature variables and rural sites had higher relative humidity variables. For prediction, we found random forest algorithms were the best fit for many microclimate variables (temperature mean, median, minimum and relative humidity mean, median), with decent prediction (R^2: 0.61—0.65). Generalized boosting models fit temperature and relative humidity variance well, as well as minimum and maximum relative humidity, with good prediction (R^2: 0.61—0.72). The best fit model for maximum temperature was a support vector machine, though this yielded moderate prediction (R^2: 0.53). Our study was limited by a small sample size over time and space, and limited availability of prediction variables at fine spatial scales. Machine learning approaches are a promising option for microclimate prediction though additional research should be conducted with validation of model predictions.

753

STUDY ON RESERVOIRS OF CUTANEOUS LEISHMANIASIS IN AN ENDEMIC AREA IN MALI

Cheick Amadou Coulibaly

University Park, PA, State College, PA, United States

Cutaneous leishmaniasis (CL) remains a major public health problem in African nations, including Mali, where no information is available on the vertebrate reservoirs involved in parasites transmission cycles. This study was carried out to determine the natural infection rates of *Leishmania* parasites in rodents, and to assess exposure of both rodents and domestic animals to sand fly bites in a cryptic focus of CL in the district of Baroueli, Mali. in Baroueli, a high prevalence (31%, May 2006) of disease was detected through leishmanin surveys though active cases were never found despite active case detection over a period of two years. The sampling of rodents using Sherman traps was carried out. a total of 42 rodents were captured and identified as *Mastomys natalensis*, *Mus musculus*, *Mastomys sp*. *Mastomys natalensis* species with 64.3% was the predominant specie. PCR and Microscopy, used for infection detection, were negative. ELISA, used to assess exposure to bites, detected antibodies against *Phlebotomus duboscqi* saliva and possibly *Leishmania* in the rodent *Mastomys natalensis.* Bloodmeal analysis from humans and domestic animals by PCR targeting cytochrome B revealed that *P. duboscqi* fed on mostly on goats, chicken and humans. To the best of our knowledge, this is the first detection of anti-*Leishmania* antibodies in *Mastomys natalensis* in Baroueli, Mali. Successful parasite isolation from lesions need to be established to confirm these rodents are disease reservoirs.

754

GENETIC DIVERSITY OF ORIENTIA TSUTSUGAMUSHI IN NORTH INDIA

Manisha Biswal, Ryumzok Targain, Jasleen Kaur, Navneet Sharma, Arun Bansal

Postgraduate Institute of Medical Education and Research, Chandigarh, India

Scrub typhus has emerged as a major cause of acute febrile illness in India in recent years. The causative agent, *O. tsutsugamushi* has more than 20 antigenic types due to a variable 56-kDa outer membrane protein. It is crucial to know the prevailing antigenic types in India for the success of diagnostic immunoassays and prospective vaccine candidates. in north India, the principal antigenic types circulating are unknown. Our tertiary care hospital caters to a large area of north India (around 8 states with ten million population). Therefore, the current study was planned to identify the genotypes of *O. tsutsugamushi* circulating in this wide area of north India. a total of 590 adults and children with presenting with suspected scrub typhus between July 2017 and March 2018 were included in this study. DNa was extracted from whole blood and eschar (where available) and a nested PCR was used to amplify a 483-bp region of the 56-kDa antigen gene of *O. tsutsugamushi*. The PCR products were purified and DNa sequencing was performed and aligned using the CLUSTAL_V program. a phylogenetic tree was constructed using neighbour- joining algorithms and analyzed using the sequences obtained in this study and those obtained from the GenBank database.a total of 52 samples were positive for PCR and 48 amplicons were sequenced and analysed. Karp-like strains predominated in all states studied (72.9%) followed by Kawasaki-like (20.8 %). We found three amplicons which were very similar to Boryong and Kuroki reference strains. We did not find any Kato or Gilliam-like strains. *Orientia tsutsugamushi* shows a great diversity in its strains over a large geographical area of north India. This has implications for both diagnostic assays and vaccines for scrub typhus.

755

BIODIVERSITY OF MOSQUITOES IN MALARIA AND DENGUE CO-ENDEMIC AREAS IN KHANH HOA AND BINH PHUOC PROVINCES, VIETNAM

Nam Sinh Vu[1], Nicholas J. Martin[2], Jeffrey C. Hertz[2], Tu Cong Tran[1], Phong Vu Tran[1], Anh Duc Dang[1], Duong Nhu Tran[1], Maysa T. Motoki[3]

[1]*National Instititue of Hygiene and Epidemilogy, Hanoi, Vietnam,* [2]*Naval Medical Research Unit-2, Singapore, Singapore,* [3]*Vysnova Partners, Maryland, MD, United States*

A collaborative study to assess the distribution and behavior of mosquitoes, with a focus on those recognized as vectors of malaria and dengue was conducted, to determine if vectors of both diseases were present in the same areas within Khanh Hoa and Binh Phuoc Provinces, Vietnam. Adults and immature (larvae) mosquitoes were collected in Khanh Thanh and Cau Ban Communes, Khanh Hoa Province, and Bu Gia Map and Dac O Communes, Binh Phuoc Province. Adult mosquitoes were collected using cow-baited net trap, light trap and aspirator, while immatures were collected by standard larval dippers. Mosquito taxa were identified based on morphological characters, PCR-based identification methods were used to aid identification for *Anopheles* (*Celia*) *dirus s.l.*, *An*. (*Cel*.) *minimus s.l.*, and *An.* (*Cel*.) *maculatus s.l.*, and the internal transcribed spacer 2 (ITS2) region of ribosomal DNa (rDNA) was generated for overall species identification. a total of 4,492 specimens were collected in the first survey, representing 30 taxa from five genera: *Aedes*, *Anopheles*, *Armigeres*, *Culex* and *Mansonia*. Among them, the primary malaria vectors, *An*. (*Cel*.) *dirus s.s.*, *An*. (*Cel*.) *minimus s.s.*, and *An.* (*Cel*.) *maculatus s.s.,* and dengue vectors, *Aedes* (*Stg*.) *aegypti and Ae.* (*Stg*.) *albopictus*, were collected in almost all the studied locations. The PCR-based identification allowed us to discriminate *An. maculatus s.l.* into two species: *An. maculatus s.s.* and *An. sawadwongporni*. Voucher specimens will be deposited into the Smithsonian Institution, National Museum of Natural History (SI-NMNH), Washington DC, USA. The PCR-based identification method was critical to the identification of *Anopheles* cryptic species. This study provides additional information about the biodiversity and ecology of mosquito fauna in Vietnam. Results demonstrate that there is a risk of infection from either malaria and dengue in study areas within Khanh Hoa and Binh Phuoc Provinces.

756

THE EFFECTS OF LANDCOVER ON *ANOPHEL*ES POPULATION DYNAMICS IN ANN TOWNSHIP, RAKHINE STATE, MYANMAR

Christopher C. Hayes[1], Sai Zaw Min Oo[2], Yannaung M. Maung[2], Zayar Han[2], Maung M. Mya[2], Christopher V. Plowe[1], Myaing M. Nyunt[1]

[1]*Duke University, Durham, NC, United States,* [2]*Department of Medical Research, Yangon, Myanmar*

Over the past several decades, developing countries such as Myanmar have increased industrialization and use of natural resources. The

results of these trends are anthropogenic environmental impacts such as deforestation, strip-mining, and land re-purposing for agriculture or housing. The effects of these human-driven land-cover changes on vector species densities, distribution, ecology, and local malaria transmission within Myanmar are largely undocumented. The objective of this study was to assess the effects of variability in the land-cover on the species-specific density of major local malaria vectors. To accomplish this, we systematically collected mosquitoes at randomized locations, both indoor and outdoor, within varying land-cover types such as deep forest, rice paddy, and forest edge. Collections were performed across multiple villages, over a period of 3-5 days within each village. Villages were divided into sectors based on similar land-cover types, and within those sectors one trap was rotated to different randomized geographic positions for several twelve-hour day and night trapping sessions. The collected mosquitoes were identified by both morphology and polymerase chain reaction (PCR). The relative species-specific densities were determined based on village-specific levels of land-cover independent of vector-control measures used. The distribution of specific species of *Anopheles* in the studied land-covers will be presented, and potential factors affecting anthropogenic variability will be discussed. Data from this study will improve the current understanding of species-specific vector dynamics in the area, and will help guide further efforts for vector reduction and infection control.

757

MICROORGANISM-BASED LARVAL DIETS AFFECT MOSQUITO DEVELOPMENT, SIZE AND NUTRITIONAL RESERVES IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

Raquel Souza[1], Flávia Vírginio[2], Thaís Riback[1], Lincoln Suesdek[2], José Barufi[3], Fernando Genta[1]

[1]*Fundação Oswaldo Cruz, Rio de Janeiro, Brazil,* [2]*Instituto Butantan, São Paulo, Brazil,* [3]*Universidade Federal de Santa Catarina, Santa Catarina, Brazil*

Mosquito larvae feed on organic detritus from the environment, particularly microorganisms comprising bacteria, protozoa, and algae. Little attention has been paid to nutritional studies in *Aedes aegypti* larvae. We investigated the effects of yeast, bacteria and microalgae diets on larval development, adult size, survivorship, lifespan and wing morphology. Microorganisms (or Tetramin® as control) were offered as the only source of food to recently hatched first instar larvae and their development was followed until the adult stage. The main macronutrients were analyzed in single larvae to correlate energetic reserve accumulation by larva with the developmental rates and nutritional content observed. FITC-labeled microorganisms were offered to fourth instar larvae, and its ingestion was recorded by fluorescence microscopy and quantitation. Larvae developed in all diets, however insects fed with bacteria and microalgae showed a severe delay in development rates and low survivorship. Diets with better nutritional quality resulted in adults with bigger wings. *Asaia* sp. and *Escherichia coli* resulted in better nutrition and developmental parameters and seemed to be the best bacterial candidates to future studies using symbiont-based control. The diet quality was measured and presented different protein and carbohydrate amounts. Bacteria had the lowest protein and carbohydrate rates, yeasts had the highest carbohydrate amount and microalgae showed the highest protein content. Larvae fed with microalgae seem not to be able to process and store these diets properly. Larvae were shown to be able to process yeast cells and store their energetic components efficiently. In conclusion. our results point that mosquito larvae show high plasticity to feed, being able to develop under different microorganism-based diets. The important role of *Ae. aegypti* in the spread of infectious diseases requires further biological studies in order to understand the vector physiology and thus to manage the larval natural breeding sites aiming a better mosquito control.

758

MATING INDUCED MRNA EXPRESSION CHANGES IN FEMALE SPERM STORAGE ORGANS OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

Carolina Camargo[1], I. Alexandra Amaro[2], Laura C. Harrington[2], Frank W. Avila[1]

[1]*Universidad de Antioquia Max Planck Tandem Group, Medellin, Colombia,* [2]*Cornell University, Ithaca, NY, United States*

The yellow fever mosquito *Aedes aegypti* is the primary vector for numerous viruses that negatively impact human health, including dengue, chikungunya, and zika viruses. Manipulation of insect reproduction has been proposed as an alternative to suppress mosquito populations or replace them with mosquitoes that are resistant to viruses. To develop such control strategies, it is necessary to understand the biological processes that enable males and females to successfully reproduce. Female sperm storage is a crucial process in insect reproduction that requires the coordination of male and female-derived proteins that regulate post-mating process and allow sperm viability during storage. Studies in *Drosophila melanogaster* have shown that secretory cells of the sperm storage organs (spermathecal secretory cells, SSCs) produce proteins that function in ovulation, egg-laying and sperm storage, and several SSC genes are regulated by mating. While *Ae. aegypti* contain SSCs, the proteins they produce, and their transcriptional response to mating alone or a blood-meal is unknown. We conducted an RNAseq analysis on the spermathecae of *Ae.aegypti* in 4-6 day-old virgin females, mated females, and mated and blood fed females at 6, 24, and 72hrs post-mating. Our goals were to 1) identify female-specific proteins likely to be involved in reproduction and 2) determine gene expression profiles of the spermathecae in response to mating and blood ingestion in this important species. This study will allow us to identify genes likely to be required for fertility in *Ae. aegypti.*

759

A MOSQUITO TRIGLYCERIDE LIPASE IS CRITICAL FOR *ANOPHELES GAMBIAE* REPRODUCTION AND FOR *PLASMODIUM FALCIPARUM* DEVELOPMENT IN THE MOSQUITO

Maurice A. Itoe[1], Kristine Werling[1], Amy Deik[2], Kathleen A. Westervelt[1], Clary Clish[2], Flaminia Catteruccia[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Broad Institute of Harvard and M.I.T, Cambridge, MA, United States*

When a female mosquito feeds on blood, dietary lipids are mobilized from the midgut epithelium and loaded onto lipid transporters for delivery to ovaries, fat body, and flight muscles where they are utilized for a number of physiological processes or can be stored and broken down. Despite the conservation of this process across blood feeding mosquito species, limited information is available on the role of lipolysis in the reproductive biology of female mosquitoes and in their interaction with the pathogens they transmit. To fill this knowledge gap, we have characterized putative key components of the lipolytic machinery in the main African malaria vector, *Anopheles gambiae,* for their role in the female's reproductive success and parasite development. An initial lipidomic time course analysis revealed a coordinated alteration of triacylglycerols (TAGs) and other neutral lipids in different mosquito tissues after blood feeding, suggesting lipid mobilization. Strikingly, RNa interference (RNAi) targeting the putative TAG lipase, *TL2* reduced the number of eggs developed by females and caused complete embryonic lethality. Moreover, depletion of *TL2* induced faster *Plasmodium falciparum* development, with parasites becoming infectious within a shorter time period. Similar results were also obtained upon RNAi knockdown of a putative lipid droplet surface protein 1(LDSP1), an orthologue of perilipin 1 in mammals known to regulate TAG lipase activity on the surface of lipid droplets for the breakdown of fat, suggesting conservation of lipolytic pathways between mosquitoes and mammals. Lipidomic analyses of female tissues after *TL2* silencing showed accumulation of TAGs, DAGs, PCs in midguts and cholesterol esters (CE)

in ovaries. Consistently, injection of candidate lipids into the hemolymph shortly after blood feeding partially rescued egg numbers and fertility in *TL2*-deficient females, highlighting the relevance of blood meal-derived fatty acid in the mosquito reproductive success. This study identifies TAG lipolysis as a critical step for the fertility of *An. gambiae* females and the development of *P. falciparum* parasites.

760

DETECTION OF ARBOVIRUSES AND PARASITES IN MOSQUITO VECTORS WITH A BEAM OF LIGHT

Maggy Sikulu-Lord[1], Gabriela A. Garcia[2], Lilha M. Santos[2], Jill N. Fernandes[3], Floyd E. Dowell[4], Rafael Maciel-De-Freitas[2]

[1]*The University of Queensland, Herston, Australia,* [2]*Instituto Oswald Cruz-Fiocruz, Rio de Janeiro, Brazil,* [3]*The University of Queensland, St Lucia, Australia,* [4]*U.S. Department of Agriculture, Kansas City, KS, United States*

To determine the effectiveness of mosquito control programs, decision-makers need timely, high throughput and cost-effective surveillance tools to determine mosquito survival, species identity or infection status prior to and after implementation of an intervention. This information is required to identify high risk areas in a timely fashion allowing for the prioritization of resources to communities most in need thus maximizing the efficiency of vector control interventions. To date, no technique has been shown practical, cost effective and rapid enough to be scaled up for surveillance of large mosquito control programs. In the last decade, our team has been applying the Near infrared spectroscopy (NIR) technique to characterise mosquito vectors that transmit human pathogens such as malaria, dengue Zika and Chikungunya. The main goal is to find a cheaper and rapid alternative technique for determining the efficacy of available control interventions and upcoming vector control tools and predict future disease outbreaks. We sought to determine the applicability of NIRS for rapid detection of a number of infections including Zika, dengue, Chikungunya and malaria. Infected and uninfected cohorts of mosquitoes were reared under controlled laboratory conditions and a Labspec NIR spectrometer (Malvern Panalytical Inc, Boulder, CO) was used to scan the mosquito samples. Machine learning algorithms were used to identify spectral signatures related to each infection in heads/thoraces and abdomens. Our collective results from a range of studies indicate that our light based technique could be used to predict mosquitoes infected with Zika, dengue, Chikungunya and malaria with accuracies ranging between 80-97% at a significantly lower cost and more rapidly than traditional techniques used for similar purposes.. Our next step is to expand the existing models for use in other regions and to apply the models in the field for predicting future disease outbreaks.

761

PYRUVATE KINASE ACTIVITY IS MODULATED IN THE FAT BODY OF *AEDES AEGYPTI* IN RESPONSE TO NUTRIENT CHANGES

Natthida Petchampai[1], Jun Isoe[2], Patricia Y. Scaraffia[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*The University of Arizona, Tucson, AZ, United States*

Glucose oxidation through glycolysis is important for ammonia detoxification in *Aedes aegypti*. Pyruvate kinase (PK) catalyzes the last step of the glycolytic pathway. Recently, two alternatively spliced mRNA variants (AaPK1 and AaPK2) that code for PKs were identified in the *A. aegypti* genome. Recombinant AaPK1 was found to be regulated by multiple allosteric effectors, including specific amino acids and phosphorylated sugars. Here, *in vivo* characterization of AaPKs was performed. First, we measured AaPK mRNA and protein expression in tissues dissected from sucrose- and blood-fed mosquitoes. Although transcriptional analysis showed differential mRNA expression patterns in mosquito tissues, the protein levels in most of the tissues remained unchanged throughout the time-course analyzed. Then, we examined AaPK enzymatic activity in fat body and flight muscles from mosquitoes maintained under four different nutritional conditions: (1) sucrose, (2) water (deprivation of sucrose), (3) blood/sucrose, and (4) blood/water. For non-blood-fed mosquitoes (conditions 1 and 2), the subtraction of sucrose increased AaPK activity in the fat body at 72 h after feeding. Moreover, western blotting revealed that AaPK protein expression was up-regulated in the fat body of non-blood fed mosquitoes that were given water for 72 h. In contrast, the AaPK activity declined in the fat body of blood-fed mosquitoes that were provided with water (condition 4) instead of sucrose (condition 3) for 96 and 120 h. The level of AaPK activity in the flight muscles was similar in all dietary conditions and time points. The results demonstrate that AaPK activity in mosquito fat body is regulated in response to specific nutritional changes. Our data also provide new insights into carbon and nitrogen metabolism in *A. aegypti* mosquitoes.

762

A RAPID SCREEN IDENTIFIES CLIPC9 AS A REGULATOR OF MELANIZATION IN THE MALARIA VECTOR *ANOPHELES GAMBIAE*

Gregory L. Sousa, Michael Povelones

University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, United States

The mosquito melanization response is a crucial immune pathway that is regulated by a network of CLIP domain serine proteases (CLIPs). CLIP cascades link pattern recognition to melanin production by activating phenoloxidase (PO), which is the rate limiting melanization enzyme. Current methods to identify regulators of melanization in the malaria vector, *Anopheles gambiae*, include PO enzyme activity assays and microscopic evaluations following microbial infections. These approaches, while robust, require large mosquito cohorts and/or technically intensive hemolymph extractions. Here, we describe the development of a rapid screen, referred to as the melanization-associated spot assay (MelASA), that is capable of identifying novel melanization regulators with far fewer mosquitoes and without biochemical or microscopic analyses. The MelASA was based on an observation that mosquitoes injected with *E. coli* excreted a quantifiable amount of melanotic material. CLIPA8 is essential for immune challenge-induced melanization, and we demonstrated that the appearance of these excretions, like PO activity, required this serine protease homolog. Using MelASA, we then interrogated the CLIPC subfamily and identified CLIPC9 as a putative melanization regulator. Follow up analyses revealed CLIPC9 as a critical player in both the melanization of *E. coli* and *Plasmodium berghei* parasites. To facilitate biochemical and immunohistochemical analyses, we developed a polyclonal antibody against CLIPC9 and report its presence within the hemolymph. Work is on-going to understand the hierarchical position of CLIPC9 within the CLIP network and to determine its relationship with other immune pathways such as mosquito complement. This screen represents a streamlined approach for dissecting the contributions of multimember gene families such as the CLIPs in melanization. We report that CLIPC9 is the first CLIPC family member involved in the melanization immune response in any mosquito.

763

INSECT ARYL-HYDROCARBON RECEPTOR (AHR) MEDIATES A NEGATIVE LOOP OF IMMUNE MODULATION

Aditi Kulkarni, Wanqin Yu, Jennifer Curtiss, Jiannong Xu

New Mexico State University, Las Cruces, NM, United States

The immune homeostasis in insects is known to be well maintained by different negative feedback mechanisms. AhR is a xenobiotic sensor and ligand-dependent transcription factor. In this study, we examined the role of insect AhR signaling in immune regulation in parallel to the classical immune pathways using the mosquito *Anopheles gambiae* and fruit fly *Drosophila melanogaster* models. In the mosquito model, we pharmacologically manipulated AhR activation using AhR agonists or antagonists, and knocked down *AhR* gene via RNAi, and then the mosquitoes were challenged by injection with a bacterium *Serratia* sp. S1. When AhR was activated by feeding the agonist kynurenine, survival

rate was reduced from 58% (in control) to 38.7% (Chi square test, P<0.01). When AhR was inhibited by the antagonist CH233191, survival was increased from 58% to 80% (Chi square, P<0.01). In addition, *AhR* gene silencing resulted in higher survival upon the bacterial challenge. The chemical inhibition of AhR increased immunity against rodent malaria parasite *Plasmodium berghei,* less oocysts were present in the treated mosquitoes (t test, P<0.01). In *Drosophila*, AhR is encoded by gene *spineless, Ss.* Bacterium *Providencia* causes lethal infection in flies when ingested. The wild type flies were more sensitive to the intestinal infection, showing a higher mortality than the two loss-of-function mutant lines, *Ss1* and *Ssa* (Mantel-Cox, P<0.01). When wild type flies were treated with the AhR antagonist CH233191, the survival was improved (Mantel-Cox, P<0.05). Furthermore, the interrogation of transcriptome of before and after bacterial infections in AhR manipulated mosquitoes revealed that AhR controlled the transcription of genes with immune-suppressive function, including *ClipA14, Tieg, Srpn6, Srpn10* and *Socs*. However, AhR manipulation did not affect the transcription of immune genes regulated by Toll and IMD immune pathways. The data suggest that insect AhR mediates an independent negative loop of immune modulation in maintaining immune homeostasis.

764

CHEMICAL DEPLETION OF PHAGOCYTIC IMMUNE CELLS IN *ANOPHELES GAMBIAE* REVEALS DUAL ROLES OF MOSQUITO HEMOCYTES IN ANTI-*PLASMODIUM* IMMUNITY

Hyeogsun Kwon, **Ryan C. Smith**

Iowa State University, Ames, IA, United States

Mosquito immunity is comprised of both cellular and humoral factors that provide protection from invading pathogens. Immune cells known as hemocytes, have been intricately associated with phagocytosis and innate immune signaling. However, the lack of genetic tools has limited hemocyte study despite their importance in mosquito anti-*Plasmodium* immunity. To address these limitations, we employ the use of a chemical-based treatment to deplete phagocytic immune cells in *Anopheles gambiae,* demonstrating the role of phagocytes in complement recognition and prophenoloxidase production that respectively limit the ookinete and oocyst stages of malaria parasite development. Through these experiments, we also define specific sub-types of phagocytic immune cells in *An. gambiae*, providing new insights beyond the morphological characteristics that traditionally define mosquito hemocyte populations. Together, this study represents a significant advancement in our understanding of the roles of mosquito phagocytes in mosquito vector competence and demonstrates the utility of clodronate liposomes as an important new tool in the study of invertebrate immunity.

765

PYRETHROID AND ORGANOPHOSPHATE INSECTICIDE RESISTANCE IN *ANOPHELES ARABIENSIS* IN WESTERN KENYA

Pauline W. Orondo[1], Harryson Atieli[2], Steven G. Nyanjom[1], Alex L. MingChieh[3], Andrew K. Githeko[4], Guiyan Yan[3]

[1]*Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [2]*International Center for Malaria Research, Homa Bay, Kenya,* [3]*University of California, California, CA, United States,* [4]*Kenya Medical Research Institute, Kisumu, Kenya*

Malaria control in Kenya primarily relies on insecticide treated nets, however, in 2018 indoor residual spraying (IRS) using Actellic®300SC an organophosphate was implemented. Malaria vector abundance has since been reduced significantly causing major shift in malaria vector species. Intensive use of insecticide based control measures may have further selected for increased insecticide resistance in the malaria vectors. The objective of the present study was to determine resistance to multiple classes of insecticide in Homa Bay, a county with mixed crop irrigation. This study determined the frequency of knock down resistance (kdr) and insecticide resistance mechanisms in *An. arabiensis* malaria vector. *Anopheles gambiae* s.l. larvae were collected in high and low transmission zones, which are approximately 15km apart. These were reared and subjected to standard WHO bioassay test against deltamethrin and melathion. Species identification (n=181) indicated 2.2% *An. gambiae* s.s. and 97.8% *An. arabiensis*. *An. arabiensis* recorded a mortality of 97.9% (n=324) in high transmission zone and 83.9% (n=114) in low transmission zone against deltamethrin, whereas both high (n=104) and low transmission zones (n=103) recorded 100% mortalities against melathion. The frequency of kdr-east was observed to be zero (0) and 0.16 (n=51) and (n=70) in high and low transmission zones respectively, while the frequency of kdr-west was 0.1 and 0.04 in the high and low transmission zones respectively. This suggests heterogeneous distribution of insecticide resistance. The frequency of ACE gene was observed to be zero (0) in both high (n=44) and low transmission zone (n=58). These findings provide important information for selecting appropriate insecticides for malaria vector control in an area with multiple classes of insecticide use.

766

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF PLANT EXTRACTS AS NOVEL LARVICIDAL AGENTS AGAINST *AEDES AEGYPTI* MOSQUITOES IN JAMAICA

Rhaheem N. Layne-Yarde, Simone L. Sandiford

The University of the West Indies Mona, Jamaica, Kingston, Jamaica

The *Aedes aegypti* mosquito is a vector for many arboviruses such as dengue, Zika and chikungunya which have all negatively impacted the tropical regions of the world. Current attempts to control mosquito populations in Jamaica have limited success because of inadequate funding of vector control agencies and lack of knowledge regarding the status of insecticide resistance in mosquitoes. Therefore, the discovery of novel agents which possess mosquitocidal activity is necessary in order to effectively assist in reducing the *Aedes aegypti* populations in the country. Essential oils of plant species from families such as Lamiaceae, Rutaceae and Verbanaceae have shown promise as potential mosquitocidal agents and the cytotoxic effects of local plant species from these families will be evaluated in this investigation. Insect cell lines have become a popular alternative cell-based research tool and are being used in this study for the initial screening of the plant extracts. Extracts that have prominent cytotoxic effects against the mosquito cells will be further analyzed using the *Aedes aegypti* larvicidal assay. To investigate the mechanism of action of the extracts, the expression of genes that have been shown to be involved in cell death and insect immunity will be investigated.

767

BACTERIAL COMPOSITION DIFFERS BETWEEN PERMETHRIN-SUSCEPTIBLE AND -RESISTANT *ANOPHELES GAMBIAE SENSU STRICTO* IN A SITE WITH INTENSE PYRETHROID RESISTANCE IN WESTERN KENYA

Diana N. Omoke[1], Ezekiel Mugendi[2], Eric Ochomo[1], Mathew Kipsum[1], Samson Otieno[1], Edward Esalimba[1], Mili Sheth[3], Audrey Lenhart[3], Nsa Dada[3]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Kenyatta University, Nairobi, Kenya,* [3]*United States Centers for Disease Control and Prevention, Atlanta, GA, United States*

Insecticide resistance poses a growing challenge to malaria vector control. Following evidence of associations between the mosquito microbiota and insecticide resistance, we characterized the microbial composition of malaria vectors in relation to their insecticide resistance profiles, in different locations with high and low levels of pyrethroid resistance. Here, we report findings from our study on *Anopheles gambiae s.s.* in Turukuyi village, Bungoma County, where high levels of pyrethroid resistance were detected. F_1 non-blood fed female *An. gambiae s.s.* that were obtained from wild-caught mosquitoes were exposed to five times (107.5µg/ml) the discriminating dose of permethrin using the CDC bottle bioassay. The microbiota of mosquitoes that were alive (resistant, n=50) or dead (susceptible, n=50) following the bioassay were characterized using high throughput sequencing targeting the universal bacterial and archaeal 16S

rRNA gene. Results showed significant differences in bacterial composition between resistant and susceptible individuals (PERMANOVA, F=2.33, P=0.001), with *Sphingobacterium* and *Streptococcus*—both comprising pyrethroid-degrading species, and the radiotolerant *Rubrobacter,* being over three folds (P<0.05) more abundant in resistant compared to susceptible mosquitoes. This first report of association between the microbiota and pyrethroid resistance in *An. gambiae s.s.* corroborates results of previous studies conducted on *Anopheles albimanus* from Peru and Guatemala. Our findings form the basis of future work on the role of mosquito microbiota in insecticide resistance, and potentially the identification of novel microbial markers of insecticide resistance in mosquito populations.

768

HABITAT CHARACTERIZATION AND INSECTICIDE SUSCEPTIBILITY OF *AEDES AEGYPTI* MOSQUITOES IN IFAKARA TOWN AND SURROUNDING WARDS

Najat Feruzi Kahamba, Alex Limwagu, Salum Mapua, Halfan Ngowo, Emmanuel Kaindoa, Fredros O. Okumu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

In past decades, significant progress has been made against malaria in Tanzania, but other mosquito-borne diseases such as dengue, yellow fever, chikungunya and zika which transmitted by *Aedes aegypti* remain neglected. The concern over these diseases in sub-Saharan Africa has risen in recent times due to outbreaks in various places and several detection of viruses in areas with no outbreak yet. *Ae. aegypti* is anthropophilic vector which breeds in artificial containers, but can be controlled by larval source management and personal protection. Though it is widely distributed in urban areas, its distribution and susceptibility to public health pesticides remain unknown in small towns and rural areas in Tanzania. This study aims to characterize aquatic habitats of *Ae. aegypti* in Ifakara and to assess the susceptibility against insecticides commonly used in public health. The study area was gridded (200m × 200m), grids with houses and buildings were randomly selected for search of aquatic habitats. Habitats were described by type, size, location, water clarity, presence of shades and surrounding environment. Larvae of *Aedes* were collected and reared in the insectary for insecticide susceptibility tests following standard WHO guidelines against pirimiphos-methyl 0.25%, deltamethrin 0.05%, permethrin 0.75%, dieldrin 4% and bendiocarb 0.1%. A total of 27541 larvae were collected, of which 63% (n=17245) were *Ae. aegypti* and 37% *Culex* mosquitoes. The most common *Aedes* habitats in the area were used tires, clay pots, flower pots, garage pits, tree holes and discarded containers. Habitats with clear water and shades were most commonly infested by *Aedes.* The *Aedes* mosquitoes were fully susceptible to all the insecticide classes in all wards except Mlabani, Viwanja sitini and Ifakara mjini where there were signs of resistance to bendiocarb, deltamethrin and permethrin but for which susceptibility was confirmed. These findings provide baseline data on habitat characteristics and insecticide susceptibility of *Ae. aegypti* in Ifakara. It also provides a basis for stronger vector surveillance against Aedes-borne infections in small towns

769

COPEPODS AS POTENTIAL BIO-CONTROLS AGAINST INVASIVE MOSQUITOES IN THE UK: A COMPARISON OF TWO LOCAL CYCLOPOID PREDATOR SPECIES

Marie C. Russell, Alima Qureshi, Chris G. Wilson, Lauren J. Cator

Imperial College London, Ascot, United Kingdom

The Asian Tiger Mosquito, *Aedes albopictus*, is a known vector of several diseases of public health importance including Chikungunya, Dengue, Yellow Fever, and Zika; and it is likely to successfully invade the UK in the coming years. We assessed the functional response and the predation efficiency of copepods *Macrocyclops albidus* and *Megacyclops viridis* from Surrey, South East England, UK against newly-hatched French *Ae. albopictus* larvae. Experimental replicates were all of a similar design with one cyclopoid copepod predator and varying numbers of mosquito larvae in 20 mL of spring water for a six-hour period of exposure to predation. The functional response experiments required varying the initial densities of mosquito larvae from a range of one to 32, and the assessment of predation efficiency was done at a prey density of 24. Predator-absent controls were included throughout the course of all experiments to account for background prey mortality. Both copepod species exhibit population-destabilizing type II functional responses that do not change significantly over temperatures likely to be experienced in UK larval mosquito habitats. The predation efficiency of *M. viridis* was approximately 7.5 percentage points higher than that of *M. albidus*. There is also a significant positive relationship between the body lengths of the copepods and their predation efficiencies. We suggest that *M. viridis* copepods have higher predation efficiency than *M. albidus* against French *Ae. albopictus* larvae, and that this difference is due in part to the fact that *M. viridis* copepods generally have longer body lengths than *M. albidus*. Our study suggests that in the event of an *Ae. albopictus* invasion in the UK, *M. viridis* would be a superior control agent to *M. albidus*.

770

A COMPARATIVE STUDY OF THE DENSITY, RESISTANCE STATUS AND SPOROZOITE RATES OF *ANOPHELES FUNESTUS* AND *AN. GAMBIAE* SPECIES COMPLEXES IN WESTERN KENYA

Rissy Makokha[1], Seline Omondi[2], Isaiah Debrah[2], Maxwell Machani[2], Bernard Abong'o[2], Jackline Kosgei[2], Silas Agumba[2], Duncan Athinya[3], Stephen Munga[2], Yaw Afrane[4], Carol Wangui Hunja[1], Eric Ochomo[2]

[1]*South Eastern Kenya University, Kitui, Kenya,* [2]*Kenya Medical Reserach Institute, Kisumu, Kenya,* [3]*University of Nairobi, Nairobi, Kenya,* [4]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana*

With the increasing use of insecticide-based vector control tools, two phenomena have occurred; one is a shift in populations of sympatric vectors and two, is the emergence and spread of insecticide resistance. We assessed the composition, *Plasmodium falciparum* sporozoite rates, resistance profiles and mechanisms, and the impact of the observed resistance on the commonly used Long Lasting Insecticidal Nets in *Anopheles* mosquitoes in western Kenya. Female mosquitoes resting indoors were randomly collected from houses in Ahero, Kisumu county and Asembo, Siaya county in western Kenya. The mosquitoes were exposed to 0.05% deltamethrin using WHO tube bioassays to determine resistance status. CDC bottle bioassay with escalating doses:1× (12.5µg/ml), 2× (25µg/ml), 5× (62.5µg/ml) and 10× (125µg/ml) was used to determine intensity of deltamethrin resistance. We also investigated the efficacy of locally available LLINs using WHO cone bioassay. Enzyme-linked Immunosorbent assay was used to determine *P. falciparum* sporozoite infection rates. *Anopheles funestus (s.s)* was the predominant mosquito species collected at both study sites. *An. gambiae (s.s)* was present in least proportions during the study period, accounting for 3.96% and 1.00% in Asembo and Ahero, respectively. Both WHO tube and CDC bottle bioassays implicated *An. funestus* as having the highest intensity of insecticide resistance to deltamethrin compared to *An. gambiae* complex. There was partial restoration of susceptibility in the resistant vectors when they were pre-exposed to PBO then to deltamethrin. *Anopheles funestus* also showed reduced bio-efficacy to the commonly used LLINs. *Plasmodium falciparum* sporozoite rates were highest in *An. funestus* sampled from Asembo and Ahero. Increasing insecticide resistance in *An. funestus* is alarming especially given the increasing densities and higher sporozoite rate compared to other malaria vectors existing in sympatry. This is a probable reason for the current increasing malaria incidence in many parts of Eastern and Southern Africa as reported in the most recent WHO malaria report.

771

SOCIO-CULTURAL AND COMMUNITY PERCEPTIONS ON PREGNANCY THAT INFLUENCE UTILIZATION OF MALARIA IN PREGNANCY INTERVENTIONS IN THE ASHANTI AND VOLTA REGIONS OF GHANA

Matilda Aberese-Ako[1], Harry K. Tagbor[1], Gifty D. Ampofo[1], Pascal Magnussen[2]

[1]*University of Health and Allied Sciences, Ho, Ghana,* [2]*University of Copenhagen, Copenhagen, Denmark*

Malaria in pregnancy (MiP) is a preventable and treatable infection. Despite efforts in sub-Sahara Africa to prevent and manage it, MiP exists with serious consequences. Ghana has implemented WHO recommended interventions such as insecticide treated bed nets, intermittent preventive treatment of malaria in pregnancy and testing and treating malaria in pregnancy, since 2005. This study sought to understand and describe how socio-cultural and community perceptions about pregnancy influence utilization of MiP interventions. We conducted 72 in-depth interviews with pregnant women, 30 opinion leaders, 10 herbalists, 10 pastors, 8 traditional birth attendants and 8 husbands in study communities. Focus group discussions were held with 28 pregnant women and 28 women who delivered recently. Observations and conversations were held with health providers and pregnant women in 8 health facilities. Ethical clearance was obtained from the University of Health and Allied Sciences' Research Ethics Committee and research participants' consent was sought. Nvivo 11 was used to support data coding. Data was triangulated and analyzed using grounded theory approach. Pregnant women favored cultural and community perceptions on prevention and treatment of maternal health diseases such as MiP, over health education and treatment options from health care facilities. Negative pregnancy outcomes such as miscarriages and still-birth from malaria and other biomedical causes, were interpreted as spiritual attacks from enemies. Pregnant women were encouraged by community members to seek physical and spiritual protection to prevent such outcomes. Thus pregnant women resorted to health shopping such as visiting hospitals, prayer centres, herbalists and self-medication with herbs. Some were not convinced of the efficacy of malaria in pregnancy interventions, so they focused more on pastors and traditional methods. Socio-cultural and community perceptions about pregnancy influence utilization of MiP interventions. MiP policy interventions should consider intensifying engagement with communities in order to ensure uptake of MiP interventions.

772

MODELING THE SPATIO-TEMPORAL SPREAD OF INSECTICIDE RESISTANCE AND ITS IMPACT ON MALARIA CONTROL

Prashanth Selvaraj, Caitlin Bever, Daniel Bridenbecker, Edward Wenger

Institute for Disease Modeling, Bellevue, WA, United States

Chemical insecticides are critical to the success of malaria control programs worldwide but increasing resistance threatens to undermine these efforts. Understanding the evolution and propagation of resistance is imperative to mitigating loss of intervention effectiveness. Here we present a multi-locus model of vector genetics that accounts for gene linkages and a many-to-many mapping of genotypes to phenotypes with which we model insecticide resistance in Anophelines. Using this model of vector genetics combined with an agent-based mathematical model of malaria transmission that simulates the interactions between human hosts and mosquitoes, we investigate deployment strategies and the choice of insecticides that are most likely to lead to the development of resistance in vector populations. From near elimination to high transmission settings in Africa, we predict the impact of resistance in Anophelines on malaria transmission and investigate various spatiotemporal deployment strategies of insecticides either sequentially or as mixtures to best manage resistance and ensure continued efficacy of ongoing control and elimination efforts.

773

SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* S.L. TO INSECTICIDES IN SIERRA LEONE

Evelyne Sampe Alyko[1], Samuel Juana Smith[2], Yemane Yihdego[3], Rebecca Levine[4], Jenny Carlson[5], Stephen Karando Mansaray[1], David Schnabel[6], Ramlat Jose[7], Frederick Yamba[2], Miriam Mokuena[3]

[1]*PMI VectorLink project, Freetown, Sierra Leone,* [2]*National Malaria Control Program, Freetown, Sierra Leone,* [3]*Abt Associates, Rockville, MD, United States,* [4]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [5]*US Agency for International Development, Washington, DC, United States,* [6]*President's Malaria Initiative, Centers for Disease Control and Prevention, Sierra Leone, Freetown, Sierra Leone,* [7]*President's Malaria Initiative, US Agency for International Development, Sierra Leone, Freetown, Sierra Leone*

Long-lasting insecticide-treated nets (LLINs) are the primary tool for malaria vector control in Sierra Leone. Indoor residual spraying (IRS) is under consideration for future deployment. Insecticide resistance in *Anopheles* is a threat to the utility of any vector control tools that rely on insecticides, so obtaining information on the insecticide resistance status of these vectors is a basic requirement. We investigated the susceptibility status of the main malaria vector in Sierra Leone, *An. gambiae* s.l., to several insecticides. Larvae were collected and reared to adults from four districts in Sierra Leone (Bombali, Kono, Bo, and Western Rural), which represent the country's four regions. Females aged two to five days were exposed to various insecticides using the World Health Organization (WHO) tube test or Centers for Disease Control and Prevention (CDC) bottle bioassay protocols. Insecticides tested included the pyrethroids deltamethrin, permethrin, and alpha-cypermethrin, the organophosphate pirimiphos-methyl, the pyrrole chlorfenapyr, and the neonicotinoid clothianidin. Synergist assays with piperonyl butoxide (PBO) pre-exposure were also conducted using deltamethrin and alpha-cypermethrin. Results showed that mosquitoes were fully resistant to the pyrethroids, with mortality ranging between 7.3% and 65.3%, but fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. After pre-exposure to PBO, the mortality rate observed for both deltamethrin and alpha-cypermethrin increased to between 58.9% and 82.5%, however, it still remained below the 90% cut-off point for confirmed resistance. These findings suggest that "next generation" LLINs impregnated with PBO or other non-pyrethroid insecticides should be considered for malaria vector control in Sierra Leone. Additionally, should IRS be used in the future, non-pyrethroid insecticides would be viable options. These findings are being used to make evidence-based decisions regarding the most appropriate vector control tools in Sierra Leone and form the basis for updating the country's insecticide resistance management plan.

774

YEAST-ENCAPSULATED ORANGE OIL EFFICIENCY AS LARVICIDE FOR *AEDES* MOSQUITOES

Bruno Gomes[1], Camila P. Jesus[1], Huarlen Ogélio[1], Fabiane Brant[1], Michael J. Workman[2], Monique Costa[1], Ademir Martins[1], Ivy Hurwitz[2], Mariana David[1], Fernando A. Genta[1]

[1]*Oswaldo Cruz Institute (IOC-FIOCRUZ), Rio de Janeiro, Brazil,* [2]*University of New Mexico, Albuquerque, NM, United States*

Aedes aegypti and *Aedes albopictus* are important vectors of arbovirus impacting human health in tropical and temperate regions. The control of larvae in aquatic environments is an important strategy for vector control programs. Essential oils have been considered as an environmental friendly alternative for chemical insecticides but they are vulnerable to rapid degradation by UV radiation. Here, we present a study that screen the larvicide activity of yeast-encapsulated orange oil in different strains of *Aedes* mosquitoes from Brazil. Larvicide activity was tested on three *A. aegypti* colonies (≈F10) originated from Macapá/AP, Oiapoque/AP and Caseara/TO, that present different resistance phenotypes for temephos (OP, RR_{50} = 0.8 - 21.8) and deltamethrin (PYR, RR_{50} = 1.6 - 143.9). For

A. albopictus, the activity was screened in two F1 colonies from Rio de Janeiro/RJ and Manaus/AM. For each strain, early L3 larvae were exposed to 10 concentrations of yeast-encapsulated essential oil in four subsets of 25 specimens per concentration. In all bioassays, the *A. aegypti* Rockefeller strain was adopted as positive control of essential oil larvicide activity and insecticide susceptible reference lineage. Dead yeast without essential oil was used as a negative control. Mortality was registered after 24h of exposure and lethal doses were calculated using the logit generalized linear model implemented in R. Bioassays were repeated at least three times on different days with mortality ranging between 10 and 95%. Preliminary results indicate that the yeast-encapsulated orange oil was effective (LD_{50} < 50 mg·L^{-1}) against all five mosquito colonies, regardless of their species, geographic origin or resistance phenotype for chemical insecticides. The LD_{50} values for *A. aegypti* (8.6 - 14.9 mg·L^{-1}) are consistently lower than those observed for *A. albopictus* (32.7 - 33.3 mg·L^{-1}). The low variation of larvicide activity among *A. aegypti* strains with different insecticide resistance phenotypes indicate a low probability of cross-resistance between the yeast-encapsulated orange oil and chemical insecticides, such as organophosphates and pyrethroids.

775

COMPARING THE ENTOMOLOGICAL IMPACT OF A NOVEL MALARIA VECTOR CONTROL INTERVENTION USING HUMAN LANDING CATCH VERSUS CDC LIGHT TRAPS

Welbeck A. Oumbouke[1], Eleanore D. Sternberg[2], Antoine M. Barrreaux[2], Alphonsine A. Koffi[3], Ludovic P. Alou[3], Jackie Cook[4], Matthew B. Thomas[2], Raphael N'Guessan[1]

[1]*Institut Pierre Richet/London School of Hygiene & Tropical Medicine, Bouake/Cote d'Ivoire, Côte D'Ivoire,* [2]*Department of Entomology and Center for Infectious Disease Dynamics, The Pennsylvania State University, University Park, PA, United States,* [3]*Institut Pierre Richet/Institut National de santé Publique (INSP), Bouake/Cote d'Ivoire, Côte D'Ivoire,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Human landing catches (HLCs) are currently the gold standard method to determine vector human biting rate (HBR), which is a key measure for assessing the impact of vector control efforts. However, a range of issues associated with the HLC method has led to the development of alternative methods, including the Centers for Disease Control and Prevention (CDC) light traps. Although this sampling method has been designed to perform comparably to HLC, evidence on whether this technique can be used as a proxy for HLC remains inconclusive. The current study utilizes data from a 40-village cluster randomized controlled trial in central Côte d'Ivoire to compare the performance of HLC vs CDC light traps. The trial has been evaluating the impact of household screening plus insecticide-treated eave tubes on mosquito density and malaria transmission, compared to a control arm in which the village clusters have LLINs alone. Exposure to mosquitoes was measured for two years in both study arms using HLC and CDC light traps. Indoor sampling by HLC was done every month for one night in four randomly selected houses per village. Indoor sampling by CDC traps was done every other month in 12 randomly selected houses per village. Mean indoor mosquito densities measured with each sampling method will be compared between study arms, and the correlation between both sampling methods for entomological indicators (mosquito density and entomological inoculation rate) will be assessed. Temporal changes in mosquito densities and species composition will also be estimated and compared for each sampling method. Findings from the study will provide evidence on whether CDC light traps can be used as a reliable surrogate for HLC in trials of new vector control tools.

776

RISK OF TRANSMISSION AND THE INSECTICIDE SUSCEPTIBILITY STATUS OF THE POTENTIAL VECTORS OF YELLOW FEVER IN THE NORTHERN, UPPER EAST AND UPPER WEST REGIONS OF GHANA

Millicent Captain-Esoah[1], Philip K. Baidoo[2], Samuel K. Dadzie[3], Joseph Chabi[4], Dorothy Obuobi[4], Godwin K. Amlalo[4], Chrysantus Kubio[5], Francis B. Veriegh[6], Martin N. Donkor[7], Sampson A. Abagale[7], Kwadwo K. Frempong[3], Daniel A. Boakye[3]

[1]*Department of Applied Biology, University for Development Studies, Navrongo, Ghana,* [2]*Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [3]*Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Accra, Ghana,* [4]*Vestergaard NMIMR Vector Labs, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana,* [5]*Ghana Health Service, Karaga District Health Directorate, Northern Region, Karaga, Ghana,* [6]*Center for Scientific and Industrial Research, Water Research, Accra, Ghana,* [7]*Department of Applied Chemistry and Biochemistry, University for Development Studies, Navrongo, Ghana*

Yellow fever (YF) is an arboviral disease transmitted by *Aedes* mosquitoes. Ghana reported YF cases in Northern part in 2011 and 2016 with twelve people infected and three deaths. This study is to assess the ecological and entomological risk in the transmission of YF within affected areas. Hundred houses were selected from Bolgatanga, Damongo, and Nadowli using stratified sampling. This was done both in the dry and wet seasons for 16 months in 2015 and 2016. Mosquito larvae were collected from indoor and outdoor containers within and around selected houses. The WHO transmission risk indices was used to assess the likelihood of transmission in study sites. Knockdown resistance gene mutation was identified in 200 *Ae. aegypti* mosquitoes using PCR. A total of 8,768 mosquitoes were collected from all the three study areas with *Aedes* spp being 6,630 (76 %). Out of the *Aedes* spp., *Aedes aegypti* formed 97 %, *Aedes vittatus* 2.8 %, and *Aedes simpsoni* 0.2 %. All the areas have been identified as high risk by larval indices and man-vector contact rates. The density of *Ae. aegypti* was conducive to promote an outbreak of YF if the virus is introduced. All the *Aedes aegypti* mosquitoes tested from Bolgatanga area showed a suspected resistance to deltamethrin, permethrin and propoxur with mortalities >90%. Out of 200 *Aedes aegypti*, 130 (65%) were found with *Kdr* gene mutation. Community-wide surveillance programmes should be implemented in all the study communities to monitor insecticide resistance and prevent future outbreaks.

777

EFFECT OF IVERMECTIN ON FERTILITY FECUNDITY AND MORTALITY OF *ANOPHELES ARABIENSIS* FED ON TREATED HUMANS

Wondemeneh Mekuriaw[1], Meshesha Balkew[2], Delenasaw Yewhalaw[3], Adugna Woyessa[1], Fekadu Massebo[4]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*PMI/Abt, Addis Ababa, Ethiopia,* [3]*Tropical and Infectious Disease Research Center, Jimma University, Jimma, Ethiopia,* [4]*Department of Biological Sciences, Arba Minch University, Arba Minch, Ethiopia*

Malaria is a disease transmitted by female *Anopheles* mosquitoes. The control program mainly relay on vector control using insecticides. However, insecticide resistance is widely spreading in vector populations, which might affect control programs. Ivermectin based vector control approaches are getting attention to control the resistant malaria vectors either by targeting humans or animals. Hence, the aim of this study was to assess the residual or delayed effect of administering a single oral dose of ivermectin to humans on survival, fecundity and fertility of *Anopheles arabiensis*. Six volunteer males with age range between 25-40 years and weight ranges 64-72 Kg were recruited after receiving informed consent. Volunteers were assigned randomly either treatment or control using lottery method. Four of them received a recommended single oral dose

of 12mg ivermectin and the other two individual did not receive the drug and were used as control. There was no significant variation in mortality rate of *An. arabisensis* fed on ivermectin group between day 1 and day 4 (P = 0.73). More *An. arabiensis* were died on day one post ingestion of ivermectin compared to day 7, 10, and 13 feedings (P < 0.001). The mean mortality of mosquitoes fed on treatment group at day 4 was significantly higher compared day 7, day 10, day 13 and control (P < 0.01). The mean survival day of mosquitoes fed on day 1 was 2.1 days, while those fed on day 4 survived 4.0 days. Anopheles mosquitoes fed on treatment group at day 7 produced lower number of eggs than those fed on day 10, day 13 and control. However, the difference was no significant between day 10, day 13 and control. The significant effect of ivermectin on fertility of mosquitoes was observed on day 7 post ivermectin treatment. In conclusion, a single oral dose of ivermectin induced significant mortality for seven days and reduced fecundity and fertility of *An. arabiensis* even after day 7 of administration. And it is recommended to conduct study on the effect of ivermectin on insecticide resistant mosquito population before public use for malaria control.

778

POTENTIAL DISTRIBUTION OF *ANOPHELES ARABIENSIS* IN THE AMERICAN NEOTROPICS

Juan C. Hernandez, Mariano Altamiranda-Saavedra, Margarita M. Correa

Grupo de Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Medellin, Colombia

Mosquitoes are well-known biological invaders able to colonize areas with environmental suitability for survival. One of the most important invasions in the context of malaria epidemiology was that of *Anopheles arabiensis* in Brazil. Therefore, this work estimated the fundamental niche of *An. arabiensis* to determine its potential distribution in the American Neotropics. Records of species occurrence and bioclimatic layers (WorldClim v2.0 ~5Km) were used. Bioclimatic layers were delimited to the area accessible to the species (M), corresponding to natural ecoregions. A potential distribution model was designed using maximum entropy algorithm in MaxEnt, defining the parameters of the model with the Akaike information criterion (AIC). The result was validated by applying the partial ROC test in Nichetoolbox and the model was reclassified in a binary map. In general, the results indicate a wide potential distribution of *An. arabiensis* in the American neotropics, with exception of Sierra Madre in Mexico and the Andean mountain range in western South America. These results are in accordance with the wide range distribution, adaptability and invasion capacity, characteristic of this species. Knowledge of suitable areas for *An. arabiensis* provides the information for the design of preventive measures in the possible event of reemergence and propagation of this vector in the Americas.

779

PRELIMINARY ENTOMOLOGICAL FINDINGS FROM ROUTINE MONITORING OF MALARIA VECTOR POPULATION IN FOUR SENTINEL SITES IN LIBERIA, WEST AFRICA

Ibrahima Baber[1], Agnes Nador[2], Chrispin Williams[2], Tuwuyor Belleh[1], Paye Nyansaiye[2], Julius Teahton[3], Harris Momo[4], Mamadou O. Diallo[5], Jessica Kafuko[6], Tiffany Clark[7], Aklilu Seyoum[7], Peter Obenauer[8], Yemane Yihdego[9], Jennifer Armistead[10]

[1]*U.S. President's Malaria Initiative (PMI) VectorLink (VL) Project, Abt Associates Inc., Monrovia, Liberia,* [2]*National Malaria Control Program, Ministry of Health, Monrovia, Liberia,* [3]*National Public Health Institute of Liberia (NPHIL), Ministry of Health, Monrovia, Liberia,* [4]*University of Liberia, Faculty of Sciences, Monrovia, Liberia,* [5]*U.S. President's Malaria Initiative, Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*U.S. President's Malaria Initiative, U.S. Agency for International Development (USAID), Monrovia, Liberia,* [7]*PMI VectorLink (VL) Project, Abt Associates Inc., Rockville, MD, United States,* [8]*PMI, Navy and Marine Corps Public Health Center Detachment, Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [9]*PMI VectorLink (VL) Project, Abt Associates Inc., Accra, Ghana,* [10]*PMI, United States Agency for International Development, Washington, DC, United States*

Mosquito surveillance was conducted from 2015 to 2018 to investigate malaria vectors in different zones in Liberia. Data were collected monthly (pyrethrum spray catches [PSC] and Centers for Disease Control and Prevention light traps) or bimonthly (human landing catches [HLC]) from four sentinel sites: Tomato Camp (Northern hinterland), Jeneta (Central hinterland), Frank Town and Bokay Town (coastal area). All are rural and non-irrigated areas with malaria prevalence of 52% - 62%. *Anopheles gambiae* s.l. was the predominant species collected across all sites (95%; 9525/10017), with *An. funestus* comprising 5% (492/10017) of the total collection. A total of 5758 *An. gambiae* s.l. were collected using PSC with the following site distribution: Frank Town (35%), Jeneta (30%), Tomato Camp (33%), and Bokay Town (2%). A greater proportion of *An. gambiae* s.l. were collected indoors (79%; 1535/1943) compared to outdoors (21%; 408/1943) using CDC light traps, however the outdoor traps were not baited. Using HLC, a higher number of *An. gambiae* s.l. were collected trying to bite outdoors (57%; 1047/1824) compared to those caught indoors (43%; 777/1824). The highest indoor human biting rates (HBRs) were reported in Tomato Camp (14 bites/person/night) and Frank Town (10 bites/person/night). The highest outdoor HBRs were observed in Jeneta (17/person/night) and Frank Town (13/person/night). *An. gambiae* s.l. sporozoite rates as determined by *Plasmodium* circumsporozoite enzyme-linked immunosorbent assay were 5.4% (107/1999) in Tomato Camp, 2.6% (49/1,863) in Frank Town, and 2.7% (46/1698) in Jeneta. The high densities and sporozoite infection rates of *An. gambiae* s.l. indicate continued malaria transmission risk and the need to scale up vector control interventions, mainly optimization of access to long-lasting insecticide treated bed nets, to improve community-wide protection from indoor exposure to infective mosquito bites.

780

SEX-SPECIFIC RESPONSES OF *ANOPHELES GAMBIAE* MOSQUITOES TO A MOSQUITO-BORNE ALPHAVIRUS INFECTION

Karen Kemirembe, Jason Rasgon

The Pennsylvania State University, University Park, PA, United States

In humans, hormone- related sex differences dictate various health aspects. For example, they affect how we metabolize medications, sex-specific treatment outcomes, and disease symptoms. In mosquitoes, sex differences are integral in important aspects of their biology; such as female blood-feeding, reproduction, and pathogen transmission making females, and not the males, the primary focus of most research. Male mosquitoes, in contrast, only feed on sugar. Because male mosquitoes can acquire some virus pathogens from their infected mothers by vertical transmission and pass these on to their female counterparts during mating, it is important not to ignore their potential role in disease transmission. Since female mosquitoes are more likely to encounter viral pathogens during blood feeding, we hypothesize that there will be sex-specific differences in the response of mosquitoes to pathogen infection. My research therefore aims to compare responses of male and female *Anopheles gambiae* mosquitoes to a mosquito- borne alphavirus (O'Nyong Nyong Virus [ONNV]). Results on survival comparisons, within- host virus multiplication, modes of transmission will be discussed. Experiments on sex-specific transcriptional differences upon ONNV infection are also underway. My results will inform on the overlooked possible contributions of male mosquitoes to virus persistence in the environment, help inform research efforts to use male mosquitoes, rather than just the females, as tools to reduce mosquito borne infections, and help predict the potential drawbacks of recently developed techniques such as mosquito sex ratio distortion for controlling vector-borne diseases.

781

ENHANCED SURVEILLANCE FOR DENGUE, ZIKA AND CHIKUNGUNYA IN THE SOLOMON ISLANDS

Tanya Russell[1], Albino Bobogare[2], David MacLaren[1], Emma McBryde[1], Paul Horwood[1], Tom Burkot[1]

[1]*James Cook University, Cairns, QLD, Australia,* [2]*National Vector Borne Disease Control Program, Honiara, Solomon Islands*

The transmission of dengue has been increasing at an alarming rate, more than doubling in the Western Pacific Region since 2008. At the same time, Zika and chikungunya have been emerging, but little is known about transmission in the Western Pacific region. Enhanced surveillance is essential to contain these threats and to respond rapidly to outbreaks. We assessed exposure to arboviruses including dengue, Zika and chikungunya, in the Solomon Islands by a cross-sectional serosurvey with a concurrent questionnaire to determine risk factors, fever histories (including malaria diagnosis) and potential dispersal though travel histories. The survey sampled almost 2400 people across at 23 villages in 4 provinces of the Solomon Islands including both the national and two provincial capitals as well rural villages. Summarised results detailing the antibody prevalence and risk factors of dengue, Zika, chikungunya and Ross River viruses will be presented together with information on the distribution of *Aedes aegypti* and *Ae albopictus*. The study population was also highly mobile with frequent domestic and international travel reported. This has implications for the spread of these important human viruses and the need for effective national and regional surveillance systems to improve regional health security.

782

MALARIA TRANSMISSION PROFILE ACROSS BENIN DEPARTMENTS: AN ESSENTIAL ELEMENT FOR BETTER PLANNING OF VECTOR CONTROL INTERVENTIONS

Filemon Tokponnon

National Malaria Control Program, Cotonou, Benin

Entomological surveillance in Benin has historically been limited to zones where indoor residual spraying is performed or where long-standing sentinel surveillance sites exist. However, significant geographical surveillance gaps exist. The National Malaria Control Program (NMCP) assessed population dynamics of *Anopheles* vectors and malaria transmission in each of Benin's 12 departments to generate an entomological profile across Benin during the rainy season. The study was carried out in two communes per department (24/77 communes) chosen to ensure diverse geographic and ecological scope. We selected two villages per commune and four households (HH) per village to collect mosquitoes using nighttime Human Landing Catches (HLCs). In each HH, an indoor and outdoor HLC session occurred between 7pm-7am for two consecutive nights during one of the months between July-September 2017. Captured *Anopheles* were identified and ovaries were dissected to determine parity. Heads and thoraces were tested for sporozoites of *P. falciparum* by ELISA. The Entomological Inoculation Rate (EIR) was calculated as the product of mosquito bite rate and sporozoite index. Bite rates from *An. gambiae s.l.,* the main vector identified in this study, varied considerably from one commune to another. Analyses revealed an average sporozoite infection index of 3.5%. The EIR ranged from 0.02 infectious bites (ib) per human per night in the departments of Ouémé and Plateau to 1.66 ib/human/night in Collines. According to the scale of transmission level, Avrankou, Sakété and Nikki are areas of low transmission (0<EIR<3 ib/human/year), Adjarra, Adja Ouèrè, Zè, Toffo, Bopa, Pehunco, Pèrèrè and Kandi are medium transmission (3<EIR<30 ib/human/year), and other districts are high transmission (EIR> 30 ib/human/year). The study showed the heterogeneous and diverse nature of malaria transmission in Benin that is not readily apparent when only assessing entomological surveillance from sentinel sites. Moving forward, NMCP will use the results of this study to stratify and plan vector control interventions in districts with high EIRs to better protect populations most at risk.

783

CONTRIBUTION OF INSECTICIDE TREATED MOSQUITO NETS DISTRIBUTION IN PRIMARY SCHOOLS IN MAINTAINING HOUSEHOLD COVERAGE, ATLANTIC DEPARTMENT, BENIN, 2018

Richard Dossou Yovo

[1]*Accelerating the Reduction of Malaria Mortality and Morbidity Project (ARM[3]), Medical Care Development International, Cotonou, Benin, Cotonou, Benin*

To maintain household (HH) coverage of insecticide-treated mosquito nets (ITN), WHO recommends continuous ITN distribution in schools and health facilities in addition to mass campaigns. In July 2018, eight months after a mass ITN campaign, Benin piloted ITN distribution in 681 Atlantique Department public schools; 36,557 and 19,512 ITNs were distributed to each CI (6-year-old) and CM2 (11-year-old) student, respectively. Pregnant women and children <1 year old continued to receive ITNs at routine health visits. To assess impact on HH coverage, surveys were performed before (December 2017) and after (September 2018) school distribution in 475 HH in Atlantique Department where school ITN distribution occurred and in Zou Department where no school distribution occurred. HH were recruited using stratified two stage cluster sampling. Departments were divided into communes. In each commune, 2-3 villages were randomly selected with the probability of selection proportional to village size for a total of 19 villages; 25 HH were randomly selected per village. Data analyses were performed with SPSS 20. Adjusted Pearson Chi2 tests to account for clustering were used to compare proportions with 95% confidence level. Most HH were in rural areas (Atlantique: 79%, Zou: 89%); 65.9% (95% CI: 61.6, 70.2) and 62.5% (95% CI: 58.14, 66.85) of HH in Atlantique and Zou, respectively, had ≥1 child enrolled in primary school. In both departments, >75% of HH had ≥1 ITN at baseline (Atlantique: 78.7% [95% CI: 75.0, 82.4, Zou: 80.6% [95% CI: 77.0, 84.2] and <50% had ≥1 ITN for every two people (Atlantique: 41.8% [95% CI: 37.4, 46.2], Zou: 45.7% [95% CI: 41.2, 50.2]). After school distribution, the proportion of HH with ≥1 ITN remained stable compared to baseline in Atlantique (from 79% to 78%) but decreased in Zou (from 88% to 81%) (P = 0.02). The proportion of HH with ≥1 ITN for two people remained stable in Atlantique (from 42% to 42%) (P=0.89) and Zou (from 48% to 46%) (P=0.09). School ITN distribution can play a role in maintaining HH ITN coverage. Further research is needed to evaluate cost-effectiveness of continuous distribution channels in combination with other strategies.

784

DIVERGENCES IN BLOOD-FEEDING AND RESTING BEHAVIORS OF *ANOPHELES GAMBIAE* AND TRANSMISSION OF MALARIA AND LYMPHATIC FILARIASIS IN RICE GROWING AREAS IN CÔTE D'IVOIRE

Julien B. Zahouli[1], Aboulaye Méité[2], Benjamin G. Koudou[1], Jürg Utzinger[3]

[1]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [2]*Programme National de Lutte contre les Schistomiases, Geo helminthiases et Filariose, Abidjan, Côte D'Ivoire,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Anopheles gambiae females' behavior patterns during their gonotrophic cycles are crucial for the transmission of malaria and lymphatic filariasis, and vector control success. We assessed blood-feeding and resting behaviors of *An. gambiae* and the transmission of *Plasmodium falciparum* and *Wuchereria bancrofti* along a rural-suburban gradient in irrigated rice growing areas in Côte d'Ivoire. Mosquitoes were collected indoor and outdoor using light-traps, man-nights and pyrethroid sprays in rural and suburban areas. The ovaries and salivary glands of *An. gambiae* females were dissected. *P. falciparum* and *W. bancrofti* were identified using PCR (Polymerase Chain Reaction). *An. gambiae* was the predominant species, representing 97.4% in suburban and 81.5% in rural areas. Biting rates significantly correlated with rice field landscape development stages and peaked during transplanting and tillering. Daily host-seeking cycles

showed that the biting rates increased gradually from 6 p.m., reached peaks around midnight and decreased progressively to reach minimums at 6 a.m. in all areas. However, biting and resting behaviors were indoor-centered in rural and house-independent in suburban areas: endophagic rate and indoor resting density were 69.4% (n = 4,798) of 14.9 females/bedroom/day (f/b/d) in rural, and 49.3% (n = 6,775) and 2.9 f/b/d in suburban areas, respectively. The parturity rates were estimated at 84.5% in suburban and 75.8% in rural areas. *P. falciparium* infection rates and inoculation entomological rates were estimated at 8.1% and 6.4 infected bites/person/night (ib/p/n) in rural, and 6.6% and 6.5 ib/p/n in suburban areas. *W. bancrofti* infection rates and average loads were of 0.53% and 1.86 L3/infective female (L3/if) in suburban, and 0.34% and 2.0 L3/if in rural areas, respectively. Tendency of *An. gambiae* females to change their blood-feeding and resting behaviors across an urbanization level may support malaria and lymphatic filariasis transmission and compromise current vector control strategies based on ITNs (Insecticide-Treated Nets) and IRS (Indoor Residual Spray). Integrated vector management should be required.

785

IMPACTS OF LARVA-ACQUIRED *AEDES AEGYPTI* MICROBIOTA ON VECTOR COMPETENCE FOR ZIKA VIRUS

William Louie, Lark L. Coffey

University of California Davis, Davis, CA, United States

The mosquito microbiome is an important factor shaping the ability of a mosquito to transmit arboviruses like Zika virus (ZIKV), termed vector competence. Proposed mechanisms for microbial influence on vector competence include but are not limited to priming of the mosquito immune system. However, our understanding of the scale and variability of microbiome-vector interactions remains in its infancy. Because mosquitoes initially acquire microbes from their aqueous environment as larvae, and a significant fraction of larvae-derived microbiota persist in the adult stage, it is expected that mosquitoes with increased microbial exposure as larvae become less competent vectors. Moreover, higher observed transmission rates by laboratory-colonized mosquitoes relative to field-caught mosquitoes, historically attributed to mosquito genetic bottlenecking, may also be explained by reduced microbial exposure in laboratory settings. The goal of this project is to determine the impacts of bacterial microbiota acquired during larval development on ZIKV vector competence in *Aedes aegypti*, the primary ZIKV vector worldwide. We observed interesting phenotypic differences in mosquitoes reared in water with reduced microbial diversity, such as delayed pupation time and darkening of fourth-instar larval bodies. Upon exposure to a ZIKV-spiked bloodmeal, 97% of these mosquitoes became ZIKV-infected while less than 40% of mosquitoes reared in microbe-rich stagnant water became ZIKV-infected. These preliminary results suggest that microbial exposure during the larval phase of development in *Aedes aegypti* mosquitoes confers resistance to ZIKV infection.

786

CHANGING EPIDEMIOLOGICAL PATTERN OF VISCERAL LEISHMANIASIS IN NEPAL

Lila Bikram Thapa[1], Surendra Uranw[2], Bibek Kumar Lal[1]

[1]*Ministry of Health and Population, Kathmandu, Nepal,* [2]*Tropical and Infectious Disease Centre/B.P. Koirala Institute of Health Sciences, Dharan, Nepal, Nepal*

On the Indian subcontinent, visceral leishmaniasis (VL) is targeted for elimination as a public health problem by 2017. Nepal has achieved the elimination target at district level in 2013 and has been sustained the situation since then. Recently, we conducted a field surveys in non-program hilly districts in Nepal where increasing number of VL cases have been persistently reported since 2000. A house-to-house survey in 14 villages from eight hilly districts documented retrospectively 54 cases of VL, predominantly males, mostly pediatric cases who were reported in the last five years. Anti-Leishmania antibodies were found in 22/23 past-VL cases, in 40/416 (9.6%) persons without VL and in 12/155 (7.7%) domestic animals. An age- and sex-matched case-control study showed that exposure to known VL-endemic areas was no risk factor for VL, but having a VL case in the neighborhood was. SSU-rDNA PCR for Leishmania sp. was positive in 24 (5%) of the human, in 18 (12%) of the animal samples and in 16 (14%) bloodfed female *Phlebotomus argentipes* sand flies. *L. donovani* was confirmed in two asymptomatic individuals and in one sand fly through hsp70-based sequencing. This study proves that there is indeed ongoing local transmission of *Leishmania donovani* in areas at an altitude above 600 meters, districts considered hitherto non-endemic for VL. This geographical expansion of cases and ongoing local transmission could challenge the aim of the VL elimination program in Nepal. Hence, policy makers should give a high priority in expanding active surveillance and control activities to achieve the realistic goal.

787

NOSOI: TRANSMISSION CHAIN SIMULATOR IMPLEMENTING WITHIN-HOST DYNAMICS TO LEVERAGE VECTOR COMPETENCE DATA

Sebastian Lequime[1], Paul Bastide[1], Simon Dellicour[2], Albin Fontaine[3], Guy Baele[1], Philippe Lemey[1]

[1]*KU Leuven, Leuven, Belgium,* [2]*Université Libre de Bruxelles, Brussels, Belgium,* [3]*Institut de Recherche Biomédicale des Armées, Marseille, France*

Vector competence (VC) defines the intrinsic ability of a vector to carry and transmit a pathogen. VC is usually scored experimentally as a prevalence of infected and infectious vectors at given times post-pathogen exposure. Each year, numerous experimental studies aim to evaluate vector species or local population's competence for vector-borne pathogens, ultimately intending to understand epidemiology and assess local risk of transmission for potentially introduced vector-borne pathogens. These experimental data however usually remain purely descriptive and poorly reflect the dynamic nature of VC. Thank to the estimation of within-host dynamics parameters when performing vector-competence assays, combined with the use of epidemiological model, vector competence studies can translate VC dynamic changes into their epidemiological consequences. Such modelling efforts are yet to become conventional: epidemiological modeling rarely consider within-host dynamics, usually relies on non-trivial mathematics and very few authors even share the practical implementation (i.e. code) of their models. To address this issue, we present Nosoi, a stochastic agent-based transmission chain simulator specifically designed to integrate within-host dynamics in a flexible framework. Nosoi is open-source and available as a R package. A series of tutorials is available on Nosoi's website (https://slequime.github.io/nosoi/), one of which is intended for vector competence studies, specifically designed to guide the user on how to use the simulator, tune it to its needs and visualize its output. Nosoi allows for user-set models of various level of complexity (including inter-individual variations, geography or the influence of other covariates), tailoring them to be as close as possible to local settings based on previous knowledge, thereby leveraging experimentally acquired data into epidemiological insights for vector-borne diseases.

788

ZIKA VIRUS SEROPREVALENCE DECLINES AND NEUTRALIZATION ANTIBODIES WANE IN ADULTS FOLLOWING OUTBREAKS IN FRENCH POLYNESIA AND FIJI

Alasdair Henderson[1], Maite Aubry[2], Mike Kama[3], Jessica Vanhomwegen[4], Anita Teissier[2], Teheipuauara Mariteragi-Helle[2], Tuterarii Paoaafaite[2], Jean-Claude Manuguerra[4], John Edmunds[1], Jimmy Whitworth[1], Conall Watson[1], Colleen Lau[5], Van-Mai Cao-Lormeau[2], Adam Kucharski[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Institut Louis Malardé, Papeete, French Polynesia,* [3]*Fiji Centre for Communicable Disease Control, Suva, Fiji,* [4]*Institut Pasteur, Paris, France,* [5]*Australian National University, Canberra, Australia*

Serosurveys published following major outbreaks of Zika virus (ZIKV) have so far shown a high level of seroprevalence from samples collected within 12 months of the first confirmed case. A common assumption is that ZIKV infection confers long-term protection against reinfection, preventing ZIKV from re-emerging in previously affected areas for many years. However, the long-term immune response to ZIKV following an outbreak remains poorly documented. We compared results from eight serological surveys, with sample sizes ranging from 49 to 700, before and after known ZIKV outbreaks in the Pacific region: five from cross-sectional studies of schoolchildren and the general population in French Polynesia over a seven-year period; and three from a longitudinal cohort in Fiji over a four-year period. We found strong evidence of a decline in seroprevalence in both countries over a two-year period following first reported ZIKV transmission. In the cohort in Fiji, there was also a significant decline in antibody titres against ZIKV. However, the decline in seroprevalence was concentrated in adults, while high seroprevalence persisted in children. To our knowledge, our study is the first to investigate long-term seroprevalence patterns and antibody dynamics following the introduction of ZIKV. Given that ZIKV outbreaks in Latin America were first reported 2-3 years after the outbreak in French Polynesia, findings from this study could be an early indication of what might subsequently be observed in Latin America. Several modelling studies have suggested that the ZIKV epidemic is over in countries affected by the recent epidemic. Our results show that long-term ZIKV neutralization antibody levels can wane substantially in adults following an initial outbreak; if this leads to reduced herd immunity to ZIKV, the potential for future re-emergence of infection may need to be reconsidered.

789

DETERMINING ENTOMOLOGICAL DRIVERS OF MALARIA TRANSMISSION IN ENDEMIC REGIONS IN NAMIBIA

Iitula Iitula[1], Stark T. Katokele[1], Deodatus Maliti[1], Ophilia Lukubwe[1], Tabeth Mwema[2], Rosali Joseph[2], Dennis Walusimbi[3], Sheila Ogoma[3], Elodie Vajda[4], Neil Lobo[5], Tara Seethaler[3], Deepa Pindolia[3], George Shirreff[3], Joseph Zvoushoma[3], Ayokunle Abogan[3], Charlotte Dolenz[3], Allison Tatarsky[4], Yasmin Williams[4], Cara Smith-Gueye[4], Davis Mumbengegwi[2], Petrina Uusiku[1]

[1]*National Vector-borne Disease Control Programme, Windhoek, Namibia,* [2]*University of Namibia, Windhoek, Namibia,* [3]*Clinton Health Access Initiative, Boston, MA, United States,* [4]*University of California San Francisco, San Francisco, CA, United States,* [5]*University of Notre Dame, Notre Dame, IN, United States*

Despite 85.5% coverage of indoor residual spraying (IRS) and improved case management, Namibia has seen a steady rise of the malaria incidence from 1.4 cases per 1000 population in 2012 to 14.8 cases per 1000 population in 2018. Transmission is characterized by a series of annual epidemics associated with seasonal heavy rains, especially in the northern districts of the country. In order to reach the goal of eliminating malaria by 2022 in Namibia, the National Vector-borne Diseases Control Programme (NVDCP) sought to investigate key entomological determinants that could be driving malaria transmission in priority regions. Entomological surveillance was conducted in 9 sentinel sites in the northern regions of the country between March and June of 2018 and 2019. Human landing catch and pyrethrum spray catch mosquito sampling methods were used to determine the malaria vector species composition, their temporal and geographical distribution, as well as the biting and resting behavior. Furthermore, behavioral interactions between humans and mosquitoes were measured based on the risk of exposure of humans to infectious bites by comparing the time humans are likely to be indoors under bed nets. Susceptibility of wild mosquito populations to insecticides used in IRS was evaluated. Molecular assays revealed that approximately equivalent numbers of *Anopheles arabiensis* and *An. gambiae* sensu stricto were found across the sites. Approximately 75% of the bites occurred outdoors throughout the night. In addition, resistance to deltamethrin and DDT was suspected or confirmed in two out of the nine sentinel sites prompting the NVDCP to introduce Actellic 300CS for IRS in some regions. This programmatic evaluation highlights the significance of determining the change in malaria vector populations, opportunities to understand the efficacy and limitations of vector control interventions, and the use of entomological data to better target interventions and reduce malaria transmission.

790

USING LIVE CATCHES OF MOSQUITOES AS A TOOL TO ASSESS MALARIA TRANSMISSION IN ONE VILLAGE OF MALI: COMMUNITY ACCEPTANCE AND EFFICACY

Daman Sylla[1], Adama Sacko[1], Jen C.C. Hume[2], Abdrahamane Fofana[1], Emily Higbee[2], Boubacar Coulibaly[1], Makan Camara[1], Lakamy Sylla[1], Salifou Kone[1], Gaoussou Fofana[1], Moribo Coulibaly[1], Moridie Sidibe[1], Karim Sawadogo[1], Cheick O. Sanogo[1], Sale Sidibe[1], Chata Doumbia[1], Boubacar Tembely[1], Issaka Sagara[1], Jennifer Kwan[2], Sekou F. Traore[1], Patrick E. Duffy[2], Mamadou B. Coulibaly[1]

[1]*MRTC/USTTB, Bamako, Mali,* [2]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States*

Understanding malaria transmission is key for the implementation of certain types of malaria prevention and control strategies such as vaccines that interrupt malaria transmission (VIMTs). Many tools are used to measure malaria transmission, however, there are very few data on the use of large scale live catches of mosquitoes for that purpose. Here we measure malaria infection rates using live catches of mosquitoes collected from human dwellings. Every month a team of 4 technicians and 32 guides collected in 63 compounds comprising 503 huts in Bancoumana village. From March 2018 to February 2019, *Anopheles* mosquitoes were collected using mouth aspirators in a number of huts varying from 372 to 494, for a total of 5267 hut collections. Mosquitoes were allowed to rest in a field insectary for seven days before dissection. The survival to dissection for oocyst detection, the percent infected (as measured by oocysts), were determined as well as the average mosquito densities per hut by month. 95% of the hut owners consented for mosquito collection. Overall, 3,447 *Anopheles* were collected ranging from 5 in February to 1257 in September with a mean of 287 mosquitoes per month. The monthly mosquito density per hut ranged from 2.9 in September to 0.01 in February with a mean of 0.7 (SD=1.0). The mean survival rate was 76% (N=3447) ranging from 40% (N=5) in February to 100% (N=6) in January. The mean infected rate was 2.05% [0-10] (N=2625). In conclusion, the live catches seem to be well accepted in the community. This lays the groundwork for exploring such a tool for measuring transmission before and after implementing a VIMT or any other transmission blocking interventions.

791

EVALUATION OF THE BLOOD FEEDING STATUS AND PARITY IN *AEDES AEGYPTI* IN IQUITOS, PERU

Helvio Astete

Naval Medical Research Unit-6, Iquitos, Peru

Aedes aegypti is considered the most important arboviral vector of public health concern because it can transmit arboviruses such as dengue to humans in urban environments. Female *Ae. aegypti* seek blood meals to obtain the required nutrients for egg maturation, but characteristically feed multiple times during each egg-laying cycle, allowing this species to infect multiple people after becoming infectious. For dengue virus, *Ae. aegypti* would need a minimum of 8 days after ingestion of virus to transmit to additional hosts during subsequent blood meals. Understanding the parameters associated with blood feeding behavior and parity status (a crude surrogate for mosquito age) may have more epidemiological significance than vector densities alone and allow for modeling to better understand virus transmission risks. We compared the feeding and parity status of mosquitoes collected during a double-blinded randomized cluster trial evaluating the efficacy of a mosquito spatial repellent product. Adult Prokopack aspirator collections were carried out at 2-week intervals in 26 clusters of approximately 150 households each. We collected 11,680 female *Ae. aegypti* from January 2017 to December 2018, and 69.85% (8,159) were scored for blood feeding status and dissected to determine parity status (nulliparous, parous, gravid). 58.11% of collected mosquitoes were fully engorged with fresh blood, 16.46% had partial or were digesting blood meals, and 25.43% had no blood meal. The majority of dissected female mosquitoes were parous (26.03%) and gravid (67.45%) with only 6.52% nulliparous. Blinding will not be broken until all laboratory testing and data cleaning has taken place, therefore results have not yet been analyzed for mosquito repellent efficacy. We will assess if association of frequency of blood feeding with mosquito age is affected by the spatial repellent product. If reduced transmission risk is observed, these endpoints could be used in future vector control intervention trials to evaluate novel products. Key words: Gravity, nulliparity, population structure, gonotrophic cycle.

792

EVOLUTION OF COLD TOLERANCE TRAITS IN THE INVASIVE MOSQUITO *AEDES ALBOPICTUS*

Alexandra Mushegian, Zachary Batz, Peter Armbruster

Georgetown University, Washington, DC, United States

The Asian tiger mosquito, *Aedes albopictus*, is a significant vector of arboviruses including dengue and Chikungunya. This mosquito has spread rapidly to all continents except Antarctica over the last 35 years. One of the key adaptations underlying this rapid spread is the ability to overwinter in the form of diapausing eggs, which are produced by adult females in response to changes in day length signaling impending winter. Previous results from our lab demonstrated that diapause timing in *Ae. albopictus* evolved rapidly during invasion and range expansion in eastern North America. As such, diapause timing plays an important role in determining the mosquito's abundance over space and time in temperate habitats. Given the ecological trade-offs involved in winter diapause, diapause incidence and timing are also expected to be important in determining how mosquito populations respond to ongoing global warming. We have found geographic differentiation among North American populations for thermal performance and traits expressed during diapause. We are also currently measuring diapause incidence and timing of populations collected along a latitudinal cline in the mosquito's native and introduced range (Japan and the U.S., respectively). This study recapitulates a previous study performed 10 years earlier with many populations collected from the exact same sites. Our results will be used to test the hypothesis that diapause timing and incidence have evolved in response to climatic shifts and repeated introductions. Understanding the evolutionary changes underpinning adaptation of Ae. albopictus to diverse climatic regimes will be crucial for modeling range shifts and local population dynamics of this vector under climate change.

793

EFFECT OF SUCROSE CONCENTRATION AND FASTING IN *PLASMODIUM VIVAX* EXPERIMENTAL INFECTIONS OF *NYSSORHYNCHUS* (AKA *ANOPHELES*) *DARLINGI*

James Beuzeville-Jaen[1], Carlos Tong[1], Manuela Herrera-Varela[1], Carmen Reategui[2], Joseph M. Vinetz[3], Jan E. Conn[4], Marta Moreno[5]

[1]*Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Iquitos, Peru,* [2]*Universidad Nacional de la Amazonía Peruana, Iquitos, Peru,* [3]*Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT, United States,* [4]*Wadsworth Center, New York State Department of Health, Albany, NY, United States,* [5]*Department of Infection Biology; London School of Hygiene & Tropical Medicine, London, United Kingdom*

Nyssorhynchus (AKA *Anopheles*) *darlingi*, major malaria vector in South America, was successfully colonized in 2013 in the Peruvian Amazon. Since then, *Ny. darlingi* has been used in experimental infections with *Plasmodium vivax* to study the biology of transmission (i.e. asymptomatic infections), in transmission blocking assays and in production of sporozoites for *P. vivax* liver stages experiments. Starving periods and sucrose concentration (daily sugar intake) of the adult females prior to blood feeding are of vital importance given that sugar availability has been correlated with the predisposition to feed and therefore related to the success of the parasite infection. Here, we sought to determine the effect of sucrose concentrations and starving period on development of *Plasmodium vivax* in *Ny. darlingi.* Six groups, of 60 one-day-old female mosquitoes, were fed on sugar concentrations, three of those with 5% and other three with 10%, then three starving periods were tested in each category (24, 48 and 72 h). To assess the effect of these treatments on *Plasmodium vivax* parasite development, direct membrane feeding assays were conducted and mosquito midguts dissected 7-8 days post infected blood meal. Experiments were carried out in five different times with five *P. vivax*-blood donor. A total of 924 mosquitoes were dissected an average of 185 (SE±55) mosquitoes per infection. A higher infection prevalence of 84.2% infected mosquitoes (60% - 100%) was detected in those fed on 5% sugar and 24h starving compared with other treatments. The highest infection intensity was found in a combination of 10% sugar and 48h starvation period, with an average of 72 oocyst per mosquito (0 - 424). Infection percentage was affected by sugar concentration and the oocyst density by starving treatment. This study provides valuable information about parasite development in the *vivax-darlingi* model with important implications for malaria transmission in the Amazon region.

794

THERMAL PERFORMANCE OF *AEDES AEGYPTI* AND IMPLICATIONS FOR CLIMATE CHANGE

Nina Dennington, Marissa K. Grossman, Janet Teeple, Matthew B. Thomas

The Pennsylvania State University, University Park, PA, United States

Many aspects of mosquito life history are influenced by environmental temperature. Accordingly, there is considerable concern that increases in temperature due to ongoing climate change could lead to shifts in the dynamics and distribution of vectors such as *Aedes aegypti*, with concomitant changes in the risk of transmission of diseases like dengue, Zika and yellow fever. Current mechanistic models that predict optimal temperatures for transmission tend to assume there are 'average' thermal performance curves that are representative for a given vector species. However, if there is thermal adaptation to a local environment this could create population-level differences in thermal performance, challenging the 'one size fits all' approach. In this study, we explored whether there

are population differences in thermal performance by generating thermal performance curves for five field populations of *Ae. aegypti* from Mexico, together with a standard laboratory strain. We reared these six populations at a range of temperatures between 13°C and 37°C and measured various life history traits including development time, fecundity, and adult survival. We found differences between populations in the temperature optima and the critical thermal minima and maxima, for a range of life history traits. Our results suggest that single species-level thermal performance curves might be insufficient for predicting the outcome of infectious disease transmission at the local level under conditions of climate change.

795

THE USE OF SPATIAL VIDEO GEONARRATIVES TO DESCRIBE LOCALIZED ENVIRONMENTAL RISK PATTERNS FOR ARBOVIRAL TRANSMISSION IN URBAN KENYA

Amy R. Krystosik[1], Andrew Curtis[2], Paul Mutuku[3], Sandra Bempah[2], Jayakrishnan Ajayakumar[2], Lorriane Odhiambo[4], Donal Bisanzio[5], Jenna Forsyth[6], Luti Mwashee[7], Beja Adamz[7], Francis Mutuku[3], A. Desiree LaBeaud[1]

[1]*Stanford University School of Medicine, Department of Pediatrics, Division of Infectious Disease, Stanford, CA, United States,* [2]*Kent State University, Department of Geography, GIS, Health and Hazards Lab, Kent, OH, United States,* [3]*Technical University of Mombasa, Environment and Health Sciences Department, Mombasa, Kenya,* [4]*Kent State University, College of Public Health, Kent, OH, United States,* [5]*RTI International, Washington, DC, United States,* [6]*Stanford University School of Earth, Stanford, CA, United States,* [7]*Vector borne disease control unit, Msambweni Field Laboratory, Kwale County, Kenya*

Urban arboviral disease mitigation depends on costly vector control targeted to high-risk areas which are difficult to identify. Disease ecology and epidemiology vary over space and time, requiring fine scale data on environmental and behavioral factors to evaluate disease risk. Spatial video geonarratives (SVGs) are a community based participatory method of capturing fine scale environmental data over time. We have captured 37/80 hours of SVGs from key informants (local vector control technicians, community health workers, leaders and residents) during the dry season in Ukunda (17 informants) and Likoni (20 informants), in Kwale County, Kenya, regions previously studied. Informants provided commentary on fine scale environmental risks during GPS and video recorded neighborhood walks with a technician. Using novel software, commentaries are spatialized to make risk maps. Commentary transcription and translation, and SVG mapping, and coding are ongoing (n=8/37). Using the SVGs, we will develop an index of vector-borne risk (SVG index) using contextualized environmental risks (e.g. trash and standing water) at 0.1km^2 sub-sections of known disease risk. SVG environmental risks hotspots will be compared with previously identified dengue and chikungunya exposure and *Aedes* spp. vector abundance hotspots by visual inspection and using a geographically weighted Poisson regression controlling for known confounders. Preliminary results from SVG data suggest trash clusters over space due to geographic and behavioral factors, and that there is a locally perceived connection to vector abundance in the rainy season. Informants identified breeding sites such as holes in the ground that fill with rainwater, trash—coconut shells, dump sites, plastic containers—and uncovered household containers and vegetation patches or plant types as spatial-micro risk environments. Micro-scale risk pattern identification will allow forecasting fine scale, locally informed, high-risk exposure areas and provide policy recommendations to more effectively target vector control efforts where public health resources are limited.

796

NANOTRAP PARTICLE EXTENDS THE DURATION OF DETECTABLE VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN HUMAN BLOOD

Shih-Chao Lin[1], Ivan Akhrymuk[1], Monique van Hoek[1], Benjamin Lepene[2], Kylene Kehn-Hall[1]

[1]*George Mason University, Manassas, VA, United States,* [2]*Cere Nanosciences Inc., Manassas, VA, United States*

Nanotrap® (NT) particles are hydrogel microparticles developed for target analyte separation and discovery applications. NT particles consist of cross-linked N-isopropylacrylamide (NIPAm) copolymers that are functionalized with a variety of chemical affinity baits to enable broad-spectrum collection and retention of target proteins, peptides, nucleic acids, and pathogens. NT particles have been shown to capture and enrich multiple pathogens including Venezuelan equine encephalitis virus (VEEV). This study describes the practical application of the NT particle technology for improved sample stability and detection of VEEV capsid protein spiked in human plasma or whole blood at elevated temperature storage conditions. We first established the baseline of stability of VEEV capsid at different temperatures in plasma or blood and evaluated the capsid levels by western blotting. We then co-incubated magnetic NT particles and VEEV at 37ºC and 40ºC and/or 54ºC for up to 72hrs. Compared to virus without NT treatment, the duration of detectable levels of VEEV in plasma and blood were remarkably increased across all temperatures. Next, we investigated the effects of humidity on the viral stability afforded by NT particles. Our results show that the humidity levels at both 16% and 98% did not cause discernible changes in NT capability over time, suggesting the suitability to utilization of NT could be extensive. Finally, we intranasally infected C3H mice with VEEV/TC83 and tested NT efficacy in mouse blood. Our findings demonstrated that NT particles could substantially increase the detectable VEEV capsid from the blood of infected animals. Taken together, our data demonstrate the ability of NT particles to preserve VEEV capsid protein in human blood samples over time and temperature. These results have implications for the use of NT particles as a tool to preserve infectious agents in clinical samples.

797

CHIKUNGUNYA AND DENGUE VIRUS SEROPREVALENCE AMONG CHILDREN IN COASTAL AND WESTERN KENYA AND RISK FACTORS FOR EXPOSURE

Shama Cash-Goldwasser[1], Jonathan Altamirano[2], Bryson Ndenga[3], Charles Muiruri Ng'ang'a[4], Said Lipi Malumbo[4], Jael Sagina Amugongo[4], Loice Lwamba[3], Francis Denga[3], Sandra Musaki[3], Francis Mutuku[5], A. Desiree LaBeaud[2]

[1]*Stanford University, Stanford, CA, United States,* [2]*Department of Pediatrics, Division of Infectious Diseases, Stanford University, Stanford, CA, United States,* [3]*Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya,* [4]*Vector Borne Disease Control Unit, Msambweni Field Laboratory, Kwale, Kenya,* [5]*Department of Environment and Health Sciences, Technical University of Mombasa, Mombasa, Kenya*

Chikungunya virus (CHIKV) and dengue virus (DENV) are recognized as endemic causes of fever among children in Kenya, where both viruses have caused increasingly large and frequent outbreaks. These diseases are poorly controlled, partly due to a lack of systematic surveillance programs. We conducted this study to determine the degree of exposure to CHIKV and DENV as well as risk factors for exposure among children in Kenya. From 2014 - 2018, we prospectively followed a cohort of children recruited from urban and rural communities in both coastal and western Kenya. We enrolled healthy children 1 - 12 years of age and followed them every 6 months. At each visit, a blood sample was taken and demographic surveys were administered. Blood was tested by IgG ELISA for antibodies to CHIKV and DENV. Over the five years of the study, 3,521 children were tested for CHIKV and DENV antibodies. Overall, 9.8% were CHIKV seropositive and 5.5% were DENV seropositive. The respective CHIKV and DENV seroprevalences by age were: 6.7% vs 3.6% in the under 5

year age group; 11.3% vs 6.2% in the 6-11 age group; and 15.5% vs 9.6% in the 12-17 age group. Differences by age were significant for both viruses (p<0.0001). The respective CHIKV and DENV seropositivities by study site were: 9.8% and 10.8% in the rural coast; 3.8% and 4.5% in the urban coast; 21.2% and 4.8% in the rural west; and 5.3% and 2.9% in the urban west. Seroprevalence in rural sites was significantly higher than in urban sites for both viruses (p<0.0001). Our results show that exposure to CHIKV is more common than DENV across all age groups of children in Kenya, and that a significant number of children < 5 years of age are exposed to both viruses. We observed an association between rural residence and seropositivity. This is in contrast with data from other settings, where the association between urban residence and disease risk is thought to be related to the habitat preference of the mosquito genus that serves as the primary vector for both viruses. Our findings have important implications for disease prevention efforts. Multivariate analysis of risk factors for infection with CHIKV and DENV is ongoing.

798

IMMUNOLOGICAL INSIGHTS BASED ON ANTIBODY BINDING EPITOPES ON THE CHIKUNGUNYA VIRUS ENVELOPE

Edgar Davidson[1], Rachel H. Fong[1], Rebecca Rimkunas[1], Jin Jing[2], Graham Simmons[2], Michael S. Diamond[3], Benjamin J. Doranz[1]

[1]*Integral Molecular, Inc., Philadelphia, PA, United States,* [2]*Vitalant Research Institute, San Francisco, CA, United States,* [3]*Washington University, St. Louis, MO, United States*

To identify the Chikungunya virus (CHIKV) structures that elicit a protective immune response, we have epitope mapped over 70 monoclonal antibodies (MAbs) against the CHIKV envelope glycoprotein E2/E1, using a comprehensive shotgun mutagenesis library of 910 E2/E1 alanine-scan mutants. Published studies used epitope maps to characterize broadly cross-reactive and ultrapotent neutralizing MAbs that blocked post-attachment steps, and whose binding mapped to functionally-important E2 domains A or B, suggesting that MAbs inhibit virus-host membrane fusion by preventing exposure of the E1 fusion loop. Other studies characterized MAbs that induce structural changes on E2 domains A and B. We also isolated 7 human MAbs against CHIKV E2/E1, including previously unreported cross-reactive, but non-neutralizing, MAbs against the highly conserved E1 fusion loop. Neutralizing epitopes are confined to the exposed topmost and outer surfaces of the E2/E1 trimer, providing a rationale for using the trimer for vaccine design and therapeutic MAb development. Our most potent MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), showed high-affinity binding (320 pM), and gave therapeutic and prophylactic protection in animal models up to 24 h post-exposure. Epitope mapping identified an inter-subunit conformational epitope on E2 domain A. Subsequent studies demonstrated that IM-CKV063 blocks both virus entry and virus release, and that optimal therapeutic activity required interaction of MAb Fc region with Fcγ receptor. We also used CHIKV E2/E1 mutants to map the binding site of cell adhesion molecule Mxra8, identified as an entry mediator for CHIKV, and other alphaviruses, by infectivity screens of cells targeted by CRISPR/Cas9 gene knockouts. Mxra8 enhanced virus attachment and internalization into cells, mapping suggests that Mxra8 binds to E2 A and B domains. Mxra8-Fc protein or anti-Mxra8 MAbs blocked CHIKV infection in multiple human cell types and in mice, suggesting Mxra8 as a target for therapeutics against CHIKV and other arthritogenic alphaviruses.

799

MAPPING AND FORECASTING CHIKUNGUNYA AT GLOBAL SCALE

Radina P. Soebiyanto[1], Assaf Anyamba[1], Bhaskar Bishnoi[1], Sarah Hutchinson[1], Mohammad Al-Hamdan[2], Muhammad Barik[2], Richard Damoah[1], Wassila Thiaw[3], Kenneth J. Linthicum[4]

[1]*NASA Goddard Space Flight Center, Greenbelt, MD, United States,* [2]*NASA Marshall Space Flight Center, Huntsville, AL, United States,* [3]*National Oceanic and Atmospheric Administration, National Centers for Environmental Predictions, Climate Prediction Center, International Desks, College Park, MD, United States,* [4]*US Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, United States*

Chikungunya has reemerged and emerged in new areas, affecting millions of people with significant economic burden, further raising concerns on preparedness for the next epidemic. A multitude of factors contribute to the (re)-emergence Chikungunya, from genetic mutation, susceptibility, travel to climate conditions. Despite these factors, suitable climate remains the background and necessary condition for an epidemic to occur. Availability of climate data allows us to continuously assess conditions suitable for chikungunya, deriving information on when, where and what is the risk for chikungunya. This information provides a tool to aid decision makers in preparedness efforts. We are developing climate-based global chikungunya risk mapping system, updated monthly and available through our CHIKRisk app. To develop the risk map, we used 1045 point-locations where chikungunya cases were reported (year 2000 - 2017), compiled from ProMED reports. 1469 chikungunya pseudo absence locations were generated while considering chikungunya seasonality in endemic areas. We used 5 climate monthly measures - land surface and air temperature, precipitation, specific humidity, soil moisture - and their derived composites (anomaly, 3-monthly, 1-month lag, etc.), a total of 90 variables. These datasets and population density were then used to calibrate machine-learning models to predict chikungunya. We found random forest model had highest accuracy in predicting locations with reported chikungunya (88.9%, 95% Confidence Interval (CI) of 86.1 - 91.2%). A forecast model was developed with air temperature and precipitation forecasts, with the model accuracy of 85.9% (95% CI of 82.9 - 88.6%). These risk maps are now produced every month and available through CHIKRisk App. The recent chikungunya outbreaks in Sudan and India were captured in both risk maps. We are validating the risk maps with reported data as we continue to compile and geo-reference chikungunya reports in 2019. Availability of these monthly risk maps can aid decision makers in making robust policies for chikungunya preparedness, prevention and control efforts.

800

POPULATION DIVERSITY-ALTERING MUTATIONS AS A METHOD FOR IMPROVING A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE

Christopher M. Weiss, Hongwei Liu, Kasen K. Riemersma, Lark L. Coffey

University of California Davis, Davis, CA, United States

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes a debilitating febrile illness marked by severe polyarthritis and polyarthralgia. Over the past decade and a half, CHIKV has rapidly spread across the globe, due in part to expanded vector competence, establishing autochthonous transmission in the tropics and subtropics, including South and Central America since 2013. While several CHIKV vaccine candidates are currently being assessed for human use, none have been approved. A previously developed live-attenuated vaccine (LAV) candidate, CHIKV 181/25 (also called TSI-GSD-218), while immunogenic, was pulled from FDA phase II clinical trials after roughly 8% of vaccinees developed complications owing to virulent reversion of the LAV. The goal of this study is to improve safety of the CHIKV LAV through the inclusion of two previously-described mutability-altering substitutions in the alphavirus replicase complex, while preserving the immunogenicity afforded by CHIKV 181/25. Our vaccine candidate, CHIKV-P2.P4, demonstrated similar replication kinetics to the parental LAV with no increase in morbidity or mortality in susceptible mouse models. Unexpectedly for a purported anti-mutator candidate, deep sequencing of replicate passaged CHIKV-P2.P4 showed a slightly elevated accumulation of both synonymous and non-synonymous mutations, with expected genomic hotspots prone to greater genetic variability. However, this augmented genetic diversity did not result in the genetic reversion of CHIKV-P2.P4 at *loci* responsible for attenuating the parental CHIKV 181/25 LAV. CHIKV-P2.P4 provided sterilizing immunity

in mice challenged with an Indian Ocean Lineage CHIKV strain, and produced comparable neutralizing antibody titers to the parental LAV. Our data indicate that CHIKV-P2.P4 is highly immunogenic, but additional ongoing work is required to assess candidate safety and reconcile LAV diversification over time.

801

THE BASIC REPRODUCTION NUMBER (R_0) OF CHIKUNGUNYA IN COLOMBIA DURING 2014-2016 AND ITS CORRELATION WITH ECO-ENVIRONMENTAL FACTORS

Víctor H. Peña-García[1], Rebecca Christofferson[2]

[1]*Universidad de Antioquia, Medellín, Colombia,* [2]*Louisiana State University, Baton Rouge, LA, United States*

Chikungunya virus arrived to Colombia in 2014 into a presumed fully susceptible population. This resulted in a quick and intense spread across numerous municipalities, resulting in an epidemic that affected an estimated of 450,000 people. We wanted to analyze the eco-environmental factors associated with the spread of CHIKV that produced significant outbreaks in different municipalities. To do this, we estimated the basic reproduction number (R_0) in 85 municipalities, which jointly were responsible of the 65.6% of reported cases in Colombia. At first, we divided municipalities into higher and lower R_0 and compared both groups across 13 different environmental and ecological variables like those related to temperature, demographic, and geographical variables. These variables were analyzed by correlation analyses to confirm their association with R_0. We found that temperature-related variables are significantly related to higher R_0 while other variables like duration of outbreak and size of the urban area are inversely related to R_0. We conclude that those municipalities with high R_0 associated with high temperatures had fast growth of cases in a shorter time period (with faster cessation of outbreak transmission), resulting in fewer cases than when transmission was associated with lower (but still >1) values of R_0 where transmission was slow and steady, resulting in a higher cumulative number of cases. We propose, then, that transmission may follow a tortoise-hare model such as proposed for viremia-based transmission by Althouse and Hanely (2015). That is, slow-and-steady wins the race.

802

EVALUATION OF TWO COMMERCIALLY AVAILABLE CHIKUNGUNYA VIRUS IGM ENZYME-LINKED IMMUNOASSAY (ELISA) IN AN ENDEMIC REGION FOR CHIKUNGUNYA, DENGUE AND ZIKA VIRUSES

Mariana Kikuti[1], Laura B. Tauro[2], Patricia S. Moreira[3], Leile Camila J. Nascimento[3], Moyra M. Portilho[3], Gubio C. Soares[4], Scott C. Weaver[5], Mitermayer G. Reis[3], Uriel Kitron[6], Guilherme S. Ribeiro[4]

[1]*University of Minnesota, St. Paul, MN, United States,* [2]*Conicet-UNAM, Puerto Iguazu, Argentina,* [3]*Fundacao Oswaldo Cruz, Salvador, Brazil,* [4]*Universidade Federal da Bahia, Salvador, Brazil,* [5]*University of Texas Medical Branch, Galveston, TX, United States,* [6]*Emory University, Atlanta, GA, United States*

In order to provide appropriate clinical management, laboratory diagnostic tools are necessary to distinguish Chikungunya virus (CHIKV) infections from other febrile illness. We evaluated the diagnostic performance of the Inbios (Seattle, US) and the Euroimmun (Luebeck, Germany) CHIKV IgM ELISAs on acute- and convalescent-phase sera from 915 patients enrolled in an acute febrile illness surveillance study performed in Salvador, Brazil from Sept/2014 to Jul/2016, a period of simultaneous CHIKV, dengue (DENV), and Zika (ZIKV) virus transmission. Using a positive CHIKV RT-PCR result as the gold standard, sensitivities for acute-phase samples (collected a median of 1 day post onset of symptoms (dpos)) were 4.0% (6/149) for the Inbios IgM-ELISA and 10.3% (15/145) for the Euroimmun IgM-ELISA. Sensitivities for the convalescent-phase samples of CHIKV RT-PCR-positive patients (collected in median 19 dpos) were 92.1% (58/63) for the Inbios IgM-ELISA and 92.9% (63/65) for the Euroimmun IgM-ELISA. Among DENV RT-PCR-positive cases, specificities of the Inbios IgM-ELISA were 83.9% (26/31) in acute-phase samples and 93.3% (14/15) in convalescent-phase samples, whereas the Euroimmun IgM-ELISA specificities were 82.8% (24/29) in acute-phase samples and 83.3% (15/18) in convalescent-phase samples. Among ZIKV RT-PCR-positive cases, Inbios IgM-ELISA specificities were 92.9% (13/14) and 100% (7/7) for acute- and convalescent-phase samples, respectively, and Euroimmun IgM-ELISA specificities were 83.3% (10/12) and 87.5% (7/8), respectively. Considering patients with negative RT-PCR for all three arboviruses, the Inbios IgM-ELISA specificities were 89.1% (631/708) and 90.2% (259/287) for acute- and convalescent-phase samples, respectively, and the Euroimmun IgM-ELISA specificities were 89.5% (153/171) and 83.3% (140/168), respectively. These findings indicate an overall good sensitivity of both tests for convalescent-phase samples. However, the Euroimmun CHIKV IgM-ELISA had lower specificity than the Inbios CHIKV IgM-ELISA, which might result in a higher rate of false positive when applied in low prevalence scenarios.

803

TRANSMISSION OF CHIKUNGUNYA IN A BRAZILIAN URBAN SLUM SETTING: SEROPREVALENCE AND ASSOCIATED FACTORS

Rosangela Oliveira dos Anjos[1], Vanio André Mugabe[2], Patrícia Sousa Moreira[1], Caroline Xavier Carvalho[1], Moyra Machado Portilho[1], Gielson Almeida do Sacramento[1], Nivison Ruy Nery Junior[1], Mitermayer Galvão dos Reis[1], Uriel Kitron[3], Albert Ko[4], Federico Costa[1], Guilherme Sousa Ribeiro[1]

[1]*Oswaldo Cruz Foundation, Salvador, Brazil,* [2]*Institute of Collective Health, Federal University of Bahia, Salvador, Brazil,* [3]*Emory University, Atlanta, GA, United States,* [4]*Yale School of Public Health, New Haven, CT, United States*

Chikungunya virus (CHIKV) was first detected in Brazil in 2014, and then spread, causing outbreaks throughout the country. This study aimed to assess the prevalence and factors associated with previous CHIKV infection in a neighborhood of Salvador in which an outbreak had occurred in 2015 and to estimate the frequency of clinical disease. Between November 2016 and February 2017, 1,776 participants with age ≥ 5 years were enrolled in a cross-sectional study. We collected demographic, socioeconomic, and clinical data by interview. Serum samples were tested for CHIKV IgG antibodies by an indirect enzyme-linked immunoassay (Euroimmun). Poisson regression with robust variance was used to calculate the prevalence ratios (PR) and 95% CI adjusted for design effect. The prevalence of IgG CHIKV antibodies was 11.8% (95% CI: 9.8-13.7%). In the multivariate analyses, we found that infections were more common among individuals who were illiterate (PR: 1.62; 95% CI: 1.03-2.54), resided in unpaved streets (PR: 1.56; 95% CI: 1.12-2.16), reported a prior medical diagnosis of chikungunya (PR: 2.77; 95% CI: 1.89-4.05), and reported an episode of arthralgia (PR: 1.91; 95% CI: 1.35-2.71) and rash (PR: 1.50; 95% CI: 1.06-2.12) after January, 2015. Among the 209 patients with CHIKV IgG antibodies, 15.3% reported having fever and arthralgia after January 2015. Our findings indicate that although CHIKV caused an outbreak in Salvador in 2015, overall seroprevalance after the outbreak remains low and may not confer sufficient herd immunity to preclude additional epidemics in the near future. We also found that even within this overall poor urban slum population, there are social heterogeneity associated with the risk for CHIKV transmission. Finally, although self-reporting of symptoms was a limitation of the study, the low frequency of reported CHIKV-associated symptoms amongst those with CHIKV IgG suggest that strain or host specific differences may determine the natural history of CHIKV infection.

804

AN EMERGING THREAT TO PUBLIC HEALTH IN PERU: DETECTION OF THE MAYARO VIRUS

Miguel A. Aguilar-Luis[1], Tamara Gil Ramirez[1], Luis J. del Valle[2], Saul Levy Blitchtein[1], Wilmer Silva Caso[1], Víctor Zavaleta- Gavidia[3], Jorge Bazán-Mayra[3], Daniel Cornejo[3], Juana M. del Valle-Mendoza[1]

[1]*Investigation Center and Innovation of the Health Sciences Faculty, Universidad Peruana de Ciencias Aplicadas (UPC), Av. San Marcos cdra* [2] *Cedros de Villa, Lima, Peru,* [2]*Barcelona Research Center for Multiscale Science and Engineering, Department d' Enginyeria Quimica, EEBE, Universidad Politecnica de Catalunya (UPC), Barcelona Tech, Barcelona, Spain,* [3]*Dirección Regional de Salud de Cajamarca, Cajamarca, Peru*

Mayaro virus (MAYV; Togaviridae; Alphavirus) is emerging infectious disease and can cause health complications similar to infections caused by dengue (DENV) or Chikungunya (CHIKV) viruses. Arbovirus infections emerged in the Americas and rapidly spread, affecting millions of people from 2013–2016, establishing high-risk areas for MAYV infection. MAYV produce severe complications, such as intermittent fever, neurological complications, myocarditis, and even death. In Peru, can to be misdiagnosed as CHIKV infection due to their similarities. The aim of this study was to detect Mayaro virus in patients with febrile illnesses from endemic areas of Peru. A total of 357 patients with suspected febrile illnesses from an endemic region of North of Peru participated in this study. Clinical symptoms were assessed, and blood serum samples were collected. The resulting cDNA from each serum sample processed was amplified by PCR analysis in a LightCycler 2.0 system using primers that amplify partial regions of the nsP1 gene. There were a total of 40 (11.2 %) cases positives to MAYV. Among this, 47.5 % (n = 19) were women and 52.5 % (n = 21) were men. The populations with age groups of 18-39 years and 40-59 years were the most affected with MAYV in 37.5 % (n = 15) and 32.5 % (n = 13) respectively. The most common symptoms reported were headache with 77.5 % (n = 31), myalgia in 55.0 % (n = 22) and arthralgias in 55.0 % (n = 20) of cases positives. In conclusion, Mayaro virus is present in endemic areas of Peru, however it may be misdiagnosed as Dengue Virus, CHIKV or Zika Virus due to the similar clinical presentation. It is an important emerging disease in South America region and requires highly sensible and specific tests to make an accurate diagnosis. This virus should be included in national surveillance programmes to have an insight on its transmission, pathogenicity, virulence and local epidemiology.

805

USING BIG DATA TO MONITOR THE INTRODUCTION AND SPREAD OF CHIKUNGUNYA, EUROPE, 2017

Joacim Rocklöv[1], Yesim Tozan[2], Aditya L. Ramadona[1], Maquines O. Sewe[1], Bertrand Sudre[3], Jon Garrido[4], Chiara B. de Saint Lary[4], Wolfgang Lohr[1], Jan C. Semenza[4]

[1]*Umeå University, Umeå, Sweden,* [2]*College of Global Public Health, New York University, New York, NY, United States,* [3]*Umeå University, European Centre for Disease Prevention and Control, Stockholm, Sweden,* [4]*European Centre for Disease Prevention and Control, Stockholm, Sweden*

With regard to fully harvesting the potential of big data, public health lags behind other fields. To determine its potential, we harnessed big data (air passenger volume from international areas with active chikungunya transmission, Twitter data, and vectorial capacity estimates of *Aedes albopictus* mosquitoes) to the 2017 chikungunya outbreaks in Europe to assess the risks for virus transmission, virus importation, and short-range dispersion from the outbreak *foci*. We derived risk maps for autochthonous chikungunya transmission by combining vectorial capacity and mobility proximity estimates for the Lazio region in Italy and the Var department in France, August-October 2017. The risk areas for the outbreak in Var were identified to be located along the French and northern Spanish Mediterranean coastlines, Mallorca, and Rome in August; the risk regions for the Lazio outbreak in August included large parts of Italy and areas in France, Spain, and Greece. The size of the area at risk contracted in September and more so in October owing to less favorable climate conditions, except in the most southern region of Italy, such as the Calabria region, where the outbreak empirically continued longer in the fall. In Lazio, an analysis of the combination of vectorial capacity and mobility proximity revealed a higher transmission potential in August, with implications for targeting surveillance and outbreak control activities to this region. The largest area of risk for spread from Anzio was Rome, but the risk for spread from Rome was more widespread in the region. The areas at risk for spread in Lazio differed in August compared to September and October. We found that indicators based on voluminous and v*elocio*us data can help identify virus dispersion from outbreak *foci* and that vector abundance and vectorial capacity estimates can provide information on local climate suitability for mosquito-borne outbreaks. In contrast, more established indicators based on Wikipedia and Google Trends search strings were less timely. We found that a combination of novel and disparate datasets can be used in real time to prevent and control emerging infectious diseases.

806

SINGLE ADMINISTRATION OF LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE CANDIDATE, KD-382, INDUCED LONG-LASTING (>3.5 YEARS) NEUTRALIZING ANTIBODY AGAINST ALL FOUR SEROTYPES IN DENGUE NAÏVE CYNOMOLGUS MONKEYS

Yasuhiko Shinmura[1], Masaya Yoshimura[1], Kazuhisa Kameyama[1], Kengo Sonoda[1], Sutee Yoksan[2], Kazuhiko Kimachi[1]

[1]*KM Biologics Co., Ltd., Kikuchi, Kumamoto, Japan,* [2]*Mahidol University, Bangkok, Thailand*

One of challenges in dengue vaccine development, there being four serotypes of dengue virus, is that a vaccine has to induce long lasting neutralizing antibody response against all four serotypes simultaneously with minimum administration number. Our tetravalent dengue vaccine candidate, KD-382, is a live attenuated and genetically unmodified vaccine using a classical host range mutation strategy and thus is expected to induce strong and comprehensive immune response similar to those induced by natural infection. To date, we have shown that dengue-naïve cynomolgus monkeys administered a single dose of KD-382 seroconverted for all four serotypes. However, it is important to show how long neutralizing antibodies induced by KD-382 can be expected to last. We administered a single dose of KD-382 (5, 5, 5, 5 Log_{10} PFU/dose) to 6 cynomolgus monkeys (male and female), and are measuring the neutralizing antibody titer against parent strains for each serotype at intervals using a standard focus reduction neutralization (FRN) assay. This study has currently been running for over 3.5 years, and we have confirmed that neutralizing antibody titers against all four serotypes ($FRNT_{50}$=1:>10) persist for at least 3.5 years. The geometric mean neutralizing antibody titers at 42 months for serotypes-1, -2, -3, and -4 were 3.2, 2.5, 2.4 and 2.9 (Log_{10} $FRNT_{50}$), respectively. This neutralizing antibody persistence for over 4 years suggested that in dengue naïve cynomolgus monkeys, with a single administration, KD-382 can induce a strong neutralizing antibody response against all four dengue serotypes simultaneously and thus has high potential as a dengue vaccine. We will continue our observations up to 5 years, but as no decrease in neutralizing antibody titer has been observed so far, we expect that neutralizing antibodies will last for even longer. A first-in-human Phase 1 clinical study for KD-382 is now ongoing in a dengue non-endemic country.

807

CO-CIRCULATION OF DENGUE, ZIKA AND CHIKUNGUNYA IN THE PERUVIAN AMAZON

Francesca Falconi-Agapito[1], Xiomara Merino[2], Karen Kerkhof[1], Kevin K. Ariën[1], Michael Talledo[2]

[1]*Institute of Tropical Medicine, Antwerp, Belgium,* [2]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru*

The similar clinical symptomatology and co-circulation of arthropod-borne viruses (arboviruses) such as Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) requires accurate diagnostics. PCR is highly specific and sensitive, although only useful during the short viremic phase of the disease. When this phase is passed, diagnosis should be based on serology. Serology tests for arboviruses suffer from low specificity due to high genetic and antigenic similarity, especially among the flavivirus family, with detection of cross-reactive antibodies. Further serological studies are needed in areas where different arboviruses co-circulate, in order to understand its utility for diagnosis. In this study, the IgM and IgG antibody responses against DENV, ZIKV and CHIKV were evaluated in dengue PCR (+) patients (n=32). The commercial Dengue duo RDT from Alere and the IgM/IgG ELISA for DENV, ZIKV and CHIKV from Euroimmun were used. The screened samples were collected in Iquitos and Yurimaguas, cities located in the Peruvian Amazon. From each patient, two samples were collected: an acute samples (AS) up to 9 days after the onset of symptoms and a convalescent sample (CS). All samples were PCR negative for ZIKV and CHIKV. For dengue, 18/25 AS were positive to NS1 antigen in the RDT. IgM antibodies against DENV were detected in 11/25 and 11/29 AS tested with RDT and ELISA assays, respectively. Eight additional patients seroconverted at the convalescent phase of the disease when tested for IgM in the ELISA. IgG antibodies against dengue were detected in 28/32 AS, suggesting that these patients had a secondary infection of dengue. The remaining four patients showed IgG seroconversion in the CS. Neither patient showed IgM or IgG responses against CHIKV and ZIKV at the acute phase of the disease. Surprisingly, 7/29 patients from Yurimaguas were IgG positive to CHIKV. The IgG levels remained positive in the CS, thus suggesting a history of CHIKV infection. Although sporadic CHIKV PCR (+) have been reported in Yurimaguas by the Ministry of Health, to our knowledge this is the first serological evidence of a past CHIKV outbreak in the area.

808

PERFORMANCE EVALUATION OF A RDT DENGUE IGG ASSAY FOR PRE-VACCINAL SEROSTATUS DETERMINATION

Anthony Palvadeau, Muriel Cardona, Catherine Bachard, Muriel Costaille, Nadia Sagot, Akram Yahia-Ammar, Gaëlle-Anne Cremer, Stéphanie Antil-Delbeke, Patrice Sarfati

Bio-Rad, Marnes-la-Coquette, France

CYD-TDV, the world first tetravalent dengue vaccine was licensed in December 2015 and has been approved in 19 countries. In high seroprevalence areas clinical trial data demonstrated overall population benefits. However, recent field data indicated that people inoculated with CYD-TDV who were seronegative at time of the first vaccination have an excess risk to develop a severe form of the disease when primary infected by Dengue virus. Today, WHO recommends performing a pre-vaccination screening in order to determine the serostatus of each patient before vaccination. In this context, we have developed the first prototype of rapid diagnostic test to detect Dengue IgG for recent and past infection for the 4 serotypes in less than 20 minutes. This rapid test can be used in whole blood, plasma and serum specimens. Performance and seroprevalence studies of this new RDT Dengue IgG rapid test were performed. Specificity has been evaluated on 127 seronegative blood bank specimens and 97 seronegative hospitalized patients from non-endemic area. Sensitivity has been evaluated on 206 seropositive anti-Dengue IgG specimens. Cross-reactivity has been measured on Yellow Fever IgG and West Nile IgG positive samples. Results have shown a specificity of 99.1% (222/224) for specimens from non-endemic area and a sensitivity of 96.1% (198/206). No cross-reactivity was observed on West Nile and Yellow Fever positive specimens. Cross-reactivity on Zika positive samples could not be evaluated as all samples came from endemic settings and were all Dengue seropositive. In the seroprevalence study, a selection of 30 serums from high prevalence endemic area (India) has also been tested. A total of 76.6% (23/30) was found positive with RDT Dengue IgG. These results are in agreement with the published range of seroprevalence found in India. In conclusion, RDT Dengue IgG shows expected performance including in high seroprevalence area and would fit with performances required by the WHO recommendation for a Dengue pre-vaccinal screening strategy.

809

HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I CHAIN-RELATED (MIC) GENE ASSOCIATIONS WITH DENGUE INFECTIONS IN BANGKOK

Henry A. Stephens[1], Panpimon Luangtrakool[2], Sasijit Vejbaesya[2], Komon Luangtrakool[2], Siripen Kalayanarooj[3], Anon Srikiatkhachorn[4], Louis Macareo[5], Stefan Fernandez[5], Richard Jarman[6], Alan Rothman[4]

[1]*University College London, London, United Kingdom,* [2]*Siriraj Hospital, Bangkok, Thailand,* [3]*Queen Sirikit National Institute of Child Health, Bangkok, Thailand,* [4]*University of Rhode Island, Providence, RI, United States,* [5]*Armed Forces Research Institute of Medical Science, Bangkok, Thailand,* [6]*Walter Reed Army Institute of Research, Silver Spring, MD, United States*

Human genome-wide association studies of dengue patients in mainland SE Asian populations have detected and replicated intron-specific associations with the non-classical MHC class I chain-related gene *MICB*, a known target for the ubiquitous activating NK receptor NKG2D which is expressed on both NK and T cells. MIC proteins are stress-related molecules expressed at the cell surface of virus infected cells, but can also be released from the cell surface as soluble proteins after proteolytic cleavage. Soluble MIC can block NKG2D bearing NK cells in the periphery before they engage with virus infected cells, which is considered a form of immune evasion. In this candidate gene association study, we have determined exon-specific *MIC* allele profiles in a discovery cohort of Bangkok patients with dengue fever (DF) and dengue haemorrhagic fever (DHF) (n=166), then compared these groups with the equivalent *MIC* frequencies in ethnically and geographically matched controls (n=149). We detected an association between allele *MICB*002* and protection from acquiring DHF after secondary dengue infections (OR = 0.41, 95% CI = 0.21<OR<0.68) and confirmed this association in a replication cohort of dengue patients (N= 222; OR = 0.43, 95% CI = 0.22<OR<0.82). *MICB*002* is a known low expresser of soluble proteins, which can block NKG2D baring cells in the periphery and impede the cytotoxic and immuno-regulatory activities of NK and T cells. Our results indicate that the expression and the likely retention of *MICB*002* encoded proteins on the surface of dengue infected cells confers a significant protective advantage in controlling this infection via NK and T cells.

810

THE CHANGING EPIDEMIOLOGY OF DENGUE FEVER IN EUROPE AS INFLUENCED BY CLIMATE CHANGE, GLOBALIZATION, AND CONFLICT-INDUCED MIGRATION

Elisabeth Nelson, Erin M. Sorrell

Georgetown University, Washington, DC, United States

The mosquito-borne virus that causes dengue fever represents one of the most significant threats to global health in the 21st century. Over half of the world's population lives in areas at risk of dengue transmission, with cases increasing 30-fold in the past 50 years. A combination of phenomena has allowed for an unprecedented global expansion of both the dengue virus and the mosquito species that act as main vectors of the disease. Europe, previously unaffected by dengue for most of the 20th century, resides on the brink of dengue re-introduction. Here we focus on the impacts of climate change and migration on the incidence of dengue

fever in Europe between 2008 and 2015 through the analysis of dengue case reports, in correlation with climate data, and flight and migration patterns. Our study indicates that since the 1990s warming winters have correlated with the *Ae. albopictus* expansion. Peaks in dengue incidence among travelers and the prevalence of outbreaks in endemic regions are correlated with peaks in travel to Europe during the summer months. This creates an extreme risk for autochthonous transmission as Europe is most climactically similar to the tropics during this time interval. Germany, France, and the United Kingdom were selected for in-depth analysis of the converging risk factors: *Ae. albopictus* habitat coverage, risk for habitat expansion, and viral introduction into local populations. These countries have the three largest airports in Europe, allowing for sustained introduction of the dengue virus through returning travelers. Our analysis suggests that active surveillance is necessary to quell autochthonous transmission. Furthermore, seasonal outbreaks appear increasingly likely however complete continental endemicity seems improbable. Previous studies have investigated the interaction between one or two of the risk factors for dengue and *Ae. albopictus* spread in Europe. This study is the first to investigate human and environmental factors, as well as the complex interplay and circumstantial overlap leading to autochthonous transmission. Further research is needed to better understand the future risks.

811

EPIDEMIOLOGIC TRENDS OF DENGUE IN U.S. TERRITORIES - 2010-2018

Kyle R. Ryff, Dania M. Rodriguez, Aidsa Rivera, Tyler M. Sharp, Stephen H. Waterman, Laura E. Adams, Gabriela Paz-Bailey

Centers for Disease Control and Prevention, Dengue Branch, San Juan, PR, United States

Dengue is a flavivirus transmitted by infected *Aedes* mosquitos. Dengue remains one of the most globally important mosquito-transmitted viral infections. In the United States, dengue became a nationally notifiable disease in 2010, and cases are reported to the Centers for Disease Control and Prevention through ArboNet, the national arboviral surveillance system. We present characteristics of confirmed and probable dengue cases in the United States territories of Puerto Rico, American Samoa and the United States Virgin Islands (USVI) during 2010 to 2018. Laboratory-positive dengue cases are classified using the Council of State and Territorial Epidemiologists (CSTE) dengue case definitions for confirmed and probable cases. Severe dengue cases were also classified following CSTE guidance. We describe cases by selected characteristics and estimate rates per 1,000 persons using 2010 US census population data. During 2010-2018, a total of 30,394 dengue cases were reported to ArboNet from Puerto Rico (28,990, 95%), American Samoa (1,054, 3%), and USVI (350, 1%). Dengue rates in Puerto Rico varied from 3.0/1,000 persons in 2010 to 0.0008/1,000 in 2018. In USVI, rates ranged from 0 in 2010 to 1.6/1,000 persons in 2013, and in American Samoa from 0 in 2010 to 9.2/1,000 in 2017. Number of cases and rates by age group were highest among 10–14 and 15–19 year-olds. Hospitalization and severe dengue followed similar patterns by age, with the highest rates among the 10—19 year olds. Puerto Rico had the highest number of severe dengue cases (n=544) followed by USVI (n=6). A total of 68 fatal cases were reported from Puerto Rico; no fatal cases were reported by USVI or American Samoa. Dengue deaths were highest in adults. Dengue is endemic in Puerto Rico; American Samoa and USVI experience periodic dengue outbreaks. The highest burden of disease is among children, although deaths mainly occurred among adults. These trends characterize risk in areas of high dengue activity in the U.S. and can be useful to guide preparedness and disease prevention activities.

812

A UNIVERSAL DENGUE VACCINE ELICITS NEUTRALIZING ANTIBODIES AGAINST STRAINS FROM ALL FOUR DENGUE SEROTYPES

Naoko Uno, Maria T. Arevalo, Ted M. Ross

University of Georgia, Athens, GA, United States

Developing a vaccine for Dengue virus (DENV) has been difficult to achieve. It is critical for a DENV vaccine to elicit protective immune responses against viruses representing all four DENV serotypes without enhancing disease. In the current study, DENV subviral particles (SVP) were designed to express an envelope (E) glycoprotein that elicits broadly reactive neutralizing antibodies. Each E antigen was designed using computationally-optimized broadly reactive antigen (COBRA) methodology. DENV E sequences were obtained from GenBank and a layered, consensus-building approach was used to derive four final COBRA DENV sequences. COBRA and wild-type SVPs (prM-E) were expressed and purified from mammalian cell lines expression vector. Two studies were performed: 1) in female C57BL/6 mice (age 6-8 weeks) and 2) in rhesus macaques (*Macca mulata*) that were immunologically naïve to DENV or pre-immune with antibodies to DENV serotype 1 or DENV serotype 2. Animals were vaccinated with DENV SVP intramuscularly individually or in a tetravalent mixture. Immune sera were collected and total IgG antibody titers to DENV E were analyzed by ELISA and the ability to prevent virus infection *in vitro* was assessed in a focus reduction neutralization test ($FRNT_{50}$) against a panel of 12 prototype and modern strains from all four serotypes. Mice vaccinated with wild type DENV SVPs expressed anti-E IgG antibodies that were specific to strains in each homologous serotype and stimulated B cells against DENV from all 4 serotypes. The elicited antibodies neutralized serotype specific viruses. COBRA DENV SVPs elicited a broader breadth of antibodies that neutralized various strains across all four serotypes. The COBRA DENV E immunogen neutralized all 12 strains of DENV *in vitro*, comparable to tetravalent SVP vaccination.

813

ANTIGENIC EVOLUTION OF DENGUE VIRUSES 1-4 IN BANGKOK, THAILAND IN RELATION TO GLOBAL DENGUE VIRUS ANTIGENIC DIVERSITY

Leah Katzelnick[1], Ana Coello Escoto[1], Nayeem Chowdhury[1], Bernardo Garcia Carreras[1], Irina Maljkovic Berry[2], Christian Chávez[1], Wiriya Rutvisuttinunt[2], Philippe Buchy[3], Veasna Duong[4], Philippe Dussart[4], Justin Lessler[5], Louis Macareo[6], Derek Smith[7], Richard Jarman[2], Stephen Whitehead[8], Henrik Salje[9], Derek Cummings[1]

[1]*Department of Biology and Emerging Pathogens Institute, University of Florida, Gainesville, FL, United States,* [2]*Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [3]*GlaxoSmithKline (GSK) Vaccines, Singapore, Singapore,* [4]*Institut Pasteur in Cambodia, Réseau International des Instituts Pasteur, Phnom Penh, Cambodia,* [5]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [6]*Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [7]*Department of Zoology, University of Cambridge, Cambridge, United Kingdom,* [8]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [9]*Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Paris, France*

Dengue viruses 1-4 (DENV1-4) are theorized to be under selective pressure to escape from neutralization but also to take advantage of prior immunity via antibody-dependent enhancement. In this study, we tracked antigenic dynamics of DENV1-4 isolated in Bangkok, Thailand in relation to temporal, regional, and global DENV antigenic diversity. Thailand DENV1-4 strains isolated from 1980-2014 (n=265) were titrated against sera from non-human primates (n=20, each inoculated with distinct DENV strains) using the Plaque Reduction Neutralization Test (PRNT). We also studied DENV1-4 strains from 19 other countries (n=57). $PRNT_{50}$ titers (n=6,843) were interpreted as antigenic distances using antigenic

cartography. On the resulting antigenic maps, Thailand DENV1-4 strains were more centrally located than strains from other countries, indicating greater sensitivity to heterotypic immunity. Generalized additive models of antigenic distances from the map center revealed that DENV1-4 had become more antigenically similar to one another between 1994-2003, followed by a period of increasing antigenic dissimilarity. The dip in antigenic distance for Thailand DENV1-4 remained when we only analyzed strains from 1994-2006, extended observations through 2014, and analyzed each DENV type separately. We tested whether DENV type-specific incidence in Bangkok correlated with antigenic distance and found the months with the largest epidemics were those during which DENV types were most antigenically central on the antigenic maps. Finally, within DENV type, antigenic and genetic distance increased linearly up to ~20 amino acid differences, whereas strains separated by greater genetic distances were actually more antigenically similar. Thus, we find DENV1-4 circulating in a highly endemic area can oscillate in antigenic similarity across time and genetic space and that antigenic similarity correlates with dengue epidemics. These findings raise new questions about paths to viral antigenic evolution and may help explain why DENV vary antigenically within type without divergence of the DENV types.

814

MOLECULAR DETECTION OF DENGUE FEVER VIRUS IN PATIENTS SUSPECTED OF EBOLA VIRUS DISEASE IN GHANA

JH Kofi Bonney[1], Hayashi Takaya[2], Samuel Dadzie[1], Esinam Agbosu[1], Deborah Pratt[1], Franklin Asiedu-Bekoe[3], Badu Sarkodie[4], Shoji Yamaoka[2]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Tokyo Medical and Dental University, Tokyo, Japan,* [3]*Disease Surveillance Department, Ghana Health Service, Accra, Ghana,* [4]*Public Health, Ghana Health Service, Accra, Ghana*

Introduction: Dengue fever (Df) is known to be one of the most common Arthropod-borne viral infectious disease of public health importance. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific with an estimated two fifths of the world's population being at risk. The notable endemic Viral Hemorrhagic Fevers (VHFs) found in West Africa, including Yellow fever, Lassa fever, Rift Valley fever, Dengue fever and until recently Ebola have been responsible for most outbreaks with fatal consequences. These VHFs usually produce unclear acute febrile illness, especially in the acute phase of infection. Aim: We set out to investigate and characterize the presence of Df and other arboviral agents in archived residual clinical specimens of serum 150 patients clinically suspected of Ebola virus disease during the Ebola Virus Disease (EVD) outbreak in West Africa. Methods: Through a surveillance system set up as part of an EVD preparedness and response plan to detect and rapidly respond to cases, clinical specimens of serum/plasma from patients suspected of EVD were submitted and tested for EVD as well as known endemic VHFs including Yellow fever, Lassa fever West Nile and Dengue fever. Results: In this study we detected the presence of 2 different serotypes (DENV-2 and DENV-3) of Df virus in 4 sera of the patients clinically suspected of EVD during the outbreak in West Africa with the use of serological and molecular test assays. The phylogenetic analysis of the envelope gene showed that the DENV-3 had close homology with serotype from Senegal and India. Conclusion: This study documents molecular evidence of an indigenous Dengue fever viral infection in Ghana and therefore necessitates the need to have an efficient surveillance system to rapidly detect and control the dissemination of the different serotypes in the population which has the potential to cause outbreaks of Dengue Hemorrhagic fevers.

815

MAPPING THE CLONAL AND FUNCTIONAL DIVERSITY OF DENV-ELICITED HUMORAL IMMUNITY USING HIGH THROUGHPUT SINGLE CELL RNA SEQUENCING

Adam T. Waickman[1], Wiriya Rutvisuttinunt[1], Gregory D. Gromowski[1], Kaitlin Victor[1], Hayden Siegfried[1], Tao Li[1], Abhinaya Srikanth[1], Benjamin Gabriel[2], Anon Srikiatkhachorn[2], Stefan Fernandez[3], Alan Rothman[2], Richard G. Jarman[1], Jeffery R. Currier[1], Heather Friberg[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Institute for Immunology and Informatics and Department of Cell and Molecular Biology, University of Rhode Island, Providence, RI, United States,* [3]*Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

Tracking the development and maturation of virus-specific humoral immunity is a highly complex technical challenge. However, the importance of understanding the isotype distribution and relative abundance of type-specific or cross-reactive antibodiesis becoming increasingly apparent, especially in the setting of complex pathogens such as dengue virus (DENV). In order to close this functional gap in our understanding of viral-specific humoral immunity, we utilized high-throughput single cell RNA sequencing to capture individual B cell plasmablasts circulating in response to both primary and secondary natural DENV infection. At least two time points with circulating plasmablasts were captured per subject (within days pre- and post- viral clearance), allowing for the temporal resolution of antibody maturation and clonal turnover. Paired full-length heavy and light immunoglobulin chains were identified for ~90% of captured plasmablasts, and monoclonal antibodies were synthesized and tested for virus binding and neutralization capacity from multiple time points per subject. Plasmablasts circulating in response to a secondary DENV infection were found to predominantly express IgG class-switched antibodies that were broadly cross-reactive to all four DENV serotypes and were extensively hypermutated. In contrast, plasmablasts circulating in response to a primary DENV infection were predominantly IgA expressing and were specific for the infecting viral serotype or weakly cross-reactive to all DENV serotypes. The observation that primary - but not secondary - DENV infection was associated with a IgA biased-humoral response was confirmed by serum ELISA, where significant levels of DENV-reactive IgA were found in early-convalescent samples in subjects with primary DENV infections, but not those with secondary DENV infections. These results highlight the utility of unbiased molecular analysis of acute virus-elicited humoral immunity, and also suggest a previously unappreciated role for IgA in controlling - or responding to - primary DENV infection.

816

PRIMARY AND SECONDARY DENGUE VIRUS INFECTIONS ELICIT SIMILAR MEMORY B CELL PROFILES BUT CROSS-REACTIVITY TO ZIKA VIRUS IS HIGHER IN SECONDARY DENGUE

Paulina Andrade[1], Josefina Coloma[1], Angel Balmaseda[2], **Eva Harris**[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua*

Dengue virus (DENV) exists as four serotypes that differ antigenically; Zika virus (ZIKV) shares high protein sequence homology with DENV. Little is known about the development of polyclonal memory B cells (MBCs) during DENV infection and the relationship of these responses to DENV and ZIKV cross-reactivity. Here we analyzed MBCs from peripheral blood mononuclear cells collected ~2-4 weeks or ~6 months after RT-PCR-confirmed DENV1, DENV2 and DENV3 infections from primary (1°) and secondary (2°) cases in a pediatric hospital study in Nicaragua. Analysis of activated MBCs was performed using a Multi-color FluoroSpot assay that enables measurement of immunoreactivity of B cells to DENV1-4

and ZIKV at a single-cell level. During 1° DENV infections, type-specific (TS) responses accounted for 37.9±15.5% (mean ± standard deviation) of total antigen-specific responses, DENV cross-reactive (CR) responses for 49±16%, and DENV&ZIKV cross-reactivity for 13±8.4%. During 2° infections, TS responses represented 26±17% and reacted to the infecting serotype but also to heterotypic serotypes from previous exposures. DENV CR responses represented 55.9±13% and DENV&ZIKV CR responses 23±10% of antigen-specific responses. Thus, CR responses were significantly higher than TS responses in both 1° and 2° infections. Interestingly, when comparing TS responses during 1° vs. 2° infections, no significant differences were observed, similarly to CR responses. However, DENV&ZIKV cross-reactivity was significantly higher during 2° infection compared to 1° infections (p=0.017), suggesting that the breadth of MBC antibodies increases after more than one DENV infection. Finally, we compared DENV&ZIKV CR responses from DENV-immune Zika patients before and after their ZIKV infection and found that these responses were similar between both temporal groups. Overall, the frequency of DENV- and DENV&ZIKV-specific responses among total antibody-secreting cells reflected antigen-specific data. These results improve our knowledge of memory B cell responses during DENV infection and their implication for the development of DENV and ZIKV cross-reactivity.

817

NEW ANTIGENIC EPITOPES ON DENGUE VIRUS SEROTYPE 3

Ellen Young[1], Daniela V. Andrade[2], Nurgun Kose[3], Fritch Ethan[4], Rob Carnahan[3], Raschel Nargi[3], Michael Doyle[3], Jennifer Munt[1], Laura White[4], Thomas Baric[1], Mark Stoops[4], Marcus Wong[2], Diego A. Espinosa[2], Magelda Montoya[2], Angel Balmaseda[5], Aravinda DeSilva[4], Eva Harris[2], James E Crowe Jr[3], Ralph Baric[1]

[1]*Department of Epidemiology, Chapel Hill, NC, United States,* [2]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [3]*Vanderbilt Vaccine Center, Nashville, TN, United States,* [4]*Department of Microbiology, Chapel Hill, NC, United States,* [5]*Laboratorio Nacional de Virología, Centro Nacional de Diagnostico y Referencia, Ministry of Health, Managua, Nicaragua*

Four serotypes of dengue virus (DENV1-4) circulate in human populations, and immunity to one serotype does not confer long-lasting immunity to the others. Rather, pre-existing DENV immunity may actually increase the risk of severe dengue after exposure to a second serotype. The possibility of antibody-mediated enhancement has complicated vaccine development because of the need to induce robust immunity to all 4 serotypes simultaneously. After a primary infection, type-specific (TS) antibodies to individual serotypes of DENV is thought to be associated with robust, life-long homotypic protection, but the full repertoire of primary neutralizing antibody epitopes in each DENV serotype remains incomplete. Currently, the only DENV3 TS neutralizing human monoclonal antibody (mAb) is 5J7, which recognizes a complex quaternary epitope spanning 3 monomers of the envelope (E) glycoprotein. Importantly, several studies in natural DENV-infected cohorts suggest only a fraction of the polyclonal response targets the 5J7 epitope and there are additional neutralizing epitopes. To test this hypothesis, we immortalized memory B cells from DENV3-infected individuals with secondary DENV infections from a cohort in Nicaragua. New DENV3 TS neutralizing mAbs were identified that do not compete with 5J7 in competition assays. We designed a panel of 4 chimeric DENV3/1 viruses containing increasingly larger transplants of the DENV1-specific 1F4 and 14c10 epitopes into the DENV3 E protein along with chimeric DENV1/3 viruses containing increasing portions of domain I of DENV3 transplanted into DENV1. Using the panels of DENV3/1 and DENV1/3 chimeras along with an existing panel of 5 recombinant viruses containing DENV3 genotype E protein swaps, we mapped 14 new human mAbs to 4 distinct areas of the E protein. When tested in mice, several of the new mAbs were highly effective in reducing viral load when challenged with DENV3. These findings provide new insights into the mechanism of DENV3 neutralization and will lead to new tools for defining the primary neutralizing epitopes associated with DENV3 protective immunity following natural infection or vaccination.

818

SEROLOGICAL CHARACTERIZATION OF HOMOTYPIC AND HETEROTYPIC REPEAT DENGUE VIRUS INFECTIONS IN A LONG-TERM COHORT STUDY

Parnal Narvekar[1], Ciara Gimblet-Ochieng[2], Magelda Montoya[1], Paulina Andrade[1], Daniela Valente Andrade[1], Leah Katzelnick[1], Sandra Henein[2], Angel Balmaseda[3], Aravinda de Silva[2], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC, United States,* [3]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua*

Dengue is the most common mosquito-borne viral disease, affecting an estimated 100 million people annually worldwide. It is caused by one of 4 related dengue virus serotypes (DENV1-4), and it is thought that infection confers lifelong immunity to the homologous serotype. However, cases of homotypic reinfection have been documented and may be more common than currently appreciated. Here, we identified instances of homotypic and heterotypic DENV repeat infections and analyzed memory B cell (MBC) and antibody responses. We selected individuals with consecutive annual samples from a 15-year on-going community-based cohort study in Nicaragua with evidence for repeat infections based on paired annual inhibition ELISA (iELISA) titers. We then conducted flow cytometry-based neutralization assays to DENV1-4 reporter virus particles using Raji-DC-SIGN cells in each annual sample and calculated NT_{50} titers. Based on a >4-fold increase in NT_{50}, we identified potential repeat infections as homotypic (increase to the previous infecting serotype only) or heterotypic (increase to >1 serotype). We also selected a control group of subjects with primary infection only. Depletion of DENV antibodies specific to all but one serotype was carried out using magnetic beads coated with purified DENV virions in 4 distinct combinations (DENV1/2/3; DENV1/2/4; DENV1/3/4; DENV2/3/4). Successful depletion was confirmed by binding ELISA, and the type-specific (TS) antibody response generated by every infection was compared at each timepoint. Homotypic repeat infections showed no evidence of a TS response from a different serotype even when increased iELISA and NT_{50} titers indicated an infection had occurred. We confirmed TS and cross-reactive MBC responses using a Multi-Color B-cell FluoroSpot that enables measurement of reactivity to DENV1-4 at a single-cell level. Using these discriminating methods, we document cases of homotypic as well as heterotypic infections in the cohort. We are currently developing simplified assays to characterize larger numbers of samples to determine the proportion at which homotypic DENV infections occur at a population level.

819

PERSISTENCE OF ANTI-ZIKA VIRUS ANTIBODIES OVER TIME IN PEDIATRIC AND ADULT COHORT STUDIES IN NICARAGUA USING DIFFERENT SEROLOGICAL ASSAYS

Magelda Montoya[1], Fausto Bustos[1], Damaris Collado[2], Tatiana Miranda[2], Guillermina Kuan[3], Angel Balmaseda[4], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Centro de Salud Sócrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua,* [4]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua*

The Zika epidemic in 2015 began in Brazil and then spread throughout the Americas. Zika virus (ZIKV) entered Managua, Nicaragua, in January 2016 and caused an explosive epidemic that peaked in July-September 2016. ZIKV seroprevalence was estimated in two different groups, a pediatric (*n* = 3,740) and an adult cohort (*n* = 1,047) in Managua, with seroprevalence values of 36% and 56%, respectively. There is debate regarding the differential stability of anti-ZIKV antibodies in both children and adults. Here, we use two serological tests, the ZIKV NS1 Blockade-of-Binding (BOB) ELISA (measuring total anti-ZIKV NS1 antibodies and highly specific

vs. anti-dengue virus [DENV] antibodies) and a ZIKV Inhibition ELISA (iELISA; measuring total anti-ZIKV antibodies). In the ZIKV NS1 BOB ELISA, a 1:10 dilution of serum is competed against a ZIKV-specific monoclonal antibody, and the % of antibody blocking is calculated, with a cut-off of 50%. In the iELISA, 4 serial 10-fold dilutions are tested, and the titer at which 50% inhibition occurs is estimated. Here, we apply these 2 assays to the Nicaraguan pediatric and adult cohorts over a 3-year timeframe: 8 months (2017), 20 months (2018) and 32 months (2019) after the peak of the Zika epidemic. This period experienced no ZIKV circulation and scant DENV infections, which can elicit cross-reacting antibodies. Using a 4-fold decrease in titer as a decay cutoff, 23.5% of iELISA titers decayed from 2017 to 2018 in the pediatric cohort. However, ZIKV NS1 BOB ELISA blocking percentages appear quite stable (1.6% sero-reversion from >50% to <50%) in the pediatric cohort study over the same time-frame, suggesting that the large decay in iELISA titers could reflect both the presence of cross-reactive anti-DENV antibodies and true anti-ZIKV antibody titer decay. Thus, conclusions from serological assays must account for the type of antibodies being measured and their respective stability. The decay rates in the adult versus pediatric cohorts over the 8-32 months post-epidemic is being compared. Our results provide insights into the stability of anti-ZIKV antibodies over time and inform future epidemiological studies.

820

THE GLOBAL BURDEN OF DENGUE FROM 1990-2017

Anum Najeem Khan, Harrison Chase Gottlich, Taren Gorman, Steph Zimsen, Martina Vargas, Amanda Deen, Jeffrey Stanaway, Robert C. Reiner, Jr., Elizabeth Cromwell

Institute for Health Metrics and Evaluation, Seattle, WA, United States

Dengue is a mosquito-borne infectious disease with the potential to develop into severe dengue, a lethal complication and one of the leading causes of disability and fatality. The global incidence of dengue has grown drastically in all regions during recent decades, with almost 50% of the world's population now at risk. Dengue incidence is also known to be underreported and many cases are misclassified. In this analysis, we estimate mortality due to dengue disease, as well as incidence and prevalence of dengue fever, severe dengue fever (dengue hemorrhagic fever & dengue shock syndrome) and post-acute chronic fatigue due to dengue from 1990 to 2017 as part of the Global Burden of Disease study. National level data were modeled using a multi-level mixed effects negative binomial model, adjusted for spatial distribution, temporal trends, and global dengue death rate. We then adjusted results for under-reporting and modeled age and sex-specific dengue incidence and prevalence. Overall, global estimates for dengue incidence, prevalence, deaths and associated Disability Adjusted Life Years (DALYs) have increased from 1990 to 2017, and we estimate 40,467 (95% Uncertainty Interval (UI): 17,620 to 49,779) deaths due to dengue in 2017. We estimate 1,388,995 (95% UI: 27,306 to 3,119,291) prevalent cases and 23,283,274 (95% UI: 453,181 to 51,840,670) incident cases in 1990, 2,061,222 (95% UI: 295,550 to 3,863,987) prevalent cases and 34,459,672 (95% UI: 5,144,944 to 62,137,081) incident cases in 2000 and 6,267,410 (95% UI: 3,416,131 to 10,611,908) prevalent cases and 104,771,911 (95% UI: 63,759,019 to 158,870,031) incident cases in 2017. In 2017, the prevalence of dengue is highest among children aged 5-14, with majority of cases estimated in India, Indonesia and Brazil. Also, in 2017, burden of dengue in DALYs is highest in India (1,480,679; 95% UI: 744,422 to 2,087,713), followed by Indonesia (666,598; 95% UI: 304,378 to 821,767) and Philippines (227,152; 95% UI: 317,742 to 112,608). Given the rise in dengue incidence, we highlight the need to improve methods that account for under-reporting to better estimate the true burden associated with dengue.

821

RE-EMERGENCE OF DENV-2 IN THE STATE OF SAO PAULO, BRAZIL

Expedito J. Luna, Gerusa M. Figueiredo, Sergio R. Campos, Jose E. Levi, Walter M. Figueiredo, Angela A. Costa, Alvina C. Felix, Nathalia S. Souza, Claudio S. Pannuti

Universidade de Sao Paulo, Sao Paulo, Brazil

A cohort study has been set up to determine the incidence of dengue in a mid-level endemicity town, Araraquara, located in the central region of the state of Sao Paulo, Southeastern Brazil. A cluster randomized sample of children and adolescents, from 2 to 16 years of age was selected and invited to participate. Parents or legal guardians that agreed to participate provided a written consent. A baseline blood sample was collected for dengue serologic diagnosis. Families have been contacted weekly for fever surveillance. If the participant reports a febrile episode, the study's nurse visits the household to collect a blood sample for dengue diagnosis. Acute cases were confirmed according to their PCR and NS1 results. Confirmed dengue cases underwent full medical examination. Yearly blood samples were collected for serology. Participants were recruited from Sept 2014 to March 2015. 3,514 participants were enrolled in the cohort. A large dengue outbreak occurred in 2015. There were 290 confirmed cases among the participants in 2015 and 14 in 2016 (cumulative incidence of 8.3% and 0.48% respectively). All cases were confirmed as DENV1. No confirmed cases were observed in 2017. By the end of the transmission season in 2018 (April - June) three cases of DENV2 were diagnosed. In the beginning of the current transmission season (Nov 2018) new cases were confirmed as DENV2. So far, 35 DENV2 cases have been confirmed in this season, a cumulative incidence of 1.3%. Of them, 10 had had previously a DENV1 confirmed diagnosis during the cohort follow up, and 57% had an IgG reagent result in their last serology before the dengue episode. The cumulative incidence was significantly higher among the previously exposed to dengue (2.27%) than among the non-exposed (0.85%), which suggests a heterogeneous exposure to the disease. So far, no severe cases have been observed among the cohort participants. After two years of record low incidence, and ten years of predominance of DENV1, a new dengue outbreak is under way in some regions of Brazil.

822

GROWING EVIDENCE THAT THE WORLD MOSQUITO PROGRAM'S *WOLBACHIA* METHOD REDUCES DENGUE TRANSMISSION

Katherine L. Anders, Cameron P. Simmons

Monash University, Clayton, Australia

The World Mosquito Program (WMP) is a not-for-profit initiative which aims to provide a safe, sustainable, and cost-effective new tool for preventing transmission of *Aedes aegypti*-borne diseases, including dengue, chikungunya and Zika. WMP's method involves introgression of *Wolbachia* bacteria into *Ae aegypti* mosquitoes. The resultant *Wolbachia* infected *Ae aegypti* have significantly reduced transmission potential for dengue, Zika and chikungunya viruses. Field releases in 9 countries have demonstrated successful and sustained *Wolbachia* establishment in local *Ae aegypti* populations. The public health impact of WMP's *Wolbachia* method is being evaluated through a suite of epidemiological studies in global field sites. A cluster-randomized controlled trial is underway in Indonesia and due to report in 2021, and a non-randomized prospective clinical study in Colombia in 2020. Consistent with predictions from mathematical modelling, pragmatic field effectiveness studies in Australia, Brazil, Colombia, Indonesia, Vietnam, and the Western Pacific have demonstrated that dengue outbreaks have ceased in locations where *Wolbachia* has been established. We used controlled interrupted time series analysis of surveillance data to quantify the reduction in dengue incidence in *Wolbachia*-treated areas, compared with historical time series and (in most sites) an untreated control area. Using segmented negative binomial regression to model monthly dengue case counts before and after *Wolbachia* deployment, with an offset for population size and

controlling for seasonal and interannual dengue variability using flexible cubic splines, we show that dengue incidence is significantly lower in *Wolbachia*-treated populations. Point estimates of the reduction in dengue incidence for the 5 sites with ≥6 months post-intervention by end 2018 ranged from 46-97%, with significance at the 0.05 level for 4/5 sites. These results, together with the efficacy trials and cost-effectiveness studies, strengthen the evidence that WMP's *Wolbachia* method can deliver large and sustained reductions in the burden of dengue in endemic and non-endemic countries.

823

COST-EFFECTIVENESS OF *WOLBACHIA* TO REDUCE DENGUE BURDEN IN MAJOR INDONESIAN CITIES

Oliver Brady[1], Lauren Carrington[2], Emilie Hendrickx[1], Dinar D. Kharisma[3], Ida S. Lakswanawati[4], Kathleen O'Reilly[1], **Donald S. Shepard**[3], Cynthia Tschampl[3], Nandyan N. Wilastonegoro[5], Laith Yakob[1], Wu Zeng[3]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,* [3]*Brandeis University, Waltham, MA, United States,* [4]*Dr Sardjito General Hospital, Yogyakarta, Indonesia,* [5]*Universitas Gadjah Mada, Yogyakarta, Indonesia*

Dengue is among the world's fastest growing vector-borne diseases, costing US$18 billion globally in 2016. Releasing mosquitoes infected with *Wolbachia* is a promising strategy to control dengue, chikungunya and Zika. As *Wolbachia* are passed on to the next generation of mosquitoes, the process is self-sustaining and generally entails minimal ongoing costs. A 24-cluster randomized trial and observational studies with *Wolbachia* are underway in Yogyakarta, Indonesia. Here we assess the cost-effectiveness over a 10-year present-value horizon of extensions to two potential sites: (1) the current control clusters of Yogyakarta (population 225,313); (2) densely populated parts of Indonesia's top 7 cities in number of predicted dengue cases (Jakarta, Surabaya, Bandung, Medan, Semarang, Palembang, and Makassar, population 37 million). We based costs (in 2018 US$) on budgets from Yogyakarta and disease impact on a dengue transmission model calibrated to entomologic data. We projected that the first site would cost US$4.4 million (with a 95% uncertainty interval of $2.9-$6.7 million) and avert 534 (138-1,213) DALYs per year, a 97.4% reduction. The incremental cost-effectiveness ratio (ICER) was $1,123 per DALY averted on a gross basis (counting only program costs), but -$82 on a net basis (i.e., showing a net savings due to medical costs averted). The second site would cost $480 ($306-$746) million, or $13.1 ($8.4-$20.3) per person protected, and avert 48,454 (12,582-110,074) DALYs annually. Its gross and net ICERs were $1,335 and -$4 per DALY, respectively. These results suggest *Wolbachia's* ICER is lower (i.e., even more cost-effective) than other widely-accepted public health interventions in Indonesia. For example, Didik Setiawan et al. estimated the ICER for visual inspection with acetic acid and human papillomavirus vaccination for girls at $1,863 per DALY averted. Because *Wolbachia* costs depend primarily on the area covered, this strategy is most cost-effective for large urban areas. If epidemiologic results confirm entomologic data, the extension of *Wolbachia* to major cities appears highly cost-effective, and perhaps cost saving.

824

LABORATORY EVALUATION OF A NOVEL MICROFLUIDIC NANOPARTICLE-BASED IMMUNOASSAY FOR THE DETECTION OF DENGUE VIRUS NS1 ANTIGEN IN A DENGUE-ENDEMIC SETTING

Michelle Ylade, Jedas Veronica Daag, Kristal An Agrupis, Leidenia Castro, Riacarl Alpay, Jacqueline Deen, Anna Lena Lopez

University of the Philippines Manila National Institutes of Health, Manila, Philippines

Dengue is an important public health problem in Asia and Latin America. There are four serotypes; individuals living in endemic areas may experience repeat dengue infections. Since dengue has non-specific manifestations, an accurate rapid diagnostic test would be useful. The flavivirus nonstructural 1 (NS1) protein is an important biomarker for early diagnosis of disease. A study evaluating commercially-available NS1 RDT kits for diagnosis of acute dengue found sensitivities ranging from 40 to 59% and specificities of 76 to 80% compared to RT-PCR and/or virus isolation. A better performing NS1 RDT, particularly with improved sensitivity, would be useful. We evaluated the performance of a novel kit, ViroTrack acute dengue NS1 antigen test used with the BlueBox reader (BluSense Diagnostics, Copenhagen, Denmark). The ViroTrack test consists of a microfluidic disc with magnetic nanoparticles coated with high-affinity monoclonal antibodies against NS1. The presence of NS1 antigen in the specimen triggers agglutination and nanocluster formation that is detected using an optomagnetic readout in the BluBox. We tested 100 stored samples from Filipino children with acute febrile illness for dengue RNA by RT-PCR and for dengue NS1 by a commercial RDT (SD BIOLINE Dengue NS1 Ag, Abbott) and the ViroTrack test. Compared to RT-PCR, the overall sensitivity and specificity of SD was 35.29% and 87.76% and Virotrack was 41.18% and 87.76%. Using RT-PCR as the gold standard, we found similar specificities but a slightly higher sensitivity of the ViroTrack test compared with the SD NS1 RDT. A larger study is ongoing to assess the performance of ViroTrack at the point-of-care.

825

GENOME MICROEVOLUTION OF DENGUE VIRUS TYPE 2 CAUSING OUTBREAKS IN THE KENYAN COAST, 2014-2017

Solomon Kipngetich Langat, Fredrick Eyase, Albert Nyunja, John Distelhorst, Rosemary Sang

U.S. Army Medical Research Directorate-Africa, Nairobi, Kenya

Dengue virus (DENV) is an important arbovirus pathogen with significant disease burden and risk potential globally. An estimated ^{390} million yearly infections occur throughout the tropical and subtropical regions. In Kenya, the virus has been associated with outbreaks since ^{1982}, especially in the Coastal towns of Malindi, Kilifi and Mombasa. DENV^{2} has been implicated on the majority of those outbreaks, with DENV^{1} and ^{3} also being reported. DENV epidemics in Kenya have increased over the recent past in terms of the frequency and severity of the disease. Information on the evolutionary dynamics of DENV in Kenya is still lacking. In this study, therefore, we complemented previous sequencing efforts of DENV^{2} by performing complete genome sequencing of samples covering the periods between ^{2014} and ^{2017}, and subsequently performed viral microevolutionary analysis of this important pathogen which has caused outbreaks at the Kenyan Coast between these periods. Newly generated DENV^{2} genomes, combined with previously published genomes resulted in a dataset of ^{19} complete and near-complete sequences isolated between ^{2014} and ^{2017}. Our findings show that DENV^{2} in Kenya is undergoing a rather rapid lineage turnover, characterized by replacement of earlier strains. The most recent outbreak strains are genetically distinct and they have acquired unique amino acid changes in the NS^{1}, NS^{2}A and NS^{2}B genes. The observed lineages do not appear to be as a result of importation of new strains, rather they appear to have arose In Situ through elaborate evolutionary processes acting on the DENV^{2} genome. Our findings suggest an interplay of natural selection and genetic drift in this process. This study demonstrates the importance of evolution in the emergence of DENV^{2} as a significant human pathogen in Kenya.

826

THE TETRAVALENT DENGUE LIVE ATTENUATED VACCINE TV003 ELICITED A CROSS-REACTIVE MEMORY B CELL REPERTOIRE IN FLAVIVIRUS-NAÏVE SUBJECTS

Huy A. Tu[1], Usha K. Nivarthi[2], Matthew Delacruz[2], Kristen Pierce[1], Stephen Whitehead[3], Beth Kirkpatrick[1], Aravinda Desilva[2], Sean Diehl[1]

[1]*University of Vermont, Burlington, VT, United States,* [2]*University of North Carolina School of Medicine, Chapel Hill, NC, United States,* [3]*Laboratory of Infectious Disease, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States*

Dengue, caused by any of the four dengue virus serotypes (DENV-1, 2, 3, 4), is the leading mosquito-borne viral disease worldwide, with approximately 400 million new infections annually. The lack of direct treatment and increasing rate of transmission result in an urgent need for safe and efficacious vaccines to protect populations living and traveling in endemic areas. The tetravalent DENV live attenuated vaccine candidate TV003 protected 100% of the subjects from clinical symptoms and viremia upon an attenuated DENV-2 challenge, and recently DENV-3 challenge. Toward the goal of defining novel correlates of protection for improved evaluation of vaccine efficacy, we interrogated the antibody response following TV003 immunization. Using longitudinal samples acquired during the six-month period following immunization, we assessed the plasmablast and memory B cell response induced by TV003. In healthy, naïve adult subjects, TV003 induced a plasmablast response within three weeks following immunization, averaging 3.0% of peripheral B cells. The magnitude of this plasmablast response was correlated to duration and level of vaccine-related viral replication, and was associated with development of serum neutralizing antibodies. At 6-month post-immunization, we estimated 0.15% of the memory B cell pool to be DENV2-specific. The majority of these DENV-2-specific memory B cells showed cross-reactivity against multiple DENV serotypes, which reflected the tetravalent nature of the TV003 formulation. Given the vaccine's ability to confer protection, our findings suggested that TV003-induced immunity may involve cross-reactive antibodies along with other immune mechanisms. In addition, the connection between vaccine-related viremia and the early plasmablast response may be further explored to determine its applicability as an immunologic parameter to inform vaccine efficacy evaluation.

827

ARBOVIRAL VULNERABILITIES OF ECUADOR: CHIKUNGUNYA ORIGINS AND NOVEL DENGUE INTRODUCTIONS FOLLOWING THE INCREASED INFLUX OF VENEZUELAN AND COLOMBIAN CITIZENS

Irina Maljkovic Berry[1], Wiriya Rutvisuttinunt[1], Rachel Sippy[2], Katherine Figueroa[1], Abhinaya Srikanth[1], Anna M. Stewart-Ibarra[2], Timothy Endy[2], Richard G. Jarman[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*SUNY Upstate Medical University, Syracuse, NY, United States*

In recent years, Ecuador and other South American countries have experienced an increase in arboviral diseases. A rise in dengue infections and severe forms of the disease was followed by introductions of chikungunya and Zika, two viruses never before seen in the country. This, coupled with the latest socioeconomic and political instability in Venezuela and the mass migration of its population into the neighboring countries, has given rise to concerns of infectious disease spillover and escalation of arboviral spread in the region. Our analyses of dengue virus (DENV) and chikungunya virus (CHIKV) genomes sampled from a surveillance site in Ecuador in 2014-2015, along with genomes from the surrounding countries, revealed several DENV introductions from Venezuela and Colombia (in years 2010, 2011 and end of 2013), some of which were subsequent to an increase in the influx of Venezuelan and Colombian citizens into Ecuador. However, we show that Venezuela has historically been a major source of DENV dispersal in this region, even before the massive contemporary exodus of its population, suggesting already established paths of viral distribution. Like DENV, CHIKV was introduced into Ecuador at multiple time points, but unlike DENV, these introductions were associated with the Caribbean strains. Findings indicate no direct CHIKV dispersal between Ecuador, Colombia, and Venezuela as of 2015, suggesting that CHIKV was not following the paths of DENV spread despite the viruses sharing a common vector in this region. Finally, our results reveal that Ecuador is vulnerable to arbovirus import from many geographic locations, highlighting the need of continued surveillance and more diversified prevention strategies.

828

CLINICAL PROFILE OF PATIENTS HOSPITALIZED WITH DENGUE DURING AN EPIDEMIC IN PUERTO RICO

Laura Divens Zambrano[1], Brenda Torres-Velasquez[1], Laura E. Adams[1], Tyler M. Sharp[1], Janice Perez-Padilla[1], Vanessa Rivera-Amill[2], Stephen H. Waterman[1], Gabriela Paz-Bailey[1]

[1]*Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*Ponce Health Sciences University, Ponce, PR, United States*

Dengue is endemic in Puerto Rico and typically causes outbreaks every 3-5 years. An epidemic dominated by dengue virus (DENV)-1 occurred during 2012-2013. Among other criteria, WHO recommends hospitalizing patients with severe dengue and warning signs to closely monitor disease progression and manage fluid intake. To assess adherence to the current WHO clinical management guidelines, we evaluated symptoms associated with hospitalization among dengue patients in 2012 and 2013 with onset of fever within 7 days prior to presentation at two sentinel sites in southern Puerto Rico. We analyzed demographic and clinical data from initial clinical assessments among patients with DENV infection detected by RT-PCR or IgM ELISA. Adjusted prevalence ratios comparing the proportion of hospitalized patients with severe dengue and dengue with warning signs was determined through log-binomial regression. Of 916 confirmed dengue patients during the outbreak period, 412 (45%) were hospitalized. Of all dengue patients, 13% (n=117) had severe dengue, 80% (n=736) had one or more dengue warning signs, and 6.9% (n=63) had dengue without warning signs. Of patients with severe dengue, 71% (83/117) were hospitalized, compared to 43% (318/736) of patients with warning signs and 18% (11/63) of dengue patients without warning signs. Compared to patients without warning signs, the likelihood of hospitalization was twice as high with warning signs (aPR: 2.19, 95%CI: 1.29-3.72) and over 3 times as high (aPR: 3.64, 95%CI: 2.08-6.39) with severe dengue. Despite their higher frequency of hospitalization, some patients with severe dengue and warning signs were not admitted. The subjectivity of key self-reported and observed severity indicators, particularly for lethargy and abdominal pain, could contribute to misclassification. Review of hospitalization practices can optimize triage of dengue patients. These data underscore the importance of sensitizing clinicians to published clinical guidance. Evaluation of existing guidance and methods used to identify warning signs predictive of severe dengue may improve dengue case management algorithms.

829

LABORATORY DIAGNOSIS ZIKA IN NEUROLOGICAL CASES DURING ZIKA OUTBREAK 2016 AND 2018

Leda Parham, Kimberly García, Isis Figueroa, **Ivette Lorenzana**

Centro de Investigaciones Genéticas, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras

Since 2016 Zika outbreak that occur in Latin America, it has been confirmed its relationship to complications such as Guillain Barré Syndrome and other neurological manifestations, representing a considerable impact to global public health.In 2016, Honduras it was reported 32,132 Zika suspected cases,corresponding to more than 45% of Arboviral infections and 167 of neurological cases.Since then, efforts have been done for laboratory evidences of Zika infection and its association neurological.We aim to describe the laboratory results obtained in neurological cases since

the introduction of Zika in Tegucigalpa, Honduras(year 2016 to 2018). Analysis of 150 neurological cases. Plasma and/or urine were tested by qRT-PCR, as reported previously. A comparison was performed between plasma and urine in a subgroup of subjects (n=82). Plasma samples from 135 cases were tested for IgM antibodies for Zika&Dengue EIA-CDC methods. A 6% (9/150) of positivity for Zika by qRT-PCR & 36% (49/135) by Zika IgM antibodies. Distribution by year: 2.5% (2/79, CI95% 1 ± 9%) for 2016 and 11.2% (7/62, CI95% 6 ± 22%) for 2018 by PCR; 59% (43/73, CI95% 49 ± 69%) for 2016 and 9.8% (6/62, CI95% 5 ± 20%) for 2018 by serological means. In 2017 only 9 cases were reported and none was positive in the molecular assays, neither by serology. About 82 cases tested in both urine&plasma, a 7.3% (6/82, CI95% 3 ± 15%) positivity was found only in urine; 3.6%(3/82, CI95% 1 ± 10%) positivity only in plasma. The temporal distribution had two peaks, first in February and then in June during the two years. A Higher frequency of was observed in young people (less than 30 years of age)&males.The strong association between Zika in neurological cases considered a real challenge because of the low probability of detecting viral RNA after acute infection, nevertheless it was possible to determine it in 6% of neurological cases by molecular means.Urine and plasma samples contribute an added value for the ARN detection of Zika virus in neurological cases.The positivity could increase to 36% if serology is used, though one must keep in mind that Dengue is endemic in Honduras and some could be due to cross reactivity

830

ENSO PHENOMENON, THE FLOW OF THE RIVER AND THE PREVALENCE OF DENGUE CASES IN PIURA-PERU. 2013.01-2017.12

Victor Raul Ocana Gutierrez, Jorge Gonzales, Luis Varona

National University of Piura, Piura, Peru

We study for the dynamics of temporal behavior of cases of Dengue virus (DENV) as well as explain the causal relationships between ENSO and the prevalence of cases of DENV in the Piura-Peru Region, for the period 2013- 2017. The Distributed Autoregressive Recesses (ARDL) model that explains the impact of ENSO on the behavior of the prevalence of DENV in the Piura Region. The limit test accepts the long-term relationship of the model variables, which is adjusted in the short term by 38% per month. Two rainy scenarios are presented with ANIÑO indicators greater than zero and regular flow of the Piura River with DENV cases over 2466 and another scenario for a dry period that tends to zero the three variables. The model with the variables of ENSO and flow of the Piura River explain the variations in the prevalence of DENV by 83%. The model is useful for the analysis of future trends in DENV or other arboviruses transmitted by *Aedes aegypti.* For health prevention, the timely design of public policies before ENSO and the monitoring of water resources to reduce and mitigate endemic or epidemic levels is recommended. The rainy scenario with ANIÑO indicators greater than zero and flow of the Piura River with DENV cases causes higher DENV and another scenario for a dry period that does not.

831

THE BURDEN OF DISEASE DUE TO DENGUE IN THE DISTRICT OF COLOMBO, SRI LANKA

M. B. Azhar Ghouse[1], Hasitha A. Tissera[1], Yeşim Tozan[2]

[1]*Epidemiology Unit, Ministry of Health, Sri Lanka,* [2]*New York University, College of Global Public Health, NY, United States*

Dengue is endemic in all districts in Sri Lanka, and the incidence of dengue has risen significantly during the last decade. The highest proportion of cases are reported from Colombo district, which is the most populated and urbanised district in the country. Assessing the underlying dengue disease burden in Colombo is key to policy decision making on the introduction of a successful vaccination strategy in the future. We evaluated the dengue disease burden by estimating the disability-adjusted life-years (DALYs) using an incidence-based approach of morbidity and mortality surveillance data from 2015 to 2017 in Colombo district and at the national level. In 2015 and 2016, the dengue incidence in Colombo was 473.5 and 742.4, while the incidence for the whole country was 128 and 242 cases per 100,000 population. In 2017, when a severe dengue outbreak was experienced in all parts of Sri Lanka, with an incidence rate of 883 cases per 100,000 population, the incidence in Colombo increased to 1,901 cases per 100,000 population. The dengue disease burden in Colombo district was estimated at 918 DALYs (39.49 per 100,000 population) in 2015, 978 DALYs (42.08 per 100,000 population) in 2016, and 2,203 DALYs (133.84 per 100,000 population) in 2017. The national disease burden was estimated at 1,918 DALYs (9.42 per 100,000 population) in 2015, 3,270 DALYS (16.06 per 100,000 population) in 2016, and 13,635 DALYs (66.97 per 100,000 population) in 2017. This is the first study on the dengue disease burden in Sri Lanka, and our findings show a significant increasing trend over time, particularly following the massive dengue outbreak in 2017. The findings of this research serve as a catalyst for future countrywide disease burden assessments and have the potential to inform the implementation of a future dengue vaccination strategy

832

GENOTYPIC DIVERSITY IMPACTS DENGVAXIA DENV4 ANTIBODY RESPONSES AND PROTECTIVE IMMUNITY

Emily Gallichotte[1], Sandra Henein[1], Usha Nivarthi[1], Matthew Delacruz[1], Matthew Bonaparte[2], Ralph Baric[1], Aravinda de Silva[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Sanofi Pasteur, Allentown, PA, United States*

Dengvaxia, Sanofi-Pasteur's licensed tetravalent dengue vaccine, has completed two large phase III trials in Latin America and Southeast Asia. While overall vaccine efficacy varied depending on baseline dengue (DENV) immune status and DENV serotype, efficacy was highest against serotype 4 (DENV4) (~77%). Recent genetic epidemiological studies revealed that two DENV4 genotypes circulated during the trials, genotypes I and II. Additionally, these studies found that vaccine efficacy was substantially higher against vaccine-matched genotype II DENV4s (83%), than vaccine-mismatched genotype I viruses (47%). Importantly, this phenomenon was not observed when only looking at older individuals, who are likely pre-immune to DENV, suggesting a difference in vaccine-elicited antibody (Abs) responses in naïve versus pre-immune individuals. In our study, using chimeric genotype I and II DENV4 viruses, we find that sera from naïve vaccinated individuals more potently neutralize DENV4 genotype II viruses than genotype I, whereas pre-immune vaccinees cross-neutralize both genotypes similarly. Using depletion techniques, we find this is driven by differences in quality of vaccine-elicited Abs. Naïve vaccinees generate high levels of DENV4 serotype-specific Abs that more potently neutralize vaccine-matched genotype II, whereas pre-immune vaccinees have lower levels of DENV4-specific Abs, revealing cross-reactive Abs are primarily responsible for DENV4 neutralization. Additionally, we map five specific amino acids within the viral envelope glycoprotein that differ between genotype I and genotype II viruses, which are responsible for the differential neutralization between the genotypes. These results suggest that the immune response to Dengvaxia vaccination is measurably different between naïve and pre-immune individuals, and that these differences in responses might allow for vaccine-mismatch genotype breakthrough infections. Additionally, these results highlight the importance of antigenic variation within DENV serotypes, and possibly provides a molecular explanation of heterotypic reinfection.

833

LINKING DENGUE VIRAL GENOMICS AND HOST CELL TRANSCRIPTOMICS IN SINGLE CELLS

Felix J. Hol, Fabio Zanini

Stanford University, Stanford, CA, United States

Viral infections originate from one or a handful of virions infecting a single host cell, yet in the laboratory this process is generally studied at vastly larger scales. Recent advances in sequencing technologies, however, enable the characterization of the host cell and its infecting pathogen at

an unprecedented level of molecular detail. Using virus-inclusive single cell RNAseq data, we present an analysis of hundreds of human cells infected with dengue virus (DENV) and characterize the joint dynamics of the host transcriptome and viral population genetics at the single-cell level. Our analysis reveals a strong heterogeneity in the viral genetic diversity generated in individual host cells, and the distribution of single nucleotide variant (SNV) frequencies across cells indicates that DENV replicates geometrically, implying an accelerated accumulation of SNVs compared to a 'stamping machine' replication mode. Detailed analysis of mutational profiles shows that the frequency and type of SNVs vary along the genome. Connecting the genetics of the infecting virus to the transcriptome of the host cell shows that standing genetic variation in the parental DENV strain and de novo mutations result in different clusters of host cells with linked viral genomic variation and distinct host transcriptional patterns. This study provides an exploratory proof-of-concept of simultaneous viral genomics and host transcriptomics in single cells at high throughput, and can be extended to virtually any virus and host cell type.

834

EVALUATION OF THE LARVAL INDEX RAPID ASSAY FOR *AEDES AEGYPTI* (LIRAA) AS A PREDICTOR OF DENGUE EPIDEMICS

Andrew William Enslen, Antonio S. Lima Neto, Marcia C. Castro

Harvard TH Chan School of Public Health, Boston, MA, United States

To retrospectively examine whether the LIRAa larval survey served as a useful tool in predicting the occurrence of future dengue epidemics within Brazilian municipalities from 2009-2015. Weighted sensitivity and specificity analysis were used to determine the validity of the LIRAa predictor in predicting epidemics across Brazil from 2009-2015. Models were stratified by year, by geographic macro-region, by municipality-population size, and temporal interval between LIRAa survey and epidemic outcome. Between 2010 and 2015, the LIRAa survey had an overall weighted sensitivity of 25.07% (CRL: 22.67, 27.47), a weighted specificity of 73.45% (CRL: 71.97 - 74.52), a weighted PPV of 42.57% (CRL: 39.84 - 45.31) and weighted NPV of 55.27% (CRL: 53.84 - 56.70) in predicting future dengue epidemics across all municipalities in Brazil. The LIRAa survey was a poor predictor of future dengue outbreaks within municipalities throughout Brazil. The marginal increases in sensitivity associated with northern macro-regions, heavily populated municipalities, and for the month following the LIRAa survey as compared to 6-months post-survey suggest that usage of LIRAa scores on a national scale requires further investigation. The Brazilian Ministry of Health, PAHO, and the WHO should re-consider widespread recommending LIRAa implementation or reevaluate strategies to optimize its use in order to better respond to future Dengue epidemics.

835

CHARACTERIZATION OF GENETIC VARIATION BETWEEN ZIKV ASIAN AND AFRICAN STRAINS

Camila R. Fontes-Garfias[1], Bruno Nunes[2], Chao Shan[1], Antonio Muruato[1], Scott C. Weaver[1], Pedro F. Vasconcelos[2], Daniele B. Medeiros[2], Pei-Yong Shi[1]

[1]*University of Texas Medical Branch, Galveston, TX, United States,* [2]*Evandro Chagas Institute, Ministry of Health, Brazil*

Zika virus (ZIKV) Asian lineage is responsible for recent epidemics in the Americans causing neurological complications and is associated with sexual transmission. However, ZIKV African lineage has not been associated with the recent epidemic outbreak or severe disease manifestations. However, in laboratory settings, the African strains seem to be more virulent than the Asian strains. To identify the determinants of virulence between the two ZIKV lineages, we engineered chimeric viruses by swapping the structural and non-structural regions between the African (DKR) and Asian (FSS) strains. We observed differences *in vivo* and *in vitro* that indicate the structural genes (C-prM-E) are responsible for the increased viral replication and virulence of the African over the Asian strain. We investigated the effect of the variations of the structural genes of the DKR strain by constructing structural chimeric viruses; in which each virus contained one structural gene from DKR strain in the backbone of FSS strain. In mouse models, the DKR prM structural gene enhanced virulence and neurovirulence. Additionally, we investigated the role of amino acid differences between the two strains located in the structural regions. We generated recombinant viruses by introducing single mutations, present in the DKR strain, into the FSS infectious clone. We identified the single mutation as one of the major determinants for neurovirulence in the CD1 mice, as evidenced by mortality, the mutant and DKR viruses caused 100% mortality at days 13 and 10 p.i., respectively. In contrast, infections FSS strain resulted in 20% mortality. Collectively, the results have identified the virulent determinant (s) between the African and Asian ZIKV lineages.

836

PERFORMANCE OF TWO ZIKA VIRUS NEUTRALIZING ANTIBODY TITER CLASSIFICATION SCHEMES AGAINST ZIKA NS1 BLOCKADE-OF-BINDING ASSAY DURING THE 2015-2016 EPIDEMIC IN GUATEMALA

Daniel Olson[1], Molly M. Lamb[2], James W. Huleatt[3], Matthew Bonaparte[3], Maria Alejandra Paniagua-Avila[4], Alma Zacarias[4], Neudy Rojop[4], Andrea Chacon-Juarez[4], Muktha Natrajan[5], Jesse Waggoner[6], Maria Renee Lopez[7], Celia Cordon-Rosales[7], Edwin J. Asturias[1]

[1]*University of Colorado School of Medicine, Aurora, CO, United States,* [2]*Colorado School of Public Health, Aurora, CO, United States,* [3]*Sanofi Pasteur, Swiftwater, PA, United States,* [4]*Fundacion para la Salud Integral de los Guatemaltecos, Los Encuentros, Guatemala,* [5]*Emory Vaccine Center, Atlanta, GA, United States,* [6]*Emory University School of Medicine, Atlanta, GA, United States,* [7]*Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala*

Neutralizing antibody (NAb) tests, including microneutralization (MN) and plaque reduction neutralizing tests (PRNT), are commonly used to determine Zika (ZIKV) and dengue virus (DENV) serostatus, though they can exhibit cross-reactivity. The ZIKV anti-NS1 blockade-of-binding (BoB) ELISA has high sensitivity (Sn) and specificity (Sp) in determining ZIKV serostatus, when evaluated against PCR. We created two ZIKV/DENV serostatus classification schemes ('Expanded' and 'Simple'), which consider various combinations of ZIKV and DENV NAb titers (<15, 15 to <100, 100 to <500, and >500) concurrently to categorize individual ZIKV and DENV serostatus into ZIKV- and DENV- 'positive,' 'possible,' 'negative,' or 'indeterminate.' Using available serum samples obtained from randomized cross-sectional, pediatric seroprevalence surveys in Guatemala during the ZIKV epidemic (Oct. 2015-Feb. 2016), we performed ZIKV/DENV 1-4 MN testing and ZIKV NS1 BoB testing on all samples. We then evaluated the performance of each NAb classification scheme (Expanded and Simple) against ZIKV NS1 BoB positivity (50% inhibition of NS1 Mab binding, titer >10 [1/dilution]). A total of 389 subjects had samples available for testing (mean age 9.9 years, 56% female), and 92 (24%) were ZIKV-seropositive by ZIKV NS1 BoB. ZIKV serostatus was classified as positive, possible, negative, and indeterminate by the two NAb schemes as follows: Expanded, 59 (15%), 48 (12%), 279 (72%), 3 (<1%); and Simple, 59 (15%), 19 (5%), 308 (79%), 3 (<1%). The Sn/Sp for positive ZIKV categorization in the Expanded and Simple schemes, compared to ZIKV NS1 BoB ≥10, were 76%/99% and 71%/99%, respectively; the Sn/Sp for positive *or* possible ZIKV categorization were 80%/89% and 75%/97%, respectively. In summary, the Expanded and Simple NAb categorization schemes for classifying individual ZIKV and DENV serostatus performed well against the ZIKV NS1 BoB assay (Sn 71-80%, Sp 89-99%), and therefore may be useful in classifying individual ZIKV/DENV serostatus. Accuracy of these schemes should be corroborated using late convalescent phase sera from PCR-confirmed ZIKV/DENV cases.

837

ZIKA VIRUS INFECTION INDUCES DETECTABLE METABOLIC ALTERATIONS IN HUMANS

Nathaniel M. Byers[1], Bryna L. Fitzgerald[1], Lyle R. Petersen[1], Amy C. Fleshman[1], Barbara Graham[2], Rebekah C. Gullberg[3], Rushika Perera[2], Michael P. Busch[4], Mars Stone[5], Claudia R. Molins[1]

[1]*Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States,* [2]*Colorado State University, Fort Collins, CO, United States,* [3]*Stanford University, Stanford, CA, United States,* [4]*Vitalant Research Institute and University of California San Francisco, San Francisco, CA, United States,* [5]*Vitalant Research Institute, San Francisco, CA, United States*

Cutting-edge untargeted metabolomics techniques can detect alterations in relative quantities of thousands of metabolites during infection, providing detailed insight into metabolic pathway disturbances indicative of disease progression. Metabolic profiling provides an orthogonal approach to nucleic acid and serologic methods and can circumvent the limitations of commonly used diagnostics. To develop metabolic biosignatures of Zika virus (ZIKV) infection, we applied liquid chromatography-mass spectrometry to well-characterized longitudinal plasma samples obtained from asymptomatic blood donors in the US (n = 53) positive for ZIKV RNA at index collection. We selected molecular features with at least two-fold changes or with statistically significant ($p < 0.05$) differences in concentrations between sample groups. Approximately 300 molecular features differentiated samples collected during the adaptive immune response to ZIKV (5-30 days post index collection) from samples post-ZIKV clearance (> 150 days post index collection). Longitudinal samples allowed us to evaluate the abundance changes of these molecular features over time and to correlate metabolic alterations to the progression of ZIKV infection. In comparing our results to findings from similar studies on dengue, we preliminarily identified several lipid metabolites that were perturbed in both infections, indicating conserved host-virus metabolic interactions between these flaviviruses. Ultimately, this study aims to understand fundamental metabolic changes occurring in ZIKV-infected patients and inform the development of novel biosignatures to predict complicated clinical outcomes of ZIKV, such as congenital Zika syndrome and Guillain-Barré syndrome.

838

CHARACTERISTICS OF A HIGH THROUGHPUT, SPECIES-INDEPENDENT PLATE-BASED ZIKA REPORTER VIRUS PARTICLE (Z-RVP) NEUTRALIZATION ASSAY

Kelly Bohning[1], Melissa Zahralban-Steele[1], Hui-Ling Chen[1], Greg Hather[2], Tim Powell[1], Hetal Patel[1], Stephanie Sonnberg[1], Hansi Dean[1]

[1]*Takeda Vaccines, Inc., Cambridge, MA, United States,* [2]*Takeda Pharmaceuticals, Inc., Cambridge, MA, United States*

Zika virus is a Flavivirus, transmitted via *Aedes* mosquitos, that causes a range of symptoms including Zika congenital syndrome. Zika has posed a challenging situation for health, public and economic sectors of affected countries. To quantitate Zika neutralizing antibody titers in serum samples, we developed a high throughput plate-based Zika reporter virus particle (RVP) assay that uses an infective, non-replicating particle encoding Zika virus surface proteins and capsid (CprME) and a reporter gene (*Renilla* luciferase). This is the first characterization of a Zika RVP assay in 384-well format using a Dengue replicon *Renilla* reporter construct. Serially diluted test sera were incubated with RVPs, followed by incubation with Vero cells. RVPs that have not been neutralized by antibodies in the test sera entered the cells and expressed *Renilla* luciferase. Quantitative measurements of neutralizing activity were determined using a plate-based assay and commercially available substrate. The principle of limiting the infection to a single round increases the precision of the assay measurements. RVP EC_{50} titers correlated closely with titers determined using a plaque reduction neutralization test (PRNT) (>95%). The plate-based Zika RVP assay also demonstrated excellent precision, reproducibility and throughput. The assay employs identical reagents for human, rhesus macaque and mouse serum matrices. Spiking studies indicated that the assay is species-independent, producing equivalent titers to monoclonal antibodies irrespective of the serum species. The assay is conducted in 384-well plates and can be automated to simultaneously achieve high throughput and high reproducibility.

839

SEX AS A BIOLOGICAL VARIABLE IN A POWASSAN VIRUS INFECTION MODEL

Erin Reynolds[1], Paul T. Massa[1], Steven G. Widen[2], Saravanan Thangamani[1]

[1]*SUNY Upstate Medical University, Syracuse, NY, United States,* [2]*University of Texas Medical Branch, Galveston, TX, United States*

Powassan Virus (POWV) is a tick-borne Flavivirus endemic to regions of the United States, Canada, and Russia. Following initial isolation of POWV from a fatal case in 1958 there has been relatively few confirmed cases in the US, although the rate of infection in the past few years appears to be increasing. The majority of POWV cases are presumed to be asymptomatic or present with mild to moderate symptoms but severe neuroinvasive disease has also been reported. In severe cases the fatality rate is approximately 10% and over 50% of survivors develop severe, long-lasting neurological sequelae. Animal models are often used to study human disease and a well-designed experiment can improve our understanding of the pathogenesis of infectious agents. Poor model selection or experimental design may lead to erroneous results while wasting animal lives. As advances are made in our understanding of the human body and how it responds to disease, as well as our understanding of animal models, it has become increasingly apparent that sex is an important biological variable. The Balb/C mouse is often used as a model for POWV infection but published research often used only one sex or has not fully evaluated differences in pathology or immune response in both sexes. To evaluate these potential differences, groups of male and female Balb/C mice were injected with either media or POWV. Animals were monitored for signs of clinical disease and blood was collected to determine viremia. At the end of the study, brains were harvested and split into two equal halves. RNA was extracted from one half for viral load and RNA-Seq analysis while the other half was sectioned and used for histopathology analysis via H&E and IHC staining. Our preliminary analysis indicates that there are differences in the temporal infection pattern of POWV in brain. We are currently investigating the pathology and immunological response to POWV infection. The outcome of this work will determine if sex is a biological variable for POWV infection and clinical outcomes, and will also provide valuable information in using male and female mice for future POWV infection and pathogenesis studies.

840

LEVERAGING MULTIPLE DATA TYPES TO ESTIMATE THE TRUE SIZE OF THE ZIKA EPIDEMIC IN THE AMERICAS

Sean M. Moore[1], Rachel J. Oidtman[1], K. James Soda[1], Amir S. Siraj[1], Robert C. Reiner, Jr.[2], Chris M. Barker[3], T. Alex Perkins[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*University of Washington, Seattle, WA, United States,* [3]*University of California Davis, Davis, CA, United States*

Since the first Zika virus (ZIKV) infection was confirmed in Brazil in May 2015, several hundred thousand cases have been reported across the Americas. This figure gives an incomplete picture of the epidemic, however, given that asymptomatic infections, imperfect surveillance, and variability in reporting rates complicate the interpretation of case report data. The infection attack rate (IAR)-defined as the proportion of the population that was infected over the course of the epidemic-has important implications for the longer-term epidemiology of Zika in the region, such as the timing, location, and likelihood of future outbreaks. To estimate the IAR and the total number of people infected, we took advantage of multiple Zika case data types from 15 countries and

territories where subnational data was publicly available. Datasets included confirmed and suspected Zika cases in pregnant women and the total population, Zika-associated Guillan Barré syndrome cases, and cases of congenital Zika syndrome. We used a hierarchical Bayesian model with empirically-informed priors that leverages the different case report types to simultaneously estimate national and subnational reporting rates, the fraction of symptomatic infections, and subnational IARs. The estimated country-level IAR ranged from 0.08 (95% CrI: 0.07-0.10) in Peru to 0.36 (95% CrI: 0.21-0.51) in Ecuador. There was significant subnational variability, with median IAR estimates ranging from 0.02 to 0.84 in Brazil and 0.03 to 0.83 in Colombia. Recently published seroprevalence estimates from Nicaragua, Ecuador, and Brazil all fell within the 95% credible intervals of our subnational model estimates. Totaling these infection estimates across the entire region, our results suggest that 118.7 million (95% CrI: 79.1-246.9 million) people in the Americas have been infected by ZIKV since 2015. These estimates represent the most extensive attempt to date to determine the size of the Zika epidemic in the Americas, and they offer an important baseline for assessing the risk of future Zika epidemics in this region.

841

STATISTICAL MODELS TO ESTIMATE THE NUMBER OF INFECTIOUS DENGUE AND ZIKA VIRUSES USING A REAL-TIME PCR

Steev Loyola, Dina Popuche, Alfredo Huaman, Zonia Rios, Maria Silva, Carolina Guevara

U.S. Naval Medical Research Unit No. 6, Lima, Peru

Dengue (DENV) and Zika virus (ZIKV) represent a major public health problem worldwide. The evaluation of the viral infection kinetic is necessary to predict clinical outcomes and to monitor the disease course. Real-time reverse transcription polymerase chain reaction (RT-PCR) is a molecular test routinely used for diagnosis and follow-up for infected patients providing a rapid viral load approximation in hours. The plaque assay method is used for the quantification of infectious viral particles. However, this assay is associated with high costs and requires resources that would not be available in developing nations. Statistical models to infer the infectious viral load in resource-limited laboratories with no access to cellular assays are required. Here, we report the correlation between the RT-PCR and plaque assay results for DENV and ZIKV and propose models to estimate the infectious load in plaque forming units (PFU) of DENV and ZIKV using the cycle threshold (Ct) value obtained from the RT-PCR. We conducted two *in vitro* infection experiments with three biological replicates each, using Vero76 cells with DENV and ZIKV inoculums, representing 38 and 32 samples, respectively. Daily, 200μL of supernatant from each replicate was collected until cells displayed a cytopathic effect of>75%. Specific RT-PCR assays for DENV and ZIKV were performed to quantitate the viral load through the Ct value. Plaques were counted and used for calculating the PFU per mL (PFU/mL). PFU/mL values were transformed to log10 (PFU/mL) for data analysis purposes. The overall correlation of the RT-PCR and plaque assay was excellent for both viruses (R>0.9580, p-value<0.001). The coefficients of determination for the DENV and ZIKV models were 0.9342 (p-value< 0.001) and 0.9178 (p-value<0.001), respectively. Additionally, both models displayed statistically significant betas coefficients. Our findings suggest that the RT-PCR could be used as a reliable infectivity assay in samples with unknown load of infectious viral particles. The proposed regression models could be used in locations with limited resources and without access to cell culture assays.

842

DETECTION OF ZIKA VIRUS IN PATIENTS WITH ACUTE FEBRILE RESPIRATORY SYMPTOMS

Dina Popuche[1], Steev Loyola[2], Zonia Rios[1], Julia S. Ampuero[1], **Carolina Guevara**[1]

[1]*U.S.Naval Medical Research Unit No 6, Lima, Peru,* [2]*Asociacion Benefica PRISMA, Lima, Peru*

Over the past 19 years the U.S. Naval Medical Research Unit No. 6 has collaborated with local authorities and universities throughout Latin America to characterize acute febrile and exanthematous illness in throughout the region. Zika virus (ZIKV) is an emerging flavivirus transmitted by *Aedes* mosquitoes and is currently circulating in the Caribbean and Central and South America. In 2016, ZIKV was declared a Global Emergency by the World Health Organization, a classification that recommends ZIKV-vulnerable countries to establish and maintain capacity to detect and confirm cases of ZIKV infection. Detection and isolation of ZIKV from blood can be challenging. In order to overcome this challenge we decided to look for the virus in other types of specimens, such as oropharyngeal swabs, as an alternative method for virus detection. Therefore, we retrospectively selected 21 respiratory samples collected during 2016-2018 from subjects presenting with a febrile illness with respiratory symptoms. All corresponding serum samples tested negative for DENV and ZIKV. All swabs were inoculated in mosquitoes cells (C6/36) and human epithelial cells (A-549) and evaluated for the presence of cytopathic effect (CPE) daily. One sample in A-549 cells produced a cytopathic effect (CPE) of 1+ on day 6 with no CPE in C6/36 cells. A second sample in C6/36 cells a produced CPE of 1+ on day 4 with no CPE in A-549 cells. Both samples were harvested and reacted with Polyclonal Flavivirus pool and MoAb4G2 when tested in immunofluorescence assays. Furthermore, supernatants from both samples tested positive by RT-PCR for ZIKV, we went back to the original oropharyngeal swab samples and both were positive by RT-PCR for ZIKV. Our results indicate that ZIKV can be isolated from oropharyngeal swabs as an alternative to identification in serum or other body fluids Most ZIKV infections are asymptomatic and detection of infectious ZIKV particles in oropharyngeal swabs may represent a person-to-person transmission, and the possibility that ZIKV transmission may not involve vectors in all cases.

843

SEROSURVEILLANCE USING A MICROSPHERE IMMUNOASSAY AND PHYLOGENETIC ANALYSIS OF FLAVIVIRUS INFECTION IN THAILAND - 2002-2014

Vivek R. Nerurkar[1], Lauren L. Ching[1], Akanitt Jittmittraphap[2], Siriporn Chattanadee[2], Jasmine Padamada[1], Narin Thippornchai[3], Madhuri Namekar[1], Axel Lehrer[1], Pornsawan Leaungwutiwong[3]

[1]*University of Hawaii at Manoa, John A. Burns School of Medicine, Department of Tropical Medicine, Honolulu, HI, United States,* [2]*Mahidol University, Faculty of Tropical Medicine, Department of Microbiology and Immunology, Bangkok, Thailand,* [3]*Mahidol University, Faculty of Tropical Medicine, Department of Microbiology and Immunology, Bangkok, Thailand*

Thailand is endemic for dengue virus (DENV), with serological evidence of Zika virus (ZIKV) as early as 1954 in a serosurveillance study conducted using sera collected from military personnel stationed in Bangkok, and epidemiological evidence of human transmission since 2011. In this study we employed a laboratory-developed flavivirus multiplex microsphere immunoassay (MIA) using color-coded fluorescent microspheres covalently coupled with ZIKV, DENV and Japanese encephalitis virus (JEV) nonstructural protein 1 (NS1), and human IgG conjugated to streptavidin peroxidase. The flavivirus MIA was used to evaluate for presence of flavivirus antibody in serum samples from two Thai cohorts of patients who presented with dengue-like symptoms, however were DENV negative by RT-PCR. The first cohort included archival samples from hospitalized patients in rural northeast Thailand collected between 2002 and 2004 (n=300). The second cohort of serum samples was collected at the

Hospital for Tropical Diseases in urban Bangkok in 2014 (n=50). In the setting of high DENV endemicity, we detected 3% (n=11) serum samples reactive for only ZIKV NS1 antibodies as early as 2002. Overall ZIKV NS1 antibodies were detected in 41% (n=142) of the serum samples, some of which were also positive for DENV (56%, n=80), JEV (3%, n=4), or DENV and JEV (33%, n=47) NS1 antibodies. When stratified by geographic location, ZIKV seropositive serum samples were found more frequently in the rural cohort, 41% (n=124), as compared to the urban cohort, 36% (n=18), while DENV (71%; n=249) and JEV (18%; n=63) seropositivity remained constant throughout the country. ZIKV phylogenetic tree was constructed using eight samples collected from febrile patients from Bangkok in 2017 with confirmed ZIKV infection. Sequence analysis of the *env* gene showed the circulating 2017 ZIKV strains in Bangkok clustered with the Asian linage of ZIKV, specifically those originating from Singapore in 2016. These data suggest transmission of ZIKV in Northeastern Thailand as early as 2002 and warrants further surveillance of ZIKV in mosquitoes and humans across Thailand to understand virus transmission.

844

ZIKA VIRUS-LIKE-PARTICLE VACCINE DEVELOPMENT BASED ON IMMATURE AND MATURE PARTICLES

Danielle Thompson[1], Lo Vang[1], Carla Uranga[2], Diego Espinosa[3], Ben Guenther[1], Jason Mendy[1], Darly Manayani[4], Shannon Beaty[1], Jon Smith[5], Eva Harris[3], Jeff Alexander[6]

[1]*Emergent BioSolutions, San Diego, CA, United States,* [2]*J. Craig Venter Institute, San Diego, CA, United States,* [3]*University of California Berkeley, San Diego, CA, United States,* [4]*CA, United States,* [5]*ClearPath Vaccines with RRD International, Rockville, MD, United States,* [6]*JL Alexander Research & Development Consulting LLC, Greater San Diego Area, CA, United States*

Zika virus (ZIKV) is a mosquito-borne flavivirus that causes an acute febrile disease with neurological complications in adults and developing fetuses. To date, there are no licensed vaccines against ZIKV infection. When overexpressed in mammalian cells, the surface structural proteins of ZIKV, pre-membrane protein (prM) and envelope protein (E), self-assemble into virus-like particles (VLPs). VLPs have a physical structure comparable to that of the native virus, with conformationally intact surface epitopes that elicit strong B and T cell responses. Since VLPs are replication incompetent, they may provide a safer alternative to attenuated and inactivated vaccines throughout manufacturing and use. Genetic sequences encoding the surface structural ZIKV proteins from a Brazil outbreak strain (insert strain designation) were selected to construct our lead VLP candidate. In addition, we also generated and characterized a VLP candidate that contains a single point mutation (residue F108A) in the fusion loop of the E protein. We hypothesized that mutation of the F108A residue would increase VLP production and indeed particle yield of the F108A mutant was approximately twice that of the lead candidate. Both constructs induced VLPs that were found to be highly immunogenic, however, the F108A mutant conferred weaker *in vivo* protection against a lethal ZIKV challenge compared to the lead candidate. The F108A mutant displayed uncleaved prM protein in purified VLP material, an indicator of particle immaturity. Previous studies on dengue virus (DENV), a related flavivirus, have shown particle immaturity to be correlated with antibody dependent enhancement (ADE). Here, we used a cell-based ADE assay to evaluate post-boost immunization sera for enhancement of ZIKV infection. Consistent with previous work on DENV enhancement, antibodies induced by the immature F108A particles enhanced ZIKV uptake *in vitro*. Together, these results indicate that there is a relationship between particle immaturity and ADE in ZIKV infection.

845

PREDICTORS OF ZIKA VIRUS SEROPOSITIVITY AMONG RURAL GUATEMALAN CHILDREN EARLY IN THE 2015-16 EPIDEMIC, USING RAPID ACTIVE SAMPLING SURVEYS AND THE ZIKV NS1 IGG BLOCKADE-OF-BINDING ASSAY

Molly M. Lamb[1], Maria Alejandra Paniagua-Avila[2], Alma Zacharias[2], Neudy Rojop[2], Andrea Chacon[2], James W. Huleatt[3], Matthew I. Bonaparte[3], Maria Renee Lopez[4], Celia Cordon-Rosales[4], Edwin J. Asturias[5], Daniel Olson[5]

[1]*University of Colorado School of Public Health, Aurora, CO, United States,* [2]*Fundacion para la salud integral de los guatemaltecos, Los Encuentros, Guatemala,* [3]*Sanofi Pasteur, Swiftwater, PA, United States,* [4]*Universidad del Valle de Guatemala, Guatemala City, Guatemala,* [5]*University of Colorado School of Medicine, Aurora, CO, United States*

Zika virus (ZIKV) swept through Central America in 2015-2016, infecting a large proportion of the pediatric population, often before surveillance systems could be established to evaluate risk factors and clinical outcomes. Rapid identification of risk factors for ZIKV infection in children could have directed public health efforts to limit transmission in children and their caretakers. We tested 383 available samples for ZIKV seropositivity using a ZIKV NS1 blockade-of-binding (BoB) ELISA assay, obtained from two cross-sectional seroprevalence surveys conducted in children (mean age: 9.9 years, 56% female) in the lowlands of rural southwest Guatemala early in the ZIKV epidemic. Multivariable generalized linear regression was used to test the following socio-demographic and epidemiologic factors for association with ZIKV seropositivity: age, gender, ethnicity, presence of standing water on property, house type and water source (socio-economic indicators), primary caregiver literacy, household crowding, number of small children in house, and school attendance (children ≥ 6 years). Overall, 89 (23.2%) children tested positive for ZIKV infection: 20/197 (10.2%) in Survey 1 (Oct-Nov 2015), and 69/186 (37.1%) in Survey 2 (Jan-Feb 2016). Older age (Prevalence Ratio (PR) for each year in age: 1.12, 95% Confidence Interval (CI): 1.07-1.17) and primary caregiver literacy (PR: 2.8, CI: 1.3-6.06) were identified as independent risk factors for ZIKV seropositivity. The results from these cross-sectional RAS Surveys in a ZIKV-naive population suggest that older children were at greater risk of ZIKV seropositivity early in the epidemic, which may be due to increased exposure to *Aedes* mosquitoes due to daily activities, or greater cross-reactivity in the ZIKV BoB assay due to cumulative DENV infections. Primary caregiver literacy may be a proxy for urban household setting, which is associated with increased risk of ZIKV exposure. These findings suggest that RAS Surveys may be a useful tool to identify risk factors for infection with emerging pathogens early in the course of an outbreak, which may then be used to direct public health messaging.

846

RECENT ZIKA INFECTION AMONG WOMEN OF REPRODUCTIVE AGE IN GUATEMALA, 2017-2018

Carol Y. Rao[1], Manuel de Jesus Sagastume[2], Carolina Martinez[3], Mireya Palmieri[3], Maria Elena D. Jefferds[1], Olga L. Henao[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Ministry of Health, Guatemala City, Guatemala,* [3]*INCAP, Guatemala City, Guatemala*

Zika infections were declared a public health emergency of international concern in the Americas in 2016. Zika infection during pregnancy can cause severe brain defects. In 2016, Guatemala reported 3,214 cases of Zika infections; 549 Zika infections were reported in 2017 with most cases reported in the first half of the year. Although official reports of Zika infections had decreased in Guatemala, Zika virus was still a potential risk for pregnant women. SIVESNU (Sistema de Vigilancia Epidemiológica de Salud y Nutrición) routinely monitors health and malnutrition among women and children in Guatemala. In the 2017 round of data collection for SIVESNU, we added questions on Zika-like symptoms and tested serum for recent Zika infection among women of reproductive age (WRA). Between July 2017 and March 2018, SIVESNU collected data

from a nationally representative sample of WRA (between 15 and 49 years of age). We administered a standardized questionnaire which asked about demographics, pregnancy status, symptoms of Zika infection in the last year and possible risk factors for infection. Venous blood was collected and sent to Instituto de Nutrición de Centroamérica y Panamá in Guatemala City for analysis. Serum was tested for IgM antibodies for Zika (Inbios ZIKV Detect 2.0). Of the 1,738 WRA enrolled in the project, 18% reported severe rash in the past 12 months. Ninety-nine women were pregnant at the time of the interview; 15.8% received information on preventing Zika infection during pregnancy. Of the WRA enrolled in the project, 1,504 participants provided a blood sample. One hundred and sixty five were IgM positive for recent Zika infection (165/1,504 = 11.0%). Seven were IgM positive for other flavivirus infection (7/1,504 = 0.5%). Although the official reports of Zika infections in Guatemala had waned considerably by late 2017, Zika infections among women of reproductive age were occurring in late 2017/early 2018. The majority of pregnant women, however, did not receive Zika prevention messaging from a healthcare provider. Countries endemic for Zika should remain vigilant in preventing Zika infection among pregnant women.

847

ASSESSMENT OF COMMUNITY SUPPORT FOR VECTOR CONTROL INTERVENTIONS IN A SOUTHERN PUERTO RICO COHORT

Emma M. Little[1], Liliana Sánchez-González[1], Laura E. Adams[1], Matthew Lozier[1], Brenda Torres-Velasquez[1], Marianyoly Ortiz[2], Grayson Brown[2], Angela F. Harris[1], Roberto Barrera[1], Ryan Hemme[1], Carmen Pérez[1], Steve Waterman[1], Vanessa Rivera-Amill[3], Gabriela Paz-Bailey[1]

[1]*Division of Vector-borne Diseases, Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*Puerto Rico Vector Control Unit, San Juan, PR, United States,* [3]*Ponce Health Sciences University and Saint Luke's Episcopal Hospital Consortium, Ponce, PR, United States*

Public support is vital to the successful implementation and sustainability of vector control interventions (VCI). Identifying factors that influence acceptance of VCI is necessary to maximize public support and inform community education campaigns. Communities Organized to Prevent Arboviruses (COPA) is a cohort study in communities with historically high rates of arboviral disease in Ponce, Puerto Rico. During 2018-2019, half (N=1,215) of adult participants were interviewed in their homes on their opinions about six VCI, including if they had heard of them and whether they would support or oppose implementation in their community. VCI included traditional (larviciding and indoor residual spraying [IRS]) and novel (autocidal gravid ovitraps [AGOs], genetically modified [GMO] mosquitoes, *Wolbachia* suppression, and *Wolbachia* replacement) methods. Binomial logistic regression with the log link was used to compute prevalence ratios (PR) with 95% confidence intervals (CI) to identify associations between support for VCI and participant demographics, practices, knowledge, and opinions on mosquitos and breeding site prevention. Overall, 51% of respondents had heard of larviciding, 35% of IRS, 26% of AGOs, 16% of GMO mosquitoes, and 5% of *Wolbachia*. Support for traditional VCI methods was high; 85% supported IRS and 90% supported larviciding. Support for AGOs was high (97%), but support for other novel methods was lower: GMO mosquitos (62%), *Wolbachia* replacement (64%), and *Wolbachia* suppression (68%). Older participants tended to be less likely to support novel VCI. Participants aged 41-50 years were less likely to support *Wolbachia* suppression (PR 0.90; 95% CI 0.82-0.99) and *Wolbachia* replacement (PR 0.80; 95% CI 0.70-0.93) than those aged 21-30 years. Greater support for *Wolbachia* suppression was found among participants that indicated arboviruses were a problem in the community (PR 1.09; 95% CI 1.001-1.19) as well as those who used store bought insecticide (PR 1.13; 95% CI 1.04-1.23). Ongoing community engagement and education efforts targeted toward specific age groups is necessary to increase community acceptance of novel VCI.

848

THE EFFECTS OF INCREASED INOCULUM ON ORAL ROTAVIRUS VACCINE TAKE AND IMMUNOGENICITY AMONG INFANTS IN DHAKA, BANGLADESH

Benjamin Lee[1], Dorothy Dickson[1], Masud Alam[2], Sajia Afreen[2], Abdul Kader[2], Faria Afrin[2], Tania Ferdousi[2], Christina Damon[1], Soyeon Kim[1], Monica McNeal[3], Daniel Bak[1], Mona Tolba[1], Marya Carmolli[1], Mami Taniuchi[4], Rashidul Haque[2], Beth Kirkpatrick[1]

[1]*University of Vermont, Burlington, VT, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States,* [4]*University of Virginia, Charlottesville, VA, United States*

Oral rotavirus (RV) vaccines have reduced immunogenicity in low-income countries, where interventions to improve vaccine performance remain elusive. Increasing vaccine inoculum might improve Rotarix vaccine immunogenicity, but this has not been formally evaluated in a low-income setting. Therefore, we performed a double-blind, randomized controlled trial among infants in Dhaka, Bangladesh randomized 1:1 to standard-(10^6 FFU) or high-dose ($10^{6.3}$ FFU) Rotarix at 6 and 10 weeks of age. Plasma RV-specific IgA (RV-IgA) was measured by EIA. Seroconversion was defined as pre-immunization RV-IgA <20 U/mL converting to seropositive (≥20 U/mL) 4 weeks post-immunization. Stool was collected 3, 7, and 14 days after each dose to detect vaccine shedding, defined as real-time qRT-PCR detection with sequence confirmation of vaccine-strain virus in any post-vaccination specimen. The primary outcome was vaccine take, defined as either seroconversion or vaccine shedding. PCR-positive specimens were also tested by stool EIA. Secretor status was determined by saliva EIA. 189 children completed the study per-protocol (97 standard-dose, 92 high-dose). Seroconversion was 49% vs 51% in the standard- vs high-dose arms (P=0.6). Post-vaccination RV-IgA geometric mean concentration was 22.6 U/mL vs 30.4 U/mL in the standard- vs high-dose arms (P=0.2) and 76.8 U/mL vs 107.9 U/mL, respectively (P=0.3), among seropositive infants. Vaccine shedding was 61% vs 55% in the standard- vs high-dose arms (P=0.4). Vaccine take was 70% vs 66% in the standard- vs high-dose arms (P=0.6). More children in the high-dose arm were non-secretors (40.2%); secretor status did not affect the primary outcome, but secretor-positive phenotype was associated with increased seroconversion (P=0.03) and vaccine take when shedding was determined using EIA-positive stools (P=0.01). Vaccination with an increased inoculum of Rotarix at 6 and 10 weeks of life did not increase seroconversion, vaccine shedding, or vaccine take among infants in Bangladesh. Larger dose increases may be effective, but limited by cost-effectiveness. Secretor status appeared to affect vaccine response.

849

DEVELOPMENT OF LASSA VIRUS GLYCOPROTEIN IMMUNOASSAY TO AID IN LASSA FEVER SURVEILLANCE AND VACCINE DEVELOPMENT

Duane Bush[1], Diana Nelson[1], Irina Aimukanova[1], Megan Rowland[2], Megan Heinrich[2], Kathryn Hastie[3], Mambu Momoh[4], Francis Baimba[4], Eghosa Uyigue[5], Adeyemi Kayode[6], John Aiyepada[5], Benevolence Ebo[5], Testimony Olumade[6], Patience Akhilomen[5], Grace Okonofua[5], Michael Airende[5], Blessing Osiemi[5], Ekene Muoebonam[5], Ikponmwosa Odia[5], Augustine Goba[7], Onikepe Folarin[6], Erica Ollmann Saphire[3], Luis Branco[2], Donald Grant[8], Christian Happi[6], John Schieffelin[9], Matthew Boisen[1], Robert Garry[9]

[1]*Zalgen Labs LLC, Aurora, CO, United States,* [2]*Zalgen Labs LLC, Germantown, MD, United States,* [3]*La Jolla Institute for Immunology, La Jolla, CA, United States,* [4]*Kenema Government Hospital, Kenema, Sierra Leone,* [5]*Irrua Specialist Teaching Hospital, Irrua, Nigeria,* [6]*Redeemers*

University, Ede, Nigeria, [7]Kenema Government Hospital, Kenema, Nigeria, [8]Ministry of Health and Sanitation, Freetown, Sierra Leone, [9]Tulane University, New Orleans, LA, United States

Lassa fever (LF) is a severe, often fatal, febrile disease endemic to West Africa. The etiologic agent is Lassa virus (LASV; family Arenaviridae) encodes several viral proteins including a membrane glycoprotein (GP) that mediates entry into host cells. The Lassa GP undergoes post-fusion conformational changes to complete cell invasion. Efforts to develop effective Lassa fever vaccines have focused on eliciting Lassa GP immune responses however these vaccine candidates have not addressed conformational stability in the prefusion state that the host must recognize for successful viral neutralization and protection. Recently point mutations have been optimized that successfully stabilize the Lassa GP in the prefusion conformation. Zalgen Labs and collaborators from the Viral Hemorrhagic Fever Consortium have utilized these stabilized prefusion GP antigens to develop the ReLASV Pan-Lassa Prefusion GP IgG/IgM ELISA to aid in the development of viable LF vaccine candidates and improve LF surveillance. The GP ELISA incorporates three recombinant prefusion GP representing LASV lineage II, III, and IV into a stabilized microwell coating for the capture of Lassa specific IgG or IgM. A Lassa GP specific human monoclonal antibody is included as an IgG Calibrator to provide a semi-quantitative estimate of anti-Lassa GP antibody in the clinical sample. Limit of detection of the IgG Calibrator is 50ng/mL. This assay was field tested during the 2018 and 2019 LF outbreaks in Nigeria and found Lassa specific IgG seroprevalence to be 22% and 25%, respectively. Long-term follow-up of Lassa survivors has demonstrated IgG reactivity at endpoint dilutions between 1:400 and 1:12,800. These results were confirmed 97.4% (38/39) positive agreement with a Lassa GP pseudovirus neutralization assays. The ReLASV Pan-Lassa Prefusion GP IgG/IgM ELISA has proven capable of detecting Lassa GP specific IgG and IgM in early convalescent LF patients and long-term LF survivors. The assays utility in LASV vaccine development and LF surveillance will provide a valuable medical countermeasure for countries affected by LF.

850

LASSA FEVER SEROPREVALENCE IN PLATEAU STATE, NIGERIA

Rashidat Adeyemi[1], **Matthew Boisen**[2], Elijah E. Ella[1], Maryam Aminu[1], Benevolence Ebo[3], John Aiyepada[3], Testimony Olumade[4], Olusola Ogunsanya[5], MacDonald Onyechi[6], Johnson Etafo[6], Matthew Afam Eke[7], Philomena Eromon[4], Andrew Hoffmann[8], Brandon Beddingfield[8], Onikepe Folarin[4], Simji Gomerep[9], Robert Garry[8], Christian Happi[4]

[1]*Ahmadu Bello University, Zaria, Nigeria, [2]Zalgen Labs LLC, Aurora, CO, United States, [3]Irrua Specialist Teaching Hospital, Irrua, Nigeria, [4]Redeemers University, Ede, Nigeria, [5]University of Ibadan, Ibadan, Nigeria, [6]FMC Owo, Owo, Nigeria, [7]FMC Abakaliki, Abakaliki, Nigeria, [8]Tulane University, New Orleans, LA, United States, [9]Jos University Teaching Hospital, Jos, Nigeria*

Lassa fever (LF) is a severe, often fatal, febrile disease endemic to West Africa. Nigeria has experience significant increases of confirmed LF in 2018 and 2019. While endemic in Nigeria, the majority of the LF cases has occurred in the southern states of Ondo, Edo, and Ebonyi. In January 2019, we conducted a LF seroprevalence study in a cohort of febrile patients admitted to two hospitals in Jos, Plateau State. While not a high transmission area, these febrile patients did satisfy the case definition of LF. Collected samples (n=278) were taken to the Africa Centre Of Excellence for Genomics of Infectious Diseases (ACEGID) laboratories at Redeemers University, Ede, Osun State, for screening of Lassa-specific IgG and IgM. Serum samples were tested using ReLASV Pan-Lassa Antigen ELISA and IgG/IgM ELISAs specific for nucleoprotein (NP) or glycoprotein (GP). Samples exhibiting antigen reactivity were also tested by ReLASV RDT and RealStar Lassa Virus RT-PCR Kit 2.0 (Altona Diagnostics GmbH, Germany). Samples exhibiting IgG or IgM reactivity were tested for endpoint titers against LASV lineage specific antigens to assess strain specificity. LASV GP pseudovirus neutralization was performed to confirm Lassa-specific antibody reactivity. One sample was positive by ReLASV Antigen ELISA and RDT (IgG, IgM ELISA negative) but was not confirmed by Altona 2.0 qPCR. Lassa NP-specific IgG seroprevalence was 6.1% (17/278; 95th% CI 3.6 - 9.6%) and GP-specific IgG seroprevalence was 20.5% (57/278; 95th% CI 15.9-25.7). Endpoint titer testing exhibited cross-reactivity toward antigen from LASV Lineage II, III, and IV with endpoint typically in the range of 1:1600 to 1:6400. Lassa-specific antibody reactivity was confirmed in a small subset of samples by LASV pseudovirus neutralization but further confirmation testing is needed using this reference method. The Lassa antigen cross-reactivity observed in this study group is consistent with findings in southern Nigeria and could facilitate an integrated LF surveillance program utilizing standardized LF diagnostics.

851

RESPIRATORY AND FEBRILE ILLNESSES IN CHILDREN DUE TO HUMAN PARAINFLUENZA VIRUS TYPE 4 (HPIV4) AND HUMAN CORONAVIRUS (HCOV) OC43 IN DHAKA, BANGLADESH

Mohammed Ziaur Rahman[1], Md. Muzahidul Islam[1], Md. Shaheen Alam[1], Mariya Kibtiya Sumiya[1], Doli Rani Goswami[1], Mustafizur Rahman[1], W. Abdullah Brooks[2]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Human parainfluenza virus (HPIV) and human coronavirus (HCoV) are globally recognized viral pathogens causing more than 15% of the overall respiratory tract infections. In Bangladesh, respiratory viral studies were mostly linked to the epidemiological and clinical impact of Influenza, RSV, HPIV-1, 2, 3, hMPV and adenovirus. However, information regarding HPIV4 or HCoV infections, its specific clinical syndromes, age distribution, seasonal patterns and the extent of genetic variation among the strains are limited. A total of 200 samples (20%) were randomly selected out of 1022 archived nasopharyngeal wash (NPW) specimens that were tested negative for a panel of viruses (Influenza, RSV, HPIV-1, 2, 3, hMPV, adenovirus). These specimens were collected under an urban rural surveillance for pneumonia, diarrhea and febrile illness among children under five years old in between 2014 and 2015. In order to detect novel paramyxoviruses, semi-nested RT-PCR was performed to amplify large polymerase (L) gene fragment and for detection of HCoV, samples were also subjected to RdRp (RNA Dependant RNA Polymease) gene nested RT-PCR followed by Sanger sequencing. Upon PCR based direct nucleotide sequencing, 10 samples were positive for either HPIV4 (n=4) or HCoV (n=6). The HPIV4 strains were phylogenetically belonged to HPIV4a (n=3) and HPIV4b (n=1) having sequence similarity of >98% with the other globally circulating strains. The sequence analysis of the RdRp gene fragment revealed that all the 6 samples that were positive for HCoV belonged to HCoV-OC43 with the sequences similarity of >99%. This study results may help to identify the contribution of HPIV4 and HCoV, causing respiratory and febrile illness in children and their circulating pattern among Bangladeshi children.

852

INCIDENCE OF FEBRILE ILLNESS EPISODES AND ASSOCIATED OUT-OF-POCKET COSTS AND SCHOOL AND WORK ABSENCES REPORTED IN A SOUTHERN PUERTO RICO COHORT

Liliana Sanchez-Gonzalez[1], Dania Rodriguez[1], Emma Little[1], Robert Rodriguez[2], Nicole Medina-Lopes[1], Olga Lorenzi[1], Janice Perez-Padilla[1], Laura E. Adams[1], Stephen H. Waterman[1], Luisa I. Alvarado[2], Vanessa Rivera-Amill[2], Gabriela Paz-Bailey[1]

[1]*Centers for Disease Control and Prevention - Dengue Branch, San Juan, PR, United States, [2]Ponce Health Sciences University and Saint Luke's Episcopal Hospital Consortium, Ponce, Puerto Rico*

Fever is one of the most common reasons for seeking health care. Delay in health care can occur when out-of-pocket and time costs are high. Communities Organized for the Prevention of Arboviruses (COPA) is a community-based cohort study in southern Puerto Rico that measures the incidence of arboviral diseases through annual serosurveys using serologic

and virologic testing. Participants aged 1-50 years (y) were recruited in 2018-2019 from randomly selected households. We administered a questionnaire to assess febrile illness, and out-of-pocket expenses. We present demographic characteristics, and time and out-of-pocket costs for persons reporting fever in the previous year. Among 3,644 participants, 15% were positive for Zika IgM, 0.4% for dengue IgM, 0.6% for chikungunya IgM, and 31% were chikungunya IgG positive. Overall, 987 (27%) reported at least one episode of fever during the last year. Most participants (60%) were female and the median age was 26y (interquartile range 17-40). Participants 31-40y and 41-50y were less likely to report fever (23% and 24% respectively, $p<0.005$) when compared with those 1-10y (32%). Reports among participants 11-20y (30%) and 21-30y (30%) were similar to those 1-10y. More than half reported seeing a doctor (58%). Viral syndrome was the most common reported diagnosis (14%) followed by influenza (7%). Reports of dengue, Zika and chikungunya diagnoses were rare (4 each). Among participants who reported fever, missed days of work among those employed were reported by 55% (207/373), median of 3 (range 0.5-80) days missed. Among students, missed days of school were reported by 45% (175/388), median of 3 (range 1-21) days missed. Most (70%) reported out-of-pocket expenses. The median out-of-pocket expense was $20 (range $1-$4,000). Episodes of febrile illness were common in this population, and resulted in a median of 3 days of school or work missed. The economic burden of disease will likely increase in areas endemic for arboviral diseases during outbreaks. Effective control and prevention strategies are needed to decrease common causes of fever such as arboviral diseases and its associated economic impact.

853

COLD BLOOD: REPTILES AND AMPHIBIANS AS RESERVOIR AND OVERWINTERING HOSTS FOR ARBOVIRUSES

Izabela K. Ragan, Airn Hartwig, RIchard A. Bowen

Colorado State University, Fort Collins, CO, United States

Arthropod-borne pathogens continue to have a significant threat to animal and human health. A majority of these pathogens are maintained in enzootic cycles where the pathogen cycles between an animal reservoir and hematophagous arthropods. Animal reservoirs like ectothermic vertebrates are ubiquitous in the environment and may be important in the maintenance and overwintering of viral pathogens. Viremia in ectothermic vertebrates has been demonstrated for emerging arthropod-borne viruses (arboviruses) like Zika, Chikungunya and West Nile virus. However, there is a limited understanding of the viral pathogenesis in ectothermic vertebrates under various environmental conditions. Our objective is to better understand the role of amphibians and reptiles in the maintenance, overwintering and amplification of arboviruses. We initially screened a diverse range of arboviruses (Zika, Dengue, Mayaro, Yellow fever, Venezuelan equine encephalitis, and Rift Valley fever virus) for infectivity and viremia in four genuses of ectothermic vertebrates (frogs, toads, snakes, iguanas). Our results show that infectious virus was detected in blood from frogs (*Lithobates)* infected with Zika virus and from toads (*Rhinella)* and iguanas (*Iguana)* infected with Venezuela equine encephalitis virus; however, the magnitude of viremia was low. Additionally, antibodies were detected in sera against Yellow fever virus in frogs and against Zika virus in iguanas. The screening of snakes (*Thamnophis)* are ongoing. After the screening, selected arbovirus-ectotherm host models will then investigate the influence of temperature changes on pathogenesis. Lastly, an artificial ecosystem will evaluate arbovirus transmission cycles among ectothermic vertebrate hosts and arthropods (mosquitos). The knowledge gained from these studies will significantly enhance our understanding of ectothermic vertebrates as reservoir hosts for several arboviruses with significant public health consequences. This in turn will aid in developing global measures to reduce or eliminate the disease risk associated with these pathogens.

854

PLACENTAL TROPHOBLASTS AND GIANT CELLS ARE TARGETED FOR INFECTION IN A RODENT MODEL OF CONGENITAL RIFT VALLEY FEVER

Devin A. Boyles, Cynthia M. McMillen, Amy L. Hartman

University of Pittsburgh, Pittsburgh, PA, United States

Rift Valley fever virus (RVFV) is endemic to Africa and infects livestock via mosquito bite. Severe cases in animals can lead to fatal hepatic necrosis and "abortion storms" that result in the loss of up to 90-100% of pregnancies. Human infection occurs from contact with livestock and manifests as a febrile illness that can advance to hemorrhagic fever, liver disease, and encephalitic disease. Two published cases of human vertical transmission resulted in fetal viremia and death. The first established rodent model of vertical transmission observed that RVFV was able to bypass the antiviral protections of the reproductive system and infect the offspring. Significant damage was observed in the maternal decidua and placental trophoblasts. Even asymptomatic dams that survived to give birth produced stillborn pups with severe abnormalities. Little is known about the mechanism of viral infection in the placenta and the resulting fetal pathology. We sought to further characterize the cellular and molecular mechanisms of congenital RVF. Pregnant Sprague-Dawley rats were inoculated at embryonic day 14. Maternal and fetal tissues were processed for RNA *in situ* hybridization of RVFV RNA and immunohistochemical (IHC) staining of immune cell markers in order to identify inflammatory cell infiltrates and resulting pathologies of infected rat tissues compared to that of pregnant uninfected controls. RVFV-infected rats showed signs of liver and placental inflammation and necrosis and contained high viral titers; however, tissues from survivors displayed evidence of tissue repair. Infected pregnant rats were more susceptible to tissue pathologies than non-pregnant rats. Infection of trophoblasts, giant cells, and infiltration of neutrophils were identified by using IHC. RVFV was found in the brains and peritoneal cavities of pups from dams that succumbed while pups from survivors exhibited fetal resorption or extreme deformities. This study gives insight into RVFV cellular targets during pregnancy which can be further investigated for development of antiviral therapies.

855

TECOVIRIMAT, A NEW FDA-APPROVED DRUG FOR TREATMENT OF HUMAN SMALLPOX - SUPPORTING PRECLINICAL DATA

Peter M. Silvera[1], Aklile Berhanu[2], Jonathan Prigge[3], Kady Honeychurch[2], Doug Grosenbach[2], Dennis Hruby[2]

[1]*Advanced Bioscience Laboratories, Rockville, MD, United States,* [2]*SIGA Technologies, Inc., Corvallis, OR, United States,* [3]*US Army Joint Program Office, Frederick, MD, United States*

The etiological agent for human smallpox is variola virus (VARV), a highly infectious virus with an estimated case mortality rate of 30%. In 1980, the WHO declared eradication of smallpox - this resulted in a cessation of routine vaccinations in the US during the 1970s. Consequently, a large proportion of the population remains unprotected in the event of a potential release of VARV as an act of biowarfare or bioterrorism. To address this biosecurity concern, the US government has procured smallpox vaccines (ACAM2000 and IMVAMUNE) into the Strategic National Stockpile. Tecovirimat (registered trade name, TPOXX®), a small molecule with antiviral activity against orthopoxviruses was developed by SIGA Technologies as a post-exposure therapeutic. Here, we present a snapshot of preclinical data that supported advance development of TPOXX® utilizing the macaque monkeypox virus (MPXV) model. Experiments were designed to simulate a potential exposure scenario in a real-life situation. Thirty-two cynomolgus macaques were randomized into four treatment groups and exposed to a lethal intravenous challenge ($5x10^7$ PFU) with MPXV, Zaire strain 79 on study day 0. On study day 3 post-infection, groups of macaques were either mock-vaccinated or vaccinated with ACAM2000 via percutaneous scarification. Oral treatment with 10 mg/kg TPOXX® or placebo was initiated on study days 3, 4, 5 or

6 post-infection and continued for 14 consecutive days. Subsequently, all animals were monitored for survival, clinical signs of disease, fever, weight loss, MPX viremia, formation of pock lesions, and development of humoral and cell-mediated immune responses. Results demonstrated that post-exposure administration of ACAM2000 alone failed to provide protection against severe MPXV disease or mortality. In contrast, post-exposure treatment with TPOXX® alone or in combination with ACAM2000 provided 100% protection. Additionally, delaying TPOXX® treatment until day 4, 5, or 6 post-infection provided 83% (days 4 and 5) and 50% (day 6) protection. These data supported the New Drug Application and subsequent approval (2018) of TPOXX® for the treatment of human smallpox.

856

EXPERIMENTAL INFECTION OF JAMAICAN FRUIT BATS (*ARTIBEUS JAMAICENSIS*) WITH BUKAKATA ORBIVIRUS, A NOVEL VIRUS FROM A UGANDAN BAT

Anna C. Fagre[1], Alex Byas[1], Ashley Malmlov[2], Nicholas Bergren[1], Erin M. Borland[1], Lauren Rice[3], Tony Schountz[1], Rebekah Kading[1]

[1]*Colorado State University, Fort Collins, CO, United States,* [2]*Colorado Parks and Wildlife, Fort Collins, CO, United States,* [3]*University of Colorado School of Medicine, Aurora, CO, United States*

In 2013, a novel orbivirus (*Reoviridae: Orbivirus*) was isolated from an Egyptian fruit bat (*Rousettus aegyptiacus*) in Uganda. To further study the effects of infection with Bukakata orbivirus in a bat host, 9 male and 5 female captive Jamaican fruit bats (*Artibeus jamaicensis*) bats were inoculated intraperitoneally with 5.3 $\log_{10}$ pfu Bukakata orbivirus and monitored daily for signs of clinical disease. One male and 1 female bat were humanely euthanized and tissues collected for processing at day 0, and the same was done for two males and one female bat at days 2, 5, 10, and 15. Organs were collected and processed for qRT-PCR, quantification of viral load in multiple organs by plaque assay, histopathology, and immunohistochemistry. Blood, rectal swabs, and oral swabs were also collected to assess viremia profiles and shedding potential. Gross pathologic findings included hyperemia in the skin, pulmonary congestion, liver pallor, and injected vasculature on serosal surfaces of abdominal organs. Histopathologic lesions included vasculitis, hemorrhage, and vacuolar changes in multiple organ systems. Further, immunohistochemistry supports antigen localization in areas of inflammation and hemorrhage. The female bat euthanized at day 15 was discovered to be late in gestation, and the fetus was collected for processing following approval by the CSU Institutional Animal Care and Use Committee. Serology is being performed to confirm seroconversion, and qRT-PCR and viral titration (PFU/mL) are being performed to assess relative abundance of virus in various organ systems. This model provides an opportunity to examine pathology induced by an arbovirus in bats, and these data are critical in the assessment of the potential role of bats as reservoirs for arboviruses.

857

A RECOMBINANT SUBUNIT LASSA VIRUS VACCINE ELICITS A STRONG ANTIBODY AND CELL-MEDIATED RESPONSE

Albert To

University of Hawaii at Ma¯noa, Honolulu, HI, United States

Lassa virus (LASV) is classified as a Category A Priority Pathogen by the NIAID and NIH due to its high mortality rate, ease of dissemination, and for lack of preventive countermeasures. The accretion of LASV infections in Nigeria eariler this year is a clear characteristic of an emerging pathogen. Due to the epidemic potential and high case fatality rate, the WHO has declared the development of a LASV vaccine as a high priority for preventative use in endemic regions. The recombinant subunit vaccine platform offers a safe, non-replicating alternative to live-attenuated and chimeric vaccine candidates. In this study, we generated recombinant LASV glycoprotein (GP) using an insect cell expression system and tested the immunogenicity of an adjuvanted antigen in an outbred mouse model. Briefly, stably transformed *Drosophila* S2 cell line excreting LASV GP was scaled up in a multi-liter bioreactor and the clarified culture supernatant containing soluble GP was purified using a single-step immunoaffinity chromatography (IAC) purification method. Coomassie blue staining of SDS-PAGE gels showed that IAC using a GP1 specific-antibody yielded protein with a high level of purity. The eluted LASV GP was composed of GP1 and GP2 subunits, the GP1-2 heterodimer, as well as trace amounts of oligomer on a Western blot. Swiss Webster mice immunized intramuscularly with 3 doses of purified recombinant LASV GP formulated with an adjuvant showed a maximal, GP-specific antibody response after two immunizations. Low neutralizing antibody titers, and a robust cell-mediated response was observed after the 3rd dose. We have successfully developed an immunoaffinity-based method to produce highly purified, insect-cell expressed recombinant LASV GP capable of eliciting high antibody titers when formulated with a suitable adjuvant after only two doses. These results indicate the potential for recombinant GP to be an efficacious vaccine candidate. Further structural enhancments and a in-detail characterization of antibody and cell-mediated responses induced by this recombinant subunit LASV candidate vaccine are the next steps towards preclinical development.

858

IMMUNOGENICITY AND EFFICACY OF RECOMBINANT SUBUNIT FILOVIRUS VACCINES IN NON-HUMAN PRIMATES AND DEVELOPMENT OF THERMOSTABLE FORMULATIONS

Axel T. Lehrer[1], Michael M. Lieberman[1], Teri Ann S. Wong[1], Chih-Yun Lai[1], Eleanore Chuang[1], Oreola Donini[2], Kendall Neuberger[3], Theodore W. Randolph[3], Thomas W. Geisbert[4]

[1]*University of Hawaii, Honolulu, HI, United States,* [2]*Soligenix, Inc., Princeton, NJ, United States,* [3]*University of Colorado Boulder, Boulder, CO, United States,* [4]*University of Texas Medical Branch, Galveston, TX, United States*

Ebola (EBOV), Marburg (MARV) and Sudan (SUDV) viruses are three filoviruses that have caused the most fatal cases in humans. Transmission from animals into the human population typically causes outbreaks of limited scale in endemic regions. In contrast, the 2013-16 outbreak in several West African countries claimed more than 11,000 lives revealing the true epidemic potential of filoviruses. This is further emphasized by the continuing difficulty in controlling the largest ever outbreak of EBOV in the DRC. Despite significant progress with the clinical development of several EBOV vaccine candidates and therapeutics during and after the West African outbreak, no vaccines targeting EBOV have received regulatory approval. Moreover, protection of a monovalent EBOV vaccine against other filoviruses has never been demonstrated in primate challenge studies. We are developing a trivalent vaccine based on recombinant filovirus glycoproteins (GP) from EBOV, MARV and SUDV produced using the *Drosophila* S2 platform. The highly purified recombinant subunits elicit potent immune responses in mice, guinea pigs and non-human primates (NHPs) and consistently produce high antigen-specific IgG and virus neutralizing antibody titers. Candidate vaccines show full protection against EBOV infection in rodent and NHP challenge models. Similarly formulated monovalent MARV or SUDV vaccine candidates can protect cynomolgus macaques completely against infection with lethal doses of MARV or SUDV and combinations with the EBOV vaccine can be formulated yielding multivalent vaccines retaining efficacy. Ongoing formulation optimization in our laboratory focuses on thermostabilization of recombinant subunits by lyophilization. Current data suggest that shelf stability of at least three months at 40°C is feasible for each of the three antigens individually. Most importantly, formulations of antigens lyophilized in the presence of adjuvant are also stable, which should enable clinical development of safe and efficacious, field-deployable vaccine candidates for protection against Ebola, Marburg and Sudan Virus Disease.

859

HOST GENES ARE DIFFERENTIALLY EXPRESSED IN DENGUE COMPARED TO INFLUENZA INFECTIONS

L. Gayani Tillekeratne[1], Sunil Suchindran[1], Emily R. Ko[1], Elizabeth A. Petzold[1], Champica K. Bodinayake[2], Ajith Nagahawatte[2], Vasantha Devasiri[2], Ruvini Kurukulasooriya[2], Megan E. Reller[1], Bradly P. Nicholson[1], Micah T. McClain[1], Thomas Burke[1], Ephraim L. Tsalik[1], Ricardo Henao[1], Geoffrey S. Ginsburg[1], Christopher W. Woods[1]

[1]*Duke University, Durham, NC, United States,* [2]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka*

The host response to viral infections such as dengue and influenza is largely characterized by interferon-related pathways. Identifying other gene sets that are variably expressed in specific viral infections may be useful in the development of diagnostics and therapeutics. We searched for genes that may be differentially expressed in patients with dengue compared to influenza, the most common viral infections resulting in hospitalization in southern Sri Lanka. We enrolled patients admitted with acute febrile illness in Sri Lanka from July 2012- May 2013 and collected nasopharyngeal samples, serum, and blood in PAXgene RNA tubes. We confirmed influenza using multiplex polymerase chain reaction (PCR; Luminex NxTAG) and dengue using a combination of virologic (PCR and virus isolation) and serologic techniques. Total RNA was extracted from peripheral blood and RNAseq was performed from samples confirmed as acute dengue or influenza. We aligned reads to a reference transcriptome (hg38) using Bowtie2, quantified at the isoform level using Express version 1.5.1, and normalized using trimmed-mean normalization. The voom method, an empirical Bayes method designed for RNA-sequencing data, was used to test for differential expression. Sequencing batch was entered as a covariate and the Benjamini-Hochberg method was used to control for multiple comparisons. Among 39 patients confirmed to have dengue and 43 patients confirmed to have influenza, the Enrichr gene-set-analysis tool was used to characterize pathway enrichment. Overall, 447 transcripts achieved a false discovery rate (FDR) less than 1%. Genes associated with T-cell chemotaxis, apoptotic cell clearance, cellular triglyceride homeostasis, and natural killer cell activation and chemotaxis were enriched among genes considered differentially expressed (adjusted p-value enrichment <0.05). We identified genes that were differentially expressed among patients infected with dengue and influenza. Further studies are required to determine if such differences may translate to diagnostics or therapeutics for these viral illnesses.

860

PLUG AND DISPLAY VIRUS-LIKE-PARTICLE VACCINES FOR OUTBREAK PATHOGENS

Iona J. Taylor[1], Yu Zhou[1], Yuanyuan Li[1], Cheryl Lee Yi-Pin[2], Siti Naqiah Amrun[2], Olga Dolnik[3], Arianna Marini[1], Stephan Becker[3], Lisa F. P. Ng[2], Sumi Biswas[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore,* [3]*Institute of Virology, Philipps University of Marburg, Marburg, Germany*

A fast and effective response to emerging and outbreak diseases requires a vaccine platform that is adaptable, highly immunogenic, rapidly produced and has a previously demonstrated safety profile. The SpyCatcher/SpyTag biochemical superglue technology enables the rapid and efficient development of highly immunogenic virus like particle (VLP) vaccines against target pathogens. VLPs are a platform technology that is used to produce vaccines against many different diseases. These technologies are particularly suited for the induction of strong antibody responses, which are achieved through presentation of an ordered antigen array to the immune system. The SpyCatcher/SpyTag technology has been used to establish a novel chimeric VLP vaccine platform technology for rapidly and irreversibly decorating VLPs simply by mixing with protein antigen in a "Plug-and-Display" manner. This approach overcomes the well-described challenges of producing VLP carriers with complex antigens genetically-fused on their surface, or low conjugation efficiency often reported when using chemical conjugation. The SpyTag-SpyCatcher technology has been used previously to enhance humoral immunogenicity by displaying malaria antigens on VLPs. The application of this technology for the development of vaccines against Ebola and Zika viruses will be discussed. Immunogenicity and neutralisation data from mice immunised with glycoproteins from both viruses will be presented along with further investigation into the protective efficacy of the Zika virus vaccine using a murine challenge model. Examination into the dose sparing effect of the VLP displayed antigens using both viruses further demonstrates the versatility and applicability of the platform to generate vaccines against new epidemics and emerging infections.

861

HEPATITIS A & E AMONG HOSPITALIZED PATIENTS: DISEASE MAPPING FROM A NATIONWIDE SURVEILLANCE IN BANGLADESH

Md. Taufiqul Islam

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are transmitted by the fecal-oral route and are responsible for epidemic and sporadic outbreaks of acute hepatitis in low-income countries like Bangladesh. The purpose of this study was to determine the burden of acute hepatitis due to HAV and HEV in different districts of Bangladesh. The nationwide foodborne illness surveillance started in 2014 at 10 different hospitals which covered seven divisions of Bangladesh. Blood samples of acute hepatitis cases were collected and transported to the central laboratory twice a week. Cases were screened for the presence of IgM specific to HAV and HEV using enzyme-linked immunosorbent assay (ELISA). Multivariate logistic regression was performed to assess the association between HAV and HEV infection with different risk factors. A total of 1016 patients were enrolled during the study period where 998 samples were tested for both HAV and HEV. Among the tested samples, 19% (191/998) were identified as HAV positive and 10% (103/998) were HEV positive. The median age was 12 years and 25 years for HAV and HEV positive patients, respectively. The prevalence of HAV was higher among the females (24.9%), whereas HEV was higher among males (11.2%). The highest occurrence of HAV was observed among children while HEV was most prevalent in the 15-60 years age group (12.4%). Through our nationwide surveillance, it is evident that infection with enteric hepatitis viruses is common in Bangladesh. These data will be useful towards planning preventive and control measures by planning future vaccination strategies in Bangladesh.

862

A SEROLOGICAL ASSAY FOR DIFFERENTIATING RIFT VALLEY FEVER (RVF) NATURALLY INFECTED ANIMALS FROM ARMP12 NSM DEL VACCINATED ANIMALS

Linda Peniel Salekwa[1], Douglas Watts[2], George Bettinger[2], Pedro Palermo[2], Mirende Matiko[1], Philemon Wambura[1]

[1]*Sokoine University of Agriculture, Morogoro, United Republic of Tanzania,* [2]*University of Texas, Texas, TX, United States*

Rift Valley fever virus (RVFV) is the cause of Rift Valley fever (RVF), a significant public health and veterinary problem in Africa and the Arabian Peninsula. An effective vaccine is needed to prevent RVF in livestock, preferably a vaccine with a biomarker to distinguish naturally (virulent) infected from vaccinated animals (DIVA). Therefore, the goal of this study is to evaluate a live attenuated recombinant RVFV vaccine with deleted the non-structural nucleotides deleted from the M RNA segment (NSm) to serve as a biomarker to distinguish RVF vaccinated from animals infected with virulent RVFV. An indirect ELISA was developed using NSm protein as a capture antigen for detecting NSm-antibody in samples from naturally infected and for possible non-detection of NSm antibody in animals vaccinated with the NSm genes deleted RVF vaccine arMP-12ΔNSm21/384.

Sera samples from animals previously infected with virulent RVFV were obtained from Kenya and Tanzania, and from experimental challenge study in Canada. Also, samples from animals vaccinated with parent RVF MP-12 and the arMP-12ΔNSm21/384 vaccine were obtained from virology laboratory at Sokoine University of Agriculture. All sera from animals infected naturally with virulent RVFV or from the challenge study had a geometric mean optical density (OD) reading of 0.88, thus demonstrating that animals were infected with virus containing the NSm antigen induced a detectable antibody response to this antigen. In contrast, animals vaccinated with RVF arMP-12ΔNSm21/384had an OD reading of only 0.17, or negative for antibody, thus demonstrating that animals vaccinated with RVF arMP-12ΔNSm21/384 can be distinguished from animals infected with virulent RVFV.

863

ENTOMOLOGICAL INVESTIGATIONS AND LABORATORY DETECTION OF A RIFT VALLEY FEVER OUTBREAK IN HUMAN POPULATIONS IN OL KALAU SUB-COUNTY OF NYANDARUA COUNTY, KENYA, 2019

Samson Limbaso Konongoi[1], Allan Ole Kwallah[2], Kizito Lubano[3], Joel Lutomiah[1], Rosemary Sang[1]

[1]*Center for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya,* [2]*Production Department, Kenya Medical Research Institute, Nairobi, Kenya,* [3]*Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya*

Rift Valley fever (RVF) is an acute mosquito-borne viral disease affecting ruminants and humans. It's associated with negative economic impacts due to abortions in animals, high mortality in young animals and trade restrictions. In the last two decades, parts of East Africa have experienced RVF epidemics. The last major outbreak of RVF in Kenya occurred in 2006/2007. Since then there have been sporadic detections in selected localities and an outbreak in Northeastern Kenya in mid-2018. Between February and March 2019 there were reports of livestock abortions in Olkalau sub-county, Nyandarua County in Central Kenya. Suspected human cases associated with close contact to sick animals were reported in health facilities and blood samples sent to the KEMRI Viral hemorrhagic fever laboratory. Real-time PCR and immunoglobulin M enzyme linked immunosorbent assay (IgM ELISA) were used to detect acute cases and the presence of IgM antibodies against RVFV. Entomological investigations were done in areas with reported human cases. A total of 139 human samples were collected and tested.12.9 % (n=18) were positive for RVF by real-time PCR while 1 tested positive for RVF IgM antibodies. A total of 2,259 mosquitoes were collected and identified to 8 diverse species, predominated by *Culex theileri*, 40% (n=903), *Cx.zombaensis*, 20.2% (n=456), *Anopheles squamossus,* 16.6% (n=375), *Cx.pipiens,* 12.6% (n=285), *Cx.vansomereni,* 7.2% (n=163), *Aedes tricholabis,* 1.5% (n=34) and others 1.9 %(n=43). Laboratory analysis is ongoing to determine infectivity. This is the first documentation of human RVF cases in Nyandarua; occurring during the dry season. Initial investigations indicate that the outbreak was of lower magnitude and severity compared to previous outbreaks in Kenya with no human mortalities reported and was quickly contained. Preliminary findings indicate that none of the mosquito species sampled belonged to the group known as primary vectors of RVF, yet there was active RVF transmission. There is need to monitor spatial and temporal vector densities and virus activity and detect changes in the epidemiology of RVF occurrence in Kenya and the region.

864

PROTOCOL FOR THE SURVEILLANCE STAGE OF A COMMUNITY-BASED RSV (RESPIRATORY SYNCYTIAL VIRUS) MORTALITY STUDY IN KARACHI, PAKISTAN

Abdul Momin Kazi[1], Asad Ali[1], Nazia Ahsan[1], Waliyah Mughis[1], Saima Jamal[1], Beryl Guterman[2], Fauzia Aman Malik[2], Saad Bin Omer[2]

[1]*Aga Khan University, Karachi, Pakistan,* [2]*Emory University, Atlanta, GA, United States*

RSV is a respiratory pathogen with potentially moderate to high disease burden in LMICs - it is estimated to annually cause 34 million episodes. This pathogen is a potential target for maternal immunization strategies to prevent disease and early death in young infants. However, due to lack of evidence, role of RSV in early mortality in young infants and neonates in LMICs is not confirmed. Most current studies estimate burden of disease in terms of hospital-based deaths, there is a knowledge gap regarding proportion of community-based deaths due to RSV. Primary study objectives are to assess and analyze the burden and determinants of RSV mortality in infants in 4 low-income settlements of Karachi and to provide a cause of death consultation for the enrolled families of deceased neonates. This is an observational surveillance study, with primary outcome measures including a laboratory (rPT-PCR) confirmed RSV and pertussis infection report for nasopharyngeal swab sample collected from deceased infant. From August 2018 to March 2019, 80 specimens have been obtained (out of 324 criteria-eligible neonatal deaths, and 161 timely death alerts) from catchment areas of Ibrahim Hyderi, Ali Akbar Shah Goth, Bhains Colony & Rehri Goth in Karachi. Field team consists of nurses (trained in nasopharyngeal swab collection and grief support), field site supervisors and site-specific community health workers and mobilizers (who engage with the community and bereaved parents and offer grief support). Following a 3-month advocacy phase (exploring the acceptance of study procedures within communities, assessing benefits to families of deceased infants for enrolling), project is currently in its 9-month pilot surveillance phase, which is expected to be phased into a 12-month scale-up surveillance. There are many challenges in generating evidence for the burden of disease, specifically for RSV, such as lack of resources for hospital and community surveillance and diagnostics, and difficulty in obtaining specimens. Religious community acceptance and obtainment of *fatwas* or religious rulings for medical research have aided the successful specimens collection.

865

GRIEF SUPPORT TRAINING FOR COMMUNITY HEALTH WORKERS AND NURSES WORKING WITH BEREAVED PARENTS ENROLLED IN A COMMUNITY-BASED RSV MORTALITY STUDY IN KARACHI, PAKISTAN

Waliyah Mughis[1], Saima Jamal[1], Ayesha Mian[1], Nargis Asad[1], Fauzia Aman Malik[2], Saad Bin Omer[2], **Abdul Momin Kazi**[1]

[1]*Aga Khan University, Karachi, Pakistan,* [2]*Emory University, Atlanta, GA, United States*

A community-based study on burden of respiratory syncytial virus (RSV) on infant mortality is being conducted in 4 catchment areas of Karachi. The study purpose is to collect nasopharyngeal specimen from deceased infants under 6 months, in order to analyze burden of RSV as cause of neonatal deaths in Pakistan. Upon receiving a death alert, community health workers, mobilizers and nurses approach household to obtain parent consent to collect nasopharyngeal sample from deceased infant before bathing/funeral process. The field team is completing training in grief support skills, conducted by project psychologist. The health workers apply these skills in follow-up home visits with enrolled infants' parents, acting as referral pathway to psychologist, who conducts grief counseling for bereaved parents with complicated grief or mental health issues that require specialist intervention. The grief support training curriculum is broadly divided into modules on mental health first aid skills, communication skills, differentiating between complicated/uncomplicated

grief; covering listening skills, counseling strategies, screening for mental health issues, and utilizing appropriate social-emotional support to prevent the development of complicated grief. The staff are trained to conduct grief support home visits for bereaved parents; during these visits (at 1, 2, 4 and 8 weeks after infant's death), CHW/nurses enquire about the infant's caretakers about their mental health and their experience of grief surrounding infant's death. From August 2018 to March 2019, 80 specimens have been obtained and over 90 sets of parents supported and counseled in their grief. Grief from bereavement often presents as a clinical disorder such as depression, anxiety, OCD, PTSD. Training field staff (the first responders) to screen for issues that could lead to complicated grief is imperative. Community-based models of social support require further examination to understand how grief support can prevent the need for counseling for complicated grief to arise.

866

NIPAH VIRUS INFECTION IN 2018-19 NIPAH SEASON IN BANGLADESH

Arifa Nazneen[1], Mohmmed Ziaur Rahman[1], Sharmin Sultana[2], Syed Moinuddin Satter[1], John D. Klena[3], Nichol T. Sturat[3], Stephen P. Luby[4], Emily S. Gurley[5], Meerjady Sabrina Flora[2], Mahmudur Rahman[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Institute of Epidemiology Disease Control & Research, Bangladesh (IEDCR), Dhaka, Bangladesh,* [3]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [4]*Stanford University, Stanford, CA, United States,* [5]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Nipah virus (NiV) has been identified as one of the most dangerous emerging pathogens by the World Health Organization, due to infections resulting in high case fatality and person-to-person transmission potential. A hospital-based Nipah encephalitis surveillance began in 2006 and event-based surveillance in 2008 in Bangladesh, identifying Nipah outbreaks and sporadic cases every year. Presently icddr,b in collaboration with Government of Bangladesh is conducting active surveillance at five medical college hospitals (Rajshahi, Rangpur, Faridpur, Chattogram, and Khulna). Through the surveillance, 521 suspected encephalitis cases were enrolled during the 2018-19 Nipah season (December to March). We collected blood and throat swab samples from the enrolled cases, tested for PCR (serum and throat swab) and serology (serum). Four laboratory confirmed Nipah (both Nipah PCR and IgM positive) cases were identified, three from Rajshahi and one from Rangpur. The Rangpur Nipah case which was detected by our surveillance was the final case in an outbreak consisting of five fatal cases. Two of the Rangpur cases were missed by our surveillance staff, as the cases were admitted to the facility in the evening after surveillance staff had gone home. These cases had fever and headache, and their condition deteriorated rapidly and they died shortly after hospital admission. Among the five Rangpur cases, four had very close contact with the first case and were his caregivers. Each became infected and subsequently died within 14 days of the death of the first case. These subsequent four cases likely developed secondary NiV infection and the first four cases were identified as probable cases. We sampled all contacts (74) of the cases (8) who became ill with fever and who had direct contact with the case's body fluids, and none had evidence of Nipah antibodies. In the 2018-19 season, we found a total of eight (four lab confirmed and four probable) cases with 87% case fatality. NiV remains an ongoing threat in Bangladesh and in other sites in Asia. Continued investment in surveillance can monitor for threatening changes in epidemiology and transmission.

867

IDENTIFICATION OF ORTHOBUNYAVIRUSES CO-CIRCULATIN DURING A RIFT VALLEY FEVER OUTBREAK IN RWANDA 2018

Marie Fausta Dutuze[1], Angélique Ingabire[2], Isidore G. Mapendo[2], Solange Uwituze[3], Manassé Nzayirambaho[4], **Rebecca C. Christofferson**[1]

[1]*Louisiana State University, Baton Rouge, LA, United States,* [2]*Animal Research Resources and Transfer Technology Department, Kigali, Rwanda,* [3]*Rwanda Agriculture Board, Kigali, Rwanda,* [4]*University of Rwanda, Kigali, Rwanda*

Bunyamwera (BUNV), Batai (BATV), and Ngari (NRIV) are arboviruses of the *Bunyaviridae* family, genus Orthobunyavirus. NRIV is a recombinant of the other two. All three cause disease in domestic ruminants and humans, presumably sharing ecological niche and clinical symptoms with Rift Valley Fever Virus (RVFV). In Rwanda, RVFV is the only Bunyavirus regularly surveilled. In 2018, there was an historic outbreak of RVFV in Uganda, Rwanda, and Kenya. Cattle showing clinical signs of RVFV were diagnosed, with few molecular confirmations. We hypothesized that clinical-based diagnosis of RVFV in Rwanda eclipses the occurrence of these Orthobunyaviruses. From May to July 2018, 185 blood samples from acutely ill cattle were collected from Rwanda. cDNA was synthesized from vRNA and amplified by conventional PCR using specific primers for RVFV. RVFV-negative samples were then tested for these Orthobunyaviruses. 56 (30.3%) were positive for RVFV by PCR and we identified BUNV and BATV for the first time in Rwanda. 7 samples (3.8%) were positive for undifferentiated Orthobunyaviruses; two samples were positive for BATV, and two were positive for BUNV. The remaining three were positive for both BUNV and BATV, which means that they were either co-infected with these two viruses or some combination of BUNV/BATV and NRIV. This is the first report of Orthobunyaviruses in Rwanda and the second report of BATV in Africa, and suggests that these may contribute to the burden of disease in cattle in the region.

868

NIPAH VACCINE TRIALS- ASSESSING THE FEASIBILITY BASED ON PREVIOUS OUTBREAKS IN BANGLADESH

Birgit Nikolay[1], Henrik Salje[1], Marc Lipsitch[2], Stephen P. Luby[3], Simon Cauchemez[1], Emily S. Gurley[4]

[1]*Institut Pasteur, Paris, France,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Stanford University, Stanford, CA, United States,* [4]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Nipah virus is an emerging, bat-borne pathogen that can be transmitted from person-to-person. Vaccine candidates are currently being developed for Nipah virus, and studies are funded to evaluate the safety and immunogenicity of these vaccines, so that they could possibly be used to contain large outbreaks. An important unanswered question is whether it will be possible to evaluate the efficacy of these vaccine candidates in phase III clinical trials in a context where spillovers are infrequent and associated with outbreaks that are small and often detected late. Ring vaccination strategies were used in trials of Ebola virus vaccines; however Nipah virus transmission, with few exceptions, results in short transmission chains. The objective of this study was to investigate the feasibility of conducting a phase III trial for Nipah vaccines in Bangladesh, the only country reporting regularly Nipah cases. We used simulations based on previously observed Nipah spillovers from Bangladesh, and an assumed vaccine efficacy of 90%, to compare two vaccine trial scenarios: (i) ring vaccinations including contacts of Nipah cases and contacts of contacts; and (ii) cluster-randomized trials in the two most affected districts in Bangladesh. The simulations showed that it would take approximately 160 years (1,230 rings per arm) to carry out a ring vaccination trial with 80% power to detect a difference between the arms. It would take about 50 years for a cluster-randomized trial (cluster size 10,000; 140 clusters by arm). Without a change in the epidemiology of Nipah, ring vaccination or cluster randomized trials will be impractical. Other trial designs such

as a vaccine campaign for all residents of the two most affected districts, followed by a case-control design, should be explored. Efforts to increase case detection through enhanced surveillance efforts would also be beneficial as the limited number of cases currently reported is the primary driver of the feasibility of these studies.

869

DETECTION OF DICISTROVIRUS RNA IN THE BLOOD OF FEBRILE TANZANIAN CHILDREN

Samuel Cordey[1], Florian Laubscher[1], **Mary-Anne Hartley**[2], Thomas Junier[3], Francisco J. Pérez-Rodriguez[4], Kristina Keitel[5], Gael Vieille[1], Josephine Samaka[6], Tarsis Mlaganile[7], Frank Kagoro[7], Noémie Boillat-Blanco[8], Mylène Docquier[9], Francisco Brito[3], Daniel Eibach[10], Peter Sothmann[10], Cassandra Aldrich[10], John Lusingu[11], Valérie D'Acremont[5], Laurent Kaiser[1]

[1]*Division of Infectious Diseases and Laboratory of Virology, University of Geneva Hospitals, Geneva, Switzerland,* [2]*University of Lausanne, Lausanne, Switzerland,* [3]*Swiss Institute of Bioinformatics, Geneva, Switzerland,* [4]*University of Geneva Medical School, Geneva, Switzerland,* [5]*Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [6]*Amana Hospital, Dar es Salaam, United Republic of Tanzania,* [7]*Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania,* [8]*Infectious Diseases Service, Lausanne University Hospital, Lausanne, Switzerland,* [9]*iGE*[3] *Genomics Platform, University of Geneva, Geneva, Switzerland,* [10]*Department of Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany,* [11]*National Institute for Medical Research, Tanga Research Centre, Tanga, United Republic of Tanzania*

Fever is the leading cause of paediatric outpatient consultations in Sub-Saharan Africa. Although most are suspected to be of viral origin, a putative causative pathogen is not identified in over a quarter of these febrile episodes. This study includes sera from 692 febrile children (aged 2-59 months) and plasma from 77 febrile adults recruited at outpatient clinics in Tanzania. These blood products were screened for novel viruses using a de novo assembly sequencing approach. We report the presence of RNA from a dicistrovirus (DicV) in 15.4% of the paediatric cohort. In contrast, DicV RNA was only detected in 1/77 (1.3%) plasma samples from febrile Tanzanian adults, suggesting that children could represent the primary susceptible population. The virus is novel to human tissue and phylogenetic analysis of the capsid region in the three full-length genomes obtained showed the presence of two clusters representing a tentative novel genus. Estimated viral load across all samples by specific quantitative real-time RT-PCR assay ranged from < 1.32E3 to 1.44E7 viral RNA copies/mL serum. Although DicV-positive cases were detected throughout the year, a significantly higher positivity rate was observed during the rainy season. Dicistrovirus is part of a family of RNA viruses that have been detected in some hematophagous insects that are known vectors of parasitic disease in humans (such as triatomes). However, these viruses have never before been detected in human blood. This study reveals that novel DicV RNA is frequently detected in the blood of Tanzanian children and works to encourage further investigations to determine whether DicV may be a novel infectious agent in humans.

870

DORMANCY OF PLASMODIAL SPOROZOITES

Miles B. Markus

University of the Witwatersrand, Johannesburg, South Africa

Hypnozoites in the primate liver appear to be derived from dormant sporozoites. Can the equivalent stage of *Plasmodium* also persist elsewhere in the body, such as dermally or in the lymphatic system, and be a source of recurrent malaria? *Plasmodium* has certainly been seen in these particular sites in the early phase of infection (references will be given on the poster). Hypnozoites of some non-plasmodial apicomplexan coccidia can persist not only in the liver, but elsewhere too. Both light and electron microscopic images of these extrahepatic hypnozoites, derived from the presenter's laboratory research (which resulted in his discovery of the apicomplexan hypnozoite in the 1970s), will be used to illustrate the point. This question of whether persisting, quiescent, extrahepatic sporozoites occur in the life cycle of primate *Plasmodium* species should be intensively investigated in both primates and primatized mice - *inter alia*, by using modern imaging techniques. Detection of any such latent forms would necessitate assessment of the implications for the elimination of malaria parasites in human populations as well as the drug treatment of malaria in individual patients.

871

PLASMA TAU AND OTHER BLOOD-BASED BIOMARKERS OF BRAIN INJURY IN CEREBRAL MALARIA AND SEVERE MALARIAL ANEMIA

Dibyadyuti Datta[1], Katrina Co[1], Peter F. Castelluccio[2], Andrea L. Conroy[1], Robert O. Opoka[3], Paul Bangirana[4], Andrew J. Saykin[5], Chandy C. John[1]

[1]*Ryan White Center for Pediatric Infectious Disease and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN, United States,* [3]*Department of Paediatrics and Child Health, Makerere University, Kampala, Uganda,* [4]*Department of Psychiatry, Makarere University, Kampala, Uganda,* [5]*Indiana Alzheimer Disease Center and Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, United States*

In children with cerebral malaria (CM), elevated admission cerebrospinal fluid (CSF) concentrations of tau, an axonal injury marker are associated with disease severity and worse long-term cognitive outcomes. In the present study, we sought to determine if brain injury markers were present in admission plasma samples of children with severe malaria (CM or severe malarial anemia (SMA)), and to determine their association with disease severity and long-term cognitive outcomes. Plasma concentrations of 4 markers of neuronal/axonal injury produced exclusively in the CNS – tau, neurofilament-light (NFL), glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl-terminal hydrolase isoenzyme L1 (UCHL1) – were assessed in 183 Ugandan children with CM, 162 with SMA, and 123 community children (CC), using the Human Neurology 4-Plex Assay on a single molecule array (Simoa) instrument. Plasma tau, NFL, and UCHL1 concentrations in children with severe malaria were higher than in CC, and plasma tau and UCHL1 concentrations were higher in CM than SMA (all $P<0.001$). In children with CM, elevated tau, NFL, and UCHL1 concentrations were associated with acute kidney injury (all $P<0.05$), elevated NFL concentrations were associated with prolonged coma duration ($P=0.001$), and elevated tau and UCHL1 concentrations were associated with number of convulsions in hospital (all $P<0.03$). After adjusting for confounders, elevated $\log_{10}$-transformed plasma tau concentrations in children with CM correlated with worse z-scores over 2-year follow-up for overall cognition (β coefficient [95 % CI]) (-0.77 [-1.31,-0.24]) in children <5 years at the time of testing, and attention (-0.64 [-1.23,-0.04]) and memory (-0.72 [-1.35,-0.08]) in children ≥5 years at the time of testing. In children with SMA, plasma tau concentrations correlated inversely with scores in cognition, attention, and memory, but no significant associations were detected. Plasma tau concentration is a promising prognostic marker for disease severity and worse cognitive outcomes after CM and possibly other forms of severe malaria.

872

LACTIC ACID SUPPLEMENTED MEDIA STIMULATES GAMETOCYTOGENESIS IN *PLASMODIUM FALCIPARUM* CULTURE

Rachel M. West, David J. Sullivan

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Malaria infection by *Plasmodium falciparum* continues to afflict millions of people worldwide, with transmission maintained by the definitive

host mosquito. Transmission is dependent upon mosquito ingestion of the gametocyte stage of the parasite. These sexually committed stages develop from the asexual stages, yet the factors behind this transition are poorly understood. *In vitro* studies have revealed that extracellular factors, such as LysoPC, present in different media and cellular environments influence gametocytogenesis. Parasites readily produce metabolites such as lactic acid in millimolar concentrations during malaria infection. We hypothesize gametocytogenesis is induced through molecular factors found in parasite- or reticulocyte-rich environments. We have demonstrated that lactic acid supplemented media significantly influences gametocytogenesis in an NF54 strain *P. falciparum* infection. Parasites exposed to different exposure times of lactic acid were monitored throughout gametocyte development *in vitro*. We found that continuous lactic acid supplementation increased parasitemia and gametocytemia after 72h and 168h, respectively. Using reverse-transcriptase qPCR, we also found that gametocyte specific gene expression was increased after 240 hours in lactic acid supplemented cultures. In a mosquito infection, we found that gametocytes continuously exposed to lactic acid were more infectious to *Anopheles stephensi* mosquitoes, increasing prevalence of infection but not oocyst density. Further, we found that gametocytes produce lactic acid throughout development, and produce it at higher levels than controls when supplemented with lactic acid. We then adapted a gametocyte-specific quantitative Pfs16 promoter luciferase assay to examine the effects of media environment on gametocyte development. Lactic acid supplemented media significantly increased gametocyte equivalent numbers after 72 hours of incubation, counted by luciferase assay. We will continue to test various acids produced by parasites or reticulocytes in a dose and time dependent to determine their effects on gametocytogenesis.

873

SEVERE ANEMIA IS ASSOCIATED WITH SYSTEMIC INFLAMMATION IN YOUNG CHILDREN PRESENTING TO A TERTIARY HOSPITAL IN UGANDA

Robert O. Opoka[1], Andrea L. Conroy[2], Ali Waiswa[3], Ronald Wasswa[3], James K. Tumwine[1], Charles Karamagi[1], Chandy C. John[2]

[1]*Makerere University, Kampala, Uganda,* [2]*Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States,* [3]*Global Health Uganda, Kampala, Uganda*

There are multiple causes of severe anemia (SA) but the etiology and prevalence of risk factors varies from region to region. We carried out a cross sectional study in which a range of etiological factors and biomarkers of host response were evaluated in children aged 0-5 years in Jinja Regional referral hospital, Uganda. Study participants included 284 children with SA (Hb < 5.0 g/dl), 63 admitted children with acute illness without severe anemia (Hb > 9.3 g/dl) and 53 healthy community controls. Descriptive statistics were used to categorize the etiological factors in the SA children, and appropriate logistic analysis performed to determine factors associated with SA. Amongst SA children, common etiological factors were *Plasmodium falciparum* (Pf) parasitemia 106 (36.4%), hemoglobinuria 93 (32.7%) and vitamin B12 deficiency 30 (10.6%). Human Immunodeficiency Virus infection, bacteremia, hookworm infection, severe acute malnutrition and folate deficiency were uncommon (each accounting for < 8%). The factors found to be associated with SA included (adjusted odds ratio (OR); 95% Confidence Interval [CI]); Pf parasitemia (OR, 3.9; 95% CI, 1.3 to 11.5), total white blood count (OR, 1.3; 95% CI, 1.1 to 1.4), C-reactive protein (OR, 1.7; 95% CI, 1.2 to 2.3), and ferritin (OR, 2.7; 95% CI, 1.9 to 3.9). There are multiples etiological factors that contribute to SA in young children in Jinja Hospital. Elevated inflammation was associated with SA in the study population. There is need for studies to confirm the immuno-pathogenic pathways of SA in malarial endemic areas in order to target intervention strategies.

874

CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* ARMADILLO-TYPE REPEAT PROTEIN

Philip Ilani[1], Emmanuel Amlabu[1], Grace Opoku[1], Prince B. Nyarko[1], Evelyn Quansah[1], Laty G. Thiam[1], Manfred Anim[1], Reuben Ayivor-Djanie[1], Ojo-ajogu Akuh[1], Henrrieta Mensah-Brown[1], Julian C. Rayner[2], Gordon A. Awandare[1]

[1]*West African Centre for Cell Biology of Infectious Pathogens, Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana,* [2]*Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom*

Nearly half of the genes in the *Plasmodium falciparum* genome have not yet been functionally investigated. We used homology-based structural modelling to identify multiple copies of Armadillo repeats within one uncharacterised gene expressed during the intraerythrocytic stages, PF3D7_0410600, subsequently referred to as P. falciparum Armadillo-Type Repeat Protein (PfATRP). Soluble recombinant PfATRP was expressed in a bacterial expression system and used to screen plasma samples from malaria endemic areas in Ghana, which revealed that malaria-infected children have naturally acquired PfATRP-specific antibodies, with prevalence varying across transmission areas. Affinity-purified α-PfATRP human and rabbit antibodies specifically recognised both native and recombinant parasite proteins. Immunofluorescence imaging established that PfATRP is a component of the inner membrane complex (IMC) and its associated microtubules, consistent with its differential solubility. Size exclusion chromatography suggests PfATRP forms part of a larger-order protein complex, the composition of which could potentially play a role in its sub-cellular distribution.

875

ANALYSIS OF NATURAL ANTIBODY RESPONSE TO NOVEL *PLASMODIUM FALCIPARUM* MEROZOITE PROTEINS

Grace Opoku, Ojo-ajogu Akuh, Prince Nyarko, Damata Ibrahim-Dey, Gordon A. Awandare, Emmanuel Amlabu

West African Centre for Cell Biology of Infectious Pathogens, Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana

Despite the enormous research into the biology of the malaria parasite, our understanding still remains limited. A panel of novel merozoite proteins that could play essential roles during erythrocyte invasion were identified using data-mining analysis of published transcriptome and proteome of the parasite combined with protein-informatics approach. Time-resolved real-time PCR analysis for the genes coding for these proteins indicated that they were optimally expressed at early to late schizogony. To produce these proteins in bacteria system with optimum recovery, codon-optimisations and gene synthesis were performed. These recombinant proteins were expressed and purified in their active forms and used in a systematic serological screen with plasma samples from malaria-exposed children. We observed that the patterns of antibody acquisition varied with age and transmission intensities across the different malaria endemic sites in Ghana. Antigen-specific immunoaffinity columns were used to purify human antibodies from the plasma of malaria-exposed children. Immunofluorescence assays using these affinity-purified human antibodies show that three (3) of the merozoite proteins were localised on the parasite surface. We are currently evaluating these antigen-specific human antibodies in invasion assays to establish that these antigens are targets for protective immunity or they are potential bio-markers for malaria exposure.

876

ELEVATED PLASMA SOLUBLE ST2 CONCENTRATIONS ARE ASSOCIATED WITH COGNITIVE IMPAIRMENT IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

Elizabeth Fernander[1], Pontian Adogamhe[2], Katrina Co[1], Dibyadyuti Datta[1], Robert Opoka[3], Chandy John[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*University of Wisconsin- Whitewater, Whitewater, WI, United States,* [3]*Makerere University, Kampala, Uganda*

Children who recover from episodes of severe malaria caused by *Plasmodium falciparum* exhibit long term cognitive impairment. The mechanisms that lead to this impairment are unknown. In murine experimental cerebral malaria (ECM), IL-33 and its receptor ST2, can act in both a protective and deleterious manner. IL-33 binding to membrane bound ST2 can lead to pro-inflammatory signaling and recruitment of T cells, which exacerbates ECM. Conversely, IL-33 can act through anti-inflammatory signaling via regulatory T cells and a Th2 response, preventing the development of ECM. Soluble ST2 (sST2) acts as a decoy receptor to neutralize the actions of IL-33 and is elevated in disease states such as sepsis or brain injury. We assessed the role of ST2 in disease severity and neurodevelopmental outcomes in a cohort of Ugandan children 18 months to 12 years of age with cerebral malaria (CM), severe malaria anemia (SMA) or asymptomatic community children (CC). Overall cognitive ability, attention and associative memory were assessed 12 months after discharge. Plasma concentrations of sST2 were measured by ELISA. Among the 515 children studied, plasma concentrations of sST2 were highest in children with CM (median [IQR], 123.23 ng/mL [72.53-177.20]) compared to SMA (81.39 ng/mL [50.17-140.89], p <0.0001) or CC (5.76 ng/mL, [4.14-7.72], p <0.0001). In children with CM who were >5 years of age at the time of testing, elevated levels of sST2 were associated with worse age-adjusted z-scores for overall cognitive ability (beta coefficient [95% CI], -1.56 [-2.72- -0.39], p=0.01) and attention (-1.17 [-2.23- -0.27], p=0.04) 12 months after discharge. Plasma sST2 concentrations increase with disease severity in malaria and are associated with worse neurocognitive outcomes in children >5 years of age. Future research will assess cerebrospinal fluid (CSF) sST2 concentrations and the mechanisms by which plasma or CSF sST2 may affect cognition.

877

DYNAMICS OF HOST CELL SURFACE REMODELING IN *PLASMODIUM* GAMETOCYTES

Priscilla Ngotho[1], Kathleen W. Dantzler[2], Brian R. Omondi[3], Franziska Hentzschel[1], Karl Seydel[4], Miriam Laufer[5], Terrie Taylor[4], Teun Bousema[6], Matthias Marti[1]

[1]*Wellcome Centre Integrative Parasitology, University of Glasgow, Glasgow, United Kingdom,* [2]*Division of Infectious Disease and Geographic Medicine, Stanford University, Stanford, CA, United States,* [3]*KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya,* [4]*Blantyre Malaria Project, University of Malawi College of Medicine, Blantyre, Malawi,* [5]*Division of Malaria Research, Institute for Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [6]*Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands*

As efforts towards malaria elimination intensify, blocking the transmission cycle becomes a crucial component of control programmes. *Plasmodium* transmissible forms, gametocytes, arise during the blood stage cycle of the parasite. A small subset of parasites switches from the disease-causing asexual cycle to produce gametocyte progeny that are taken up by mosquitoes during blood meals, completing the transmission cycle. While asexual parasites sequester in various vascular niches, causing pathology such as cerebral malaria, gametocytes sequester and develop in the extravascular niche of the bone marrow and spleen. We have recently characterized immune responses targeting immature but not mature gametocytes in *P. falciparum* and identified putative target surface antigens. We hypothesize that such antigens play a role in gametocyte sequestration and contribute to acquired transmission reducing immunity. Indeed, we demonstrate that immune sera contain anti-gametocyte IgG that mediate opsonic phagocytosis by macrophages, a plausible mechanism of gametocyte clearance *in vivo*. Interestingly, the dynamics of antigen surface expression, phagocytosis induction and surface membrane remodeling (phosphatidylserine exposure on the outer membrane of host RBC) show the same dynamics during asexual and gametocyte maturation, suggesting that these processes might be interlinked. Moreover, parasite reverse genetics and drug perturbations suggest conserved mechanisms of surface remodeling in asexual and sexual *P. falciparum* stages and across *Plasmodium* species. These findings have important implications for our understanding of parasite biology and novel intervention strategies to simultaneously reduce parasite burden and transmission.

878

IS THAT A REAL OOCYST? IDENTIFICATION OF *PLASMODIUM FALCIPARUM* OOCYSTS FROM MIDGUTS OF *ANOPHELES* MOSQUITOES FED ON INFECTED HUMAN BLOOD IN TORORO, UGANDA

Alex Kashaija Musiime[1], Joseph Okoth[1], Melissa Conrad[2], Daniel Ayo[1], Ismail Onyige[1], John Rek[1], Joaniter I. Nankabirwa[3], Emmanuel Arinaitwe[1], Moses R. Kamya[3], Grant Dorsey[2], Geert-Jan van Gemert[4], Sarah G. Staedke[5], Chris Drakeley[5], Teun Bousema[4], Chiara Andolina[4]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, United States,* [3]*Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda,* [4]*Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

The human infectious reservoir for malaria consists of individuals capable of infecting mosquitoes. Oocyst prevalence and density are typical indicators of infectivity of human-to-mosquito transmission. However, identification of oocysts is challenging, particularly in areas of low malaria transmission intensity where few individuals may infect mosquitoes, and infected mosquitoes tend have few oocysts. Although molecular techniques like polymerase chain reaction (PCR) allow accurate detection of low- level *Plasmodium falciparum* oocyst infections in mosquito midguts, they are expensive, require highly trained personnel and do not allow quantification of oocysts. Here, we explain and illustrate features that differentiate oocysts from other structures seen under light microscope in mosquito midguts that may resemble oocysts to help guide oocyst detection in low transmission settings. In 2014, an infrastructure was developed and equipped with *Anopheles gambiae s.s.* mosquitoes to support infectivity experiments from participants enrolled in a large cohort study in Tororo, Uganda. Venous blood drawn from participants who were naturally infected with malaria parasites was used for membrane feeding assays, using 60-80 mosquitoes per experiment. Approximately 9-10 days after feeding, mosquitoes were dissected, and midguts were stained in mercurochrome and examined by light microscopy for *Plasmodium falciparum* oocysts and similar structures. A total of 16458 mosquitoes were successfully fed on infected blood and dissected of which 330 were infected (2.0%).Oocysts and oocyst-like structures were observed in mosquito midgut walls. Oocysts were characterized by; presence of malaria pigment, clearly defined edge, round shape within the mosquito midgut or on the peripheral tissue and always attached to the epithelium. Distinguishing real oocysts from oocyst-like structures may be challenging for inexperienced microscopists due to overlapping features. The characteristics and guidelines outlined here support identification of oocysts and reliable detection at low oocyst densities.

879

VARIATION IN THE *CD40* PROMOTER PREDICTS LONGITUDINAL SUSCEPTIBILITY TO MALARIAL ANEMIA AND ALL-CAUSE MORTALITY IN KENYAN CHILDREN

Elly Munde[1], Samuel B. Anyona[2], Evans Raballah[3], Qiuying Cheng[4], Christophe G. Lambert[1], Benjamin H. McMahon[5], Collins Ouma[6], Nick Hengartner[5], Douglas Perkins[4]

[1]*University of New Mexico-Kenya Global Health Programs, Albuquerque, NM, United States,* [2]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya,* [3]*Masinde Muliro University of Science and Technology, Kakamega, Kenya,* [4]*University of New Mexico Center for Global Health, Albuquerque, NM, United States,* [5]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [6]*Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Kisumu, Kenya*

Molecular mechanisms influencing pediatric severe malaria anemia (SMA, Hb<5.0g/dL and any density parasitemia) are only partially defined. Investigation of pathways leading to severe disease has the potential to aid in understanding the complex pathogenesis. Cluster of differentiation 40 (CD40) expressed on immune and non-immune cells can stimulate inflammatory reactions. Therefore, we hypothesized that polymorphisms within the *CD40* promoter will impact on the production of downstream inflammatory molecules and clinical outcomes of pediatric malaria during the development of naturally-acquired immunity. The influence of *CD40* variation [(-580G>A; rs1800686, -245C>T rs752118, and -1C>T rs1883832) on cross-sectional and longitudinal outcomes (over 36 months) were determined in children (n=1,370, aged 6-36mos.)] from Siaya County, western Kenya, a *P. falciparum* holoendemic transmission area. *CD40* genotypes were generated by Taqman® genotyping assays. Transcriptional expression of IL-1β, TNF-α, and IL-6 were determined by qPCR, while protein levels were quantified by ELISA. Bivariate logistic regression analyses (cross-sectional), controlling for anemia-promoting covariates, revealed that inheritance of the *CD40* -580G/-245C/-1C (GCC) haplotype protected against SMA (OR=0.31, 95%CI=0.14-0.67, *P*=0.0030), while carriage of the GCT haplotype enhanced susceptibility to SMA [OR=5.24, 95%=3.31-8.82, *P*<0.001]. Longitudinal analyses showed that carriage of the ATC haplotype increased the risk of malaria (RR=1.09, 95%CI=1.00-1.19, *P*=0.044), and that the GCT haplotype increased the risk of all-cause mortality (HR=2.11, 95%CI=1.08-4.15, *P*=0.030) over a 36month period. Additionally, phagocytosis of malarial pigment (*Pf*Hz) increased the mRNA and protein expression of IL-1β, TNF-α, and IL-6 in human peripheral blood mononuclear cells. Collectively, these results demonstrate that variation in CD40 influences susceptibility to malaria disease outcomes and all-cause mortality, potentially through altering the inflammatory milieu.

880

ARGININE METABOLISM DRIVES THE ADAPTIVE PROLINE RESPONSE TO HALOFUGINONE IN *P. FALCIPARUM*

Lola Fagbami

Harvard T.H. Chan School of Public Health, Boston, MA, United States

We have previously identified the cytoplasmic prolyl tRNA synthetase (cPRS) in *Plasmodium falciparum* as the functional target of the natural product febrifugine and its synthetic analogue halofuginone. HFG treatment triggers a novel mode of drug resistance wherein intracellular proline levels are increased by 30 fold prior to any alteration in the target cPRS gene. This metabolic adaptation, termed the Adaptive Proline Response (APR), persists after drug withdrawal and renders the parasite tolerant to HFG treatment. We investigated the molecular basis of the APR by identifying the source of the increased proline using a multiplexed high-resolution mass-spectrometry (HRMS) based assay. After culturing HFG-induced parasites in the presence of orthogonally labeled proline and proline precursor amino acids (15N proline, 13C-15N arginine, and 13C5 glutamine), we observed predominantly 13C-15N-labeled proline, indicating that arginine is a major contributor. We then probed the essentiality of the arginine biosynthesis of proline by using CRISPR/Cas9 technology to generate independent parasites lines that lack ornithine δ-aminotransferase (PF3D7_ 608800), a key enzyme in this metabolic pathway. Functional analysis of the ornithine δ-aminotransferase (Dd2 ΔOAT) knockout parasites revealed a complete absence of the arginine derived 13C-15N-labeled proline. Dd2 ΔOAT parasites exposed to HFG remain sensitive to drug and do not increase their intracellular proline levels. This observation that ornithine δ-aminotransferase knockout parasites do not activate the APR demonstrates that arginine metabolism is required for the adaptive proline response to halofuginone.

881

EXPRESSION AND LOCALIZATION PROFILES OF RHOPTRY PROTEINS IN *PLASMODIUM BERGHEI* SPOROZOITES

Tomoko Ishino[1], Naohito Tokunaga[1], Mamoru Nozaki[1], Takafumi Tsuboi[2], Motomi Torii[1]

[1]*Ehime University, Toon, Japan,* [2]*Ehime University, Matsuyama, Japan*

The apical structure of infective forms of apicomplexan parasites is highly conserved as containing the secretory organelles named rhoptries and micronemes. Proteins localized to the rhoptry neck region are secreted from the apical-end of infective-stage parasites to form the tight junction between parasites and target cells prior to invasion. In the *Plasmodium* lifecycle, two infective forms, merozoites and sporozoites, efficiently infect different target cells: erythrocytes, hepatocytes and mosquito salivary glands. It has been questioned whether the invasion mechanisms are conserved between merozoites and sporozoites. Recently, we demonstrated that rhoptry neck protein 2 (RON2), one of the components of RON complex mediating tight junction formation, has an important role during sporozoite invasion of mosquito salivary glands by generating sporozoite-stage *ron2* knockdown parasites (Ishino et al., 2019, *Cell Microbiol*). For further elucidation of the molecular mechanisms of sporozoite invasion, the expression and localization profiling of rhoptry proteins was investigated in *Plasmodium berghei* sporozoites. Nine of 12 genes, known as merozoite rhoptry molecules, are transcribed also in oocyst-derived sporozoites at the similar or higher level compared to those in schizonts. For the comprehensive localization analyses, transgenic parasites expressing each rhoptry protein tagged by c-Myc at the C-terminal were generated. Immuno-electron microscopy demonstrates that eight proteins localize to rhoptries in sporozoites. It is noteworthy that the neck and body compartment in rhoptries was not clearly determined in sporozoites. Most rhoptry proteins except components of the high-molecular mass rhoptry protein complex are commonly expressed in merozoites and sporozoites, suggesting that a certain part of invasion mechanisms is conserved between infective forms independently of their target cells. Combined with sporozoite-stage specific gene silencing systems, roles of rhoptry proteins during invasion will be elucidated.

882

PREVALENCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANT MUTATIONS IN 2012 AND 2017 COMMUNITY SURVEILLANCE IN WESTERN KENYA

Zhiyong Zhou[1], Simon Kariuki[2], Sheila B. Sergent[1], Kephas Otieno[2], Benard Abong'o[2], Ying Liu[3], Winnie Chebore[2], John E. Gimnig[1], Edward D. Walker[4], Aaron M. Samuels[1], Meghna Desai[1], Ya Ping Shi[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [3]*Parasitic Diseases Control and Prevention Institute, Henan Provincial Centre for Diseases Control and Prevention, Zhengzhou, China,* [4]*Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States*

Scale-up of malaria control over the past decades has reduced childhood morbidity and mortality and decreased malaria transmission in Kenya. However, widespread use of anti-malarial drugs, such as artemisinin-

based combination therapies (ACTs), may select for the development of anti-malarial drug resistance, potentially impeding this progress. Community surveillance and monitoring for drug-resistant parasites in human blood and mosquitoes is important to decide when a change in antimalarial regimens is necessary. The present studies in 2012 and 2017 extend our previous analysis in 1996, 2001, 2007 for tracking markers of resistance to sulfadoxine-pyrimethamine (SP), chloroquine (CQ) and ACT in Asembo Bay. Parasites from 225 and 110 blood samples (2012 and 2017, respectively) were sequenced. Additionally, 66 oocyst-positive mosquito midguts from the same area in 2012 were genotyped for the same markers. Results indicate that SP resistance mutations remained high, with the frequency of N51I, C59R, S108N of *Pfdhfr* and A437G, K540E of *Pfdhps* approaching fixation (96-100%), while the prevalence of *Pfdhps* S436H mutations increased significantly from 0% in previous 3 surveys to 9.8% in 2012, and 33.6% in 2017. The CQ resistance marker *Pfcrt* K76T declined over time, from 81.6% in 1996, 81.8% in 2001, 94.6% in 2007 to 17.3% in 2012 and 0.9% in 2017. The multi-drug resistant marker, *Pfmdr1* N86Y dropped from 74.8% in 1996, 73.1% in 2001, 71.0% in 2007 to 9.6% in 2012 and 0% in 2017, but *Pfmdr1* Y184F prevalence increased from 17.9% in 2007 to 53.4% in 2012 and 55.9% in 2017. Importantly, no mutations in the *K13* propeller domain associated with artemisinin resistance were found. Parasites from human blood and mosquito oocysts showed similar prevalence for all markers. The increased prevalence of *Pfdhps* S436H may confer increased resistance to SP. The present study finds no evidence of artemisinin resistance in western Kenya. However, the increase in Y184F prevalence may be due to the selection by the partner drug in ACT, lumefantrine. Therefore, regular surveillance and further evaluation of resistance markers is pivotal for effective treatment of malaria.

883

EXTENSIVE CUTANEOUS ULCERATIONS IN A PATIENT FROM MYANMAR: AN OLD ENEMY WHICH SHOULD NOT BE FORGOTTEN

Khin R. Ko[1], Aye A. Win[1], May Zabe[1], Aye M. Win[1], Mya Paing[1], Cho C. Nwe[1], Soe A. Thu[1], Moe M. San[1], Patricia F. Walker[2]

[1]*University of Medicine [1], Yangon, Myanmar,* [2]*University of Minnesota, St. Paul, MN, United States*

A 23 year old female presented with multiple painful erythematous skin papules and nodules over the hands, forearms, lower extremities, trunk and face of eight months duration. Lesions ulcerated for 1 month before admission. High grade fever, joint pain, loss of appetite and weight loss were associated with onset of skin lesions. All biochemical parameters were within normal limits with the exception of low albumin and proteinuria. Screening tests for connective tissue diseases and infective serology tests were negative. Wound swab culture revealed growth of *Klebsiella pneumoniae*. Slit skin smear for AFB showed multiple bacilli. Skin biopsy was not done. Patient was admitted and treated as severe erythema nodosum leprosum (ENL) (type 2 lepra reaction), as her presenting symptom complex for Hansen's disease.

884

SEASONAL MALARIA CHIMIO PREVENTION 2017 IN THE HEALTH DISTRICT OF GOUDOMP SENEGAL COST-EFFECTIVENESS ANALYSIS OF TWO TREATMENT STRATEGIES FOR CHILDREN AGED 3-120 MONTHS

Malick Anne[1], Mamadou Mactar Leye[2], Doudou Sene[3], Abdel Kader Dieye[4], Ibrahima Mbamby Keita[5], Youssoupha Ndiaye[5]

[1]*Senegal Health Ministry and Social Action, Kaolack, Senegal,* [2]*Institute Health Development of Mbour Sénéngal, Mbour, Senegal,* [3]*National Malaria Control Program, Dakar, Senegal,* [4]*Senegal Health Ministry and Social Action, Sedhiou, Senegal,* [5]*Senegal Health Ministry and Social Action, Dakar, Senegal*

Despite a good coverage rate for the 2017 Seasonal Malaria Chemo-Prevention (SMC) campaign, more than 95% treatment for children aged 3 to 120 months, a major challenge persists regarding the complete treatment in three days of these children. . The pilot study conducted in the Goudomp district serves as a basis for analyzing the cost-effectiveness ratio of these two approaches. The overall objective of the study is to conduct cost-effectiveness analysis of seasonal malaria prevention strategies for children aged 3-120 months in the Goudomp health district. It was a mixed estimate: an economic evaluation of the three-day and one-day directly observed treatment (DOT) strategies at the 2017 SMC and a qualitative study for the community actors, nurses and the executive team. The quantitative study included 12-health post: six who completed the full treatment strategy under DOT and six who completed the one-day DOT strategy. The choice of posts that had led the strategy was based in a reasoned manner taking into account certain criteria: the location of health posts, the size of the population, the accessibility of structures, the motivation of providers. For the qualitative study, 240 community actors All the head nurses from the 12 health posts and 4 members of the executive team were also interviewed. The average cost in the three-day DOT treatment strategy compared to that of the one-day DOT was respectively passing from the first to the third passage of 1.69$, 1.38$ and 1.39$ against 1.41$, 1.11$ and 1.25$. Note a variation if we compare the two strategies respectively of +0.28, +0.28 and 0.14. The ratio cost effectiveness is 1052.97$ per case avoided for the full treatment strategy compared to 2223.72$ per case avoided for the one-day DOT strategy. Conferring on the most cost-effective three-day TDO strategy. The qualitative study showed a deficiency in the delivery of three doses to children and strong adherence of providers and community relays in the comprehensive treatment strategy to increase the effectiveness of drugs. Comprehensive TDO strategy strengthens child protection, reduces risk of antimalarial drug resistance

885

NEW INSIGHTS INTO THE MODE OF ACTION OF THE ANTIMALARIAL DRUG PROGUANIL

Kathy Andrews[1], Gillian Fisher[1], Andrews Riches[2], Oliver Hutt[2], Karen Jarvis[2], Tony Wilson[2], Mark von Itzstein[3], Pradeep Chopra[3], Yevgeniya Antonova-Koch[4], Stephan Meister[4], Elizabeth Winzeler[4], Mary Clarke[1], David Fidock[5], Jeremy Burrows[6], John Ryan[2], Tina Skinner-Adams[1]

[1]*Griffith University, Nathan, Australia,* [2]*CSIRO, Clayton, Australia,* [3]*Griffith University, Gold Coast, Australia,* [4]*University of California San Diego, La Jolla, CA, United States,* [5]*Columbia University Medical Center, New York, NY, United States,* [6]*Medicines for Malaria Venture, Geneva, Switzerland*

Malarone® is a combination of atovaquone and proguanil that is used for malaria prophylaxis and treatment. Atovaquone has potent anti-plasmodium activity as a cytochrome bc1-inhibitor while dogma is that proguanil does not have potent intrinsic activity. Proguanil can, however, potentiate atovaquone activity and its cyclization-metabolite (cycloguanil) is a dihydrofolate reductase inhibitor with potent activity. We have recently found that proguanil, and an analogue that cannot convert to cycloguanil (tBuPG), have potent slow-acting activity *in vitro* against asexual *P. falciparum* parasites. This activity is folate-metabolism and isoprenoid biosynthesis-independent. In yeast DHODH-expressing parasites, proguanil and tBuPG slow action activity remains, however bc1-inhibitor activity switches from fast to slow-acting. Proguanil and tBuPG both act synergistically with bc1-inhibitors, while cycloguanil antagonizes activity. Overall, our data suggest that proguanil has potent slow-acting activity against asexual-stage *P. falciparum*, that bc1 is essential to parasite survival independent of DHODH-activity and that Malarone® may act as a triple-drug combination (including antagonistic partners). These findings raise the possibility that a cyclization-blocked proguanil may be a better *in vivo* combination partner for antimalarial bc1-inhibitors.

886

GENOTYPES AND PHENOTYPES OF RESISTANCE IN ECUADORIAN *PLASMODIUM FALCIPARUM*

Gabriela Valenzuela[1], Luis E. Castro[2], Julio Valencia[3], Petra Rohrbach[4], **Fabian E. Saenz**[1]

[1]*Pontificia Universidad Católica del Ecuador, Quito, Ecuador,* [2]*Ministerio de Salud Pública del Ecuador, Guayaquil, Ecuador,* [3]*Ministerio de Salud Pública del Ecuador, Esmeraldas, Ecuador,* [4]*McGill University, Montreal, QC, Canada*

Malaria continues to be endemic in the coast and amazon regions of Ecuador. Clarifying the current situation of *P. falciparum* resistance in the country will support malaria elimination efforts. Molecular analyses of 69 samples were performed to search for mutations in known resistance markers (*pfcrt*, *pfdhfr*, *pfdhps*, *pfmdr1* and *k13*) and *Pfmdr1* copy number was determined by qPCR. PFMDR1 transporter activity was characterized in live parasites using live cell imaging in combination with the Fluo-4 transport assay. Chloroquine, quinine, lumefantrine, mefloquine, dihydroartemisinin and artemeter sensitivities were measured by *in vitro* assays. The majority of samples from this study presented the CVMNT genotype for *pfcrt* (72-26), NEDF SDFD mutations in *pfmdr1* and wild type genotypes for *pfdhfr*, *pfdhps* and *k13*. The Ecuadorian *P. falciparum* strain ESM-2013 showed *in vitro* resistance to chloroquine, but sensitivity to quinine, lumefantrine, mefloquine, dihydroartemisinin and artemeter. In addition, transport of the fluorochrome Fluo-4 from the cytosol into the digestive vacuole (DV) of the ESM-2013 strain was prevented. All analyzed samples revealed one copy of *Pfmdr1*. This study indicates that Ecuadorian parasites presented the genotype and phenotype for chloroquine resistance and were found to be sensitive to sulfadoxine-pyrimethamine, arthermeter-lumefantrine, quinine, mefloquine, and dihydroartemisinin. The results suggest that the current malaria treatment employed in the country remains effective. Our results clarify the status of antimalarial resistance in Ecuador and aide to best inform the *P. falciparum* elimination campaigns in the country.

887

USING AMPLICON-BASED NEXT GENERATION SEQUENCING TO DETECT DRUG RESISTANCE MARKERS IN *PLASMODIUM FALCIPARUM* FROM AFRICA

Brooke M. Clemons, Erica Lasek-Nesselquist, Susan Madison-Antenucci

New York State Department of Health Wadsworth Center, Albany, NY, United States

Each year ~1,700 malaria cases are diagnosed in the United States and are almost exclusively associated with travel. Cases identified in New York State (NYS) alone contribute ~20% of this total and are largely caused by *Plasmodium falciparum* (*Pf*). For this species, drug resistance is recognized in all currently available antimalarial classes. To investigate *Pf* drug resistance markers in NYS patients with reported travel history, we used amplicon-based next generation sequencing (NGS) to detect single nucleotide polymorphisms in six genes that are known to cause, or are strongly associated with, treatment failure. The genes targeted were *pfcrt*, *pfdhfr*, *pfdhps, pfmdr, pfcytb* and *pfk13*. All *Plasmodium* positive samples in NYS are required to be confirmed by the Wadsworth Center Parasitology Laboratory. As a result, we were able to sequence hundreds of blood samples containing *Pf* from patients who had traveled to over 30 different endemic countries. These data were compared to generate a maximum likelihood phylogeny based from a concatenated gene alignment. Antimalarial resistance markers were also detected and compared within and between countries. Data thus far show ~25% of African samples have *pfcrt* mutations associated with chloroquine resistance, ~95% have *pfdhfr* mutations linked to pyrimethamine and proguanil failure and >99% of samples have *pfdhps* mutations impacting sulfadoxine efficacy. The data indicate that ~70% of patient samples have mutations in the *pfmdr* gene, reported to cause resistance to multiple drugs including quinine, amodiaquine and artemether-lumefantrine. Less than 1% of samples contain *pfcytb* mutations, therefore overall susceptibility for atovaquone remains high. Lastly, we have not observed *pfk13* mutations that cause artemisinin resistance. Using NGS to detect *Pf* drug resistance markers in NYS's population allows for a better understanding of antimalarial efficacy. These data can assist in more accurate recommendations for prophylaxis in travelers to endemic countries and can assist clinicians in choosing treatment to lessen the risk of recrudescence once infected.

888

EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE, ARTESUNATE-AMODIAQUINE AND DIHYDROARTEMISININ-PIPERAQUINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO IN 2017: AN OPEN-LABEL RANDOMIZED TRIAL

Gauthier Masia Kahunu[1], Joris Likwela Losimba[2], Eric Mukomena Sompwe[2], Junior Matangila[3], Hyppolite Muhindo Mavoko[3], Ntamabyaliro Nsengi Yumva[1], Papy Mandoko Nkoli[1], Albert Kutekemeni Kaputu[2], Aboubacar Sadou[4], Leah F. Moriarty[5], Eric Halsey[5], Pascal Ringwald[6], Jean Jaques Muyembe Tanfum[7]

[1]*Unit of Clinical Pharmacology and Pharmacovigilance University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [2]*National Malaria Control Programme, Ministry of Health DRC, Kinshasa, Democratic Republic of the Congo,* [3]*Faculty of Medicine University of Kinshasa DRC, Kinshasa, Democratic Republic of the Congo,* [4]*United States Agency for International Development, President's Malaria Initiative, Kinshasa, Democratic Republic of the Congo,* [5]*President's Malaria Initiative, Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*World Health Organization Global Malaria Programme, Geneva, Switzerland,* [7]*Institut National des Recherches Bio-médicales (INRB) DRC, Kinshasa, Democratic Republic of the Congo*

Regular monitoring of artemisinin-based combination therapy (ACT) efficacy is important for timely detection of the emergence of antimalarial drug resistance. In a study conducted in 2012-13, artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ), the first line treatments for uncomplicated *Plasmodium falciparum* malaria in the Democratic Republic of Congo (DRC), met WHO standards for efficacy and safety. In 2017, we conducted a study of the efficacy of dihydroartemisinin-piperaquine (DP) in addition to AL and ASAQ. In a three-arm, open label randomized trial, 1613 children aged six to 59 months with uncomplicated *Plasmodium falciparum* malaria were enrolled from six sites representing the diversity of malaria epidemiologic settings in DRC (same sites as 2012-2013 study). After randomization and receiving an ACT for three days, participants were followed for 28 (AL and ASAQ arms) or 42 (DP arm) days. Clinical outcome was classified according to the standard WHO protocol published in 2009. The uncorrected adequate clinical and parasitological response (ACPR) rates for AL, ASAQ, and DP, respectively, were 79.8%, 72.0%, and 58.6% in Rutshuru, 63.0%, 76.2%, and 55.7% in Mikalayi, 85.1%, 92.7%, and 90.1% in Kimpese, 75.3%, 100%, and 64.1% in Kapolowe, and 88.0%, 100%, and 90.5% in Kabondo. Results are not available for Bolenge. The analysis to determine PCR-corrected results is ongoing. Five serious adverse events were reported, none of which were attributed to the ACT. Based on the uncorrected results, the efficacy of AL, ASAQ and DP appears to vary by site in DRC. PCR correction of the recurrent infections, which is currently pending, will offer more insight into the low uncorrected efficacy in some of the sites.

889

IDENTIFICATION OF NOVEL ANTIMALARIALS BY HIGH THROUGHPUT SCREENING OF *PLASMODIUM FALCIPARUM* PROTEASOME

Lydia Mata Cantero[1], Alvaro Cortés[1], Mercedes García[1], Stanley Xie[2], David Gillett[2], F. Javier Gamo[1], Esther Fernández[1], Leann Tilley[2], Maria G. Gómez Lorenzo[1]

[1]*GlaxoSmithKline, Tres Cantos, Madrid, Spain,* [2]*University of Melbourne, Melbourne, Australia*

The most severe form of malaria is caused by the protozoan parasite *Plasmodium falciparum*. Current first-line treatments are based on artemisinin combination therapies (ACTs). However, the malaria parasite has developed resistance against all widely used antimalarials, and ACTs are not an exception. Increasing drug resistance has led to an urgent need for developing novel therapies acting though new parasite targets. Ideally, new drugs should be effective not only on acute infection, but also on different stages of the parasite lifecycle to block transmission. Targeting proteasome fulfill these criteria, as is one of the best validated targets in malaria parasites. Human proteasome inhibitors used for cancer treatment are active on all parasite stages, liver, blood (both sexual and asexual) and mosquito. Moreover, in blood stages they show a synergistic behavior against sensitive and resistant parasites when combined with artemisinins. Although *P. falciparum* proteasome exhibits a high degree of homology with its human counterpart, significant structural divergences have been found, including an unusually open β2 active site. These divergences are being exploited to develop inhibitors with selectivity for the parasite proteasome, thus limiting the potential side effects derived from human proteasome inhibition. Here, we present a high throughput screening campaign where around 550K compounds from GSK screening collection have been tested using purified *P. falciparum* proteasome. Chymotrypsin-like activity was used as primary assay to filter the compounds, followed by secondary assays to test trypsin and caspase-like activities, as well as evaluation of selectivity using purified human proteasome. Results from the primary screening, validation in secondary and selectivity assays, as well as the progression cascade to validate both biochemically and biologically the hits will be presented.

890

QUIESCENT ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* PARASITES ARE ABLE TO SURVIVE MOST ANTIMALARIAL DRUGS, INCLUDING ARTEMISININ PARTNER DRUGS: WHAT CONSÉQUENCES?

Lucie Paloque[1], Thibaud Reyser[1], Manel Ouji[1], Sandie Ménard[2], Benoit Witkowski[3], Jean-Michel Augereau[1], Françoise Benoit-Vical[1]

[1]*CNRS; LCC (Laboratoire de Chimie de Coordination), Toulouse, France,* [2]*CPTP (Centre de Physiopathologie de Toulouse Purpan), INSERM U*[1043]*, CNRS UMR*[5282]*, Université de Toulouse III, Toulouse, France,* [3]*Malaria Unit, Pasteur Institute in Cambodia, Phnom Penh, Cambodia*

Quiescence phenomenon is an unconventional drug resistance mechanism of *Plasmodium falciparum* that occurs in *pfk13* mutated parasites in response to artemisinins treatment. During artemisinin-based combination therapies (ACTs), due to the respective pharmacokinetics properties of the drugs, artemisinin-resistant parasites are first exposed to artemisinins which induce the quiescence phenomenon of a parasite subpopulation. The ability of these quiescent parasites to also survive artemisinin partner drugs may lead to parasite recrudescence. We designed a novel *in vitro* phenotypic assay, named Quiescent-stage Survival Assay, to assess the efficacy of drugs on quiescent artemisinin-resistant parasites. In this assay, as it happens in patients, parasites are first exposed to a 6h-pulse of artemisinins inducing quiescence entry, then exposed, during 48h, to a combination of both artemisinins, to maintain the quiescent state, and the drug to be evaluated. In this way we investigated the efficacy of artemisinin partner drugs currently used in ACTs, but also other antiplasmodial drugs recommended by the World Health Organization for malaria treatment. These experiments were achieved on relevant quiescent artemisinin-resistant parasites from lab strains and Cambodian field isolates. Our results show for the first time that the drug activity on proliferating artemisinin-resistant parasites cannot be predictive of its efficacy level against quiescent artemisinin-resistant parasites. Indeed, we highlighted that because of their mode of action, some drugs highly active on dividing artemisinin-resistant parasites, lost their activity during the parasite quiescent state. These results are particularly interesting since artemisinin resistance was proved as a first step to multi-resistance acquisition responsible for clinical failures. This study provides a valuable tool for taking into account chemosensitivity of quiescent artemisinin-resistant parasites during drug discovery and development process of new partner drugs especially in the current context of artemisinin resistance.

891

CHANGES IN ANTIMALARIAL DRUG SENSITIVITY OVER TIME IN EASTERN UGANDA

Patrick K. Tumwebaze[1], Marvin Duvalsaint[2], Victor Asua[1], Oswald Byaruhanga[1], Thomas Katairo[1], Martin Okitwi[1], Stephen Orena[1], Jennifer Legac[2], Brett Bayles[2], Melissa Conrad[2], Samuel L. Nsobya[1], Roland A. Cooper[3], Philip J. Rosenthal[2]

[1]*Infectious Disease Research Collaboration, Kampala, Uganda,* [2]*University of California, San Francisco, CA, United States,* [3]*Dominican University, San Rafael, CA, United States*

Malaria remains a large problem in Uganda, although disease incidence varies greatly across the country, in part due to varied use of malaria control measures in different districts of the country. The first line therapy for malaria, artemether-lumefantrine, and the alternatives artesunate-amodiaquine and dihydroartemisinin-piperaquine, have all shown excellent treatment efficacy. We have monitored the ex vivo drug sensitivity and prevalence of key genetic determinants of resistance of *Plasmodium falciparum* from patients presenting with malaria in Tororo and Busia districts in eastern Uganda since late 2015. Parasite isolates have shown good sensitivities to chloroquine (CQ; median IC_{50} 20 nM), monodesethylamodiaquine (7.3 nM), piperaquine (5.3 nM), lumefantrine (4.9 nM), mefloquine (8.8 nM), pyronaridine (1.1 nM), atovaquone (0.3 nM), and dihydroartemisinin (1.5 nM). The prevalence of parasites with ex vivo IC_{50} consistent with resistance to CQ (IC_{50} >80 nM) has decreased over time (isolates with IC_{50} >80 nM 24% (24/100) in 2016 and 1.9% (3/161) in 2018 ($p < 0.0001$). Consistent with this finding, the prevalences of key mutations associated with CQ resistance have decreased over time (mixed/mutant *pfcrt* K76T 39% (37/96) in 2016 and 4.7% (8/170) in 2018; *pfmdr*1 N86Y 5% (4/99) in 2016 and 0.6% (1/177) in 2018; *pfmdr*1 D1246Y 19% (19/98) in 2016 and 8.3% (14/169) in 2018. Interestingly, comparing samples collected over the same time frame (May-June, 2018), the prevalence of the *pfcrt* 76T mutation was greater in parasites collected in Tororo District, where district-wide indoor residual spraying of insecticide (IRS) has greatly reduced malaria transmission, compared to that in parasites collected in nearby Busia District, where IRS has not been used (mixed/mutant 33.3% in Tororo; 3.6% in Busia; $p=0.0027$). In summary, sensitivity of *P. falciparum* to standard antimalarials remains excellent in eastern Uganda, but parasites have evolved over time, most likely in response to the pressure of wide use of artemether-lumefantrine, and changes in malaria epidemiology appear to impact on prevalences of resistance markers.

892

CORRELATIONS OF EX VIVO ANTIMALARIAL DRUG SENSITIVITIES BETWEEN STANDARD AND NEW ANTIMALARIAL COMPOUNDS IN TORORO, UGANDA

Thomas Katairo[1], Patrick K. Tumwebaze[1], Oswald Byaruhanga[1], Martin Okitwi[1], Sam L. Nsobya[1], Brett R. Bayles[2], Benjamin Blasco[3], Didier Leroy[3], Philip J. Rosenthal[4], Roland A. Cooper[2]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*Dominican University of California, San Rafael, CA, United States,* [3]*Medicines for Malaria Venture, Geneva, Switzerland,* [4]*University of California, San Francisco, CA, United States*

The efficacies of artemisinin-based combination therapies (ACTs) are threatened by resistance to both artemisinins and partner drugs, which has emerged in southeast Asia. Of great concern is spread of ACT resistance to Africa, where the greatest burden of falciparum malaria is present. As part of a long-term study to characterize potencies of existing antimalarials and experimental lead compounds, we have monitored the ex vivo sensitivities of *Plasmodium falciparum* collected from patients presenting with malaria in Tororo and Busia Districts, eastern Uganda, since 2015. Ex vivo sensitivities for standard antimalarial drugs and lead compounds from the Medicines for Malaria Venture portfolio were assessed with 72 h growth inhibition (IC_{50}) assays for 518 clinical isolates collected from 2015-18. In order to gain insights into shared mechanisms, we measured the strength and direction of associations between IC_{50} values of tested antimalarials using the Spearman rank-order correlation coefficient. We observed moderate to strong correlations between compounds predicted to have shared mechanisms of action. For aminoquinolines and related compounds, positive correlations were observed for chloroquine, monodesethylamodiaquine, AQ13, pyronaridine and ferroquine but, interestingly, not for the bisquinoline piperaquine. Positive correlations were also seen among drugs targeting the parasite mitochondrion (proguanil and the cytochrome B inhibitors atovaquone and ELQ300), the putative transporter PfATP4, dihydroorotate dehydrogenase, and phosphatidylinositol 4-kinase. Interestingly, activities of a number of compounds with unknown mechanisms of action correlated with those with known mechanisms, suggesting novel insights regarding mechanisms of action or resistance. Additional studies assessing correlations in activities between compounds and the genetic bases of shared mechanisms are underway.

893

SEASONAL MALARIA CHEMOPREVENTION AND COMPLIANCE DURING FOUR MONTHLY TREATMENTS WITH SULFADOXINE-PYRIMETHAMINE AND AMODIAQUINE AT 3 STUDY SITES IN MALI

Lansana Sangare[1], Oumar Kone[1], Youssouf Diarra[1], Lassina Doumbia[1], Haidara D. Bouye[1], Vincent Sanogo[1], Bassi Coulibaly[1], Amadou Bouare[1], Abdoul K. Diallo[1], Zakaria Haidara[1], Modibo Telly[1], Jules Mihigo[2], Erin Eckert[3], Moustapha Coulibaly[1], Etienne Coulibaly[1], Mouctar Diallo[1], Ababacar Maiga[1], Donald J. Krogstad[4], Ousmane A. Koita[1]

[1]*University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*United States Agency for International Development, President's Malaria Initiative, Bamako, Mali,* [3]*United States Agency for International Development, President's Malaria Initiative, Washington, DC, United States,* [4]*Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

Seasonal Malaria Chemoprevention (SMC) consists of giving preventive doses of Sulfadoxine-Amodiaquine (SP/AQ) monthly during the peak malaria season. The purpose of this study was to assess the relationship between the number of rounds of SP/AQ received and the mean parasite densities in children during and after an SMC campaign. During the 2018 SMC campaign SP/AQ was delivered door to door on designated days in August, September, October and November of 2018. During each round, the first dose (of three) was administered to children from 3 to 59 months of age during door-to-door visits. Instructions were given for the other two doses to be administered by the child's caregiver on the subsequent 2 days. The approach of this study was to screen children who presented to the health center with malaria symptoms. Between October 2018 and March 2019, 864 children from 3 to 59 months of age were screened and blood smears were prepared to identify *P. falciparum*. 428 of the 864 children screened had parasitemias ≥ 25 per μl. We examined the relationship between parasitemia and the number of rounds of SP/AQ that a child had received based on the hypothesis that children who received all 4 rounds of SP/AQ should have lower mean parasite densities than children who received less than 4 rounds of SP/AQ. Analysis of these data indicate there were no differences in mean parasite densities between children who received four rounds of SP/AQ and children who received less than four rounds of SP/AQ ($p = 0.493$). In addition, at one study site, children who reported receiving all four rounds of SP/AQ had higher mean parasite densities ($p = 0.039$) than children who received fewer rounds of SP/AQ with a marked impact on children more than 48 months of age. These results suggest there is a need to reinforce drug administration compliance by caregivers during days 2 and 3 of each round of SMC treatment.

894

FUNCTIONAL ANALYSIS OF THE ANTIMALARIAL TARGET *PLASMODIUM FALCIPARUM* PHOSPHATIDYLINOSITOL 4-KINASE

Anna R. Sternberg, Matthew R. Hassett, Paul D. Roepe

Georgetown University, Washington, DC, United States

Artemisinin-based combination therapy (ACT) is the front-line treatment recommended for uncomplicated *Plasmodium falciparum* infections by the World Health Organization (WHO). With the rise in delayed clearance phenotype (DCP) infections due to reduced ACT efficacy in Southeast Asia, much research has been focused in identifying novel, potent antimalarial agents. Recent high-throughput screening efforts have identified phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositol 4-kinase (PI4K) inhibitors as very potent antimalarial drugs. The *P. falciparum* malarial parasite genome encodes a single PI3K and sequence analysis suggests that the enzyme is a "class III" or "Vps34" PI3K. Sequence analysis of the *P. falciparum* PI4K antimalarial target suggests that it is a PI4K "type III beta" isoform. Both enzymes are likely to be essential genes in *P. falciparum*, which makes them attractive antimalarial drug targets. Previously, we heterologously expressed, purified, and characterized the PfVps34 enzyme as a class III PI3K, as well as a target for artemisinin-based antimalarials. Consequently, we optimized the *pfpi4k3b* gene for heterologous expression in yeast, purified the protein to homogeneity, and adapted a recently validated quantitative assay for PIP production from PI to characterize enzyme activity and to quantify drug inhibition.

895

TARGETING PHOSPHATIDYLINOSITOL 3' KINASE TO DESIGN NOVEL COMBINATION THERAPIES AGAINST ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM*

Kalpana Iyengar, Paul Roepe

Georgetown University, Washington, DC, United States

Resistance to artemisinin (ART) combination therapies in *Plasmodium falciparum* malaria is a continuously growing problem. Previous results from our laboratory have shown that *P. falciparum* parasites encode an autophagy - like process, that this pathway is triggered in response to external stressors such as starvation or cytocidal drug treatment, and that the process is impaired in drug resistant parasites. Phosphatidylinositiol 3' kinases (PI3K) are key regulators of autophagy. Not surprisingly then, we have found that PI3K inhibitors are extremely potent antimalarial compounds that both inhibit the autophagy response and are synergistic with artemisinin (ART) - based drugs. We have also shown that the synthetic trioxolane OZ439 is equally potent against both ART resistant (ART-R) and sensitive (ART-S) *P. falciparum* strains. Taken together, these

findings led us to further investigate the autophagy pathway in both ART-S and ART-R parasites. Results from these experiments further support the conclusion that there is a distinct autophagy pathway in *P. falciparum* parasites, and that this pathway is further dysregulated in ART-R parasites relative to chloroquine resistant (CQR) parasites studied previously. Significant cytostatic and cytocidal synergy was found for drug combinations involving ART - based drugs and PI3K inhibitors. Based on these data, we suggest novel endoperoxide-based combination therapies for the treatment of ART-R parasites.

896

THE SUBSTANDARD ARTEMISININ EPIDEMIC - ACCELERATING RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA?

Matthew R. Hassett, Paul D. Roepe

Georgetown University, Washington, DC, United States

The acceleration of antimicrobial resistance (AMR) is often hypothesized to be, in part, an unfortunate consequence of more frequent use of subtherapeutic dosages of drugs. In 2017, the World Health Organization (WHO) estimated that nearly 1 in 10 medical products tested in low- and middle-income countries failed to meet quality standards. Artemisinin-based combination therapies (ACTs) are currently the frontline treatment for malaria infections recommended by WHO, and currently our last line of defense to effectively combat malaria in the clinic. Troublingly, use of substandard ACTs has been well-documented over the last decade. Perhaps relatedly, there has been an increasing number of cases of delayed clearance of parasites in patients who were treated with ACTs, suggesting decreasing ACT efficacy. Effectively understanding any link between substandard adherence and/or dosing of drug and subsequent proliferation of ACT resistant parasites is critical for future treatment and containment of ACT resistance. We have developed a tissue culture-based approach for testing possible connections between substandard drug use and the spread of ACT resistance. Via sequencing of *Pfkelch13*, a molecular marker that is predictive for artemisinin resistance in *Plasmodium falciparum*, we are able to monitor competition of mixed populations of sensitive and resistant strains over time and under various conditions. We will present data related to subtherapeutic drug use that may promote spread of ACT resistance in *P. falciparum* malaria.

897

ANALYSIS OF PIPERAQUINE TRANSPORT BY *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER ISOFORMS HETEROLOGOUSLY EXPRESSED IN *S. CEREVISIAE* YEAST

Bryce E. Riegel, Paul D. Roepe

Georgetown University, Washington, DC, United States

Emerging resistance of *Plasmodium falciparum* malaria to artemisinin derivatives and partner drugs in Southeast Asia represents a major health challenge. Piperaquine (PPQ) is a bisquinoline antimalarial drug that is used as a partner drug with dihydroartemisinin (DHA) in artemisinin combination therapy (ACT). DHA/PPQ (Artekin, Eurartesim) has been recommended by WHO and widely used since 2009. However, evolving resistance to both PPQ (PPQR) and DHA has been reported in South East Asia. Resistance to the former front-line quinoline drug chloroquine (CQ), as well as to other quinoline - based compounds, is caused by multiple mutations in the *P. falciparum* chloroquine resistance transporter (PfCRT). While multiple markers for PPQR have been reported - including amplification of plasmepsins 2 and 3, a non-synonymous SNP in Exonuclease1, and several mutations in PfCRT - a better understanding of the mechanism(s) of resistance is needed. Here we have tested whether specific PfCRT mutations recently detected in *P. falciparum* Cambodian isolates contribute to PPQR. Using a galactose inducible expression system, we monitored PPQ transport in *S. cerevisiae* yeast expressing PfCRT at the plasma membrane. Elevated levels of PPQ transport were found for yeast expressing multiple PPQR-associated PfCRT isoforms relative to wild type, suggesting these mutations in PfCRT promote altered PPQ transport and contribute to PPQR.

898

USING A SMALL FLUORESCENT PROBE TO MEASURE REDOX POTENTIAL IN THE *PLASMODIUM FALCIPARUM* DIGESTIVE VACUOLE

Andreas V. Willems, Paul D. Roepe

Georgetown University, Washington, DC, United States

The recent emergence of artemisinin (ART) resistance in *Plasmodium falciparum* malarial parasites is of major concern and has focused attention on the mechanism of action of ART - based drugs. During the intraerythrocytic stage of parasite development, copious amounts of free ferriprotoporphyrin IX (FPIX) heme is released upon hemoglobin catabolism within the parasite digestive vacuole (DV). Recently, we have shown that ART-FPIX adducts are formed within ART treated parasites, and that the abundance of these toxic adducts is altered for ART resistant *P. falciparum*. Adduct formation is dependent upon glutathione (GSH) balance within the DV. For these reasons we have synthesized novel fluorescent small molecule GSH probes and have endeavored to localize them specifically to the DV using our previously reported approach. With specific localization we are able to use these methods to compare the redox environment within the DV of ARTS vs ARTR *P. falciparum.* Data quantifying DV [GSH] will further elucidate the mechanism of ART - based drug action and ART resistance.

899

IN VIVO EFFICACY AND FREQUENCY OF MOLECULAR MARKERS OF RESISTANCE OF *P. FALCIPARUM* TO SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE IN BOUGOUNI, MALI AND HOUNDÉ, BURKINA FASO

Issaka Sagara[1], Issaka Zongo[2], Irene Kuepfer[3], Matthew Cairns[3], Modibo Diarra[1], Amadou Barry[1], Frederic Nikiema[2], Amadou Tapily[1], Samba Coumaré[1], Ismaila Thera[1], JeanBosco Ouedraogo[4], Paul Milligan[3], Daniel Chandrahoman[3], Abdoulaye Djimde[1], Brian Greenwood[3], Alassane Dicko[1]

[1]*Malaria Research and Training Center/University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, Bobo Dioulasso, Burkina Faso,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso*

Seasonal malaria chemoprevention (SMC) is being implemented widely in Sahelian countries. The World Health Organization recommends continued monitoring of drug resistance in areas where SMC is being deployed. As part of the large trial of the SMC with or without Azithromycin, we assessed the frequency of the molecular markers of resistance of *P. falciparum* to Sulfadoxine-Pyrimethamine (SP) and Amodiaquine (AQ) in children after one and three years of SMC and the *in vivo* response of *P. falciparum* to SP and AQ after three years of SMC in Bougouni, Mali and Hounde in Burkina Faso. In 2016, after three years of implementation of SMC, the PCR uncorrected proportion of adequate clinical and parasitological response (ACPR) was 94.8% (95% CI 85.0 - 98.3) in Burkina and 96.1% (95% CI 91.5 - 98.2) in Mali. After PCR correction the ACPR was 99.3% (95% CI 95.4 - 99.9) in Mali and remained unchanged in Burkina. The frequency of the three mutations dhfr-59I + dhps-437G + dhps-540E associated with resistance to SP in Mali was 0.8% (1/125) in 2014 and 4.2% (5/120) in 2016, p =0.09. The corresponding figures in Burkina Faso were 0 % (0/61) in 2014 and 1.5% (2/134) in 2016 p =0.34. Similarly, the frequency of the double mutation (Pfcrt-76T + pfmdr1-86Y), associated with resistance to AQ, remained similar in both countries; 16.3% (20/123) in 2014 and 12.8% (15/117) in 2016 in Mali p =0.44, while in Burkina double mutations were detected in 6.8% (4/59) children in 2014, but not seen in 113 children in 2016. The frequency of these

markers in schoolchildren aged 6-12 years in the same villages were similar to those seen in the study children. In summary, SMC implementation was not associated with evidence of an increase in frequencies of molecular markers of resistance to SP and AQ or a reduced 28 days *in vivo* efficacy after three years of the SMC.

900

GUT MICROBIOME PREDICTS LUMEFANTRINE PHARMACOKINETICS IN HEALTHY MICE

Matthew M. Ippolito[1], Joshua Denny[2], Elizabeth Nenortas[1], Theresa A. Shapiro[1], Nathan Schmidt[2]

[1]*Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*University of Louisville, Louisville, KY, United States*

The antimalarial drug lumefantrine exhibits highly variable pharmacokinetics—absorption, distribution, and clearance—between individuals with up to 16-fold differences in drug exposure. Differences are due, in part, to poor absorption which is mitigated by administration with fatty food. We hypothesized that the intestinal microbiome may also play an important role in lumefantrine disposition. Four cohorts of isogenic mice from different vendors (n=24) previously shown to harbor distinctive enterotypes were administered a humanized dose of lumefantrine (150 μg/g) by gavage. Gut microbiome was characterized by 16s rRNA amplicon sequencing of fecal pellets and enterotypes were classified according to β-diversity using weighted UniFrac distance. Plasma was collected at 0, 0.5, 1.5, 10, and 24 h post dose for drug analyte quantitation by liquid chromatography tandem mass spectrometry. Drug exposure measured as maximal concentration (C_{max}) and area under the drug concentration-time curve (AUC_{0-24}) was estimated by the linear-log trapezoidal method in non-compartmental analyses. Oral clearance (Cl/F) and apparent volume of distribution (Vd/F) were estimated using covariate analysis of enterotype with stepwise selection in population-based compartmental models. Four discrete enterotypes were identified. Enterotypes 1 and 2 had significantly greater taxonomic abundance than 3 and 4, and β-diversity plots showed clustering of the same pairs. Mice with enterotypes 1 and 2 had higher lumefantrine exposure than enterotypes 3 and 4 (C_{max} = 542 and 768 vs. 392 and 380 ng/ml, $P \leq 0.04$ for pairwise comparisons; AUC_{0-24} = 511,000 and 657,000 vs. 344,000 and 333,000 ng*h/ml, $P \leq 0.02$). Results of compartmental analyses suggest that enterotype-related differences in Cl/F but not Vd/F correlated with drug exposure. The gut microbiome has previously been shown to contribute to inter-individual variation in metabolism of orally administered drugs. Here, we demonstrate that gut microbiome differences may partially account for variation in lumefantrine exposure.

901

EXPANSION OF CHLOROQUINE SENSITIVE HAPLOTYPES IN THE *PLASMODIUM FALCIPARUM* RESERVOIR IN BONGO DISTRICT, GHANA

Charles A. Narh[1], Kathryn E. Tiedje[1], Michael F. Duffy[1], Anita Ghansah[2], Abraham R. Oduro[3], Kwadwo A. Koram[2], Karen P. Day[1]

[1]*School of Bioscience/Bio*[21] *Institute, The University of Melbourne, Melbourne, Australia,* [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana,* [3]*Navrongo Health Research Centre, Navrongo, Ghana*

In the early 2000s widespread resistance to chloroquine (CQ) prompted Ghana to change its first-line treatment policy to artemisinin-based combination therapies (ACTs) for uncomplicated *P. falciparum* malaria. Following this withdrawal of CQ, ongoing surveillance of clinical infections in children has shown that sensitivity to CQ has rebounded. In Ghana, the genomic signatures suggest that ACTs, artesunate-amodiaquine and artemether-lumefantrine, exert differential selection at the two drug transporter genes, *Pfcrt* and *Pfmdr1*. What is happening to the spread of drug-resistance–associated mutations in the large reservoir of asymptomatic infections has been poorly studied. This study set out to monitor this neglected reservoir in Bongo District, Ghana at the end of the 2012 dry season by genotyping the *Pfcrt* and *Pfmdr1* mutations associated with chloroquine (CQ) resistance and the microsatellite *loci* flanking these genes. For the isolates sequenced at *Pfcrt* (N=170) and *Pfmdr1* (N=198), the majority carried CQ-sensitive haplotypes, specifically *Pfcrt* CVMNK (88.2%) and/or *Pfmdr1* NY (17.7%) or *Pfmdr1*NF (69.7%). To investigate selection of these haplotypes, isolates with single genomes were typed at 11 microsatellite *loci* flanking *Pfcrt* (N=55) and *Pfmdr1*(N=46). For *Pfcrt*, 93.8% of isolates with the CQ sensitive CVMNK haplotype (N=48) had unique multilocus microsatellite haplotypes that were diverse (*He* = 0.75). This result indicates that CVMNK has expanded in the *P. falciparum* reservoir via soft selective sweeps from genetically diverse parasites. For *Pfmdr1* NY (N=8) and NF (N=35), all isolates had unique multilocus microsatellite haplotypes, and there was no significant difference in the *He* (NY: 0.85, NF: 0.83), suggestive of equal selective advantage. We have shown that this asymptomatic *P. falciparum* reservoir, which has limited exposure to antimalarial drugs during the dry season, harboured highly diverse *Pfcrt* and *Pfmdr1* haplotypes. The change in treatment policy to ACTs has influenced this underlying diversity and as such it is important to prioritize this reservoir during monitoring programs to inform policy.

902

LABORATORY EVALUATION OF INTRODUCED PROCEDURAL ERRORS ON MALARIA RAPID DIAGNOSTIC TEST PERFORMANCE

Christina M. Carlson, Yong Ah, Scott Wilson, Jeffrey A. Glenn, Michael Aidoo

Centers for Disease Control and Prevention, Atlanta, GA, United States

Malaria rapid diagnostic tests (RDTs) account for ~75% of all malaria testing in sub-Saharan Africa and are often used by community health workers or other health workers in peripheral facilities. These workers are often trained using job aids extracted from manufacturer's instructions for use (IFU). Imperfect compliance with IFUs could lead to procedural errors during RDT administration, with unknown consequences on diagnostic result accuracy. We aimed to systematically determine the consequences of procedural errors on RDT outcome in a laboratory setting. Cultured samples containing *Plasmodium falciparum (Pf)* parasites at 1,000, 200, and 50 parasites/μL (5 μL total volume) were tested with 6 separate RDT products that met the WHO RDT procurement criteria and target either 1) *Pf*-specific HRP2 and the pan-malaria pLDH antigens or 2) *Pf*-specific HRP2 and *P. vivax*-specific pLDH antigens. Results of RDTs conducted according to IFUs were compared to results of the same products for which the following errors were deliberately introduced: higher and lower buffer volumes and extended and shortened incubation times. Common consequences of introduced errors on RDT performance across tested products included incomplete blood clearing or red background resulting from shortened incubation times or excessive buffer volume. For some tests at lower parasite density, poor blood clearing obscured weak positive test lines and could produce a false-negative test result. For 3 of 6 products tested, insufficient buffer volume resulted in failed migration of sample across the length of the test strip, producing an invalid test result. For the same 3 products, extended incubation time and/or the combination of extended incubation time and increased buffer volume improved test result reads from tests run as per IFU. Minor incubation time and buffer volume deviations from IFU can result in RDT performance anomalies that may lead to incorrect test result interpretation and reporting, with serious implications for patient care and the success of malaria control programs. Efforts should be made to communicate product and IFU changes to non-laboratory users.

903

DIAGNOSTIC PERFORMANCE OF ULTRA-SENSITIVE RAPID DIAGNOSTIC TESTS FOR MALARIA IN PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN WESTERN KENYA

Aaron M. Samuels[1], Oliver Towett[2], Brian Seda[2], Kelvin Onoka[2], Kephas Otieno[2], Winnie Chebore[2], Kammerle Schneider[3], Patrick Walker[4], Titus Kwambai[2], Meghna R. Desai[5], Laurence Slutsker[3], Simon K. Kariuki[2], Feiko ter Kuile[6]

[1]*Centers for Disease Control and Prevention, DPO, AE, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*PATH, Seattle, WA, United States,* [4]*Imperial College, London, United Kingdom,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

In settings with moderate to high malaria transmission, infection with *Plasmodium falciparum* can cause adverse outcomes in pregnant women and their fetuses, such as maternal anemia and low birthweight neonates. In these settings, infections during pregnancy may have a low density of parasites or associated antigens in peripheral blood; particularly amongst multi-gravid women. Such infections are more likely to be below the detection limit of blood smear microscopy (BS) and standard rapid diagnostic tests (RDTs). Diagnostic tests with increased sensitivity, and subsequent treatment, may prevent adverse pregnancy outcomes. In the high transmission setting of western Kenya, we compared the diagnostic characteristics of expert BS, First Response Malaria Ag. (pLDH/HRP2) Combo RDTs (RDT), and the ultra-sensitive Alere™ Malaria Ag P.f. RDT (uRDT), using polymerase-chain reaction (PCR) as the gold standard for detection of *P. falciparum* infections in women attending their first antenatal clinic (ANC) visit in 9 ANC clinics. From May-September, 2018, 488 individual women attended these clinics for their first ANC visits and 483 had all 4 tests performed. The median parasite density per μL determined by qPCR was 148 (Interquartile range: 11-1260); 122 (25.2%) were positive by PCR; 105 (21.7%) were positive by uRDT; 96 (19.9%) were positive by RDT; and 66 (13.7%) were positive by BS. Compared to PCR, the sensitivity and specificity for BS were 52.5% (95% Confidence Interval [CI]: 43.2-61.6) and 99.5% (CI: 98.0-100); RDT 63.1% (CI: 53.9-71.7) and 94.7% (CI: 91.9-96.8), and uRDT 69.7% (CI: 60.7-77.7) and 94.5% (CI: 91.6-96.6). When stratified by gravidity, the sensitivity of RDTs and uRDTs in pauci-gravidae (primi- and secondi-gravidae) were 69.9% (CI: 58.0-80.1) and 79.5% (CI: 68.4-88.0), respectively. In multi-gravid women, the sensitivity of RDTs and uRDTs were 53.1 (CI: 38.3-67.5) and 55.1% (CI: 40.2-69.3), respectively. In this setting, the performance of uRDTs in pregnant women at first ANC visit was marginally better than that of a standard RDT. Evaluation of other more sensitive point-of-care tests in the ANC setting should be considered.

904

INTEGRATING VERTICAL AND LATERAL FLOW ASSAYS FOR IMPROVED DIAGNOSIS OF ASYMPTOMATIC MALARIA INFECTIONS

Carson P. Moore, Nathaniel Z. Piety, David W. Wright

Vanderbilt University, Nashville, TN, United States

A major challenge for malaria control and elimination programs is sensitive and specific diagnosis of patients within the asymptomatic transmission reservoir. Because many commercially available rapid diagnostic tests (RDTs) have detection limits around 200 parasites/μL, low-level asymptomatic patients can be missed during point-of-care (POC) diagnosis. These patients have been reported as having average parasite burdens as low as 5 parasites/μL in some endemic settings. In order to detect these infections, the limit of detection of available tests must be improved. We report a method to increase the signal generated on malaria RDTs by utilizing a vertical flow sample preparation method consisting of a metal functionalized cellulose membrane in tandem with a lateral flow assay. This method allows for extraction and enrichment of both primary biomarkers of malaria: *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) and *Plasmodium* lactate dehydrogenase (*P*LDH). Integration of the proposed enrichment and enhancement system allows for concentration of the antigens from large sample volumes directly onto POC-ready RDTs. This system exploits the ease of functionalization of cellulose and the innate affinity between the histidine residues of *Pf*HRP2 and immobilized divalent metal ions. The functionalization of these membranes with chelating ligands and divalent metalions was first optimized for the capture of *Pf*HRP2. More than 99% of the *Pf*HRP2 was extracted from a 250 μL blood sample in a simple flow-through assay and 89% of the captured protein was eluted from the membrane using ethylenediaminetetraacetic acid. Use of this enhancement protocol on an in-house HRP2 lateral flow assay (LFA) yielded a limit of detection of 3 parasites/μL. A histidine-rich *P*LDH-specific capture agent (*P*CA) was then developed to allow for capture of both *P*LDH and *Pf*HRP2 on the surface of the modified membrane. Using the novel *P*CA, 80% of *P*LDH was immobilized on the membrane. Future work will include optimization of *P*LDH elution from the membrane and integration with a PLDH-specific LFA for point-of-care detection of both biomarkers.

905

ATYPICAL *PLASMODIUM FALCIPARUM* SEEN IN GIEMSA-STAINED SMEAR

Mamadou Alpha Diallo, **Ndeye Anna Seck**, Khadim Diongue, Mouhamadou Ndiaye, Aida Sadikh Badiane, Mame Cheikh Seck, Daouda Ndiaye

Cheikh Anta Diop University, Dakar, Senegal

A 4-year-old boy was admitted to a health post of Kedougou, Senegal, for fever, headache and vomiting. As the patient was suspected of uncomplicated malaria he was tested using an HRP-2 based RDT which came out positive for *Plasmodium falciparum*. Giemsa-stained thick and thin smear was then performed. However, the microscopic examination showed *Plasmodium*-infected erythrocytes that have features of both *P. falciparum* and *P. ovale* - both of which are endemic in Senegal. This could lead to misdiagnosis for an inexperienced microscopist. In fact, it was observed that many of infected red blood cells (RBCs) were oval in shape with fimbriated edges which is consistent with *P. ovale*. For differential diagnostic, additional details were observed; the presence of Maurer clefts alone was indicative of *P. falciparum*. In addition, the smear was monotonous with high parasitemia. The diagnosis of *P. falciparum* malaria was confirmed by PCR; no other malarial species were associated. The patient was treated effectively with artemether/lumefantrine and he had significant clinical improvement over the next three days. The diagnosis of *P. falciparum* is usually made by microscopy, demonstrating the presence of young trophozoites in Giemsa-stained thick and thin films. Infected red blood cells (RBCs) are usually normal in size with no significant change. *P. falciparum* rings are typically small with regular small and curved cytoplasm. In well-stained thin films Maurer clefts may be observed in RBC infected with mature trophozoites. In some cases mature trophozoites of *P. falciparum* may cause red blood cell deformation which may lead to confusion with another species. Here, the unusual elongated infected RBCs resembled the ones of *P. ovale* infection. Maurer's clefts may resemble the Schüffner's dots seen in *P. vivax* and *P. ovale*, but are usually larger and more coarse. Additionally, Maurer's clefts in this case are few in number and do not cover the whole infected RBC as it should be seen in *P. ovale* and *P. vivax*. Accurate identification of the *Plasmodium* species may be difficult or uncertain due to variations in morphological characteristics within *P. falciparum* specie.

906

IMPROVEMENT OF MALARIA DIAGNOSIS THROUGH OUTREACH TRAINING AND SUPPORTIVE SUPERVISION (OTSS) IN BENIN FROM 2015 TO 2018

Augustin Kpemasse

National Malaria Control Program, Cotonou, Benin

Malaria is the leading cause of morbidity and mortality in Benin. In 2015, the National Malaria Control Program (NMCP), with the support of the

U.S. President's Malaria Initiative (PMI), launched Outreach Training and Supportive Supervision (OTSS) to improve malaria microscopy diagnostics in Benin. A total of 123 health facility laboratories were enrolled into the OTSS scheme. OTSS consisted of 1) updating national malaria microscopy guidelines to reflect World Health Organization recommendations and 2) expert microscopists performing semiannual supervision in all enrolled health facilities. Every six months, OTSS supervision teams verified the availability of laboratory equipment, supplies, and consumables in all enrolled facilities, performed quality control assessments of a random sample of stored slides from the facility through a double reading process, and made recommendations for improvement. Each supervision visit was followed with close monitoring of the implementation of recommendations. From 2015 to 2018 significant improvements in OTSS indicators occurred. The percentage of functional microscopes in enrolled laboratories increased from 64% to 70%. The percentage of health facilities with stock-outs in methanol decreased from 23% to 3%. The overall sensitivities for all participants against expert microscopists increased from 75% to 92% and the overall specificities increased from 89% to 100%. In addition, the rate of correct identification of *Plasmodium falciparum* species rose from 82% to 100%. OTSS improved the quality of malaria diagnosis by microscopy through the rigorous monitoring of adherence to protocols, standards, and guidelines. OTSS also helped enhance provider performance. OTSS should be maintained and scaled-up to maintain a high level performance of malaria microscopy in Benin.

907

A SYSTEMATIC REVIEW OF NOVEL BIOMARKERS FOR MALARIA DIAGNOSIS

Ewurama D. Owusu, Augustina Frimpong, **Seda Yerlikaya**, Xavier Ding

FIND, Geneva, Switzerland

Prompt diagnosis and treatment of malaria have consistently been shown to be the most effective way of preventing an uncomplicated case of malaria from developing into a severe one. Increasingly the most common diagnostic tests used to detect malaria among suspected patients are rapid diagnostic tests (RDT) which rely on the identification of parasite-specific biomarkers in bodily fluids like blood and urine. Plasmodial antigens histidine-rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase and aldolase are the three traditional biomarkers currently in use, but there are challenges with their clinical performance in the context of changing malaria epidemiology. Efforts to identify novel biomarkers are ongoing for diagnostic development a. We report here the results of a systematic review of published data on biomarker candidates for malaria diagnosis and provide, for the first time, a comprehensive analysis of the current landscape of biomarkers with the potential to overcome the limitations of HRP2 and pLDH. A comprehensive search term was composed and used to perform a systematic search of MEDLINE, EMBASE, Web of Science, and CENTRAL databases, as well as grey literature to identify original research, which report on biomarker candidates for malaria diagnosis. After removing duplicates, 4103 records remained for screening. Data from studies eligible for inclusion have been extracted and sorted by pre-defined variables, providing a comparative analysis of malaria biomarker candidates and identifying those well-suited for the development of novel diagnostic tests for uncomplicated malaria diagnosis based on nature and origin of biomarker, diagnostic developmental stage, and method/sample type used for detection. This study will enable a comprehensive database of malaria biomarker candidates and help synergize product development efforts globally.

908

EVALUATION OF THE PERFORMANCE OF SD-BIOLINE MALARIA RAPID DIAGNOSTIC TEST CARRIED OUT BY COMMUNITY MEDICINE DISTRIBUTORS AGAINST MICROSCOPY AND PCR IN THE DIAGNOSIS OF MALARIA IN SOUTHWEST NIGERIA

Catherine Olufunke Falade[1], IkeOluwapo Oyeneye Ajayi[1], Ayodele Samuel Jegede[1], Chinenye Afonne[1], Tolulope Ogunsesin[1], Roland I. Ibenipere Funwei[1], Olusola Ojurongbe[2], Jan Singlovic[3], Melba Gomes[3]

[1]*University of Ibadan, Ibadan Nigeria, Ibadan, Nigeria,* [2]*Ladoke Akintola University of Technology, Oshogbo, Nigeria,* [3]*UNICEF/UNDP/World Bank/WHO/Special Programme for Researchmand Training in Tropical Disease, World Health Organization, Geneva, Switzerland*

The WHO recommends that malaria diagnosis be parasite-based as much as possible. Malaria rapid diagnostic test (MRDT) is the preferred option for programmatic use. This study set out to evaluate the performance of SD-Bioline™, an HRP-II based MRDT performed by trained voluntary community medicine distributors (CMD) during a pilot study of parasite-based diagnosis of malaria in a rural community in south west Nigeria, where malaria transmission is intense. MRDT, thick blood smears and dried blood spots (DBS) on filter paper were performed from finger prick blood samples during a cross-sectional study of febrile children aged 3 to 59 months presenting to CMD in Ona-Ara Local Government Area, Southwest Nigeria. MRDT was read at study site by trained CMDs according to manufacturer's instructions. Blood smears and DBS were processed in the laboratory using standard procedures. More than half, (52.4%; 547) of 1043 enrollees were females. The mean age of the study participants was 17.86 ±10.6 months (range 3-59 months). Malaria parasite was detected in 821 (78.7%) by MRDT, 574 (55.0%) by microscopy and 721 (69.18%) by PCR. Over 90% of the blood smears were readable. The Geometric mean parasite density was 6,735/μL; range: 40 - 317,200/μL. The sensitivity, specificity, PPV. NPV, accuracy and κ-value for SD-Bioline MRDT using microscopy as gold standard were 92.5%, 38.2%, 64.8%, 80.6%, 68.15 and 0.322 respectively. Corresponding figures when PCR was used as the gold standard were 88.2%, 42.6%, 88.2%, 61.7% 74.0% and 0.336 respectively. When microscopy was compared to PCR, corresponding values were 93.9%, 61.6%, 79.8%, 89.4%. 79.2% and 0.567 respectively. Speciation revealed that *Plasmodium falciparum* constituted 97.8% (707/721) of detected malaria parasites while *P. malariae constituted* 4.4% (32/721) and *P. ovale,* 2.5% (18/721). *P. malariae* and *P. ovale* occurred as co-infections with *P. falciparum* in 71.9% (23/32) and 72.2% (13/18) of cases. *P. vivax* was not detected. Trained community medicine distributors successfully performed MRDT, Blood smears and DBS thus providing parasite-based diagnosis of malaria at the community level.

909

ASSESSMENT OF COMPETENCE OF PARTICIPANTS BEFORE AND AFTER 6-DAY INTENSIVE MALARIA MICROSCOPY TRAINING IN RWANDA

Noella Umulisa[1], Veneranda Umubyeyi[1], Tharcisse Munyaneza[2], Ruzindana Emmanuel[2], Aline Uwimana[3], Stephen Mutwiwa[1], Aimable Mbituyumuremyi[3]

[1]*Maternal and Child Survival Program/Jhpiego, Kigali, Rwanda,* [2]*National Reference Laboratory (NRL), Rwanda Biomedical Centre (RBC), Kigali, Rwanda,* [3]*Malaria and Other Parasitic Diseases Division (Mal & OPDD), Rwanda Biomedical Centre (RBC), Kigali, Rwanda*

Microscopic diagnosis of Giemsa stained thick and thin blood films by skilled microscopists has remained the gold-standard laboratory method for the diagnosis of malaria. Sufficient training of laboratory staff is paramount for the correct microscopic diagnosis of malaria. The aim of this assessment was to evaluate laboratory technicians' performance diagnosing malaria by microscopy at health facility level within 6 districts in Rwanda. In May 2018, the Maternal and Child Survival Program (MCSP),

in collaboration with the Rwanda Biomedical Center (RBC), conducted a 6-day malaria microscopy training for laboratory technicians from 75 health facilities located in six districts in Rwanda. The training puts emphasis on practical compared to theoretical sessions when determining parasite density and detection of malaria species. In August 2018, the MCSP with RBC conducted a follow-up assessment. Of the 75 technicians trained, 35 were randomly chosen and assessed at their respective health facilities. Assessment teams distributed standardized pre-validated slide-panels to each technician. The laboratory technicians were requested to report parasite densities using the semi-quantitative (+) method. During the training, a significant improvement was found between pre and post-tests scores, with median scores improving from 52% to 85%. The overall post-training sensitivity and specificity of laboratory technicians in detecting malaria parasites were 99.6% and 85.16% respectively while positive predictive value and negative predictive value were 98.5% and 95.8% respectively. Species identification and parasite quantification accuracy were 90% and 88% respectively. The percent agreement between laboratory professionals and expert microscopists in the detection of malaria parasites was 90.7% with a Kappa index of 0.89. This assessment demonstrates laboratory technicians' satisfactory performance concerning parasite species and density detection while illustrating the importance of continuous capacity building of laboratory technicians to ensure accurate diagnosis for the appropriate treatment of malaria.

910

PERFORMANCE OF A HIGH SENSITIVITY MUSE® MALARIA *P.F.-P.V.* DETECTION ASSAY IN A STUDY IN LAGOS, NIGERIA

Wellington Oyibo[1], Chinonye Anabike[1], Bummi Olalekan[1], Ginika Onuachwusi[1], Adeyanju Adeyinka[1], Gracemary Ndidium[1], Uche Igbasi[1], Julie Clor[2], James Mulry[3], **Kamala Tyagarajan**[2]

[1]*ANDI Centre of Excellence for Malaria Diagnosis, Lagos, Nigeria,* [2]*Luminex Corporation, Hayward, CA, United States,* [3]*Merck Global Health Institute, Germany, Germany*

Effective malaria diagnosis is critical in the implementation of current malaria case management strategies. The need for development of high sensitivity detection methods which also provide typing of malarial parasites has been amplified. While microscopy is considered a gold standard, it requires a high degree of operator expertise and training to provide reliable and sensitive results. A new method has recently emerged, which uses microcapillary cytometry on the low cost Muse® Cell Analyzer along with the Muse® Malaria *P.f.-P.v.* Detection Assay. The assay uses multiplexing to confirm the presence of malarial parasite antigens by detecting *P.f.* HRP2, *P.f.* LDH, and *P.v.* LDH antigens in parallel with high detection sensitivity down to a few parasites/μL. In this study, we evaluated the research use only Muse Malaria *P.f.-P.v.* Detection Assay with fresh EDTA-whole blood samples in our laboratory in Lagos, Nigeria. In total, 62 adult and pediatric samples with parasitemia of 19-450,000 parasites/μL were examined. Data on the samples was obtained using three methods: malaria microscopy of Giemsa-stained blood films; malaria RDT (SD Bioline P.f/Pan); and the Muse Malaria *P.f.-P.v.* Detection Assay. Comparison of results from the Muse Assay demonstrated excellent agreement with expert microscopy as well as RDTs. Clear shifts were observed from low to high parasitemia, making interpretation simple. Compared to microscopy, the sensitivity of the assay was 100% and the specificity of the assay was 95.24% for the limited sample set. It was noted that while Muse HRP2 and RDT HRP2 test results were similar to microscopy, pLDH RDT results were much less sensitive compared to microscopy. Muse *P.f.* LDH results showed correspondence to microscopy results. The assay with dual high sensitivity *P. falciparum* markers provided high confidence when analyzing *P. falciparum* samples, and also ensured that *P. vivax* LDH antigens were not being missed. The Muse Malaria *P.f.-P.v.* Detection Assay can be a valuable high sensitivity tool in research laboratories to ensure *Plasmodium* antigens are appropriately detected and classified.

911

DIAGNOSIS OF RED CELL GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN BURKINA FASO: COMPARISON OF QUANTITATIVE AND QUALITATIVE TESTS

Edith C. Bougouma[1], Emelie Badoum[2], Sam Coulibaly[1], Samuel Serme[1], Issiaka Soulama[1], Alphonse Ouedraogo[1], Alfred B. Tiono[1], Sodiomon B. Sirima[1]

[1]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [2]*Université de Ouagadougou, Ouagadougou, Burkina Faso*

G6PD deficiency is the most widespread enzyme defect that can result in red cell breakdown under oxidative stress when exposed to certain medicines including antimalarials. Development of reliable, easy-to-use, rapid diagnostic tests (RDTs) to detect G6PD deficiency at point of care is essential to deploying primaquine therapies as part of malaria elimination strategies We compared CareStart™ test kits for G6PD deficiency screening test (CSG) and G6PD BinaxNow text kits to by comparing its performance to quantitative G6PD enzyme activity using a Fluorescent spot test (FST) a quantitative G6PD enzyme activity test and colorimetric method. The study took place in Banfora, Participant with G6PD deficiency status was checked in study participants in the field conditions (capillary blood: CBS) with Diagnostic CSG Test then invited to the laboratory where venous blood samples (VBS) were collected and tested for G6PD deficiency two qualitative G6PD RDTs Tests (CSG and BinaxNow) and semi quantitative method (FST). Results were compared with the quantitative spectrophotometric analysis of G6PD activity. Comparison was also done between CBS and VBS. Sensitivity (SE) of CSG (97.14%), BinaxNow (97.06%) and FST (97.7%) tested with venous blood were comparable ($p>0.05$).The specificity (SP) scores for the three tests (CSG, BinaxNow and FST) compared to Colorimétric were 53.70%, 52.72% and 59.18% respectively. When the CSG tests were performed on capillary blood samples or on venous blood samples, SE of CSG was not comparable ($p< 0.05$). In conclusion, all the three test formats had a highest SE when performed on VBS and was comparable to the Colorimetric. The FST had the highest SP among all tests formats. The performance and operational characteristics of the CSG performed on VBS suggest the test to be a good alternative to the Semi quantitative and quantitative where basic laboratory equipment are lacking. Our observations underscore the need for improved CSG test sensitivity using CBS. Ensuring safe use of 8-aminoquinolines depends on highly sensitive and specific G6PD deficiency diagnostic tests suitable for routine use in the field.

912

PERFORMANCE OF THE PFHRP2 BASED RAPID DIAGNOSTIC TEST (CARESTART™) IN THE DETECTION OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN BOUGOUNI, MALI

Modibo Diarra[1], Amadou Tapily[1], Issaka Sagara[1], Hama Yalcouyé[1], Amadou Barry[1], Aly Tiama[1], Seydou Goro[1], Samba Coumaré[1], Mohamed Koné[1], Ismaila Thera[1], Irene Kuepfer[2], Matthew Cairns[2], Paul Milligan[2], Daniel Chandrahoman[2], Brian Greenwood[2], Alassane Dicko[1]

[1]*Malaria Research and Trainig Center/University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Rapid Diagnostic Tests are used routinely for the diagnosis of clinical malaria by health care workers. However, their ability to detect asymptomatic *Plasmodium falciparum* infection has not been adequately studied in endemic areas. Here we assessed the performance of a PfHRP2 based Rapid Diagnostic Test (RDT) CareStarT^{M} for the detection of asymptomatic malaria infections in Bougouni, Mali. A total of 2001 children, aged 6 to 59 months enrolled in a large trial of seasonal malaria chemoprevention (SMC) with or without Azithromycin were surveyed in December 2016, 4-6 weeks after the last SMC cycle. RDT were undertaken and blood smears collected from all children. Overall, 10.1% (203/2001) of children had a positive malaria blood smear and 16.8% (337/2001) had

a positive RDT. Compared to blood smear, the sensitivity of RDT was low 69.9 % (95% CI 63.6%- 84.9%) while the specificity was high 88.9%, (95% CI: 87.4%- 94.9%). The negative predictive value was high 96.3% (95% CI: 95.3% -100.0%) while the positive predictive value was low 42.1% (95% CI: 36.8%- 50.4%). The PfHRP2 based Rapid Diagnostic Test CareStarT^{M} test has good specificity and good negative predictive value for the detection of asymptomatic malaria, but the sensitivity and the positive predictive values were low. More sensitive RDTs are needed for the detection of asymptomatic malaria to support the malaria elimination initiative in Sahelian countries.

913

MALARIA PRESCRIBING PRACTICES AT AN URBAN HEALTH CENTER IN KUMASI, GHANA

Mariah Owusu-Agyei[1], Ernest Adjei[2], Christian K. Addai[2], Michael K. Addei[2], Peter K. Brenya[2], Roland Abbey[2], Tsiri Agbenyega[3], Michael Cappello[4]

[1]*University of Pennsylvania, Philadelphia, PA, United States,* [2]*HopeXchange Medical Centre, Kumasi, Ghana,* [3]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [4]*Yale University, New Haven, CT, United States*

Up to 90% of patients presenting with fever to healthcare centers in sub-Saharan Africa are treated presumptively for malaria. Since overtreatment could lead to antimalarial drug resistance, the WHO launched the "Test-Treat-Track (T3)" initiative to encourage diagnostic testing for every suspected case of malaria prior to treatment. This study aimed to characterize the diagnostic features and prescribing practices for malaria at HopeXchange Medical Center, a recently established public-private facility in Kumasi, Ghana (pop. 2.06 million). We conducted a retrospective chart review of patients prescribed antimalarials between January 2017-December 2018. Records were reviewed for malaria test results, fever, and co-treatment with antibiotics. Data were analyzed using descriptive statistics and Chi-square tests, followed by logistic regression modelling to assess factors associated with diagnostic testing . Over 24 months, 5040 patients were prescribed antimalarials. Among this group, 22.7% (1145/5040) had fever at presentation and 63.7% (3208/5040) had a diagnostic test performed. Treated patients were more likely to have a malaria diagnostic test performed if they were younger, admitted to the inpatient service, febrile, and covered by insurance. Inpatient status was the strongest predictor of receiving a malaria test (Odds Ratio=4.99 [3.88,6.42]). Among patients tested for malaria, 68.9% (2209/3208) had a positive result, while the remainder were treated despite testing negative. In addition, 38.7% (1950/5040) of patients were co-treated with antibiotics. The number of patients who received treatment in the absence of a positive diagnostic test result is high (56.2%), suggesting that clinicians frequently rely on clinical signs and symptoms to guide therapeutic decisions for malaria. However, a minority of treated patients presented with fever, suggesting that other clinical features drive decision making at HopeXchange. Further study of prescribing practices is needed in order to define barriers to WHO guideline adherence in the management of malaria in high-transmission urban settings.

914

EVALUATING SEROLOGY-BASED RAPID DIAGNOSTIC TESTS AS A TOOL TO IMPROVE *PLASMODIUM FALCIPARUM* SURVEILLANCE IN LOW-TRANSMISSION SETTINGS

Monique Ambrose[1], Victoria M. Hunt[2], Christine M. Bachman[2], David Cate[3], Bernhard H. Weigl[3], David Bell[3], Chris Drakeley[4], Caitlin Bever[1], Jaline Gerardin[5]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Global Good, Intellectual Ventures, Bellevue, WA, United States,* [3]*Intellectual Ventures, Bellevue, WA, United States,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Northwestern University, Evanston, IL, United States*

Surveillance programs in low-transmission settings may fail to accurately estimate local transmission levels due to the scarcity of active infections detectable by conventional rapid diagnostic tests (RDTs). To increase surveillance power in these settings, diagnostic tests capable of detecting antimalarial antibodies that persist for months after parasite clearance are under development. Here, we use a mechanistic model of malaria transmission (EMOD) to quantify the performance characteristics that would allow an RDT based on serological biomarkers to outperform existing HRP2-based case-management RDTs. We consider two *Plasmodium falciparum* surveillance use cases for these 'serology RDTs': stratifying villages for targeted interventions and certifying areas as free of recent local transmission. Our analyses reveal that the surveillance season and geography strongly influence the relative performance of the two types of RDTs: compared to the HRP2-based RDT, the serology RDT performs best when surveillance occurs during the low-transmission season in regions with high seasonality. For example, to outperform HRP2-based RDTs in both use cases, a serology RDT that detects infection for a mean of 240 days after parasite clearance and has 98% specificity would need ≥60% sensitivity when surveillance is conducted in the wet season but only ≥40% sensitivity when surveillance is conducted in the dry season. We also find that serology RDTs that detect infection for a mean of 240 days after parasite clearance improve surveillance power compared to those with shorter detection periods (60 or 120 days). By contrasting the performance of existing HRP2-based RDTs and hypothetical serology RDTs, this modeling analysis will inform target product profiles and guide development of high-value diagnostic tools.

915

IMPACT OF ULTRASENSITIVE MALARIA DIAGNOSTICS ON ASYMPTOMATIC PREGNANT MOTHERS IN AMHARA REGION, ETHIOPIA

Banchamlak Tegegne[1], Gizachew Yismaw[1], Ranmalee Amarasekara[2], Abu Naser Mohon[2], James Cheaveau[2], **Dylan R. Pillai**[2]

[1]*Amhara Public Health Inst, Bahir Dar, Ethiopia,* [2]*University of Calgary, Calgary, AB, Canada*

According to the WHO, in Africa, 30 million women living in malaria-endemic areas become pregnant each year. Up to 200,000 newborn deaths each year result from malaria in pregnancy. Diagnosis of malaria in pregnancy is limited by microscopy with poor sensitivity. Malaria, particularly due to *P. falciparum*, in pregnant women increases the risk of maternal death, miscarriage, stillbirth and neonatal death. Ethiopia currently requires diagnosis of malaria in pregnancy in order to treat with antimalarials and does not employ IPTP. We conducted a comparative diagnostic study with LAMP (Illumigene Malaria) compared to traditional diagnosis with microscopy in both asymptomatic and symptomatic pregnant mothers at Jawi Health Centre in Amhara Region during peak transmission season (2018). In the study population, 29 of 36 mothers (mean age 24.2 years) who presented to the antenatal clinic were asymptomatic. Of the 29 asymptomatic mothers, 23 were in the first trimester and 17 were multigravida, Malaria positivity amongst asymptomatic mothers was 2/29 (6.9%) by microscopy, 1/29 (3.4%) by RDT, 9/29 (31%) by LAMP, and 9/29 (31%) by ultrasensitive qRT-PCR. The sensitivity of microscopy in asymptomatic pregnant mothers was 25.0% (95% CI 3.19% - 65.09%), RDT 12.5% (0.32% - 52.65%), LAMP 87.50% (47.35% - 99.68%) and qRT-PCR 87.50% (47.35% - 99.68%) using a consensus gold standard. These data suggest that ultrasensitive diagnostics are able to detect malaria far more frequently than currently approved diagnostic tools. Current efforts are focused on following mothers treated with asymptomatic carriage to determine the impact on maternal and fetal outcome including anemia and birthweight of the infant.

916

PLATE-BASED ASSAY FOR TYPING AND CHARACTERIZING *PLASMODIUM* ANTIGENS USING MICROCAPILLARY CYTOMETRY

Julie Clor, Xuemei Wan, Kamala Tyagarajan

Luminex Corporation, Hayward, CA, United States

Malarial research spans a wide variety of researchers, from labs using simplified technologies that are more proximal to samples, to labs involved in large scale, complex studies that are interested in the parallel analysis of a large number of samples. We recently demonstrated the performance of the Muse® Malaria *P.f.-P.v.* Detection Assay, a research use only, bead-based immunoassay for the detection of *Plasmodium falciparum*, *Plasmodium vivax,* and mixed infection samples, on the simple, low cost Muse Cell Analyzer for simplified yes/no callouts. In this study, we explore the application of the Muse Malaria *P.f.-P.v.* Detection Assay on the Guava® easyCyte™ 12HT System, a benchtop flow cytometer based on principles of microcapillary cytometry that enables analysis in a 96-well plate format. The system allows for automated walkaway acquisition of samples in a plate after sample preparation. Further, the InCyte™ Software package associated with the platform contains a heat mapping feature that allows for rapid and easy detection of all three malarial antigens, providing an immediate view of experimental "hits". In addition, quantitative comparisons can be obtained by exporting MFI information with standards of known concentration included to predict antigen concentration in samples being studied. Dynamic range data and the application to frozen blood samples from *P. f.*, *P. v.,* and mixed infection donors using the easyCyte 12HT System will also be presented. The plate-based adaptation of the assay provides excellent performance across a wide range of parasitemia and exhibits superior detection sensitivity of a few parasites/µL for both *P.f.* and *P.v.* detection, when compared to traditional methods such as microscopy and RDTs. The availability of simple, plate-based approaches for the rapid analysis of multiple antigens in a large number of malarial samples can enhance speed and detection capability for malarial researchers spanning all kinds of labs.

917

OPERATIONAL PERFORMANCE OF A HIGHLY-SENSITIVE DIAGNOSTIC METHOD FOR DETECTION OF MALARIA INFECTIONS IN PREGNANCY IN PAPUA NEW GUINEA

Benishar Kombut[1], Pele Melepia[2], Ruth Fidelis[2], Elma Nate[3], Lina Lorry[3], Livingstone Tavul[3], Maria Ome-Kaius[4], Michelle JL Scoullar[5], Philippe Boeuf[5], Shazia Ruybal-Pesántez[4], Michaela Riddell[6], Lisa Vallely[6], Andrew Vallely[6], Chris Morgan[5], Freya JI Fowkes[5], James Beeson[5], Jack Richards[5], Benedict Mode[7], William Pomat[8], Ewurama Owusu[9], Sandra Incardona[9], Xavier C. Ding[9], Stenard Hiasihri[2], Moses Laman[3], **Leanne J. Robinson**[5]

[1]*PNG Institute of Medical Research; Burnet Institute, Kokopo, Papua New Guinea,* [2]*Burnet Institute, Kokopo, Papua New Guinea,* [3]*PNG Institute of Medical Research, Madang, Papua New Guinea,* [4]*Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia,* [5]*Burnet Institute, Melbourne, Australia,* [6]*Kirby Institute, Sydney, Australia,* [7]*East New Britain Provincial Government, Kokopo, Papua New Guinea,* [8]*PNG Institute of Medical Research, Goroka, Papua New Guinea,* [9]*FIND, Geneva, Switzerland*

Plasmodium falciparum infections during pregnancy contribute to adverse outcomes, such as maternal and neonatal anaemia, as well as low birth weight. These infections are often sub-clinical and difficult to diagnose due to placental parasite sequestration giving rise to low-level peripheral parasitaemia. Studies using sensitive polymerase-chain reaction (PCR) techniques indicate that at least half of all infections in maternal venous blood are missed by light microscopy or conventional rapid diagnostic tests. The aim of this study is to assess the performance of a new highly sensitive rapid diagnostic test (HS-RDT) for the detection of *P. falciparum* infections during pregnancy in Papua New Guinea (PNG). The study is ongoing and will screen 940 pregnant women attending ante-natal care clinics in Gazelle, Kokopo and Rabaul Districts of East New Britain Province (ENB), PNG. The HS-RDT and conventional malaria HRP2/pLDH(Pf/Pan) Combo test are being performed in the clinic, with Pan/Pf/Pv loop-mediated isothermal amplification (LAMP) performed in the laboratory on the same day, and light microscopy and quantitative real-time PCR (qPCR) performed retrospectively. The performance of the HS-RDT will be compared with Pf/Pan RDT, light microscopy and LAMP in peripheral blood samples, with qPCR as reference standard. The performance of LAMP for the detection of *P. falciparum* and *P. vivax* infections in pregnancy will similarly be evaluated, using qPCR as reference test. We are also assessing health service measures of feasibility and acceptability of point-of-care options. Preliminary data indicates a moderate burden of malaria and a high burden of anaemia in pregnancy in ENB and full results will be available for presentation at the meeting. This study will provide critical evidence to guide the improved detection of malaria infections in pregnancy in PNG and other co-endemic settings.

918

FACTORS ASSOCIATED WITH SEVERE MALARIA DEATHS: LESSONS FROM A MORTALITY AUDIT CONDUCTED IN HEALTH FACILITIES IN UGANDA

Patrick Bukoma[1], David O. Salandini[1], Viola Nampera[1], JohnBaptist Bwanika[1], Ruth N. Kigozi[1], Sam S. Gudoi[1], Jane Nabakooza[2], Julius K. Kuule[2], Kassahun Belay[3], Gloria Ssebikaari[3], Mame Niang[4], Peter mukobi[5], James Tibenderana[6], Peter Thomas[4]

[1]*PMI Malaria Action Program for Districts Project, Uganda, Kampala, Uganda,* [2]*Uganda National Malaria Control Program, Kampala, Uganda,* [3]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda,* [4]*US President's Malaria Initiative, Malaria Branch, Centers for Diseases Control and Prevention, Atlanta, GA, United States,* [5]*Ministry of Health, Kampala, Uganda,* [6]*Malaria Consortium, London, United Kingdom*

Malaria is among the leading causes of morbidity and mortality in Uganda. In 2016, Ministry of Health (MoH) adopted clinical audit guidelines to improve quality of severe malaria management in health facilities (HF), aiming to achieve 'near zero' deaths by June 2020. Between 2017 and 2019, to help strengthen severe malaria management, the US President's Malaria Initiative supported the PMI Malaria Action Program for Districts (MAPD) Project to conduct a criteria-based review—based on MoH malaria guidelines in HF, seeking to identify factors associated with malaria deaths in two hospitals and one HC, selected purposively having reported the highest numbers of malaria deaths in the national health management information system. Joint MAPD and MoH audit teams collected data on HF systems, patient satisfaction, clinical and laboratory diagnosis and, care and treatment practices. We used WHO definitions of complications to assess diagnosis, yet they were not in the national treatment guidelines. Descriptive data analysis was conducted. The overall average score of 66% on HF functionality, competency and patient satisfaction indicators was well below the 80% clinical audit target. Health worker (HW) recognition of danger signs of complications was very low—24%; notably dyspnoea—8%, convulsions—20% and altered sensorium—23%. Half—54%, the deaths were treated for malaria without laboratory confirmation. 45% of deaths had investigations for alternative diagnoses and biochemistry; no death had tests to confirm treated bacterial infections. 93% deaths had received first line treatment for severe malaria according to MoH guidelines; a third—32% concurrently received antibiotics, while 94% deaths had not received intensive care. The Systems and practices in place were inadequate to ensure quality healthcare in the three HF. Thus, inadequate recognition and treatment of complications appear to be key factors in malaria deaths, and should be addressed at both system and HW levels. Adoption of WHO definitions of malaria complications in national treatment guidelines is needed to better guide diagnosis and treatment of severe febrile illness.

919

AN ULTRA-SENSITIVE PF-HRP2 ELISA FOR THE DETECTION OF LOW-DENSITY FALCIPARUM MALARIA

Diane D. Hall, Neil C. Marshall, Anthony M. Smithyman, G. H. Rajasekariah

Cellabs, Sydney, Australia

The WHO 2015 Global Technical Strategy for Malaria 2016-2030 made significant progress through the period 2015-2018, with often dramatic declines in malaria deaths and overall incidence, resulting in an estimated 10 countries primed for malaria elimination by 2020. However effective elimination strategies may also significantly reduce malaria parasite densities in the population, below the limit of detection of the conventional rapid diagnostic tests being used in elimination campaigns. The new challenge for the elimination effort is to determine whether these lower parasite densities still allow transmission of infection, as seen in past eradication programmes, where resurgence has been observed to confound the gains in eradication. But without sufficiently sensitive diagnostic tools this will be difficult. Thus there is now an urgent need for higher sensitivity tests to study the dynamics of transmission in low-density settings. We report here the development of a next-generation, ultra-sensitive and quantitative ELISA based on the long-established WHO reference kit, the Malaria Ag CELISA. The newly developed Quantimal Ultra-sensitive Pf-HRP2 CELISA was designed to provide a limit of detection as low as 10 picogram per mL Pf-HRP2, estimated to represent 1 parasite per mL. Included is a reference positive control calibrated using a *Plasmodium falciparum (Pf)* recombinant antigen. This positive control is an essential component used to construct a standard curve, allowing the quantitative determination of PfHRP2 in samples. This ultra-sensitive assay may be useful in supporting low-density *Pf* transmission surveillance and elimination activities.

920

MALARIA RAPID DIAGNOSTIC TESTS AS DNA STORAGE TOOL TO QUANTIFY *PLASMODIUM SPP* INFECTION

Etienne A. Guirou[1], Tobias Schindler[1], Maximilian Mpina[1], Salome Hosch[1], Glenda Cosi[1], Anna Deal[1], Silvan Wehner[1], Kamaka Ramadhani[2], Jongo Said[2], Carlos Cortes[3], Wonder Phiri[3], Jose Osa Osa Nfumu[3], Charity Okoro Eribo[3], Olivier Tresor Donfack[3], Guillermo A. Garcia[4], Claudia Daubenberger[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Medical Care Development International, Silver Spring, MD, United States*

Rapid and accurate diagnosis of malaria is essential for control and relies on Rapid Diagnostic Tests (RDTs) and microscopy. Most commercialized RDTs detect *Plasmodium falciparum* Histidine Rich Protein 2 (PfHRP2) but are limited in detecting asymptomatic, low parasitemic *P. falciparum* infections. RDTs do not discriminate between the different non-falciparum malaria species. Quantitative polymerase chain reaction (qPCR) is more sensitive and enables to distinguish the different *Plasmodium* species. We developed and evaluated a novel, cheap, high-throughput method to extract DNA from RDTs followed by qPCR assays to identify and quantify *P. falciparum* and non-falciparum infections. Our approach is supported by novel software solutions for the identification of barcoded RDTs and automated analysis pipelines to quality control qPCR data generated. We applied our new DNA extraction and qPCR data analysis pipeline in a subset of RDTs collected during the 2018 Malaria Indicator Survey conducted on the island of Bioko in Equatorial Guinea. RDTs collected from 227 pregnant and 209 non-pregnant, age matched women were included. We found that the proportion of *Plasmodium* infection was higher in non-pregnant (13.46%, 28/208) compared to pregnant women (6.61%, 15/227) (p=0.02). Nine *P. falciparum* infections were detected by qPCR that were missed by RDTs. Ten malaria positive RDTs were identified lacking qPCR amplification, indicating that circulating PfHRP2 after successful malaria treatment might be detected. Parasite density was low in both groups of women (range 1 - 2165 parasites/μL). Among the pregnant women, we detected more infections in first trimester (n=6) compared to second (n=4) and third trimester (n=4) of pregnancy. Importantly, *Plasmodium* positive pregnant women had lower mean hemoglobin levels compared to non-infected pregnant women. *P. malariae* was detected in one pregnant and in two non-pregnant women. Here, we demonstrate the feasibility of extracting DNA from used RDTs to identify and quantify *Plasmodium spp.* at high throughput facilitating unprecedented malaria surveillance at the molecular level.

921

PERFORMANCE OF A NOVEL HEMATOLOGY ANALYZER FOR MALARIA DIAGNOSIS IN AN ENDEMIC REGION OF COLOMBIA

Tatiana Maria Lopera-Mesa[1], Lina Zuluaga-Idarraga[1], Alexandra Rios[1], Veronica Sierra[1], Edwar Garzón[1], Ikki Takehara[2], Yuji Toya[2], Chiaki Takeuchi[2], Kinya Uchihashi[2], Alberto Tobón-Castaño[1]

[1]*Universidad de Antioquia, Medellin, Colombia,* [2]*Sysmex Corporation, Kobe, Japan*

Early and accurate detection of *Plasmodium* is critical to reduce malaria morbidity and mortality. Microscopic parasite identification is the diagnostic gold standard; however, it requires expert personnel and has limited sensitivity. Performance of Sysmex's analyser XN-30 for malaria diagnosis is described and compared to microscopy and real-time PCR (RT-PCR) in an endemic area of Colombia where *P. falciparum* and *P. vivax* are present. Acute febrile patients were enrolled between July 2018 and March 2019 in Quibdó city (Pacific region). Malaria diagnosis was done by microscopy / RDT in the field, and later confirmed by RT-PCR. Venous blood in EDTA was processed on the XN-30 within 4h of collection. Sensitivity, specificity, positive/negative predictive values (PPV, NPV), and likelihood ratio of positive and negative tests (LRP and LRN), were calculated. The intra-class correlation coefficient (ICC) and Bland-Altman plot were used to evaluate concordance of parasitemia. A total of 1,389 enrolled subjects were screened. The mean age was 29,2 (SD ± 19,3); 90% were black, 84% from urban areas and 1% were pregnant. *Plasmodium* was confirmed by RT-PCR in 24.8% of the subjects (345/1389). All cases were uncomplicated, attributed to *P. falciparum* (74.2%), *P. vivax* (23.2%) and mixed infections (1%). Compared with microscopy, the XN-30 showed a sensitivity of 95.4% (CI_{95} 92.9 - 97.8), specificity of 99.7% (CI_{95} 99.3 - 100), PPV of 99.0% (CI_{95} 97.8 - 100) and NPV of 98.5% (CI_{95} 97.7 - 99.3); concordance ICC 0.90 (CI_{95} 0.87 - 0.92) and average of the differences -1475 parasites/uL compared with thick smear, and ICC 0.97 (CI_{95} 0.96 - 0.97) and average of the differences -0.0021% compared with thin smear. Using RT-PCR as a reference, the XN-30 showed a sensitivity of 95.1% (CI_{95} 92.6 - 97.6), specificity of 100% (CI_{95} 99.9 - 100), PPV of 100% (CI_{95} 99.8 - 100), NPV of 98.4% (CI_{95} 97.5 - 99.2) LRN 0,05 (CI_{95} 0,03 - 0,08), and concordance ICC 0.97 (CI_{95} 0.96 - 9.70). The XN-30 analyzer offers a rapid and accurate alternative method for malaria diagnosis in areas where *P. falciparum* and *P. vivax* co-circulate, and it can eventually contribute to reducing the impact of this disease.

922

SYNTHESIS AND EVALUATION OF METABOLITES OF ANTIMALARIAL PYRIDO[1,2-A]BENZIMIDAZOLES

Richard Ferger[1], Kelly Chibale[2]

[1]*University of Cape Town, Cape Town, South Africa,* [2]*South African Medical Research Council Drug Discovery and Development Research Unit, Cape Town, South Africa*

Malaria constitutes one of the leading causes of deaths world-wide. Despite recent decades of strategic implementations aimed at intervening the drastic mortality and morbidity rates, malaria still heavily impacts the public health and economies of lesser developed countries. A series of novel metabolites of antimalarial pyrido[1,2-*a*]benzimidazole (PBI) compounds containing Mannich base side chains were designed, synthesized and evaluated for antiplasmodium activity, inhibition of

hemozoin formation, and tested for turbidimetric solubility. Some of the Mannich base side chains were designed to overcome the rapid bioactivation and metabolism of the currently prescribed antimalarial, amodiaquine. The most potent analogue exhibited sub-micromolar activity (IC_{50} value of 0.81 μM) against the drug-sensitive *NF*54 strain of *P. falciparum*. Meanwhile, three of the compounds showed moderate inhibition of the late stage gametocytes with >70% inhibition at 5 μM and >50% inhibition at 1 μM. All but two of the synthesized analogues were potent inhibitors of the formation of beta-hematin with IC_{50} values ranging between 6.5 and 43.9 μM.

923

EVALUATION OF ANTIOXIDANT AND ANTIMALARIAL ACTIVITY OF LEAF EXTRACTS OF *LUFFA CYLINDRICA*

Oluremi Aduke Saliu[1], Ayodeji Oluwafemi Idowu[2], Musbau Adewunmi Akanji[2], Biodun Noel Saliu[3]

[1]*Department of Environmental Science, Faculty of Health Science, National Open University, Abuja, Nigeria,* [2]*Department of Biochemistry, University of Ilorin, Kwara State, Ilorin, Nigeria,* [3]*Accreditation Unit, Nigerian Universities Commission, Abuja, Nigeria*

Malaria still remains one of the life threatening diseases despite receiving global attention over the years. It is endemic in sub-Saharan Africa where the level of poverty is quite high. The most vulnerable population are the young children, pregnant women and non-immune travelers or immigrants. Resistance to antimalarial drugs has contributed to the prevalence of this menace. Due to the frequency of malaria infection and the current trend of resistant malaria parasites, the use of local herbs in the treatment of malaria especially in developing countries like Nigeria is becoming more acceptable. *Luffa cylindrica* is one of such medicinal plants traditionally used in the treatment of malaria by the people of Nigeria. *Luffa cylindrica* belongs to the family of *Cucurbitaceous* and it is commonly called sponge gourd vegetable. There are ethnobotanical reports on the use of the plant in treating malaria. This study therefore aimed to investigate the antimalarial potential of extracts of *L. cylindrica* leaf in *plasmodium berghei*-infected Wistar mice and the *in vitro* antioxidant activity. Fresh leaves of *Luffa cylindrica* were successively extracted with n-hexane, ethylacetate, methanol solvents and distilled water (aqueous). Preliminary phytochemical evaluation of the four extracts was carried out using standard methods. Each extract was subjected to *in vitro* antioxidant study using different assay. The antimalarial study of the extracts was assessed *in vivo* at a calculated dose of 100, 200 and 400 mg/kg b.w and were compared with chloroquine and artesunate used as the reference antimalarial drugs. Acute oral toxicity evaluation was also carried out. Saponins, flavonoids, tannins, triterpenes, phenolics, alkaloids and cardiac glycosides were present in appreciable amounts. All the extracts showed varying *in vitro* antioxidant activity. The methanolic extract among other extracts exhibited the most potent antimalarial activity and also showed significance in the mean survival time of mice comparable with chloroquine and artesunat thus justify the acclaimed use of the plant in treating malaria.

924

USE OF AN *IN VITRO PLASMODIUM CYNOMOLGI* LIVER MODEL FOR RAPID DISCOVERY OF NEXT-GENERATION ANTIMALARIAL DRUGS

Alison E. Roth[1], Samantha O. Aylor[1], Erica C. Penn[1], Ratawan Ubalee[2], Gregory A. Reichard[1], Susan E. Leed[1], Norma E. Roncal[1], Brian A. Vesely[2], Silas A. Davidson[2], Norman C. Waters[2], Mara Kreishman-Deitrick[1], Brandon S. Pybus[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

Malaria eradication is critically dependent on novel anti-relapse drugs which specifically target the dormant liver-stage form (hypnozoite) in *Plasmodium vivax*. Additionally, new tools are sorely needed for military personnel and travelers going into and returning from *P. vivax* endemic areas. To date, the 8-aminoquinoline (8-AQ) drugs are the only effective anti-hypnozoite drug class. However, 8-AQs are toxic to G6PD-deficient individuals and CYP2D6 polymorphisms alter drug metabolism thus emphasizing the need for improved chemodiversity with retained clinical efficacy. Preclinical drug screening focused on malaria liver-stage parasites, especially hypnozoites, has been hampered by the inefficiencies of current models and the intrinsically difficult task of obtaining infectious *P. vivax* sporozoites. To discover new leads for anti-hypnozoite drugs, we used our validated and high-throughput standardized *in vitro P. cynomolgi* liver-stage model to screen >10,000 small molecule compounds comprised of the CIS 9000 library (an analytically filtered subset of the WRAIR drug repository), novel 8-AQs, and natural products. Compounds were initially screened in single-point concentration (3-10 μg/ml) examining both chemoprophylactic and curative (radical cure) activity against liver-stage schizonts and hypnozoites in this system. Several hits were further evaluated for hepatocyte toxicity (MTT) where nontoxic compounds were subsequently tested in dose-response studies. Further, hits were assessed for multistage activity using our SYBR Green blood-stage assay and compounds of interest were subjected to efficacy studies using an *in vivo P. berghei* mouse model. In summary, our data reveals novel chemotypes with prophylactic and curative activity against hypnozoites enabling new approaches and potential targets for development of next-generation anti-relapse drugs.

925

A NEW AND IMPROVED COLLABORATIVE SYSTEM TO EFFICIENTLY EVALUATE NATURAL PRODUCT EXTRACTS FOR ANY THERAPEUTIC AREA: PROOF-OF-CONCEPT IN MALARIA, MULTI-DRUG RESISTANT BACTERIA AND LEISHMANIASIS

Susan Leed[1], Brandon Pybus[1], Chad Black[1], Barry O'Keefe[2], Tanja Grkovic[3], Diana Caridha[1], Qigui Li[1], Robert Campbell[1], Jason Rohde[1], Mozna Khraiwesh[1], Erica Penn[1], Alison Roth[1], Norma Roncal[1], John Goulart[1], Tesfaye Teshome[1], Gustave Bonkoungou[1], Lisa Xie[1], Qiang Zeng[1], Hsiu Ling Lin[1], Jing Zhang[1], Ping Zhang[1], Malik Raynor[1], Thomas Langowski[1], Benjamin Sullivan[1], Samantha Aylor[1], Mara Kreishman-Deitrick[1], William McCalmont[1], Patricia Lee[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*National Institutes of Health, National Cancer Institute, Developmental Therapeutics Program Division of Cancer Treatment and Diagnosis, Center for Cancer Research and Natural Products Branch, Molecular Targets Program, Frederick, MD, United States,* [3]*Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Natural Products Support Group, Frederick, MD, United States*

Malaria, multi-drug resistant bacteria, and leishmaniasis are serious infectious disease threats, and there is an urgent need for new therapeutics to combat emerging resistance to currently available drugs. Given the scarcity of truly novel chemical classes that do not cause inherent toxicity, the research community is reconsidering the cost-benefit ratio of natural product interrogation. Historical obstacles, such as pure compound elucidation from crude extracts have made natural product screening problematic. The Walter Reed Army Institute of Research (WRAIR) Experimental Therapeutics Branch (ET) and the National Cancer Institute's (NCI) Program for Natural Products Discovery (NPNPD) have a unique opportunity to use established *in vitro* high-throughput screens (HTS) to assess NPNPD's diverse, >230,000 natural product extract library against malaria, multi-drug resistant bacteria, and leishmaniasis. This collection of natural products has never been systematically tested against any infectious disease targets. WRAIR/ET and NCI/NPNPD have completed a proof-of-concept of the cooperative system for each therapeutic area. During the malaria proof-of-concept, NCI/NPNPD provided 88 crude extracts and 616 primary fraction extracts for testing in the WRAIR/ET *in vitro* efficacy and toxicity assays. Secondary fractionation was conducted on the hits and sent back to WRAIR/ET for assessment. Fractions that showed potency were then purified into discrete compounds by NCI/NPNPD and confirmed by WRAIR/ET for malaria blood-stage efficacy, 3 compounds had IC50s between 1.2 – 16.6 nM in sensitive and resistant

Plasmodium falciparum strains and no cytotoxicity (NCI 60 cell-line panel). Potent compounds from this effort will be first evaluated for drug-like attributes and synthetic tractability, and promising compounds will then be considered for entry into already established *in vivo* efficacy, toxicity, and pharmacokinetics animal models at WRAIR/ET. This ground-breaking collaboration can be adapted to any therapeutic area of interest to discover and develop new natural product derived drugs for advancement to FDA approval.

926

TNRND315, A TORIN-2 DERIVATIVE TARGETS PI4K AND HAS POTENT ACTIVITY AGAINST *PLASMODIUM FALCIPARUM* ASEXUAL, SEXUAL AND MOSQUITO STAGES

Karthik Mosur Krishnan[1], Peter Ziniel[1], Hao Li[2], Wei Sun[2], Wei Zheng[2], Xiuli Huang[1], Nita Gombakomba[1], Sandra Mendoza Guerrero[1], Daniel Hupalo[1], Wenwei Huang[2], Philip Sanderson[2], Clifton Dalgard[3], Matthew Wilkerson[3], Kim C. Williamson[3]

[1]*Henry M Jackson Foundation, Bethesda, MD, United States,* [2]*National Center for Advancing Translational Sciences (NCATS, NIH), Rockville, MD, United States,* [3]*Uniformed Services University, Bethesda, MD, United States*

Recent efforts to control and ultimately eradicate malaria have stimulated interest in the development of safe and effective transmission blocking drugs. A large screen for compounds active against sexual stage parasites required for transmission, called gametocytes, identified Torin-2 as effective in both *in vitro* and *in vivo* rodent malaria models. Here we describe functional analysis of a Torin-2 derivative TRND000384315 (TRND315). Assays to determine stage specificity establish that the compound has potent activity against early *Plasmodium falciparum* schizonts as well as gametocytes. Using the *P. berghei*/mouse malaria model *in vivo*, administration of two 40 mg/kg doses was found to completely block transmission by inhibiting the development of ookinetes and oocysts in the mosquito. Whole genome sequence analysis of independently derived Torin-2 resistant *P. falciparum* lines found mutations in the region containing phosphatidylinositol 4-kinase (PfPI4K). The whole gene was duplicated in one line and the other had a single non-synonymous mutation in the enzyme active site. Interestingly, qRT-PCR indicated that both lines exhibited up-regulation of PI4K transcript. In addition, preliminary PI4P quantification by competitive ELISA suggests that treatment with TRND315 decreases cellular PI4P levels. Together the results support PI4K as a potential target for TRND315.

927

ARTESUNATE RESPONSE OF *PLASMODIUM FALCIPARUM* K13 MUTANT (C580Y) IN HUNSG MOUSE MODEL

Shulin Xu, Debora R. Casandra, Courtney Herman, Samantha J. Barnes, John H. Adams

Center for Global Health and Infectious Diseases Research, College of Public Health, University of South Florida, Tampa, FL, United States

Artemisinin-based combination therapies (ACTs) are the most effective treatment to reduce the *Plasmdium falciparum* infection burden and deaths, but point mutation C580Y in K13 propeller have been reported associations with ART resistance (ART-R). In this study we investigated K13 mutants artesunate responses in a humanized NSG mouse (HuNSG) model, using two parasite lines (76H10C580Y, 76H10C580Rev) previously characterized in *Aotus*, as an alternate *in vivo*model for studies of ART-R. For clearance studies, infected mice with parasitemia more than 0.5% were treated with artesunate (4mg/kg/day) for 3 daily injections and monitored at 0, 6, 12, 24, 36, 48, 72, 96 and 120 hours and then transfer to new HuNSG mouse "1 to 1" to monitor for parasite recrudescence weekly. Ring-stage Survival Assay (RSA) was used to evaluate in parallel the *ex-vivo*susceptibility of parasites cultivated in HuNSG mice to DHA. 76H10C580Y did not have longer half-life or more times of recrudescence than 76H10C580Rev parasite after infection of HuNSG mice and artesunate treatment. Parasites showed similar clearance times in primary infection and recrudescence. These results support the previous findings in NHP model infections, which create doubt about correlations between K13 mutations and altered parasite clearance half-life (t1/2) as markers of clinical resistance to ART. This study also indicates that the *P. falciparum*-HuNSG mouse model may provide a more accessible *in vivo*method for analyzing ARTs parasite clearance profiles. This model provides an additional tool for study of drug mechanisms of action and potential ART drug combinations.

928

IDENTIFYING COMPOUNDS THAT TARGET RESISTANT PARASITES AS A STRATEGY TO SUPPRESS THE EMERGENCE OF ANTIMALARIAL RESISTANCE

Rebecca Mandt[1], Maria Jose Lafuente-Monasterio[2], Madeline R. Luth[3], Matthew Reynolds[4], Sabine Ottilie[3], Elizabeth A. Winzeler[3], Javier Gamo[2], Dyann F. Wirth[1], Amanda K. Lukens[1]

[1]*Harvard TH Chan School of Public Health, Boston, MA, United States,* [2]*Tres Cantos Medicines Development Campus, GlaxoSmithKline, Madrid, Spain,* [3]*University of California San Diego, San Diego, CA, United States,* [4]*Harvard College, Cambridge, MA, United States*

Resistance has arisen to every therapy used in the clinical treatment of malaria, necessitating the continuous development of new drugs targeting different aspects of parasite biology. Unfortunately, even novel therapeutics can be stymied by the rapid emergence of resistance in parasite populations. For example, resistance to the *Plasmodium* dihydroorotate dehydrogenase (DHODH) inhibitor DSM265 arose during Phase 2 clinical trials. Our lab and others found that DHODH can accommodate a variety of mutations that decrease the parasites' sensitivity to DSM265, and that several of these mutations have no negative impact on parasite fitness *in vitro*, explaining why resistance to this drug arises so readily. However, we have also previously demonstrated that mutations in DHODH that give resistance to one inhibitor can cause increased sensitivity to other classes of DHODH inhibitors, a phenomenon generally termed 'collateral sensitivity'. Here, we tested several DSM265-resistant parasites against a range of compounds identified in our recently-published Tres Cantos Open Lab screen, and observed different patterns of cross-resistance and collateral sensitivity. We identify one compound, TCMDC-125334, that is active against all mutant lines tested. In particular, the DHODH C276Y mutant which arose in clinical trials with DSM265 is ~10-fold more sensitive to TCMDC-125334 than wildtype parasites. In attempts to select for resistance to TCMDC-125334 *in vitro,* we have thus far been unable to isolate parasites with high level (>2-fold) resistance. Treating the C276Y line with TCMDC-125334 selects for parasites with increased resistance, but which are still relatively sensitive compared to wildtype. We hypothesize that, because resistance to TCMDC-125334 occurs less frequently, this compound could be particularly effective in combination with DSM265, and experiments to test this are ongoing. Overall, this research demonstrates the value of screening for compounds that target resistant parasites, and more broadly of utilizing our understanding of resistance evolution in designing strategies for preventing or reducing resistance.

929

SMALL MOLECULE SCREEN OF EPIGENETIC INHIBITORS ON *PLASMODIUM FALCIPARUM* BLOOD STAGE REPLICATION AND GAMETOCYTE MATURATION

Leen Vanheer, Björn F. C. Kafsack

Department of Microbiology and Immunology, Weill Cornell Medicine, New York, NY, United States

The regulation of gene expression by histone modifications is critical for parasite survival in both asexual and sexual blood stages of *Plasmodium falciparum*, and changes substantially as parasites differentiate from one to the other. This makes inhibitors targeting these pathways intriguing in terms of their potential as multi-stage active anti-malarials. Therefore, we screened two cherry-picked libraries of small molecule epigenetic inhibitors from Selleckchem (142 compounds) and Cayman Chemicals

(139 compounds) for their effect on asexual blood stage replication and gametocyte development. These compounds include inhibitors of epigenetic writers, readers, and erasers known in mammalian systems, including histone deacetylases (HDAC), histone demethylases (HDM), histone methyl transferases (HMT) and histone acetyl transferases (HAT). We identified 74 epigenetic inhibitors with EC90s below 10 μM for inhibition of gametocyte development, of which 27 compounds retained their >90% inhibitory effect at 1 μM . Asexual stage growth was blocked >90% by 73 compounds at 10 μM , of which 30 compounds maintained their inhibitory effect at 1 μM . Dose-response curves were determined for compounds with potent activity at 1 μM, with several that had EC50 concentrations in the low nano-molar range against both asexual and sexual blood stages. These include inhibitors previously identified to be active against *P. falciparum* blood stages as well as several that had not previously been tested. Additionally, we identified multiple compounds that displayed higher activity against gametocyte maturation compared to asexual replication. The emergence of drug resistance in *P. falciparum*, the most virulent human malaria parasite, necessitates the development of novel anti-malarial drugs. These findings provide support for the development of new classes of anti-malarials that target the parasite's epigenetic regulation machinery.

930

REPURPOSING FDA APPROVED DRUGS TO TREAT MALARIA: UNDERSTANDING THE MECHANISM OF ACTION

Steven Goicoechea[1], Yash Gupta[2], Jessica Simpson[1], Whelton A. Miller III[2], Brijesh Rathi[3], Ravi Durvasula[2], Prakasha Kempaiah[2]

[1]*Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States,* [2]*Loyola University Chicago Stritch School of Medicine and Department of Medicine, Loyola University Medical Center, Maywood, IL, United States,* [3]*Department of Chemistry, Hansraj College University Enclave, University of Delhi, Delhi, India and Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States*

Emerging drug resistance in the *Plasmodium falciparum* parasite imperils malaria control and urges for the development of new antimalarial drugs. While the development of new drugs is a lengthy process that requires considerable financial investment, repurposing of FDA approved drugs that are effective against the malaria parasite can circumvent initial cost and time needed to introduce a drug to market. In the current study, we shortlisted compounds from the ChEMBL database, a manually curated database of bioactive molecules with drug-like properties. Nearly 100 compounds with possible secondary targets were tested against PfD6 and PfDd2 strains using an IC_{50} test in the erythrocyte stage of the malaria parasite. Nearly twenty compounds strongly inhibited growth of both PfD6 and PfDd2 (IC_{50} <2μM). Further, we selected those leads that have homologous targets in *P. falciparum*: Amlodipine Besylate (voltage-dependent calcium channel), Azithromycin (23S rRNA, arginine deiminase type-4, bacterial 50S ribosomal subunit), Balsalazide (peroxisome proliferator-activated receptor gamma, prostaglandin G/H synthase, arachidonate 5-lipoxygenase), Benzapril (angiotensin-converting enzyme), and Escitalopram (sodium-dependent serotonin transporter). The drugs were subjected to comparative docking analysis to assess the conservancy in the mechanism of action in parasite and host. The differences observed in target, homologue function, and interacting amino acids were further exploited to derive parasite-specific pharmacophore. We are currently performing specific assays to confirm the drug targets to understand the mechanism of action against *P. falciparum*.

931

A BIOINFORMATICS PIPELINE TO REPURPOSE APPROVED DRUGS AS ANTI-PARASITIC LEADS

Yash Gupta[1], Whelton A. Miller III[1], Samuel K. Kwofi[2], Brijesh Rathi[3], Ravi Durvasula[1], Prakasha Kempaiah[1]

[1]*Loyola University Chicago Stritch School of Medicine and Department of Medicine, Loyola University Medical Center, Maywood, IL, United States,* [2]*Department of Biomedical Engineering, School of Engineering Sciences, College of Basic and Applied Sciences, University of Ghana and Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States,* [3]*Department of Chemistry, Hansraj College University Enclave, University of Delhi, Delhi, India and Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States*

Two of the most important human diseases caused by protozoan parasites; malaria and leishmaniasis, have a combined disability adjusted life years of approximately 42 million. Many of the currently used medications are becoming less effective due to the development of drug resistant strains of parasites, necessitating the continued search for new drugs. Recently drug Lansoprazole, a proton pump inhibitor has been shown to have activity against tuberculosis through *in vitro* and animal studies. While similar compounds Omeprazole and pantoprazole have no such activity and the target of Lansoprazole is cytochrome bc1 complex (QcrB), acts through intracellular sulfoxide reduction to lansoprazole sulfide. Due to presence of similar sulfoxide reduction pathway in malaria parasite, *Plasmodium falciparum* (Pf; methionine sulfoxide reduction), which is important for amino acid uptake and redox balance. Most importantly, earlier reports of use of 'Morpholino Thiophenes' being highly active against Pf (Target; dihydroorotate dehydrogenase) also Mtb; QcrB Inhibitors. Therefore, we tested the drug against Pf and found it to be highly active against both PfD6 (sensitive, 0.8μm±0.1) and PfDD2 (resistant, 0.8μm±0.1) strains. However, the current dosage range for Lansoprazole for an adult human is 15-30mg which is far less from dosage required for successful antibiosis. Therefore, to identify the target of Lansoprazole in Pf and improve dose restrictions, we performed docking analysis with 'cytochrome bc1 complex' and 'dihydroorotate dehydrogenase' modelled proteins with Lansoprazole. Based on interacting docked posed validated by MD simulations, we deciphered pharmacophores and subjected the leads to a 'ligand based virtual screening' populating a curated library prescreened by in silico ADMEtox predictions. The retention of activity in the hits was again determined by molecular dockings and MD simulation validations. Thus, we have successfully pipelined from a novel lead to putative drug candidates while mitigating the shortcomings of the former drug.

932

DISRUPTION OF *PLASMODIUM FALCIPARUM* DIHYDROFOLATE REDUCTASE AND THYMIDYLATE SYNTHASE INTERACTIONS IS LETHAL TO MALARIA PARASITES

Devaraja G Mudeppa, Bennett Guo, SooNee Tan, Shiva Kumar, Pradipsinh K Rathod

University of Washington, Seattle, WA, United States

The target of selective antifolate drugs*, Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (*Pf*DHFR-TS), is a single 71 kDa polypeptide which forms an obligated dimer for TS function. Previously we demonstrated that folding and function of *Pf*DHFR is independent of *Pf*TS. Folding of *Pf*TS protein into a functional enzyme, however, is dependent on upstream *Pf*DHFR. We demonstrate that the activation of *Pf*TS domain requires the assistance of pre-folded *Pf*DHFR. Crystal structure of *Pf*DHFR-TS displayed several key contacts between *Pf*DHFR and *Pf*TS. Site-directed mutagenesis at the contact sites between *Pf*DHFR residues and *Pf*TS disrupted TS activity. A competing peptide derived from the contact residues disrupted *Pf*TS activity in cell-free protein synthesis reactions, was lethal to bacteria that were dependent *Pf*DHFR-TS, and to malaria parasite. This approach to attack parasite-specific protein-protein interactions opens an opportunity to design small molecule inhibitors against this previously unknown parasite vulnerability. Beyond that, this new approach may be of general interest for disrupting pathogen functions in a species-specific manner.

933

EXPLORING FUNGAL METABOLITES FOR MALARIA TRANSMISSION BLOCKING AGENTS

Manpreet Kaur, Guodong Niu, Yue Hao, Jun Li

Florida International University, Miami, FL, United States

Malaria remains one of the major concerns in public health sector, with an estimated 219 million clinical cases in 87 countries. Malaria transmission can be controlled by inhibiting *Plasmodium* development in mosquitoes, mainly by vector controls including insecticidal-treated mosquito nets or spraying insecticides. Due to increased insecticidal resistance, there is a growing need for alternate approaches for blocking malaria transmission. Fibrinogen-related protein 1 (FREP1) has been identified as one of the critical midgut proteins for *Plasmodium* invasion. Eliminating parasite infection by disrupting the FREP 1- parasite interaction using small molecules might be an effective solution for stopping malaria transmission. Although plant species have been extensively studied for novel small molecules against malaria transmission, fungi are one of the understudied natural resources. Based on the results of an earlier ELISA-based screening method developed by our group for a fungal extract library, the candidate fungal extract from our *Cladosporium Cladosporioides* isolate had been identified that inhibited the interaction between FREP1 and *P. falciparum* infected cells by 95%. The crude fungal extract was tested on mosquitoes using a standard membrane feeding assay (SFMA) at 100 μg/mL blood concentration, showing complete inhibition of the development of oocysts in mosquitoes. The crude fungal extract was then further fractionated using flash column chromatography and the collected fractions were continuously screened using the SFMA to eliminate inactive fractions. The active fractions at each stage were further fractionated and tested, leading to an active compound which shows 100% inhibition at 20 μg/mL blood concentration. The structural elucidation of the active compound was done using a combination of LCMS and NMR data analysis techniques. This fungal metabolite is an effective active lead candidate for malaria transmission blocking drugs.

934

DIFFERING RISK FACTORS FOR MALARIA AMONGST ADULTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Molly Deutsch-Feldman[1], Nicholas F. Brazeau[1], Kyaw Thwai[1], Melchior Kashamuka[2], Antoinette Tshefu[2], Jonathan J. Juliano[1], Steven R. Meshnick[1]

[1]*University of North Carolina - Chapel Hill, Chapel Hill, NC, United States,* [2]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo*

Malaria remains a significant public health problem worldwide, and especially in sub-Saharan Africa. Adults are frequently infected and may serve as a reservoir for further transmission. Yet, we know very little about risk factors for infection in adults. In the Democratic Republic of the Congo (DRC), malaria prevalence amongst adults is approximately 30%. In this study, we assessed malaria in adults using over 17,000 samples from the nationally representative 2013-2014 Demographic and Health Survey (DHS) conducted in the DRC. *Plasmodium falciparum* infection was determined by PCR. Covariates were drawn from the DHS and used to build a multi-level model to identify both individual and community level risk factors for infection amongst adults. Additionally, we included the community level proportion of drug resistant infections, determined by genetic sequencing, as potential risk factors. All identified risk factors were then assessed for modification by urbanicity category as designated in the DHS (large cities, small cities, towns, rural areas). Individual risk factors include: younger age, male sex, lower education, and lower wealth. Community level risk factors include: decreased community bed-net use, decreased average education, and decreased average wealth. Decreased malaria prevalence was also associated with increased cluster level antifolate-resistance mutations, probably due to increased antimalarial use. The modification analysis indicates no protective effect of individual or increasing cluster level net use in cities, but highly protective effects elsewhere. Both increasing individual and community level education are highly protective in large cities and showed little effect in towns or rural areas. A similar trend is seen for wealth. Modification of risk factors by urban status may be due to differences in transmission intensity, brand of bed-nets used, and access to resources. The findings from these analyses will help the DRC identify individuals and communities at higher risk for malaria infection. They also shed light on differences in the epidemiology of malaria by different urban settings.

935

THE PREVALENCE OF ASYMPTOMATIC, SUBMICROSCOPIC AND UNCOMPLICATED MALARIA IN A HIGHLAND AREA OF KENYA WITH LOW TRANSMISSION

Lindsey B. Turnbull[1], George Ayodo[2], Kavitha Udumula[1], Travis Putzke[1], Tuan Tran[1], Chandy C. John[1]

[1]*Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya*

Continued progress towards malaria elimination requires a greater understanding of the prevalence of submicroscopic infections among healthy individuals and those with mild, non-specific symptoms to inform interventions at the local level. We have conducted longitudinal surveillance of clinical malaria in the Kenyan highland sites of Kipsamoite and Kapsisiywa, areas of unstable transmission, since 2002. Symptomatic individuals (with fever or headache) were prospectively enrolled at the site health centers and tested for malaria by microscopy. Those with microscopy confirmed malaria were treated with artemisinin combination therapy. All others were diagnosed with non-malarial illness and provided appropriate clinical care. To investigate and disentangle clinical malaria from other infections in which the patient may also have asymptomatic parasitemia, we retrospectively assessed malaria surveillance samples collected from 2015 - 2017 using quantitative PCR (qPCR) and determined the number of individuals with submicroscopic malaria. Cross-sectional blood collections from asymptomatic individuals were also conducted in August 2016, January 2017, and June 2017 to assess for asymptomatic parasitemia. Monthly prevalence of submicroscopic malaria among symptomatic individuals ranged from zero to 20.9%. In Kapsisiywa, symptomatic individuals were more likely to have submicroscopic parasitemia than asymptomatic individuals (OR= 5.44, 95%CI 1.89, 15.60, $p < 0.01$). Symptomatic *P. falciparum* qPCR positive individuals did not seek follow-up care at a higher rate than symptomatic qPCR negative individuals and only 2 of 26 symptomatic qPCR positive individuals analyzed thus far had a visit within 30 days in which they were microscopy positive, suggesting that malaria infection in most sub-microscopic individuals is controlled by the host immune response. The study data indicate that in low, unstable transmission settings, testing of febrile individuals for *P. falciparum* infection by qPCR may be a high-yield method to detect individuals sub-microscopically infected with *P. falciparum*.

936

SUBPATENT MALARIA IN PREGNANCY: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS

Anna Maria van Eijk[1], Subpatent Malaria in Pregnancy Study Group[2]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*WWARN, Oxford, United Kingdom*

Malaria in pregnancy causes adverse pregnancy outcomes. In semi-immune pregnant women, many *Plasmodium* infections are asymptomatic and below the level of detection (subpatent) by microscopy (submicroscopic) or rapid diagnostic tests (RDT). Previous studies to determine the clinical relevance of low-grade infections provided conflicting results. We conducted an individual-participant data meta-analysis to determine the impact of subpatent malaria infections on adverse pregnancy outcomes. We searched the Malaria in Pregnancy Library (updated 3 times/year from over 20 databases) to identify eligible studies using the keywords

"Polymerase Chain Reaction", "PCR", "subpatent" or "submicroscopic" published between January 1997 and January 2019. Studies involving pregnant women in malaria endemic regions were eligible if they reported diagnostic results based on PCR and either microscopy or RDT and information on birth outcome or maternal haemoglobin (prespecified endpoints). Authors of eligible studies were approached from March 2017 to March 2019. Datasets were standardized, merged and grouped by study and location. Overall, 487 records were screened, and 80 potential studies identified. By March 2019, datasets from 32 studies were received covering 66 substudies. Preliminary results using two-stage random-effects meta-analysis and microscopy/PCR showed that, compared to women without malaria at delivery, women with microscopic and submicroscopic placental malaria had lower mean haemoglobin levels (patent: mean difference [MD]=0.8g/dL, 95% CI 0.7-0.9, p<0.001, 32 substudies, *I2*=0.0%, N=9,431; submicroscopic: MD=0.2g/dL, 0.1-0.3, p=0.003, 34 study sites, *I2*=19.5%, N=10,342). Results were similar with maternal blood at delivery. The difference in mean birthweight was: microscopic, MD=150gr decrease, 97-202, p<0.001, 33 substudies, *I2*=34.6%, N=9,647; submicroscopic MD=34gr, -1-68, p=0.059, 36 substudies, *I2*=19.5%, N=10,690). Preliminary results show that submicroscopic malaria infections detected at delivery are associated with decreased maternal haemoglobin levels and possibly birthweight.

937

MALARIA AMONG INDIVIDUALS AND COMMUNITIES ACROSS SEASONS IN SITES WITH VARYING ENDEMICITIES IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

Alpha Oumar Diallo[1], Seungwon Kim[2], Varun Goel[3], Alison Poffley[1], Kyaw Thwai[1], Mvuama M. Novo[4], Joseph A. Bala[4], Marthe Nkalani[4], Georges Kihuma[4], Joseph Atibu[4], Michael Emch[3], Jonathan B. Parr[5], Jonathan J. Juliano[6], Steven R. Meshnick[1], Melchior Kashamuka Mwandagalirwa[4], Antoinette Tshefu[4], Margaret Carrel[2]

[1]*Department of Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Department of Geographical and Sustainability Sciences, University of Iowa, Iowa City, IA, United States,* [3]*Department of Geography, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [4]*Ecole de Santé Publique, Faculté de Médecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [5]*Division of Infectious Diseases, School of Medicine, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [6]*Division of Infectious Diseases, School of Medicine, University of North Carolina-Chapel Hill, Chapel Hill, NC, Chapel Hill, NC, United States*

Globally, the Democratic Republic of the Congo (DRC) has one of the highest malaria burdens with a prevalence of over 30% in both children and adults. To better understand the individual and household factors associated with malaria, a prospective longitudinal study was conducted across seven sites (242 households and 1,565 individuals) with varying endemicities in Kinshasa Province. Participants were sampled at four separate visits from 2015-2016 (baseline and three follow-up visits) at 6-month intervals during which questionnaires were completed, participants were tested for *Plasmodium falciparum* via rapid diagnostic tests (RDT) and dried blood spots were collected for polymerase chain reaction (PCR) testing. Participants with malaria symptoms between scheduled visits attended local health centers (unscheduled visits), where their axillary temperatures and RDT results were recorded and dried blood spots were collected for PCR testing. Seventy-four percent of participants had malaria outcomes recorded across all four visits, with a further 13% having recordings for three of four visits. Malaria prevalence was highest at follow-up two (37% via RDT and 40% via PCR); malaria prevalence measured by PCR was consistently higher than RDT. The urban Kinshasa site had the lowest malaria prevalence (3-10%) in all four time periods, while sites with the highest malaria prevalence varied across visits, ranging as high as 60% via PCR in a rural village during the rainy season, 2016. Children aged 6-15 years had the highest malaria prevalence (45-50%) in most sites across time and reported consistently low bednet use. No consistent seasonal trends for malaria and bednet use were observed. There were 3,024 unscheduled visits to the local health clinics amongst 1,045 study participants during the study, with a median of 2 visits per participant per year. Of these, 43% had malaria diagnosed via RDT and 59% had PCR-positive malaria outcomes. These results and future analyses of these data that incorporate household characteristics will elucidate factors associated with malaria prevalence across varying endemicity levels in the DRC.

938

THE IMPACT OF OLYSET® NET AND DAWAPLUS® 2.0 ON THE RISK OF *PLASMODIUM* INFECTION IN GEMBE EAST, WESTERN KENYA

Noriko Tamari[1], George O. Sonye[2], Beatrice Awuor[2], James O. Kongere[3], Muneaki Hashimoto[4], Masatoshi Kataoka[4], Stephen Munga[5], Noboru Minakawa[1]

[1]*Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan,* [2]*Ability to Solve by Knowledge Project, Mbita, Homa Bay, Kenya,* [3]*Centre for research in Tropical Medicine and Community Development, Nairobi, Kenya,* [4]*National Institute of Advanced Industrial Science and Technology (AIST), Health Research Institute, Kagawa, Japan,* [5]*Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya*

Olyset® Net is a long-lasting insecticidal bed net (LLIN) incorporated with permethrin and DawaPlus® 2.0 is coated with deltamethrin. While the World Health Organization (WHO) has recommended various types of LLINs for vector control, the effectiveness of different types of LLINs are unclear in the field settings. We assessed the impact of Olyset® Net and DawaPlus® 2.0 on the risk of *Plasmodium* infection in Gembe East, western Kenya. Olyset® Net and DawaPlus® 2.0 were distributed in September and October of 2014 and June and July of 2017 respectively at the study site. Children ⩽15 years of age were tested for *P. falciparum* infection using with polymerase chain reaction (PCR). We directly observed their sleeping location, number of persons sharing a net, types of the bed net they used, material of wall and gap of eaves (open/close) in their sleeping rooms. The proportional hole index (PHI) was estimated for each net following the WHO guideline, and indoor resting female anophelines were collected from their sleeping rooms. We also characterized socioeconomic status (SES) for each household. Of 254 examined, 74 (29.1%) and 180 (70.9%) children slept under Olyset® Net and DawaPlus® 2.0, respectively. The PCR-positive prevalence was 37.8% for Olyset® Net-users and 57.8% for DawaPlus® 2.0-users. The difference was statistically significant (bivariate analysis: p<0.01). Similarly, number of persons sharing a net (sharing with none or one person/sharing with two or more), PHI on the sides, SES and material of wall (mud/others) were statistically significant between use of Olyset® Net and DawaPlus® 2.0. Multivariate Bayesian logistic regression with spatial dependency confirmed that children using Olyset® Net were at lower risk than DawaPlus® 2.0-users (OR=0.67, 95% Credible Interval: 0.45-0.97). Although the mechanism that makes the difference was not clear, the results from this study suggest that Olyset® Net after three years use was more effective for preventing from *Plasmodium* infection, compared to DawaPlus® 2.0 after a half year use.

939

MALARIA IMPORTED INTO PERU: THE ROLE OF VENEZUELAN CITIZENS

Fiorela Alvarez[1], Ana Pilar Ramos[1], Karim Pardo[2], Verónica Soto[2], Juan José Contreras[1], Dionicia Gamboa[3], Alejandro Llanos-Cuentas[1]

[1]*Unidad de Leishmaniasis and Malaria, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima,*

Peru, [2]Ministerio de salud Perú, Lima, Peru, [3]Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

Between 2000 to 2017 there was a decrease of 14.4% of malaria cases in the Americas, mainly due to control interventions. During the last years, the increment of malaria in Venezuela has generated concern in neighboring countries. This country has showed a 365% increase of malaria between the period 2000 and 2017 and 71% in 2017 in comparison with 2016. In 2017, the American continent reported 975,700 cases of malaria of which 53% corresponded to Venezuela. Until December 2018 more than 1 million of Venezuelans arrived in Peru, with multiple health problems, including malaria. This abstract describes the situation of imported malaria cases reported in the Malaria Program of the Cayetano Heredia Hospital, a national reference center located in Lima city where 100% of the cases of malaria that have been attended at this health center came from endemic areas of malaria in Peru or from abroad. The first Venezuelan with vivax malaria at our hospital was diagnosed in March 2017, and since then the number of cases has increased steadily. In that year 10.5% (15/70) of malaria cases were registered in Venezuelan citizens and in 2018 more than half of the cases (50.46%, 50/107) were Venezuelans. 84.06% of them had vivax malaria and 15.94% mixed malaria and22.8% were recurrent episodes. Currently in Lima there is no active transmission of malaria and all of them arrived in Peru through the Pan-American highway, reason why we think that most of the recurrent cases correspond to relapses. The majority of this population lives in temporary places, works in the informal sector and does not have or refuse to give a telephone number to follow them up. For all these characteristics, malaria imported by Venezuelan citizens represents a public health problem in Peru and the MHO and other ministries must collaborate and work together to implement adequate strategies to decrease the burden of imported malaria and assuring that Peruvian malaria elimination process remain unaffected.

940

EXISTENCE OF MALARIA HOTSPOTS IN ZANZIBAR: POTENTIAL CONTRIBUTION TO ONGOING MALARIA TRANSMISSION

Mohamed Haji Ali

Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania

Malaria prevalence in Zanzibar has remained less than 1% since 2008; however, transmission continues, and there is a need to implement activities to continue progress toward elimination. Malaria hotspots are geographically clustered cases and potential sources of malaria infection where targeted interventions might interrupt transmission. The objectives of this analysis were to identify and characterize malaria hotspots in Zanzibar. Hotspots were defined as a village with more than 5 total cases per 1000 population per year. Malaria surveillance data between 2015 and 2018 from all 10 districts (383 Shehias) were analyzed. Overall, there were 43 malaria hotspots (39 Unguja island; 4 Pemba island). The highest incidence among all the hotspots was 56.5/1000 for 2015, 29.7/1000 for 2016, 22.5/1000 for 2017 and 44.7/1000 for 2018. In Unguja, 30 (77%) hotspots were located in three districts (16 Magharibi, 8 Mjini, and 6 Kati). In Pemba, 3 (75%) hotspots were located in Micheweni district. Among the 43 hotspots, households within 17 (40%) hotspots in 2016 and 19 (44%) hotspots in 2017 were targeted for indoor residual spraying (IRS) based on incidence and available resources to conduct IRS; 15 hotspots were targeted for repeat IRS between 2016 and 2017. The majority of malaria hotspots in Zanzibar are located in four districts and are potential sources of ongoing malaria infection where targeted interventions might interrupt transmission. Characters (with spaces): 1,456

941

EVALUATION OF ASYMPTOMATIC MALARIA IN DAK NONG PROVINCE IN THE HIGHLANDS OF VIETNAM FOR THE MALARIA ELIMINATION ROADMAP

Huynh H. Quang[1], Marina Chavchich[2], Nguyen T. Trinh[1], Nguyen D. Manh[3], Michael D. Edstein[2], Nicholas J. Martin[4], Kimberly A. Edgel[4]

[1]*Institute of Malariology, Parasitology and Entomology, Quy Nhon, Vietnam,* [2]*Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia,* [3]*Military Institute of Preventive Medicine, Hanoi, Vietnam,* [4]*U.S. Naval Medical Research Unit Two, Singapore, Singapore*

Malaria cases have been steadily declining in Vietnam since 2006, providing the opportunity for malaria elimination by 2030. A significant challenge for elimination is asymptomatic parasite carriers, which represent a silent reservoir for malaria transmission and contribute to the persistence of malaria. Gaining an understanding of the prevalence and distribution of asymptomatic malaria will help design intervention strategies to achieve elimination. We conducted a survey, where finger prick blood samples were collected from two cohorts of asymptomatic people each with approximately 1,415 subjects residing in three communes of Tuy Duc district, Dak Nong Province, Vietnam. The communes Dak Buk So, Dak Ngo, and Quang Truc were stratified using criteria from the National Malaria Control Program as low, moderate and high malaria endemic areas, respectively. The first and second cohorts of asymptomatic people were surveyed at the end of the wet season (November-December 2018) and at the start of the dry season (February-March 2019), respectively. All subjects were free of malaria symptoms and negative by rapid diagnostic testing and microscopy. RNA was extracted from 50 μL of blood and preserved in RNAprotect®. Malaria parasites were detected by one-step RT-qPCR targeting 18s ribosomal RNA transcripts of *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. Preliminary data for the first cohort showed asymptomatic parasite prevalences of 1.5%, 3.6%, and 12.8% for the low, moderate and high endemic areas, respectively. All subjects were also screened for glucose-6-phosphate dehydrogenase (G6PD) deficiency (Carestart Biosensor) revealing 1.5% and 3.8% with severe and intermediate G6PD deficiency, respectively. This information is essential for future elimination strategies using anti-hypnozontocidal drugs (primaquine or tafenoquine) for treating *P. vivax* malaria. The study is ongoing and more information on *Plasmodium* species identification and gametocyte prevalence will be presented. These data will be discussed in the context of devising targeted strategies for malaria elimination.

942

CHILDREN WITH CLINICAL *PLASMODIUM FALCIPARUM* INFECTION HAVE INCREASED SHARING OF HAPLOTYPES WITH HOUSEHOLD MEMBERS AS WELL AS TEMPORALLY-PROXIMAL, SYMPTOMATIC PEERS

Cody S. Nelson[1], Kelsey M. Sumner[2], Betsy Freedman[1], Andrew A. Obala[3], Judith N. Mangeni[3], Steve M. Taylor[1], Wendy P. O'Meara[1]

[1]*Duke University, Durham, NC, United States,* [2]*University of North Carolina, Chapel Hill, NC, United States,* [3]*Moi University, Eldoret, Kenya*

Falciparum malaria transmission has failed to decline in proportion to control efforts in certain regions such as Bungoma county, western Kenya. One proposed strategy to eradicate malaria is ring testing and treatment, however it remains unknown whether infections spread locally or if asymptomatically-infected household members are a risk factor for clinical disease. From April 2013 to June 2014, we enrolled 442 cases (RDT+ children hospitalized with malaria) and 442 matched controls; all household members of cases and controls were also enrolled and tested, of which 13.6% (n=608/4449) were RDT+. From each RDT+ participant, parasite gDNA was PCR-amplified at both Pf circumsporozoite protein (*csp*) and apical membrane antigen 1 (*ama1*) *loci*, amplicons sequenced on an Illumina Miseq, and haplotypes inferred using dada2. We identified

120 *csp* and 180 *ama1* unique haplotypes. We evaluated genetic distance between infected individuals using three novel indices: sharing of parasite haplotypes on binary and proportional scales and the L1 norm. Case children median [IQR] binary/proportional sharing of both *csp* and *ama1* haplotypes was significantly increased with members of their origin household (e.g. *csp* binary sharing: origin = 50.3 [0-87.5] vs. similar household = 0 [0-50.3]; p = 0.01; Wilcoxon Sign-Rank test), indicating that cases are more likely to share haplotype-identical parasites with members of their own household. We also computed population-level haplotype sharing indices for all pairs of case children and observed no association between genetic relatedness and geographic distance. In contrast, we identified a strong inverse relationship between haplotype sharing and temporal distance, which we exploited to identify the molecular signature of an outbreak. Overall, these findings suggest that, although haplotype sharing is more common within households, temporal rather than geographic proximity predicts parasite genetic similarity. The observation that identical haplotype combinations are found nearly simultaneously across the study area implies that ring testing approaches may not effectively reduce transmission.

943

MALARIA MORBIDITY AND MORTALITY TRENDS IN CENTRAL UGANDA

Daniella Busharizi[1], Ruth Kigozi[1], Emily Goodwin[1], Patricia Mukose[1], Gloria Ssebikaari[2], Peter Thomas[2], Paul Oboth[1], Patrick Bukoma[1], Damian Rutazaana[3], Thomson Ngabirano[1], Godfrey Magumba[4], James Tibenderana[5], Sam S. Gudoi[1]

[1]*PMI Malaria Action Program for District Project, Uganda, Kampala, Uganda,* [2]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda,* [3]*Uganda National Malaria Control Program, Kampala, Uganda,* [4]*Malaria Consortium, Kampala, Uganda,* [5]*Malaria Consortium, London, United Kingdom*

Malaria remains a global heath challenge with 3.2 billion people at risk. In 2016, there were 216 million cases of malaria and 445,000 deaths globally. In Uganda, malaria is among the leading causes of morbidity and mortality, contributing to 30-50% of outpatient department attendance and over 20% of inpatient hospital admissions. In the last quarter of 2016, the US President's Malaria Initiative (PMI)-supported Malaria Action Program for Districts (MAPD), instituted several interventions to prevent and control malaria in nine high burden districts in Uganda: Kyotera, Sembabule, Lwengo, Rakai, Lyantonde, Bukomansimbi, Kalangala, Kalungu and Masaka. These included: distributing long lasting insecticidal nets through schools and health facilities, improving data reporting and use, and training and mentoring health workers in malaria diagnosis and case management. This study sought to assess the effect of these interventions by comparing trends in malaria morbidity and mortality at the start (October - December 2016: pre-I) and during the intervention period (October - December 2017: post-1yr and October -December 2018: post-2yr). Analysis of District Health Information System 2 data from all 400 district health facilities showed that while the proportion of malaria that was confirmed did not change from 76% in pre-intervention (pre-I), to 75% post-1yr intervention (post-1yr), it improved to 89% in post-2yr. The proportion of OPD attendance due to malaria fell from 41% in Pre-1 to 27% in post-1yr and 15% in post-2yr (p-value = 0.003). Similarly, the proportion of inpatient admissions due to malaria reduced from 31% to 21% and then to 15% (p-value = 0.001) in pre-1, post-1yr and post-2yr periods respectively. The number of reported malaria deaths per 100,000 population in the region has fallen from 4 (pre-I) to 3 (post-1yr) to 2 (post-2yr). Malaria morbidity and mortality in this region appears to have fallen significantly over the last 2 years and lessons related to implementing multi-channel vector control and reinforcing health worker capacity should be well noted to guide national control efforts.

944

AN OUTBREAK OF *PLASMODIUM FALCIPARUM* MALARIA IN AN ELIMINATION SETTING IN SABAH, MALAYSIA

Giri S. Rahajram[1], Matthew J. Grigg[2], Timothy William[3], Danshy Alaza[4], Joseph Benedict[4], Rashidah Mohammad[5], Jenarun Jelip[6], Nicholas Anstey[2], **Bridget E. Barber**[2]

[1]*Queen Elizabeth Hospital, Kota Kinabalu, Malaysia,* [2]*Menzies School of Health Research, Darwin, Australia,* [3]*Gleneagles Kota Kinabalu Hospital, Kota Kinabalu, Malaysia,* [4]*Infectious Diseases Society Sabah, Kota Kinabalu, Malaysia,* [5]*Sabah Department of Health, Kota Kinabalu, Malaysia,* [6]*Ministry of Health Malaysia, Putrajaya, Malaysia*

Malaysia aims to eliminate human malaria by 2020, and in the eastern state of Sabah, incidence of *Plasmodium falciparum* malaria has fallen substantially in recent years. In Kudat District, there has been no case of *P. falciparum* reported since 2015. However, an outbreak of *P. falciparum* has occurred on Banggi Island, Kudat District, in 2019, with a total of 111 cases reported during January to March. To evaluate the effect of waning immunity on the clinical and epidemiological features of falciparum malaria, we compared patients with falciparum malaria in 2019, to patients presenting in the same region with falciparum malaria during 2012 – 2015 (n=96). During 2012 – 2015 median age was 16 years (IQR 10 – 31 years), and 31 (32%) were children ≤ 12 years. In contrast, in the 2019 outbreak to-date the median age of falciparum malaria patients is 11 years (IQR 8 - 24 years), and 58% of all cases are children (chi2: p<0.0001). Median parasite densities were also lower, with a median parasitemia among adults of 1,372/μL (IQR 519 – 3,600/μL) in 2019 compared to 9,924/μL (IQR 2,522 – 22,860/μL) in 2012 – 2015, and 2,740/μL in children in 2019 compared to 7,392/μL (IQR 1,462 – 36,546/μL) during 2012 – 2015. These data are consistent with previous reports demonstrating clinical disease at lower parasite densities in low-transmission settings, with this finding particularly marked in adults. Genomic analysis of *P. falciparum* isolates from 2012 – 2015, and from the 2019 outbreak, will be conducted to investigate the changing *P. falciparum* population structure in the elimination setting.

945

PROACTIVE COMMUNITY CASE MANAGEMENT OF MALARIA IN CHILDREN AGED 6-59 MONTHS: RESULTS OF A PILOT PROJECT IN NORTHERN BENIN, WEST AFRICA, 2018

Happy Hezouwe Awide

Peace Corps Benin, Cotonou, Benin

Malaria is the leading cause of mortality in children <5 years old in Benin. WHO estimates that the average Beninese family spends 25% of their annual income on malaria-related costs. To address this burden, the National Malaria Control Program implemented a community case management strategy in 2011 that involved provision of free malaria testing with rapid diagnostic tests (RDTs) and treatment with artemisinin-based combination therapies (ACTs) to children aged 6-59 months who are brought to Community Health Workers' (CHW) homes by their parents (passive case detection). Despite these efforts, early malaria care seeking remained low in rural communities. To address barriers to care seeking, Benin Peace Corps Volunteers piloted a ProActive Community Treatment (ProACT) model in 2018 in eight villages in the northern districts of Cobly and Natitingou (Nati). CHWs were trained to conduct weekly household visits to test and treat symptomatic children aged 6-59 months for malaria. Villages in Cobly and Nati were on average 8 and 16 km away from the nearest health centers, respectively. Between June-August 2018, 26 (Cobly: 14, Nati: 12) participating CHWs made 12 weekly visits to all households in their communities. They tested 1,077 (96%) of 1,126 symptomatic children aged 6-59 months with RDTs; 1,002 (89%) tested positive for malaria and were treated with ACTs. Most children (759, 80%) who tested positive were 12-59 months old. CHWs referred 135 (12%) symptomatic children to health centers for concerning symptoms or negative RDTs. During the same time period, CHWs passively (in their own homes) treated 642 children who tested positive for malaria with

ACTs. Cobly CHWs treated more children through ProACT (658) than with the passive approach (307); Nati CHWs treated similar numbers of children through the ProACT (296) and passive (335) approaches. This pilot demonstrated that ProACT can increase the number of children who are tested and treated for malaria by CHWs when compared to standard passive malaria case detection.

946

SIMPLE CALCULATION TO IMPROVE ROUTINE HEALTH FACILITY MALARIA INCIDENCE ESTIMATES

Julie I. Thwing[1], Alioune Camara[2], Baltazar Candrinho[3], Rose Zulliger[4], James Colborn[5], John Painter[1], Mateusz Plucinski[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*National Malaria Control Program, Conakry, Guinea,* [3]*National Malaria Control Program, Maputo, Mozambique,* [4]*Centers for Disease Control and Prevention, Maputo, Mozambique,* [5]*Clinton Health Access Initiative, Maputo, Mozambique*

With the increased emphasis on malaria surveillance as an intervention, malaria incidence as reported through health information systems is increasingly used to understand trends in malaria burden and to target and monitor interventions. However, incidence data are sensitive to changes in rates of testing of suspected cases, care-seeking rates, and reporting completeness, and must be interpreted with care. Burden estimates have historically relied on community-based prevalence surveys or modeling, which are neither available frequently nor practical for programmatic decision making. We derived an algebraic formula to convert crude incidence rates to a corrected estimate of incidence. It applies a correction factor to adjust for differential testing of febrile illness by care providers, and uses the ratio of community incidence of non-malaria fever to health facility-reported incidence of non-malaria fever to account for care-seeking rates and data completeness. This formula was applied to district level data from Guinea and Mozambique from 2016-2018, and to aggregate data from sub-Saharan African countries for 2015 to estimate corrected incidence and continent-wide needs for malaria tests and treatments, assuming universal testing of febrile illness and current care-seeking rates. Maps of corrected incidence were more consistent with maps of survey prevalence than was crude incidence in Guinea and Mozambique. In areas prioritized for case management support, crude incidence showed increases from 2016-2018 (+11% annual increase in Guinea and +16% in Mozambique), while the corrected incidence showed the opposite trend (-7.2% in Guinea, -2.5% in Mozambique). Countries in southern and eastern Africa reporting recent increases in malaria incidence generally had lower corrected incidence than countries in central and west Africa. The unmet need for malaria tests was 160 M (IQR: 139-188) and for malaria treatments was 37 M (IQR: 29-51 M). Malaria incidence should be interpreted in the context of biases in testing and care-seeking rates. Adjusting for these biases provides insight into true spatiotemporal trends of malaria burden.

947

LONGITUDINAL OBSERVATIONAL STUDY EXAMINING COMMUNITY DYNAMICS OF MALARIA TRANSMISSION IN MALI

Jen C. Hume[1], Issaka Sagara[2], Daman Sylla[2], Jennifer Kwan[1], Mahamadou A. Maiga[2], Abdoulaye Katile[2], Emily Higbee[1], Amatigue Zeguime[2], Mamadou Coulibaly[2], Patrick E. Duffy[1]

[1]*National Institutes of Health/National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [2]*MRTC/USTTB, Bamako, Mali*

In 2018 a three-year longitudinal cohort study was initiated in the villages of Bancoumana and Doneguebougou in Mali to assess transmission dynamics. Approximately 1500 individuals aged 6 months to 65 years of age across the two communities were enrolled into two distinct cohorts: Direct Skin Feeding (DSF) cohort and Parasite Surveillance (PS) cohort. All individuals are seen monthly for malaria smears in conjunction with live and spray mosquito collections in/around residencies while DSF participants also undergo a single direct skin feeding assay at each visit using insectary-raised mosquitoes. In the first year of the study valuable parasitemia data through the dry and rainy seasons was generated indicating that children aged 11-17 years old carry the highest burden of infection throughout the year with parasitemia levels over 10% even in the dry season. This preponderance of infection is borne out in DSF data where positive skin feeding assays were observed most frequently in children aged 11-17 years old. To date, almost 7000 DSF have been completed and 73% of the positive skin feeds have been observed in 9-18 year olds, 13% in 5-8 year old children and 13% in adults. Over 7000 mosquito collections have been conducted with >4000 live bloodfed mosquitoes collected and dissected for infection. In this presentation we will examine dynamics of natural malaria infection and transmission over time in different age groups, the relationship between transmission measured by DSF assays of laboratory-raised mosquitoes, and by speciation assessment of live wild-caught mosquitoes.

948

STRONG PROTECTIVE EFFECT OF MEDITERRANEAN TYPE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AGAINST VIVAX MALARIA

Ghulam R. Awab[1], Fahima Aaram[2], Natsuda Jamornthanyawat[3], Kanokon Suwannasin[1], Watcharee Pagornrat[1], James A. Watson[1], Charles J. Woodrow[1], Arjen Dondorp[1], Nicholas P. Day[1], Mallika Imwong[3], Nicholas White[1]

[1]*MORU, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [2]*Kabul Medical University, Kabul, Afghanistan,* [3]*Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

G6PD deficiency (G6PDd) is the most common enzyme abnormality of humans (prevalences across the malaria endemic world as high as 35%). G6PDd is X-linked so males are either normal or deficient while women can be normal, fully deficient (homozygotes) or partially deficient (heterozygotes). The high prevalence in tropical areas suggests protection against malaria but this is controversial. Claims have been made that there is protection in female heterozygotes only, in hemizygotes only, in both or in neither! We conducted a retrospective analysis of data from clinical studies on vivax malaria and epidemiological studies of G6PDd conducted over the past ten years in Afghanistan where G6PD "Mediterranean" variant is the main cause of G6PDd. We conducted a meta-analysis using all previously published data on G6PDd in people of Pashtun ethnicity living in malaria endemic areas mainly in the eastern Afghanistan, where the clinical malaria studies were conducted. Samples were tested on site by the fluorescent spot tests, stored and transferred to MORU Thailand for genotyping of G6PD Mediterranean variant (563C>T) by PCR-RFLP. The proportions of G6PD Mediterranean male hemizygotes and female homozygotes and heterozygotes were significantly lower in vivax malaria patients than in controls (people visiting clinics or vaccination centers who did not have vivax malaria). In patients with *P. vivax* malaria 2.9% (95% CI 1.3-4.7) were male hemizygotes compared with 7.3% (5.3-9.9) in controls. For the female heterozygotes the respective proportions were 7.4% (5.1-10.2) vs. 13.6% (10.1-17.8). Thus there was a gene dose related protective effect of G6PD "Mediterranean" variant against vivax malaria; in male hemizygotes and female homozygotes 60.4% (28.7-82.2) versus 45.2% (15.6-65.2) for heterozygotes.

949

LOW INCIDENCE OF CLINICAL MALARIA IN UNDER-FIVES IN BANCOUMANA, MALI, A MALARIA VACCINE TESTING SITE

Kourane Sissoko[1], Issaka Sagara[2], Mahamadoun H. Assadou[2], Sibiri Sissoko[2], Mamady Kone[2], Seydou Sankare[2], Sadio K. Diarra[2], Boukary Togo[2], Abdoulaye Djiguiba[2], Amatigue Zeguime[2], Sintry Sanogo[2], Moussa B. Kanoute[2], Aissata D. Doumdia[2], Bourama Samake[2], M'Bouye Doucoure[2], Drissa Dembele[2], Aly Togora[2], Adama Ouattara[2], Modibo Traore[2], Jen C.c. Hume[3], Agnes Mwakingwe-Omari[3], Mamadou S. Sissoko[1], Patrick E. Duffy[3], Ogobara Doumbo[2]

[1]*National Institutes of Health/National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [2]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [3]*Laboratory of Malaria, Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Despite an estimated 20 million fewer malaria cases worldwide in 2017 than in 2010, no significant progress occurred between 2015-2017 (WHO 2018). An efficacious vaccine remains an urgent priority to bolster malaria reduction. Here, clinical malaria incidence in a general population living in a malaria-endemic area was characterized to inform future evaluations of vaccine candidates. A total of 875 volunteers aged 6 months to 65 years old were enrolled in this study of community dynamics of malaria transmission and mosquito feeding in Bancoumana, Mali, a village located 60 km southwest of Bamako (population ~10,000 people). From Feb 2018 to Jan 2019, malaria blood smears and/or rapid diagnostic tests (RDTs) were performed monthly or upon observation of clinical symptoms to confirm clinical malaria prior to treatment. Incidence rate was calculated as the number of confirmed malaria episodes divided by the number of participants for each age group followed. The overall incidence rate of clinical malaria was 0.62 (546/875)/person/year. The incidence rate of clinical malaria by age group was 0.31 (50/160)/child/year for ages 6 months - 4 years, 0.77 (186/239)/child/year for ages 5-10 years, 0.87 (184/210)/child/year for ages 11-17 years, and 0.47 (126/266)/adult/year for ages ≥ 18 years. The seasonality of malaria incidence by age group in the dry (from Jan to Jun) and wet (from Jul to Dec) seasons was respectively 0.01 (3/160) vs 0.29 (47/160)/child/season for ages 6 months - 4 years, 0.07 (17/239) vs 0.70 (169/239)/child/season for ages 5-10 years, 0.05 (12/210) vs 0.81 (12/210)/child/season for ages 11-17 years, and 0.03 (8/266) vs 0.44 (118/266)/adult/season for ages ≥ 18 years. Malaria incidence in Bancoumana is highly seasonal with the greatest burden in children aged 11-17 and 5-10 years. The disproportionately low incidence in under-fives could be explained by seasonal malaria chemoprevention distributed during the high malaria transmission season (Jul/Aug to Oct/Nov). The seasonal and age patterns of the malaria burden should be considered when developing new malaria control strategies and evaluating new interventions in a community.

950

CHARACTERIZATION OF ANTIBODY KINETICS IN TRAVELLERS WITH *PLASMODIUM FALCIPARUM* INFECTION ALLOWS FOR IDENTIFICATION OF SEROLOGICAL MARKERS OF CUMULATIVE AND RECENT MALARIA EXPOSURE

Victor Yman[1], James Tuju[2], Michael T. White[3], Gathoni Kamuyu[2], Kennedy Mwai[2], Nelson Kibinge[2], Muhammad Asghar[1], Christopher Sundling[1], Klara Sondén[1], Matteo Bottai[4], Linda Murungi[2], Dan Kiboi[2], Rinter Kimathi[2], Timothy Chege[2], Emily Chepsat[2], Patience Kiyuka[2], Lydia Nyamako[2], Simon J. Draper[5], Faith H. Osier[2], Anna Färnert[1]

[1]*Division of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden,* [2]*Kenya Medical Research Institute - Wellcome Trust Research Program, Centre for Geographical Medicine Research-Coast, Kilifi, Kenya,* [3]*Department of Parasites and Insect Vectors, Institut Pasteur, Paris, France,* [4]*Unit of Biostatistics, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,* [5]*Jenner Institute, University of Oxford, Oxford, United Kingdom*

Robust tools to evaluate *Plasmodium* exposure on both a population and individual level are needed to guide malaria control efforts and accelerate progress towards elimination. Antibody responses have been used to assess exposure on a population level but serological surveillance could be greatly improved by reliable markers that can detect recent exposure on an individual level. Identification of such markers requires detailed knowledge of the kinetics of the antigen-specific response. We examined antibody kinetics in 65 adult travellers with different levels of prior malaria exposure who were followed longitudinally in Sweden, a malaria free country, for 1 year after a natural *P. falciparum* infection. First we quantified antibody responses to 8 blood-stage antigens using a bead-based assay and applied a mathematical model that captures the boosting and biphasic decay in antibody levels to characterise the kinetics of the response. We found that prior malaria exposure was associated with a greater longevity of the response to vaccine-candidate antigens MSP1, MSP2, MSP3, AMA1 and RH5. For these antigens, the half-life of the long-lived component of the response ranged from 1.8–3.7 years and individuals with prior exposure maintained 2–9-fold greater antibody levels throughout follow-up. We then proceeded to evaluate a larger set of 111 blood-stage antigens using a protein microarray (KILchip V1.0) developed in Kenya. We applied the same antibody kinetics model and performed classification analysis to identify responses predictive of recent exposure. Antibody responses to GAMA, PTEX150, PF3D7_1136200 and PfSEA1 were most informative in predicting if an individual had been infected within the last three months and exhibited shorter half-lives and more consistent boosting and decay independent of the degree of prior exposure. Using a state-of-the-art assay developed in a southern endemic country, we characterised the antibody kinetics after a successfully treated *P. falciparum* infection in travellers and identified serological markers of both cumulative and recent exposure that can improve current tools for malaria surveillance.

951

SPATIAL AND TEMPORAL CLUSTERING OF *PLASMODIUM FALCIPARUM* INFECTION: A LONGITUDINAL COHORT AND GIS-BASED STUDY IN WEST AFRICA

Jeffrey G. Shaffer[1], Seydou O. Doumbia[2], Daouda Ndiaye[3], Ayouba Diarra[2], Jules F. Gomis[3], Davis Nwakanma[4], Ismaela Abubakar[5], Abdullahi Ahmad[6], Muna Affara[6], Mary Lukowski[7], James C. Welty[1], Joseph Keating[1], Frances J. Mather[1], Donald J. Krogstad[1]

[1]*Tulane University Health Sciences Ctr, New Orleans, LA, United States,* [2]*University of the Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [3]*University Cheikh Anta Diop, Dakar, Senegal,* [4]*Medical Research Council Units, Fajara, Gambia,* [5]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [6]*Medical Research Council Units, Fajara and Basse, Gambia,* [7]*ScienceTRAX, Austin, TX, United States*

The long-term goal of these studies is to identify and resolve obstacles to the control and ultimate elimination of malaria. Its specific aims are to test for spatial, temporal and spatio-temporal clustering of plasmodial infection and to examine the relationship between clustering of infection and the epidemiology of *P. falciparum*. This study was performed at four sites: two rural sites in Mali, one rural site in The Gambia and a peri-urban site in Senegal. Active detection of *P. falciparum* infection was based on twice-yearly blood smear surveys at the start and end of the transmission season. A total of 834 households participated in this study (n=239 in Dangassa, n=308 in Dioro, n=141 in The Gambia and n=146 in Senegal). Thick smears were used to identify infected individuals and to estimate the household prevalence of infection. Moran's I was used to quantitate spatial correlations. The Local Moran's I and SaTScans were used to identify spatial, temporal and spatio-temporal clusters of individuals with *P. falciparum* infection. Spatial cluster analyses yielded insignificant Moran's I results for both Mali sites, consistent with random spatial patterns for *P. falciparum* infection in rural Mali. There was spatio-temporal clustering

at the rural sites in Mali and The Gambia but not at the Senegal site. Although spatio-temporal clustering of *P. falciparum* infection was observed at all study sites, larger spatio-temporal clusters (≥ 3 households) were observed only at the Mali sites in 2014 and 2015. In contrast, smaller clusters of *P. falciparum* infection (≤ 3 households) were observed in The Gambia and Senegal and there was a cluster of households with a higher prevalence of infection in Senegal adjacent to a forested area in the peri-urban community of Madina Fall. These studies provide evidence for spatial clustering of infection in The Gambia, temporal clustering in Dioro and spatio-temporal clustering at the Dangassa and Dioro sites in Mali. In contrast, there was no evidence for spatial clustering in Madina Fall (Senegal).

952

SEROLOGICAL ASSESSMENT OF MALARIA AND LYMPHATIC FILARIASIS IN THE DOMINICAN REPUBLIC

Justin Willingham[1], Eric Griggs[1], Hunter Keys[2], Manuel Gonzales[3], Gregory S. Noland[4]

[1]*Emory, Atlanta, GA, United States,* [2]*University of Amsterdam, Amsterdam, Netherlands,* [3]*Centro de Prevención y Control de Enfermedades transmitidas por Vectores y Zoonosis, Ministerio de Salud Pública, Santo Domingo, Dominican Republic,* [4]*The Carter Center, Atlanta, GA, United States*

The island of Hispaniola, comprised of the Dominican Republic and Haiti, is the only island in the Caribbean with active malaria transmission and accounts for 95% of the lymphatic filariasis (LF) burden in the Americas. A cross-sectional, household cluster survey was conducted in *bateyes*—agricultural settlement villages home to Haitian migrant workers, their descendants, and ethnic Dominicans—in the Dominican Republic from March to April 2016 to determine the prevalence of malaria and LF by rapid antigen test and microscopy. The study also included collection of dried blood spots from one adult and one other randomly selected household resident of any age to determine the seroprevalence of malaria and LF antibodies using a multiplex bead assay. This study reports serology results from 1,331 samples with matched diagnostic results (median age: 34 years; range: 2—96). Although no (0%) person was *Plasmodium*-positive by RDT or microscopy, overall seroprevalence to *P. falciparum* antigens MSP-1, AMA-1, CSP, and LSA-1 was 16.9%, 10.0%, 1.5%, and 1.2%, respectively. Seroprevalence of long-lived antibodies MSP-1 and AMA-1 increased with age from 2.5% and 1.3%, respectively, in those < 10 years of age to 23.8% and 19.1% in those ≥60 years of age. Seroprevalence of short-lived antibodies CSP and LSA-1 was uniformly low across age groups. For LF, six individuals were FTS-positive (0.5%), but none (0%) were microfilariae-positive. Overall seroprevalence to Wb123, Bm14, and Bm33 antibodies was 1.3%, 16.2%, and 7.7%, respectively. Seroprevalence to Bm14, but not Bm33 or Wb123, increased with age. Univariate analysis for malaria seropositivity (PfMSP-1) revealed significantly higher odds of infection in the Southwest region and among those without access to a bednet, but no significant difference by country of birth or ethnicity. Age was the only significant predictor for LF seropositivity identified. These results indicate very low recent exposure to malaria and LF in the Dominican Republic and provide important data to prospectively monitor transmission elimination in Hispaniola.

953

UNDER THE RADAR: EPIDEMIOLOGY OF *PLASMODIUM OVALE* INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Cedar Mitchell[1], Nicholas F. Brazeau[1], Kashamuka Mwandagalirwa[2], Antoinette K. Tshefu[2], Kyaw Thwai[1], Jonathan B. Parr[1], Jonathan J. Juliano[1], Steven R. Meshnick[1]

[1]*University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [2]*University of Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo*

There are six species of the malaria parasite known to infect humans, two of which, *Plasmodium ovale wallikeri* and *{. ovale curtisi*, form a species complex known as *Plasmodium ovale.* While *P. ovale* has been shown to be prevalent throughout sub-Saharan Africa, little is known about the true burden of disease or the epidemiological factors associated with infection. Knowledge of the distribution of all malaria species is critical to malaria elimination efforts. Understanding the impact of *Plasmodium falciparum*-targeted interventions on non-falciparum species is particularly important. We conducted a risk factor analysis to characterize the epidemiology of *P. ovale* infections in the Democratic Republic of the Congo (DRC) using the nationally representative, cross-sectional 2013 DRC Demographic and Health Survey. Of 18,149 Congolese adults tested in our study sample, 143 prevalent *P. ovale* infections were detected, yielding a point prevalence estimate of 0.8% (95% CI: 0.59, 0.98). Using log-binomial regression models with generalized estimating equations to account for correlation among sampling clusters, we detected risk factors positively associated with increased ovale prevalence. These included male sex (prevalence ratio [PR] 2.12, 95% CI: 1.38, 3.26), co-prevalent *P. falciparum* infection (PR:3.52, 95% CI: 2.06, 5.99), and rural residence (PR: 2.19, 95% CI:1.31, 3.66). We found insufficient evidence for an effect of long lasting insecticidal net use on *P. ovale* prevalence (PR: 1.02, 95% CI:0.63, 1.62). Preliminary molecular analyses of our study sample identified both *P. ovale spp.* circulating in DRC. Further analyses will fully characterize the prevalence of *P. ovale curtisi* and *P. ovale wallikeri* among our full study sample size and elucidate risk factors associated with each species to build on our understanding of this neglected malaria species in one of the most malarious countries in the world. To our knowledge, this is the largest nationally representative survey of *P. ovale* conducted to date. Findings will provide insight into its epidemiology in the DRC and elsewhere in sub-Saharan Africa.

954

SEVERITY OF MALARIA IN A BIRTH COHORT OF INFANTS LIVING IN A HIGHLY ENDEMIC AREA OF UGANDA

Nicholas August Zehner[1], Teddy Andra[2], Richard Kajubi[2], Isaac Ssewanyana[2], Melissa Conrad[3], Felistas Nankya[2], Harriet Adrama[2], Tamara D. Clark[4], Moses Kamya[4], Grant Dorsey[3], Prasanna Jagannathan[1]

[1]*Stanford School of Medicine, Stanford, CA, United States,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*University of California San Francisco, San Francisco, CA, United States,* [4]*Makerere University, Kampala, Uganda*

Progress towards controlling *Plasmodium falciparum* malaria has stalled in high transmission settings, where the burden of severe malaria and death falls largely on infants and young children. A better understanding of the natural history of malaria in the setting of insecticide treated bednets (ITNs) and artemisinin-based therapy (ACT) will assist in deployment of prevention efforts. We enrolled a birth cohort of children from Busia, Uganda, an area with very high and perennial malaria transmission, and followed them through one year of age. Mothers were enrolled during pregnancy, given an ITN, and both mothers and children received all care at a study clinic, including ACT for smear-positive cases of symptomatic malaria. Routine assessments were performed every 4 weeks, including evaluation for parasitemia by microscopy. Of 678 live births, 6.5% were preterm, 8.9% low birth weight, and sickle cell trait prevalence was 17%.

Overall, there were 1,131 incident episodes of malaria (1.8 episodes ppy, range 0-10), increasing from 0.66 episodes ppy from 0-3 months to 2.9 episodes ppy from >9-12 months of age. Parasite prevalence at monthly visits increased from 5% from 0-3 months to 26% from >9-12 months of age. There were 8 episodes of severe malaria in 8 children (0.01 episodes ppy) - 1 severe anemia and 7 episodes of respiratory distress, including one death in a 9 month-old girl who had experienced 2 prior uncomplicated episodes. Episodes of severe malaria were accompanied by higher objective temperatures (P=0.04) and a trend towards longer duration of reported fever (P=0.08) and higher parasite densities (P=0.08) than uncomplicated cases, and 4 of 8 children had >=2 episodes of uncomplicated malaria before having severe malaria. Of 581 children followed through one year of age, 26% had no evidence of malaria parasitemia, 39% had only symptomatic malaria when infected, and 35% had at least one episode of asymptomatic parasitemia. Together, these data suggest that, in a high transmission setting where children are given ITNs and ACT, although the risk of malaria is high, severe malaria is preventable with prompt access to antimalarial therapy.

955

IS MALARIA ELIMINATION POSSIBLE IN UGANDA: EVIDENCE FROM POPULATION-BASED SURVEYS AND HEALTH FACILITY DATA 2014 AND 2019

Bosco B. Agaba[1], Damian Rutazaana[1], Paul Mbaka Mbaka[2], Jimmy Opigo Opigo[1], Monica Nabatanzi[1], Daniel Kyabayinze[1], Catherine Maiteki[1], Henry Katamba[1], Belay Kassahum[3]

[1]*Department of Disease Control, Ministry of Health, Kampala, Uganda,* [2]*World Health Organization, Uganda Country Office, Kampala, Uganda,* [3]*US President Malaria Initiative, PMI Uganda, Kampala, Uganda*

Malaria remains a major public health problem in Uganda despite increased intervention coverage. However malaria indicator surveys and health facility data have shown consistent decline in parasite prevalence, incidence, and deaths. In this study, we show evidence of declining malaria burden and increasing heterogeneity of malaria transmission pattern in Uganda. Using population-based survey data, we compared estimates of parasitemia for 2009, 2014 and 2018 using a sample of children aged 0-59 months at comparable time points coinciding with second peak transmission season. We determined differences in proportions of parasitemia between three time points of 2009, 2014 and 2018. Next we used multiple data sources to measure changes in malaria mortality and morbidity over time and constructed malaria incidence, risk and stratification maps to identify high and low transmission zones. Based on survey data, there was a reduction in malaria parasitemia from 42%, 19% and 16% (RDT) in 2009, 2014 and 2018 respectively. Based on Risk stratification, malaria transmission was highly heterogeneous across the country ranging from <5%, 5%-50% and >50% for low, medium and high transmission respectively. Health facility data showed reduction in malaria cases from 393 cases per 1,000, 396 cases per 1,000 and 241 per 1,000 populations in 2009, 2014 and 2018 respectively. There was also a reduction in malaria deaths from 21 deaths per 100,000 in 2009, to 17 deaths per 100,000 in 2014 and 8 deaths per 100,000 in 2018 respectively. Malaria morbidity and mortality is declining in Uganda. Transmission pattern is highly heterogenous ranging from very high to extremely low transmission areas. Low transmission areas could be targeted for malaria elimination.

956

DETECTION OF THE ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIOUS RESERVOIR AMONG SCHOOLCHILDREN IN TANZANIA USING MOSQUITO SKIN FEEDING ASSAYS

Billy Ngasala[1], Vincent O. Nyasembe[2], Christopher Basham[3], Mwajabu Loya[1], Zackary Park[3], Brian Tarimo[4], Feng-Chang Lin[3], Andreas Mårtensson[5], Jonathan Juliano[3], Rhoel R. Dinglasan[2], Derrick K. Mathias[2], **Jessica T. Lin**[3]

[1]*Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [2]*University of Florida, Gainesville, FL, United States,* [3]*University of North Carolina, Chapel Hill, NC, United States,* [4]*Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania,* [5]*Uppsala University, Uppsala, Sweden*

We are conducting a three-year field study to characterize the asymptomatic infectious reservoir in an area of declining malaria endemicity in Bagamoyo district, Tanzania. In October-November 2018, we screened 530 schoolchildren ages 8-16 for *Plasmodium falciparum*, finding that 21% were positive by RDT, 15% were positive by microscopy, and 33% were positive by an 18s rRNA real-time PCR (qPCR) assay with a limit of detection of ~1 parasite/l blood. Fifty-five RDT-positive children (median parasite density = 227 parasites/l) were enrolled for mosquito feeding assays. Of these, 69% (38/55) had gametocytes detectable by Pfs25 qRT-PCR. We used direct skin feeding assays (DFAs), which more closely mimic Anopheline sampling of gametocytes within capillaries, to evaluate the infectiousness of these individuals to mosquitoes and observed that 60% (28/47) were infectious to insectary-reared *Anopheles gambiae IFAKARA strain*. Mosquito infection was sporadic, with a low median infection rate of 7% (IQR 4-10%), yet infected midguts often harbored many oocysts. In 17 DFAs where only 1-2 mosquitoes were infected, 40% of positive midguts (10/25) harbored >100 oocysts. It is unclear if this "jackpotting effect" is due to gametocyte clustering in capillary blood. Gametocyte densities in capillary and venous blood as determined by Pfs25 RT-qPCR were closely correlated (r=0.92, p<0.001); however, gametocytes detected in 6 capillary blood samples were not detected in the corresponding venous samples, without the converse scenario. Of the 28 subjects who were infectious to mosquitoes, 21% were gametocytemic by microscopy, 68% were gametocytemic by RT-qPCR, 61% were slide-positive, and 86% were positive by 18s qPCR at the time of mosquito feeding. Thus, molecular tests appeared incompletely sensitive to detecting the entirety of the infectious reservoir in this school-age survey. By July 2019, we plan to recruit ~100 schoolchildren with submicroscopic malaria to measure transmission from lower parasite densities. Results will inform models of malaria transmission and shape strategies for interrupting transmission in areas moving towards malaria elimination.

957

CARRIAGE OF *PLASMODIUM FALCIPARUM* AND NON-*P. FALCIPARUM* INFECTIONS AND GAMETOCYTES IN ASYMPTOMATIC POPULATION IN WESTERN KENYA

Carolyne M. Kifude[1], Deborah Stiffler[2], Stephen Ocholla[3], John Waitumbi[1], Janet Oyieko[1], Shirley Luckhart[4], V.Ann Stewart[2]

[1]*US Army Medical Research Directorate-Africa, Kisumu, Kenya,* [2]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [3]*US Army Medical Research Directorate, USAMRD-Africa, Kisumu, Kenya,* [4]*University of Idaho, Moscow, ID, United States*

Accurate identification of malaria species is important in malaria control efforts. However, in asymptomatic malaria, diagnosis of *non*-falciparum submicroscopic malaria species can be difficult. This is due to mixed infections with other *Plasmodium* species and low parasitemia levels. Lowland western Kenya in particular is holoendemic for malaria transmission and has high rates of asymptomatic malaria infections. However, the true burden of submicroscopic non-*P. falciparum* infections has not been determined in this population. Using panels of highly sensitive and specific qPCR assays, we report the prevalence of malaria

species including *P. falciparum, P.malariae, P.ovale* and *P.vivax* either as single or mixed infection. In addition, we determined the magnitude of gametocyte carriage in this asymptomatic population. Using 1762 dried blood spots (DBS) samples collected in a HIV/malaria co-infection study in a malaria endemic region in western Kenya, the presence of any species of malaria was first determined using an improved assay based on genus-conserved sequences of the *Plasmodium* 18S ribosomal gene. If positive for malaria, panels of highly species-specific qPCR assays were developed to determine the presence and quantity the four malaria species. In addition, we used three stage- gametocyte specific RNA transcripts to quantify gametocyte carriage. Preliminary analysis shows malaria prevalence of about 69.5% as detected by sensitive molecular techniques. Of these, *P.falciparum* was the most prevalent species (59.1%), followed by *P.malariae* (4.7%) and *P. ovale* (2.6%). We have so far not detected any *P.vivax* in our population. Of the 18S positive samples by genus assay, only 66.4 % could be speciated suggesting that there is need for even more sensitive species assays for detection of sub-microscopic malaria. We will report on the magnitude of sexual malaria parasitemia using our gametocyte panel. The relevance of this study is that asymptomatic malaria carriage of both *falciparum* and non-*falciparum* species is an efficient reservoir of malaria hence the need for higher public health priority.

958

THE POTENTIAL IMPACT OF MATERNAL DEPRESSION ON PARENT-CHILD INTERACTIONS AND PARASITIC INFECTION IN BENINESE INFANTS

Amanda Garrison[1], Joanna 'Asia' Maselko[2], David Courtin[3], Roméo Zoumenou[4], Achille Massougbodji[4], Michel Cot[3], Suzanne Maman[5], Florence Bodeau-Livinec[6]

[1]*INSERM UMR*[1153] *Equipe de recherche en Epidémiologie Obstétricale, Périnatale, et Pédiatrique (EPOPé), Center for Epidemiology and Statistics, Sorbonne Paris Cité (CRESS); Sorbonne Universités; Ecole des Hautes Etudes en Santé Publique (EHESP), Paris, France,* [2]*Department of Epidemiology, University of North Carolina: Chapel Hill, Chapel Hill, NC, United States,* [3]*Mère et enfant face aux infections tropicales (MERIT), l'Institut de Recherche pour le Développement (IRD), Université Paris* [5]*, Sorbonne Paris Cité, Paris, France,* [4]*Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Cotonou, Benin,* [5]*Department of Human Behavior, University of North Carolina: Chapel Hill, Chapel Hill, NC, United States,* [6]*INSERM UMR*[1153] *Equipe de recherche en Epidémiologie Obstétricale, Périnatale, et Pédiatrique (EPOPé), Center for Epidemiology and Statistics, Sorbonne Paris Cité (CRESS); Ecole des Hautes Etudes en Santé Publique (EHESP), Paris, France*

Post partum maternal depression occurs in an estimated 13-19% of women following childbirth, with women from developing countries particularly at risk. Numerous studies in low-income settings have found links between maternal depression and neonatal and child health outcomes such as stunting, underweight, inhibited neurodevelopment, and diarrheal disease. However, few studies further investigated the potential consequences in terms of offspring morbidity. Our objective was to explore the relationship between maternal depression one year after birth and parent-child interactions, risk of *Plasmodium falciparum* malaria, and risk of soil-transmitted helminth infection in infants 1-2 years of age in Benin. Our population included mothers and their children enrolled in a clinical trial during the second trimester of pregnancy (MiPPAD). Maternal depression was assessed using the Edinburgh Postnatal Depression Scale (EPDS) in mothers of children one year post-partum. Parent-child interactions were assessed through the HOME subscales at one year post-partum. Blood, urine, and stool samples were taken from children to diagnose malaria and helminth infection prospectively from 1-2 years of age. Crude and adjusted linear and negative binomial regression models tested associations. Of the 303 children in analyses, 39 (12.87%) had mothers with depressive symptoms according to a cut-off of >=13 points. Mean HOME score was 27 (SD=2.4). From 1-2 years of age, median number of malaria episodes per child was 3 (0-14) and 82/240 (34.17%) children had at least one helminth infection. Adjusted linear regression analyses revealed post partum maternal depression to be significantly correlated to a reduced HOME score (Coefficient=-0.12 95% CI: -0.19--0.05). Adjusted negative binomial models did not reveal significant associations between post-partum maternal depression and increased risk of malaria episodes (IRR=1.00, 95% CI: 0.97-1.02) and increased risk of helminth infection (IRR=0.99 95% CI: 0.93-1.05). As expected, parent-child interactions were affected by post-partum depression, but this did not seem to affect offspring morbidity.

959

SHIFT OF DEMOGRAPHIC BURDEN OF MALARIA CASE OF RWANDA USING HMIS

Michee Kabera Semugunzu[1], Jean Louis Mangala[1], Monique Mulindahabi[2], Noella Umulisa[3], Aimable Mbituyumuremyi[1], Jeanine U. Condo[1]

[1]*Rwanda Biomedical Center, Kigali, Rwanda,* [2]*CDC President's Malaria Initiative, Kigali, Rwanda,* [3]*Maternal and Child Survival Program (MCSP), Kigali, Rwanda*

Malaria is a major cause of out patients' consultation in Sub Saharan African country and particularly in Rwanda where malaria constituted more than 30% of outpatient's consultation in year 2017-2018. The Rwanda National Malaria Program Annual Report 2017-18 shows that the number of malaria deaths reduced from 529 cases in 2016-2017 to 382 cases in 2017-2018 (28% reduction), and in the same period, severe malaria cases reduced from 14,033 cases in 2016-2017 to 10,894 cases in 2017-2018 (22.4% reduction). We analyzed demographic characteristic of all 2018 malaria cases and deaths captured through the Rwanda Health Management Information System (HMIS) by age group and gender. All inpatients malaria cases were 16,501: 8, 040 (49%) males and 8,461 (51%) females. A total of 7,688 severe malaria cases were reported, with 4,056 (53%) male and 3,632 (47%) female (p value<0.0001). From Jan to Dec 2018, 331 malaria deaths were notified with171 (52%) male and 160 (48%) female. The proportion of malaria deaths among inpatients male (2.1%) was not different to the proportion of malaria deaths among female (1.9%), p value>0.05. Inpatients malaria cases were 6,662 (40.4%) in under5; 4,663 (28.3%) aged 5 to 19 years and 5, 176 (31.4%) 20 years and above. A total of 3,496 (45%) severe malaria cases were found in children under5; 2,502 (33%) aged between 5 to 19 years and 1,690 (22%) 20 years and above. Malaria deaths were 86 (26%) for under5; 74 (22%) aged between 5-19 years and 171(52%) for 20 years and above. The proportion of malaria deaths to total malaria inpatients was respectively 1.3% for under5; 1.6% for 5 to 19 years and 3.3% for patients of 20 years and above (p-value<0.0001). The Routine malaria data shows that male are most likely to develop severe malaria and adults of 20 years and above have higher mortality risk.

960

DEMOGRAPHIC SURVEILLANCE TO MONITOR PREGNANCY OUTCOMES IN MALARIA ENDEMIC AREA IN OUELESSEBOUGOU, MALI

Gaoussou Santara[1], Naissem Andemel[2], Amadou Barry[1], **Almahamoudou Mahamar**[1], Moussa Traore[1], Seydou Traore[1], Mamoudou Samassekou[1], Ibrahim H. Soumbounou[1], Oumar Attaher[1], Djibrilla Issiaka[1], Halimatou Diawara[1], Patrick E. Duffy[2], Michal Fried[2], Alassane Dicko[1]

[1]*Malaria Research and Training Center (MRTC), Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, MD, United States*

Placental malaria is associated with poor outcomes for both women and their babies. To reduce poor pregnancy outcomes associated with malaria infection, WHO recommends monthly anti-malarial treatment with sulfadoxine pyrimethamine (SP) during the second and third trimester and the use of insecticide-treated bed nets. However, due to the spread of SP-resistant parasites in some parts of Africa, new interventions such as a vaccine to prevent placental malaria is needed. Prior to testing a

vaccine that may be given as a primary vaccine series or as a boosting dose during early pregnancy baseline information on pregnancy outcomes in the target population is needed. We assessed the frequency of poor pregnancy outcomes and their relation to malaria in pregnant in women in Ouelessebougou Mali from February 2017 to March 2019. Pregnant women were recruited during the antenatal consultations at the community health centers and information on malaria and pregnancy outcomes were collected four weeks post pregnancy termination (delivery, miscarriage or stillbirth). The proportion of pregnancy loss (defined as miscarriage, stillbirth and early neonatal death) and preterm delivery (PTD) were 3.9% (70/1777 and 4.7% (73/1777) respectively. The proportion of low birth weight deliveries was 6.5%. In multivariate logistic regression model adjusted for age, SP-IPTp, and bed net use, young age (<18), was associated with increased odds of pregnancy loss (OR 1.86 (95%CI 1.01-3.43, p=0.04); SP-IPTp, and bed net use were associated with reduced odds of pregnancy loss: 0.34 (0.14-0.83), p=0.02 and 0.48 (0.22-1.03) p=0.06 respectively. Women aged <18 and >35 years had increased odds of PTD, 2.98 (1.77-5.02) p<0.0001 and 2.26 (1.04-4.90) p=0.04 respectively. SP-IPTp and bed net use did not reduced the odds of PTD. The rates of pregnancy loss and preterm birth were high in area with high malaria transmission. Younger age is associated with pregnancy loss, and both young age and age >35 years are associated with increased odd of PTD.

961

PREVALENCE OF MALARIA INFECTION IN PREGNANT WOMEN IN OUELESSEBOUGOU, MALI

Moussa Traore[1], **Almahamoudou Mahamar**[1], Gaoussou Santara[1], Seydou Traore[1], Mamoudou Samassekou[1], Ibrahim H. Soumbounou[1], Seydina O. Maguiraga[1], Oumar Attaher[1], Adama Sissoko[1], Sekouba Keita[1], Bacary S. Diarra[1], Djibrilla Issiaka[1], Halimatou Diawara[1], Patrick E. Duffy[2], Michal Fried[2], Alassane Dicko[1]

[1]*Malaria Research and Training Center (MRTC), Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, MD, United States*

Malaria during pregnancy is associated with low birth weight, preterm delivery, severe maternal anemia, and maternal and perinatal death. Despite ongoing control efforts, the burden of pregnancy malaria remains high in endemic countries in sub-Saharan Africa such as Mali. We assessed the frequency of malaria infection among women appearing for antenatal consultations by rapid diagnostic test (RDT) in community health centers in Ouelessebougou health district during the malaria transmission season. Information collected included gestational age, gravidity, residence area as well as the use of intermittent preventive treatment of malaria in pregnancy with sulfadoxine–pyrimethamine (IPTp-SP) and insecticide treated nets (ITN). Among 496 pregnant women (PW) screened from August 2018 to January 2019, 39.2% were infected. The prevalence of malaria infection was higher in the rural versus semi-urban health center (57.9% (92/159) vs. 30.4% (102/336) respectively (p = 0.0002)). The frequency of positive RDT decreased from 49.4% (84/170) in primigravidae to 40.7% (59/145) in secundigravidae to 28.3% (51/180) in multigravidae women (p = 0.0003). Based on the women's report and review of antenatal clinic cards, only 38.4% (164/428) of women in their second or third trimester had received IPTp-SP. In multivariate analysis malaria infection was significantly higher in primigravidae women (OR: 2.54, 95% CI: 1.28-5.04, p=0.008), in secundigravidae women (OR: 2.67, 95% CI: 1.35-5.25, p=0.004). The odds of malaria infection was lower in pregnant women living in urban area (OR: 0.30, 95% CI: 0.17-0.53, p<0.001), who received IPTp-SP (OR: 0.30, 95% CI: 0.14-0.54, p<0.001) and always sleep under ITN (OR: 0.21, 95% CI: 0.08-0.53, p=0.001). Malaria infection is pervasive among pregnant women in Mali. Efforts are needed to improve the coverage of the preventive measures such as IPTp-SP. An effective strategy such as an easily administered malaria vaccine would be an important addition to reduce this huge burden of malaria in pregnancy.

962

IMPROVING THE SPATIAL GRANULARITY FOR TARGETING INDOOR RESIDUAL SPRAYING ON BIOKO ISLAND

Olivier Tresor Donfack[1], Guillermo A. Garcia[2], Jordan M. Smith[1], David L. Smith[3], Carlos A. Guerra[2]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Medical Care Development International, Silver Spring, MD, United States,* [3]*Institute for Health Metrics and Evaluation, Seattle, WA, United States*

Since the launch of the Bioko Island Malaria Control Project 16 years ago, 26 rounds of indoor residual spraying (IRS) have been completed on the island. Between 2015 and 2018 IRS was targeted based on risk stratification at the community level (fourth administrative division). Communities, however, vary in area and population and malaria transmission within them is highly heterogeneous. This has raised concerns regarding the efficiency of allocating a costly intervention such as IRS. For round 26, conducted in 2019, we adopted a new approach using a grid of 100x100 m sectors as the units for targeting. The aim was to target ~15,000 households considering 80-85% spray coverage. First, we identified transmission hotspots using kernel smoothing of individual *Plasmodium falciparum* parasite rate (*Pf*PR) data from the 2018 malaria indicator survey after excluding individuals with a history of travel. This allowed us to estimate sector-level *Pf*PR assumed to represent locally acquired malaria. We chose an arbitrary threshold of *Pf*PR>10% for targeting. There were 1443 such sectors containing 24,402 households. Further selection of target sectors was needed to keep within the preset number of households to spray. To do so, we ranked sectors based on a combination of *Pf*PR, population, sample size, and an urban/rural divide. The latter was used such that 80% of households would be selected from urban and 20% from rural areas. The highest ranked sectors were selected until the ~15,000 household mark was reached. Higher population sectors were preferred because the total spray area would be smaller. Higher sample size sectors were preferred because *Pf*PR estimates would have narrower confidence intervals. A total of 692 sectors were selected for spraying, most of which were clustered. Coverage will be estimated at the sector level to achieve the desired 80-85% mark. This new approach allowed IRS to be more focalized by increasing the spatial granularity of the target units, improving the efficiency and, hopefully, the effectiveness of this costly intervention.

963

SELECTIVE WHOLE GENOME AMPLIFICATION OF DNA IN LOW PARASITEMIA SAMPLES OF *PLASMODIUM VIVAX* FROM PERU

Mac Pholo Aguirre Huamaní[1], Paulo César Manrique Valverde[1], Christopher Delgado Ratto[2], Jean-Pierre Van geertruyden[2], Dionicia Gamboa Vilela[1], Dionicia Gamboa Vilela[3], Dionicia Gamboa Vilela[4]

[1]*Laboratorio de Malaria, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Global Health Institute, University of Antwerp, Antwerp, Belgium,* [3]*Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [4]*Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru*

The low recovery of parasite DNA in low parasite density *Plasmodium vivax* infections (=sub-microscopic infections) restricts the inclusion of sub-microscopic infections in population genetics and population genomic studies. The under-estimation of the genetic diversity biases the understanding of *P. vivax* transmission especially in low endemic settings where submicroscopic infections are predominant. Different methods have been developed to overcome this problem, being the Selective Whole Genome Amplification (SWGA) one of the most promissory tools. However, its effectiveness in low parasite density *P. vivax* infections has not been reported yet. This work aims to evaluate the performance of SWGA in *P. vivax* sub-microscopic infections. The protocol reported by Cowell *et al.* 2017 was adapted to improve the yield of the method while assessing

the minimum amount of DNA sample required for amplification (LOD) and its application on 20 field samples. Changes in different components and parameters of the SWGA reaction were tested. Parasite and human DNA quantity were measured by real-time PCR before and after the SWGA to determine the parasite DNA enrichment. The best performance of the method SWGA occurred at 24 hours of incubation, with 1 μL of Phi29 enzyme from illustra GenomiPhi V2 DNA amplification kit, 1.25 mM of dNTPs as final concentration, 6.4 μM of the pvset1920 primer set, and 3.4 μM of the pvset1 primer set. When two rounds of SWGA were applied, the method generated lower parasite DNA concentrations (~0.6 times) but the percentage of parasite DNA was greater (~3.5 times) than using one round. The minimum amount of parasite DNA required to produce at least 1200 parasite DNA molecules/μL was between 0.75 - 1 parasite DNA molecules/μL and 17 out of the 20 field samples amplified correctly. Our results showed that SWGA could be used to enrich *P. vivax* DNA in low parasitemia samples allowing larger coverage of samples to be included in genetic studies and also we provide a protocol to determine the fidelity of SWGA.

964

AMPLICON DEEP SEQUENCING VERSUS TRADITIONAL GENOTYPING OF MSP1 AND MSP2

Daniel Castaneda-Mogollon, Ranmalee Amarasekara, Conrad Izydorczyk, Dylan R. Pillai

University of Calgary, Calgary, AB, Canada

Malaria infection is thought to be polyclonal in nature. Microscopic or polymerase chain reaction methods are only able to detect the predominant clone due to inherent limitations. Detection of the predominant clone may not reveal the true complexity of infection which may include minority variants that are more virulent or resistant to anti-malarials. We sought to compare traditional PCR methodology with amplicon deep sequencing based on next generation sequencing. We analyzed data from 15 PCR-confirmed *Plasmodium falciparum*-infected patients and 5 control samples using the merozoite surface protein 1 and 2 (msp1 and msp2) genes as markers. Amplification of the MAD20, RO33, and K1 msp1 and FC27 and 3D7 msp2 alleles was performed using nested PCR, followed by an agarose gel electrophoresis to determine the presence and number of alleles per locus in each sample. Deep sequencing of both msp genes was performed using an Illumina MiSeq platform, followed by determination of the number of alleles present per locus per sample using SRST2 (Short Read Sequence Typing), a reference-based DNA read mapping and SNP calling bioinformatics software. Multiplicity of infection was determined by estimating the number of alleles per locus and compared against a bioinformatics haplotype-based approach. Deep sequencing found 11 K1 alleles across the 15 patient samples, 9 for MAD20, 5 for RO33, 9 for FC27, and 13 for 3D7, whereas nested PCR followed by gel electrophoresis found 9 for K1, 6 for MAD20, 3 for RO33, 3 for FC27, and 10 for 3D7. Deep sequencing found 3 K1 alleles across the 5 control samples, 2 for MAD20, 3 for RO33, 4 for FC27, and 5 for 3D7, whereas nested PCR found 3 K1 alleles, 2 for MAD20, 3 for RO33, 2 for FC27 and 4 for 3D7. Additionally, the agarose gel confirmed a MOI average and standard error of the mean (SE) of 1.4 ± 0.18 across the 20 samples by employing conventional methods, whereas deep sequencing yielded a value of 1.85 ± 0.19 (p value of 0.03). Our findings suggest deep sequencing yields significantly higher number of alleles per locus, and thus, MOI per sample which may have implications for drug treatment and diagnostics.

965

EVOLUTION OF *PLASMODIUM FALCIPARUM* AFTER AN OUTBREAK FACILITATES LOW ENDEMICITY MALARIA TRANSMISSION IN ECUADOR

Shazia Ruybal-Pesántez[1], Fabián E. Sáenz[2], Claudia A. Vera-Arias[2], Kathryn E. Tiedje[1], Karen P. Day[1]

[1]*School of BioSciences/Bio*[21] *Institute, University of Melbourne, Melbourne, Australia,* [2]*Centro de Investigación para la Salud en América Latina, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, Ecuador*

In Latin America, *Plasmodium falciparum* outbreaks are common. In the coast of Ecuador, an outbreak in 2012-2013 documented 150 *P. falciparum* clinical cases after less than 10 annual cases in previous years, pointing to the risk of "epidemics". Following the outbreak, the number of reported *P. falciparum* cases in Ecuador increased by 38% in 2014 and 78% in 2015. We aimed to understand how malaria could be sustained by characterizing *P. falciparum* transmission dynamics using the "*var*code", a genotyping tool based on the *var* multigene family encoding the major variant surface antigen. The *var* genes encoding this antigen are highly polymorphic and *var* repertoires or haplotypes are rapidly evolving, enabling high discriminatory resolution to define transmission networks in real-time. We apply this tool to characterize *P. falciparum* during and after the outbreak from 2013-2015 to detect parasite genetic *var*codes circulating locally. We then reconstructed transmission networks to identify clusters of genetically-related parasites in space and/or time. Our findings reveal the "real-time" evolution of *P. falciparum* after an outbreak, consistent with an endemoepidemic population structure whereby low but sufficient levels of recombination among parasites can generate new *var*codes and sustain transmission in a local population. Comparisons to other South American parasite populations elucidate possible origins of *var*codes circulating in Ecuador. This study confirms that the *varcode* provides a useful surveillance tool to characterize a rapidly evolving *P. falciparum* parasite population.

966

VARIED PREVALENCE OF MARKERS OF ANTIMALARIAL DRUG SENSITIVITY ACROSS UGANDA

Victor Asua[1], Marvin Duvalsaint[2], Ozkan Aydemir[3], Jennifer Legac[2], Samuel L. Nsobya[1], Melissa D. Conrad[2], Jeffrey Bailey[3], Philip J. Rosenthal[2]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*University of California San Francisco, San Francisco, CA, United States,* [3]*Brown University, Providence, RI, United States*

Antimalarial drug resistance, mediated in part by known *P. falciparum* genetic polymorphisms, is of concern. Chloroquine/sulfadoxine-pyrimethamine (SP) was replaced as the Ugandan treatment regimen by artemether/lumefantrine in 2006. SP is used to prevent malaria in pregnant women. Recent reports showed changing prevalence of key parasite polymorphisms. To continue surveillance, 50 samples were collected in 2018 from children (6 mo-10 yr) diagnosed with malaria by microscopy or rapid test at each of 13 sites across Uganda. Polymorphisms associated with resistance were characterized by ligase detection reaction fluorescent microsphere (LDR) and molecular inversion probe (MIP) assays, copy number (CN) variations were assessed by qPCR, and the K13 propeller domain was sequenced. Agreement between LDR and MIPs assays was excellent. Preliminary analysis shows that prevalences of polymorphisms varied across the country. For transporter mutations, prevalences (by MIPs analysis) were 0-42% for *pfcrt* 76T, 0-3% for *pfmdr1* 86Y, 38-86% for *pfmdr1* 184F, and 3-46% for *pfmdr1* 1246Y; the highest prevalences of 76T were at sites near international boundaries. For antifolate mutations, 5 mutations (*pfdhfr* 51I, 59R, 108N;*pfdhps* 437G, 540E) were at nearly 100% prevalence, as seen previously, and the prevalence of mutations mediating higher-level resistance was high at multiple sites (*pfdhfr* 164I 15-75% at 7 sites; *pfdhps* 581G 15-65% at 6 sites), with the highest prevalences in western Uganda. Polymorphisms associated elsewhere

with altered sensitivity to lumefantrine (increased *pfmdr1*CN), piperaquine (increased *plasmepsin-2* CN), and artemisinins (K13 mutations) were uncommon. Comparing our results to older data, prior increases in the prevalence of *pfcrt* K76 wild type parasites appear to have stalled, especially in border areas. Overall, although genetic evidence suggesting ACT resistance is lacking, recent data suggest incomplete loss of resistance to chloroquine and increasing high-level resistance to antifolates, with varied patterns across the country. Continued surveillance for drug resistance markers is an important priority.

967

SEEKING GENES AND THEIR EXPRESSION PROFILES THAT CONTROL SEXUAL ASSIGNMENT IN *PLASMODIUM FALCIPARUM*

Margaret R. Smith, Kazutoyo Miura, Ababacar Diouf, Bingbing Deng, Luwen Zhou, Carole A. Long

National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

The sexual stage of *Plasmodium* parasites is a bottleneck in the malaria life cycle, and blocking the development process could potentially stop disease transmission. Although AP2-g transcription factor is known to be important for sexual stage commitment, what is not known is what genes or gene expression profiles control the sexual assignment of gametocytes (i.e., either developing to male or female gametocytes). We are addressing this question using two lines of *P. falciparum* NF54, SA and N1. N1 is a female gametocyte dominated strain with a female to male ratio of ~20 while SA has a ratio of ~2. In turn, due to the female dominancy, N1 gives fewer oocysts than SA when doing standard membrane-feed assay. Whole genomic sequencing and RNA-sequencing were performed to determine the factors which contribute to the different sex ratios. DNA-Sequencing was performed using PacBio. N1 and SA were compared with the reference strain 3D7 individually. N1 had 3847 variants from 3D7 and SA had 4030 variants from 3D7. This came down to 112 variants that were recognized as high or moderate impact of N1 and SA based on the definition from the ensembl.org glossary. For the RNA-sequencing, RNA samples were collected at 0, 8, 16, 24, 32, 40 and 48 hours after induction of synchronized gametocyte cultures. Data from DNA-sequencing and RNA transcriptomics will be presented. This study will help better understand the sexual assignment in gametocytes in *Plasmodium falciparum.*

968

MAPPING COMPETITIVE GROWTH OF MALARIA PARASITES TO ASSESS THE FITNESS IMPACT OF ARTEMISININ RESISTANCE

Katelyn M. Vendrely[1], Lisa A. Checkley[1], Marina McDew-White[2], Ian H. Cheeseman[2], Ashley M. Vaughan[3], François H. Nosten[4], Timothy J. Anderson[2], Michael T. Ferdig[1]

[1]*Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States,* [2]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [3]*Seattle Children's Research Institute, Seattle, WA, United States,* [4]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Mae Sot, Thailand*

In vitro competitive growth assays measure the relative growth between co-infecting *Plasmodium falciparum* parasites during the blood stage and can serve as an index of fitness, revealing fitness disparities between the co-infecting parasites. Differences in blood stage fitness costs often result from drug resistance mutations which may be mitigated by compensatory mutations. Drug resistance can be conferred by a single point mutation or after a complex series of mutations that either enhance resistance or compensate for fitness costs. With the recent independent emergences of artemisinin resistance and the number of mutations associated with resistance, it is important to understand the relative competitive fitness of the blood stage parasites with different sets of mutations. Genetically distinct drug resistant parasites with different sets of mutations are likely to vary in blood stage proliferation rate, contributing to their chance of transmission to the mosquito vector. Quantitative trait locus (QTL) mapping has long been used as a way to identify genetic *loci* alone or in combination that correlate with a specific phenotype. Using QTL mapping, we can determine what mutations allow drug resistant parasites to be fit in the blood stage, as measured by *in vitro* competitive growth assays. By genetically crossing two parasites that differ in their drug resistance and fitness phenotypes, we can use the recombinant progeny to map *loci* using QTL. Using our optimized competitive growth assays, we can generate a quantitative phenotype for a full progeny set to infer mechanisms that alleviate fitness costs associated with drug resistance. Improved understanding of the fitness costs of different parasites proliferating in human blood and the role different resistance mutations play will contribute to an understanding of the potential for specific mutations to spread in populations.

969

ATG10 GENETIC VARIANTS ARE NOVEL PREDICTORS OF LONGITUDINAL SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA AND ALL-CAUSE MORTALITY IN KENYAN CHILDREN

Caroline Ndege[1], Samuel B. Anyona[2], Elly Munde[2], Nick Hengartner[3], Benjamin H. McMahon[3], Paul Fenimore[4], Qiuying Cheng[5], Christophe G. Lambert[5], Collins Ouma[6], Douglas J. Perkins[5]

[1]*University of New Mexico-Kenya Global Health Programs, Albuquerque, NM, United States,* [2]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya,* [3]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [4]*University of New Mexico, Albuquerque, NM, United States,* [5]*University of New Mexico Center for Global Health, Albuquerque, NM, United States,* [6]*Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Kisumu, Kenya*

Plasmodium falciparum severe malarial anemia [(SMA, hemoglobin (Hb) <5.0 gdL^{-1}] is a leading cause of morbidity and mortality among children under 5 years residing in *P. falciparum* holoendemic areas. The pathogenesis of SMA is multifactorial and polygenic. To identify novel molecular pathways that influence development of SMA, whole genome and transcriptome profiling were performed in a subset of Kenyan children (n=144, 3-36 months) with discrete non-SMA and SMA phenotypes. Although not previously investigated in malaria, autophagy related gene-10 (*ATG10*) emerged as a significant predictor of enhanced susceptibility to SMA (2.126-fold increase, *P*=0.024). To validate these findings, the relationship between *ATG10* polymorphisms [-7723T/G (rs2406905), -4322T/A (rs4391141), and -2442G/C (rs1023969)] and malarial outcomes were investigated in children (n=1,512, aged<5 years) at enrollment and over a 36-month follow-up. Inheritance of the -7723 recessive genotype (GG) increased susceptibility to SMA at enrollment (OR=1.690, *P*=0.037), and longitudinally (RR=1.630, *P*=0.049). At enrollment, carriage of the -4322 TA genotype protected against SMA (OR=0.59, *P*=0.014), while the -2442 GC genotype enhanced development of SMA (OR=1.550, *P*=0.009). Cross-sectionally, haplotypes (-T7723G/-T4322A/-G2442C) enhanced susceptibility to SMA in carriers of GTC (OR=1.37, *P*=0.056), GTG (OR=2.34, *P*=0.003), and TTC (OR=1.641, *P*=0.005), whereas protection was present in carriers of TAG (OR=0.570, 95%CI: 0.35-0.94, *P*=0.027). Longitudinally, the GTG (RR=1.63, *P*=0.013) and TTC (RR=1.87, *P*=0.012) haplotypes predicted increased risk of SMA. Transcriptional analyses revealed that variants which protected against SMA had lower *ATG10* expression, while those associated with enhanced risk had elevated expression. Inheritance of -7723 TG and GG genotypes increased all-cause mortality by 78% (*P*=0.050) and 117% (*P*=0.056) respectively, while GTC haplotype carriage increased susceptibility by 89% (*P*=0.018). Collectively, these novel findings show that variation in *ATG10* predicts susceptibility to SMA and all-cause mortality.

970

POINT MUTATIONS IN COMPLEMENT C3 ALTER LONGITUDINAL RISK PROFILES FOR MALARIA AND SMA EPISODES IN KENYAN CHILDREN

Evans Raballah[1], Samuel B. Anyona[2], Qiuying Cheng[3], Tessa LeCuyer[3], Elly Munde[2], Caroline Ndege[2], Nick Hengartner[4], Paul Fenimore[4], Benjamin McMahon[4], Christophe G. Lambert[3], Collins Ouma[5], Douglas J. Perkins[3]

[1]*Masinde Muliro University of Science and Technology, Kakamega, Kenya,* [2]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya,* [3]*University of New Mexico Center for Global Health, Albuquerque, NM, United States,* [4]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [5]*Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Kisumu, Kenya*

Severe malarial anemia (SMA) is a leading cause of childhood morbidity and mortality in holoendemic *Plasmodium falciparum* transmission regions such as Siaya, Kenya. To enhance the molecular understanding of factors that predispose children to SMA, we explored the relationship between non-synonymous point mutations in C3 [rs2230199 (2307C>G, Arg^{102}→Gly) and rs11569534 (34420G>A, Gly^{1224}→Asp)] and malaria disease outcomes in children (n=1,597, <3 years) at enrollment and during a 3-year longitudinal follow-up. At enrollment, there was a reduced risk of malaria in carriers of the 2307CG genotype (OR=0.714, 95%CI: 0.523-0.974, *P*=0.033) and GG haplotype (OR=0.633, 95%CI: 0.467-0.857, *P*=0.003), while inheritance of the CG haplotype reduced the risk of SMA (OR=0.623, 95%CI: 0.394-0.985, *P*=0.043). Longitudinal analyses revealed an increased risk of malaria in carriers of the AA genotype at locus 34420 (RR=1.124, 95%CI: 1.021-1.237, *P*=0.017) and carriers of the GA haplotype (RR=1.063, 95%CI: 1.018-1.110, *P*=0.006). Conversely, there was a reduced risk of malaria over the follow-up period in children who inherited the CG (RR=0.884, 95%CI: 0.817-0.958, *P*=0.002) and GG (RR=0.945, 95%CI: 0.982-1.001, *P*=0.054) haplotypes. Longitudinal susceptibility to the development of SMA in children with malaria was enhanced in carriers of the 2307GG genotype (RR=2.095, 95%CI: 1.175-3.734, *P*=0.012), and reduced in children who inherited the CG haplotype (RR=0.628, 95%CI: 0.451-0.875, *P*=0.006). Taken together, the novel results presented here show that non-synonymous point mutations in C3 at Arg^{102} and Gly^{1224} alter the risk of contracting malaria, and the development of SMA once an individual becomes infected with *P. falciparum*.

971

USE OF MOLECULAR INVERSION PROBES TO ELUCIDATE WITHIN-COUNTRY POPULATION STRUCTURE OF TANZANIAN *PLASMODIUM FALCIPARUM* ISOLATES

Kara A. Moser[1], Rashid Madebe[2], Mercy G. Chiduo[2], Celine I. Mandara[2], Ozkan Aydemir[3], Susan F. Rumisha[4], Frank Chacky[5], Madeline Denton[1], Patrick Marsh[3], Jeffrey Bailey[3], Sigsbert Mkude[5], Renata Mandike[5], Fabrizio Molteni[6], Ritha Njau[7], Ally Mohamed[5], Deus Ishengoma[2], Jonathan J. Juliano[1]

[1]*Institute for Global Health and Infectious Diseases, University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [2]*National Institute for Medical Research, Tanga, United Republic of Tanzania,* [3]*Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, United States,* [4]*National Institute for Medical Research Headquarters, Dar es Salaam, United Republic of Tanzania,* [5]*National Malaria Control Programme, Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, United Republic of Tanzania,* [6]*Swiss Tropical and Public Health Institute, Basal, Switzerland,* [7]*World Health Organization Country Office, Dar es Salaam, United Republic of Tanzania*

Genetic and genomic information is increasingly useful in assessing prevalence of clinically important mutations, parasite transmission patterns, and the impact of interventions. In order to leverage these analyses, a current understanding of local and regional parasite diversity and population structure is needed. However, many genotyping methods, such as whole genome sequencing, can be cost-prohibitive for large surveys needed for such discrimination. Here, we use two molecular inversion probe (MIP) panels (one designed around 1,800 variable single nucleotide polymorphism positions across the genome; the other on SNPs associated with drug resistance in 14 genes) to investigate *P. falciparum* population structure in Tanzania. We used over 1,000 clinical isolates collected as dried blood spots in 13 councils across the country. After removing markers and samples with high missingness (greater than 90% missing genotypes), preliminary principal component analysis (PCA) and Admixture analyses using 1,617 markers and 744 samples revealed segregation between samples collected from north/north-west and south/south-east. At the region level, average multiplicity of infection estimates ranged from 1.28 to 1.65 and tracked with increasing estimates of malaria prevalence, suggesting the ability of MIPs to inform estimates of transmission. Population structure appears to be driven by both non-drug resistance and drug-resistance associated mutations. Allele frequencies for certain *pfdhps* and *pfcrt* mutations segregated between geographic regions, and mutations on chromosome 2 and 11 (not previously shown to be related to drug resistance) contributed to the structure seen by PCAs. Further analyses with this data will attempt to discriminate parasite populations on the council-level using different subsets of variants (based on genomic position, snps verses indels, and allele frequencies). These analyses will provide an understanding of the genetic diversity and population structure of Tanzanian parasites to the National Malaria Control Program, on which assessments of the impact of control interventions can be made.

972

MLMOI - AN R-PACKAGE TO ESTIMATE MULTIPLICITY OF INFECTION

Kristan A. Schneider, Meraj Hashemi Eshkiki

University of Applied Sciences Mittweida, Mittweida, Germany

The co-occurrence of multiple genetically distinct parasite lineages within one infection, referred to multiplicity of infection (MOI), is recognized as an important quantity in malaria. Still MOI is often estimated by biased ad-hoc methods. Furthermore, accurate estimates of lineage frequencies are hampered by MOI. Here, an easy-to-use R package is introduced that allows to accurately estimate MOI and lineage frequencies from parasite molecular data by maximum likelihood. In particular, maximum-likelihood estimates for the distribution of MOI, lineage frequencies and prevalence are provided. To facilitate its usability, the package provides a flexible import function that allows in various entry formats to be imported and converted into one standard format appropriate for estimating MOI, lineage frequencies and prevalence. These formats include microsatellite, SNP or codon data, entered in various ways, e.g., one- or three-letter amino acid code. In particular, users do not need to provide their data in a particular pre-defined format. Rather, users can choose from a large variety of formats that will require only little adjustments by hand. During data import, potential issues and data entry errors are detected in an additional step to check data quality.

973

ASSOCIATION OF ALPHA GLOBIN VARIANTS WITH *PLASMODIUM KNOWLESI* MALARIA DISEASE SEVERITY AND INFECTION SUSCEPTIBILITY

Matthew J. Grigg[1], Jessica Nino De Rivera[2], Bridget E. Barber[1], Timothy William[3], Tsin W. Yeo[4], Christopher J. Drakeley[5], Nicholas M. Anstey[1], Hans Ackerman[2]

[1]*Menzies School of Health Research and Charles Darwin University, Darwin, Australia,* [2]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*Clinical Research Centre, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia,* [4]*Lee Kong Chian School of*

Medicine, Nanyang Technological University, Singapore, Singapore, Singapore, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom

Plasmodium knowlesi is a monkey parasite that is increasingly reported to infect humans across Southeast Asia. Importantly, *P. knowlesi* causes severe disease in around 6-9% of clinical cases in areas of Malaysia, and can result in case fatalities. Previously highly endemic malaria species such as *P. falciparum* and *P. vivax* in this region have exerted considerable selection pressure on the human genome, resulting in well established red blood cell (RBC) polymorphisms known to mediate disease severity, such as seen with alpha globin variants for *P. falciparum*. For *P. knowlesi*, phenotypic G6PD deficiency has also been associated with protection against susceptibility to symptomatic infection; however to date other RBC polymorphisms have not been evaluated in this context. This study aimed to evaluate the association between common alpha globin variants in Southeast Asia and infection susceptibility and disease severity in clinical cases of *P. knowlesi* malaria. Samples were utilised from a population based case-control study was conducted from 2012-15 in Sabah, Malaysia, enrolling PCR confirmed *P. knowlesi* cases and village matched health controls. Additional *P. knowlesi* malaria patients enrolled in an ongoing Sabah prospective study were also included. In total 1,176 patients with confirmed *P. knowlesi* infection, including >120 meeting WHO severe malaria criteria, and 860 malaria negative controls are expected to be included in the final analysis. High throughput digital droplet PCR technology will evaluate the 5 most common Southeast Asian alpha globin variants: -3.7 deletion, -4.2 deletion, Constant Spring mutation, Cd59 (Adana) mutation, and --FIL deletion, in addition to the inferred --SEA deletion. Results will be presented at the ASTMH meeting and include the number and proportion for each alpha globin variant, including subsets of copy number variant, and relationship with; a) disease severity, and b) disease acquisition, using descriptive statistics and logistic regression models. The final outcome will assess whether relevant alpha globin variants alter disease susceptibility and severity to zoonotic *P. knowlesi* malaria.

974

MULTIPLEXED AMPLICON SEQUENCING OF *PLASMODIUM FALCIPARUM* FOR DRUG RESISTANCE GENOTYPING AND BARCODING

Christopher Jacob[1], Naomi Park[1], Eleanor Drury[1], Kirk Rockett[2], Scott Goodwin[1], Carol Scott[1], Victoria Simpson[3], Sonia Goncalves[1], Olivo Miotto[1], Dominic Kwiatkowski[1]

[1]*Wellcome Sanger Institute, Cambridge, United Kingdom,* [2]*Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom,* [3]*MRC Centre fo Genomics and Global Health, Big Data Institute, University of Oxford, Oxford, United Kingdom*

Every malaria endemic country requires systems to monitor and survey the state of disease in their respective nation. While the goals of surveillance may be different, key metrics like proportions of drug resistant parasites and transmission intensity are valuable pieces of information that can inform policy and help make decisions related to the control of disease. Specific assays can detect resistance to a single drug based on a molecular marker(s), but the time and cost increase is high when information about multiple drugs is needed, as in areas with high proportions of multi-drug resistant parasites. Estimates of transmission intensity using genetics can be done by looking at the proportion of mixed infections in a subset of parasites from a given location, either through model-based methods using multi-locus "barcodes" or from deep sequencing of single highly polymorphic *loci*. Our method uses multiplex PCR to generate a panel of amplicons for detection of resistance to first line treatments including artemisinin, piperaquine, chloroquine, sulfadoxine, and pyrimethamine. We also genotype a 101 SNP barcode which can be used to estimate multiplicity of infection using multi-locus model-based programs. In addition to drug resistance and barcode genotyping, a set of assays can also detect the presence of other *Plasmodium* parasites including *P. vivax*, *P. knowlesi*, P. Ovale, and P. malariae. Using ~140 amplicons across 3 PCRs we reliably genotype 384 samples in a single sequencing reaction, generating an average of >100x coverage per amplicon. This method is optimized for use on DNA extracted from either whole blood or filter paper blood spots. So far more than 10,000 *P. falciparum* parasites have been genotyped from South America, Asia, Africa, and Oceania.

975

MOLECULAR AND IMMUNOLOGICAL CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTE-SPECIFIC GENES

Jonas A. Kengne-Ouafo[1], Yaw Aniweh[1], Saikou Y. Bah[1], Collins M. Morang'a[1], Lucas Amenga-Etego[1], Britta C. Urban[2], Gordon A. Awandare[1], Bismarck Dinko[3]

[1]*WACCBIP, University of Ghana, Accra, Ghana,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*Department of Biomedical Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Sciences, Ho, Ghana*

Plasmodium falciparum has an inherent ability to rapidly evolve resistance to drugs/vaccines. Therefore, the need for increased efforts towards drug/vaccine development. However, much of the parasite genome remains uncharacterized. This study sought to identify and characterize gametocyte-specific genes and determine their immunogenicity in malaria endemic regions. Genes were prioritized based on gametocyte specific expression or uniform expression pattern across different erythrocytic stages using available published and unpublished *P. falciparum* laboratory strain transcriptomics and proteomics data. Genomes from the Pf3k (https://www.malariagen.net/projects/pf3k) database were used to assess the genetic diversity of selected genes while differential gene expression in Ghanaian field isolates was determined by q-PCR. Prioritization led to the identification of 2 genes: gametocyte-specific PF3D7_051300 and PF3D7_115800 with expression across all erythrocytic stages. Moreover, peptides corresponding to predicted B-cell epitopes of the corresponding protein sequences of the prioritized genes were commercially synthesized to study the naturally acquired humoral immune response in malaria-infected children in Ghana. Genomic sequence analysis revealed the genes were relatively conserved (FsT values of 0.08 and 0.115 respectively) across continents and were found to be under directional selection based on the negative Tajima's D values obtained, -2.35 and -2.33 respectively. These values were higher in South East Asia (SEA) than in Africa. Further RT-qPCR of field isolates using asexual (rings, trophozoites and schizonts) and gametocyte (gametocytes III, IV & V) stages showed that, PF3D7_051300 had a gametocyte-biased expression while PF3D7_115800 was expressed across stages. These expression patterns were consistent with observations in laboratory strains that were used for prioritization. Ongoing immunological investigations will describe the natural immunity to these candidates and determine their potential as vaccine candidates for transmission blocking interventions.

976

POPULATION GENETICS, SEQUENCE DIVERSITY, SELECTION AND COMPLEXITY OF INFECTION OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1 GENE IN TWO ECOLOGICAL ZONES IN GHANA

Benedicta Ayiedu Mensah[1], Benjamin Abuaku[1], James Myers Hansen[1], ozkan Aydemir[2], Patrick Marsh[2], Francis Anto[3], Jeffrey Bailey[2], Anita Ghansah[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Brown University, Providence, MA, United States,* [3]*University of Ghana School of Public Health, Accra, Ghana*

One major challenge to the global agenda for malaria elimination is the extensive genetic diversity of the parasite population, resulting in the development of drug resistance and variation in antigens targeted for vaccine development. The aim of this study was to determine how much the increased use of artemisinin-based combination therapies (ACTs) and other control interventions are shaping the genetic diversity of the *Plasmodium falciparum* population in two ecologically distinct populations

in Ghana. A total of 803 dried blood spots (DBS) on filter paper were collected from symptomatic children, aged 6 months to 14 years, with *P. falciparum* mono-infection in the coastal savanna (Cape-Coast) and the forest (Begoro) zones of Ghana from 2014 to 2017. The study leveraged the high specificity and relatively low-cost of targeted next generation sequencing using molecular inversion probes for targeting and sequencing on the illumina MISEQ platform for sequencing of *Plasmodium falciparum* Apical Membrane Antigen 1 (*PfAMA1*) gene. The result showed high genetic diversity in *PfAMA1* in Ghanaian sequences with a total of 164 *PfAMA1* haplotypes and a haplotype diversity of 0.993. The overall nucleotide diversity of the *PfAMA1* sequences was 0.015. There was no significant genetic differentiation between the two study populations in Ghana. Parasite isolates from the two ecological zones in Ghana showed a moderate level of genetic differentiation with sequences from Thailand (Fst=0.054) and low differentiation with sequences from Kenya (Fst=0.004). The results also showed balancing selection which might have contributed to the high diversity in *PfAMA1*. Seventy three percent of the infections were monoclonal with an overall complexity of infection of 1.30. This study provides new data on genetic diversity of *PfAMA1* gene in Ghana and gives valuable information or the development of an effective *PfAMA1*-based malaria vaccine, thus strengthening the importance of investigating genetic diversity of *P. falciparum* and evolutionary history of parasite populations in the field of malaria vaccine development.

977

RELATEDNESS BETWEEN MALARIA PARASITES: PORTABLE INSIGHTS ACROSS SETTINGS

Aimee R. Taylor[1], Pierre E. Jacob[2], Daniel E. Neafsey[1], Caroline O. Buckee[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Harvard University, Cambridge, MA, United States*

Increasingly, genetic surveillance of malaria parasites is being used in near-elimination settings to describe patterns of importation and monitor drug resistance. Studies of parasite relatedness based on identity-by-descent (IBD) have generated demographic insight on a temporal-spatial scale relevant for disease control. However, questions remain about what data should be collected and the requirements for reliable inference of relatedness, with most efforts to date being relatively ad hoc. Using a globally diverse set of published data sets of single-genotype *Plasmodium falciparum*and *Plasmodium vivax* parasite samples, we demonstrate the superior portability of IBD-based relatedness estimates relative to those based on allele sharing (i.e. identity-by-state). Under a hidden Markov model framework, we characterize the number and type of genetic markers required for specified error around relatedness estimates, demonstrating that reliable inference can be made using genetic markers without requiring whole genome sequences. Using the parametric bootstrap, we demonstrate how confidence intervals around estimates can aid interpretation across results based on vastly different data types. Finally, we show how concepts from transport theory can be adopted for a more generalized approach to demographic inference. These results provide a basis for statistically informed prospective study design and surveillance strategies. Given a robust foundation, analyses of genetic data from the surveillance of malaria parasites can achieve portability across different data types, accommodating the diversity of different experimental approaches extant in the field and allowing insights from meta-analyses to move the field forward.

978

PROTECTION AGAINST MALARIA IN HETEROZYGOUS GIRLS FOR G6PD DEFICIENCY IN ANGOLA

Miguel Brito, Chissengo Tchonhi

CISA - Health Research Centre in Angola, caxito, Angola

G6PD deficiency has become more prevalent in malaria endemic regions because genetic variants can confer protection against *Plasmodium*. However, these conclusions are still in debate. The aim of this work is to evaluate the prevalence of G6PD deficiency in an African holoendemic region for *Plasmodium falciparum*, estimating the genotype and phenotype of the enzyme, and evaluating the risk of malaria associated with the G6PD genotype. A prospective longitudinal cohort study, involving 1692 children selected in the maternity ward and monitored over quarterly medical consultations for two years. The G202A and A376G genotypes were determined through Real Time PCR methods. For enzyme activity, we applied the NeoLISA kit for Neonatal G6PD deficiency screening to measure the NADPH produced calorimetrically in the kinetic mode. The prevalence of the A-allele was 19.4%, with 19% hemizygous boys and 4.5% A-homozygous girls. Enzyme deficiency, measured by enzyme activity, was highly prevalent (32.7% in males and 30.5% in females). The average enzymatic activity was also low for A-hemizygous boys (1.66U/ gHb) and for homozygous girls (0. 97U/gHb). Heterozygous girls would seem to hold some protection against malaria, when compared to the other genotypes, mainly A-/A- (X^2=14.35, p=0.014). The prevalence of G6PD deficiency among children in Bengo is high. Heterozygous girls, as proposed elsewhere, may be the driving force for positive selection. This data may serve for the ministry of health in taking safe and appropriate decisions regarding the usage of potentially unsafe drugs for G6PD deficient individuals.

979

HUMAN ANTIBODIES TO AN EPITOPE IN PVDBP BLOCK ADHESION OF *PLASMODIUM FALCIPARUM* PLACENTAL PARASITES VIA CRYPTIC EPITOPES IN VAR2CSA

Catherine J. Mitran[1], Angie Mena[1], Hazel Lugo[1], Ali Salanti[2], Francis B. Ntumngia[3], John H. Adams[3], Eliana M. Arango[4], Amanda Maestre[4], Michael F. Good[5], Stephanie K. Yanow[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Copenhagen, Copenhagen, Denmark,* [3]*University of South Florida, Tampa, FL, United States,* [4]*University of Antioquia, Medellín, Colombia,* [5]*Institute for Glycomics, Griffith University, Gold Coast, Australia*

During Plasmodium falciparum infection in pregnancy, parasites express the PfEMP[1] surface antigen VAR^{2}CSA that mediates sequestration of infected red blood cells (iRBCs) to the placenta. Vaccines that target the DBL domains within VAR^{2}CSA are currently in clinical trials and the goal is to elicit antibodies that will block sequestration by interfering with the interaction of VAR^{2}CSA and chondroitin sulphate A (CSA) in the placenta. We are pursuing a novel strategy to develop a vaccine against P. falciparum placental malaria that is based on cross-reactivity between PvDBP from P. vivax and cryptic epitopes within VAR^{2}CSA. This approach is based on our previous findings that a monoclonal antibody (mAb) raised against PvDBP cross-reacts with VAR^{2}CSA and blocks P. falciparum adhesion to CSA in vitro. Also, we discovered that human antibodies to VAR^{2}CSA can be acquired outside of pregnancy and arise from exposure to PvDBP. Here, we identified an epitope within PvDBP that is the target of the mAb and showed that a peptide of this epitope completely blocks antibody recognition of VAR^{2}CSA. To determine whether this same epitope is involved in cross-reactivity of human antibodies to VAR^{2}CSA, we affinity-purified antibodies specific to this epitope from pools of sera from Colombian men and children. These purified antibodies recognized VAR^{2}CSA by ELISA, and strongly blocked adhesion of P. falciparum iRBCs to CSA in vitro. Furthermore, sera from multigravid African women or from rabbits immunized with VAR^{2}CSA do not recognize the epitope from PvDBP, demonstrating that the epitope in VAR^{2}CSA is cryptic. Together, these findings identify key epitopes in PvDBP elicited by natural exposure to P. vivax and mouse immunization that cross-react with cryptic epitopes in VAR^{2}CSA.

980

MEASUREMENT OF *PLASMODIUM FALCIPARUM*- AND *P. VIVAX*-SPECIFIC ANTIBODY PROFILES ON PROTEIN MICROARRAYS FROM DRIED BLOOD SPOTS

Christine F. Markwalter[1], Myat Htut Nyunt[2], Zay Yar Han[2], Aarti Jain[3], Omid Taghavian[3], Philip L. Felgner[3], Christopher V. Plowe[1], Kay Thwe Han[2], Myaing M. Nyunt[1]

[1]*Duke University, Durham, NC, United States,* [2]*Department of Medical Research, Yangon, Myanmar,* [3]*University of California Irvine, Irvine, CA, United States*

Sensitive and field-deployable detection tools are needed for malaria surveillance and elimination in low-prevalence settings. An ideal such tool would not only measure parasite prevalence, but also estimate recent aggregate malaria exposure, providing a more robust characterization of malaria transmission in a population. Serological biomarkers are promising targets for malaria surveillance because they can indicate cumulative recent exposure and are easily integrated into existing point-of-care platforms. Serological responses to *Plasmodium falciparum* and *P. vivax* antigens, identified and measured using protein microarrays, may serve as useful tools for identifying antibody biomarkers of exposure. Typically, serum samples are probed on protein microarrays to measure antibody responses to malaria antigens. The complexity of sample processing and requirement of cold-chain limit the usefulness of this method, particularly in remote hard-to-reach places where malaria is prevalent. Dried blood spots are potentially useful alternatives to serum; they are easy to collect, durable, easy to transport, and present little biohazard risk. In this study, protein microarrays were used to measure antibody profiles against 250 *P. falciparum* and 250 *P. vivax* antigens or fragments in matched serum and DBS from 100 individuals from Ingapu Township, Myanmar and found good correlation between serological responses derived from the two sample matrices. We applied the same DBS elution methodology and protein microarrays to measure antibody responses of individuals with low-density malaria infections and matched uninfected individuals from Injangyang and Ann Townships in Myanmar. Thus, using dried blood spots can extend protein microarray sero-surveillance to remote areas where cold chains required to collect and transport frozen samples do not exist.

981

ANTIBODY LEVELS TO *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES INCREASE OVER SUCCESSIVE PREGNANCIES

Oumar Attaher[1], Ahamadou Youssouf[1], Bacary Soumana Diarra[1], Sekouba Keita[1], Almahamoudou Mahamar[1], Moussa Traore[1], Gaoussou Santara[1], Alassane Dicko[1], Patrick Duffy[2], Michal Fried[2]

[1]*Malaria Research & Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Sciences Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States*

During pregnancy, *Plasmodium falciparum* parasites sequester in the placenta & adhere to the receptor chondroitin sulfate A (CSA). Infected erythrocyte (IE) adhesion to CSA is mediated by VAR2CSA, a member of the *P. falciparum* erythrocyte membrane protein family (PfEMP1) that is preferentially expressed by placental parasites. Here we examined the levels & breadth of antibodies reacting with surface IE proteins of heterologous parasite isolates collected from malaria-infected pregnant women in Ouelessebougou, Mali. Antibody levels to IE surface proteins were measured by flow cytometry & included samples collected from 177 women between 2012 to 2015 at delivery. IE surface recognition was defined as the ratio of the proportion of IE recognized by the women's plasma to the proportion of IE recognized by plasma from naïve donors (negative control). Antibody levels to surface IE were significantly higher in multigravidae compare to primigravidae & secundigravidae ($p < 0.0001$), & similar between primigravidae & secundigravidae. In addition to comparison by gravidity groups, we compared antibody reactivity with IE surface proteins by gravid number. Antibody levels were similar between women in their 3rd pregnancy (G3) & secundigravidae & significantly lower than women with >=3 pregnancies (G4 and higher). The breadth of reactivity with IE surface proteins positively increased with the number of pregnancies ($\beta=0.43$, $p<0.0001$). The associations between plasma IE surface reactivity, malaria infection during pregnancy & pregnancy outcomes are currently under investigation & will be presented.

982

ANTIBODY PROFILES INDUCED BY IMMUNIZATION WITH RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ VACCINE) IN MALARIA NAIVE VOLUNTEERS

Freia-Raphaella Lorenz[1], Rolf Fendel[1], Philip L. Felgner[2], B. Kim Lee Sim[3], Stephen L. Hoffman[3], Peter G. Kremsner[1], Benjamin Mordmüller[1]

[1]*Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany,* [2]*Vaccine R&D Center, University of California Irvine, Irvine, CA, United States,* [3]*Sanaria Inc., Rockville, MD, United States*

Immunization with attenuated, live sporozoites has been shown to evoke sterile protection against *P. falciparum* malaria in multiple clinical trials. Yet, the mechanism of protection and the contribution of the humoral immune response remain unclear. In a recent study (MAVACHE) conducted at the Institute of Tropical Medicine, University of Tübingen, healthy malaria naive volunteers were vaccinated by direct venous inoculation of three doses of $9x10^5$ irradiated, live *P. falciparum* sporozoites (PfSPZ Vaccine) each. Vaccination efficacy was assessed by two controlled human malaria infections (CHMI) of both homologous (NF54) and heterologous (7G8) *P. falciparum* strains. Significant sterile immunity was seen in both CHMIs. IgG and IgM antibody profiles of the study participants were assessed by probing serum samples on a protein microarray representing 228 unique antigens selected from previous full proteome microarray analyses. Levels of specific IgG and IgM antibodies directed against differentially recognized antigens were determined throughout the study by ELISA. On the microarray, sera of fully protected vaccinees recognized a higher number of different plasmodial antigens and displayed elevated levels of specific IgG compared to incompletely protected vaccinees. Sterile immunity was furthermore associated with a subset of specific IgG and IgM antibodies, including various surface, transport and export proteins of the sporozoite and liver stage parasite. Strong immunogenicity of the predominant sporozoite surface protein CSP (circumsporozoite protein) was observed in all vaccinees but anti-CSP antibody titers did not correlate with the outcome of protection in CHMI. In general, the antibody repertoire predicting exposure to the vaccine substantially differed from the identified markers of protection. Profiles of IgG responses are likewise distinct from those of IgM reactions. However, recurring patterns are observed between individual samples. Thus, breadth and composition of the humoral immune response may serve as predictors for sterile protection from malaria following immunization with PfSPZ Vaccine.

983

GUT MICROBIOTA MODULATION OF GERMINAL CENTER REACTIONS IMPACTS SEVERITY OF *PLASMODIUM* INFECTIONS

Morgan Duff[1], Whitney Powell[1], Joshua Denny[1], Nathan Schmidt[2]

[1]*Department of Microbiology and Immunology, University of Louisville, Louisville, KY, United States,* [2]*Ryan White Center for Pediatric Infectious Diseases and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States*

Infections by the parasite *Plasmodium* result in approximately 200 million cases of malaria and 400,000 deaths each year. Though over 40 percent of the world population live in areas affected by malaria, there is currently no effective vaccine and resistance to antimalarial drugs is continuing spread. Our lab has previously identified that the composition of the gut microbiota in mice can modulate the severity of malaria, leading to

differences in blood stage parasite burden and the duration of infection. Humoral immunity is known to be critical in mediating clearance of *Plasmodium* blood stage infections, prompting the hypothesis that mice with enhanced control of *Plasmodium* will exhibit better germinal center responses. In support of this hypothesis, mice with decreased parasite burdens show an increase in numbers of germinal center B cells, T follicular helper cells, and parasite-specific antibody titers compared to mice with high parasite burdens. Additionally, germ-free mice colonized with cecal contents from mice that display low or high parasitemia and that were subsequently infected with *Plasmodium* phenocopy differential germinal center responses. These data demonstrate that the gut microbiota is sufficient to shape the magnitude of germinal center reactions. Mechanistically, when mice that develop low parasite burdens were treated with antibodies that disrupt germinal center reactions, they had similarly high parasite burdens as control mice. These findings suggest that microbiome-mediated modulation of germinal center reactions may be a mechanism by which the gut microbiota impacts the development of severe malaria. Enhanced germinal center reactions in mice with low parasitemia during the acute infection also impacted memory, as these mice were protected against challenge with a heterologous, lethal *Plasmodium* species. This research provides mechanistic insight into both how the microbiota interacts with the adaptive immune system as well as how the immune system interacts with *Plasmodium* in order to clear infections, providing knowledge that can be used in efforts to provide optimal treatments for malaria.

984

VACCINE OPTIMIZATION BY IDENTIFICATION, CHARACTERIZATION, AND DOWNSELECTION OF HUMAN T CELL EPITOPES FROM *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN

Amy R. Noe[1], Frances E. Terry[2], Brian C. Schanen[3], Pooja Hindocha[2], Leonard Moise[2], Jayne M. Christen[1], Kenneth D. Tucker[1], Timothy W. Phares[4], William D. Martin[2], Anne S. DeGroot[2], Donald R. Drake III[3], Lorraine Soisson[5], Carter Diggs[5], Robin Miller[5], Susan Youll[5], David R. Milich[6], David C. Whitacre[6]

[1]*Leidos Life Sciences, Leidos Inc., Fredrick, MD, United States,* [2]*EpiVax Inc., Providence, RI, United States,* [3]*Sanofi Pasteur, VaxDesign Campus, Orlando, FL, United States,* [4]*ExGloH, Leidos Inc., Fredrick, MD, United States,* [5]*Malaria Vaccine Development Program, United States Agency for International Development (USAID), Washington, DC, United States,* [6]*VLP Biotech Inc., San Diego, CA, United States*

An effective malaria vaccine must prevent disease in a wide range of populations living in regions with vastly different transmission rates and protect against genetically diverse *Plasmodium falciparum* strains. Helper CD4+ T cells, in particular those of the Th1 lineage, are central to development of protective immune responses to *P. falciparum*, playing roles in B cell activation and maturation processes as well as in cytokine production. Therefore, we took advantage of recent in silico modeling advances to predict and analyze the HLA-restricted CD4 (class II) epitopes (peptide sequences that bind HLA-DR) relevant to achieving broad human population coverage in a Pf circumsporozoite protein (CSP) vaccine, utilizing cognate T cell help. Based on in silico analysis, several class II epitope clusters are predicted in the N- and C-terminal regions of PfCSP. Further, one predicted class II cluster overlaps with the highly-variable region 2 (R2) in the C-terminus of PfCSP (3D7 strain). To identify additional predicted class II epitopes, 478 PfCSP R2 peptide sequence variants were analyzed for HLA-DR binding. Of these, nine predicted R2 variant class II clusters were identified through the silico analysis. The resulting peptide sequences were synthesized and assessed for HLA-DR binding *in vitro* as well as for the ability to prime/activate lymphocytes resulting in generation of cytokine responses using human PBMCs. The latter was conducted using MIMIC® technology (Modular IMmune In vitro Construct), a surrogate human immune system enabling researchers to test human immune responses to antigen. We found sufficient differential cellular activation and cytokine profiles among HLA-DR-matched PBMC donors to downselect class II epitope clusters for inclusion in a vaccine targeting PfCSP.

985

MALARIA-SPECIFIC B CELL RESPONSES IN CHILDREN AND ADULTS FROM UGANDA

Jake Gonzales[1], Isaac Ssewanyana[2], John Rek[2], Sebastiaan Bol[1], Bryan Greenhouse[3], **Evelien M. Bunnik**[1]

[1]*The University of Texas Health Science Center, San Antonio, TX, United States,* [2]*Infectious Disease Research Collaboration, Kampala, Uganda,* [3]*University of California San Francisco, San Francisco, CA, United States*

Individuals living in malaria-endemic regions over the years develop an immune response that protects against disease. This naturally acquired immunity targets the blood stage of the parasite's life cycle and is at least in part dependent on antibody-mediated inhibition. Protective antibodies are likely to be a small part of the total antibody repertoire. By studying immune responses at the single B cell level, we aim to understand how protective immunity develops and which antibody characteristics are important for protection. We have used B cell tetramers to isolate memory B cells specific for two blood stage malaria antigens (MSP1 and AMA1) in non-immune children and immune adults living in Tororo, Uganda, a region of high malaria transmission. Children harbored both antigen-specific IgM+ and IgG+ memory B cells, while around 80% of the antigen-specific memory B cells in adults were IgG+. The antibody variable regions of these memory B cells were amplified and analyzed by Sanger sequencing. In parallel, we performed B cell receptor sequencing to study the broader B cell receptor landscape. In comparison to the full repertoire of IgG+ memory B cells, MSP1 and AMA1-specific memory B cell receptors in adults contained high levels of somatic hypermutations (on average 27 amino acid changes in the V_H segment). We observed clonal expansion of antigen-specific B cell lineages and found evidence of convergent antibody evolution within an individual. Collectively, these results highlight the strong selection pressure of malaria exposure on antibody responses and suggest that extensive affinity maturation may be required for strong parasite inhibition. Ongoing experiments are aimed at testing antibody reactivity in various functional assays. The results of this project will contribute to our understanding of the development of anti-malaria antibody responses and the antibody features that are necessary to achieve protection.

986

PLASMODIUM-DERIVED HEMOZOIN IMPAIRS ANTIBACTERIAL INNATE IMMUNITY TO SYSTEMIC INFECTIONS

Chris Harding[1], Nicolas Villarino[2], Evelin Schwarzer[3], **Nathan W. Schmidt**[4]

[1]*University of Louisville, Louisville, KY, United States,* [2]*Washington State University, Pullman, WA, United States,* [3]*University of Torino, Torino, Italy,* [4]*Indiana University, Indianapolis, IN, United States*

Plasmodium species cause nearly 200 million episodes of malaria annually. One complication of malaria is increased susceptibility to invasive bacterial infections. Prior studies have shown that *Plasmodium* infections impair host immunity to non-Typhoid *Salmonella* (NTS) through both activation of heme-oxygenase I and the subsequent release of immature granulocytes from the bone marrow and myeloid cell-derived IL-10. Yet, it is not known if these mechanisms are specific to NTS or if they represent general defects to systemic bacterial infections. Moreover, it is not known if a parasite-derived factor is responsible for *Plasmodium*-induced immunosuppression. We show here, that *Plasmodium yoelii* 17XNL infected mice had impaired clearance of systemic *Listeria monocytogenes* during both acute parasitemia and up to 2-months after resolution of *P. yoelii* infection that was independent of heme-oxygenase I and IL-10. *P. yoelii*-infected mice were also susceptible to *Streptococcus pneumoniae* bacteremia, a common malaria-bacteria co-infection, with higher blood and spleen

bacterial burdens and decreased survival compared to naïve mice. Mechanistically, splenic phagocytes from *P. yoelii* infected mice exhibit an impaired ability to kill intracellular *L. monocytogenes*, and neutrophils from *P. yoelii*-infected mice produce less reactive oxygen species in response to *L. monocytogenes* or *S. pneumoniae*. Finally, treating splenic phagocytes with *Plasmodium*-derived Hz (*P. yoelii* or *P. falciparum*) impaired killing of intracellular *L. monocytogenes*. Moreover, treating naïve mice with *Plasmodium*-derived Hz was sufficient to impair bacterial clearance. Collectively, we have demonstrated *P. yoelii* infections impair host immunity to diverse bacterial pathogens by Hz-dependent suppression of innate immunity.

987

PEDIATRIC PARTICIPATION RATES IN A LONGITUDINAL MALARIA IMMUNOLOGY STUDY

Anushay Mistry[1], Nelly Koskei[2], Jonathan Kurtis[3], John Michael Ong'echa[2], Ann Moormann[1]

[1]*University of Massachusetts Medical School, Worcester, MA, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*Brown University Warren Alpert Medical School, Providence, RI, United States*

The resurgence of drug resistant *Plasmodium falciparum* parasites continues to motivate work toward developing a safe and efficacious malaria vaccine. Immuno-epidemiologic studies of naturally acquired immunity (NAI) have been a useful strategy to identify new malaria vaccine targets. However, retention of pediatric participants throughout longitudinal studies is essential for gathering comprehensive exposure and outcome data. Within the context of a 3-year, 400-patient pediatric cohort study involving monthly finger pricks and bi-annual venous blood sample collections, we are conducting qualitative surveys to assess factors impacting participant retention. Phase One was conducted in July 2018, 3 months after study initiation; 236 parents/guardians participated in focus groups, 3 withdrawn participants in key informant interviews and 10 community health volunteers (CHVs) in individualized interviews. Qualitative analysis indicates overall satisfaction with the study, with 136 guardians (57%) reporting no concerns. Focus group discussants associated attendance with benefits including improved access to comprehensive healthcare services and perceived reputation of healthcare quality. Participants also expressed appreciation for the thorough and extensive consent process. CHVs reported that uninvolved community members perpetuated rumors of inappropriate use of study samples, dangers associated with venous blood draws, and complaints of insufficient incentives provided in return for study participation. Another concern expressed was the volume of blood drawn, expressed by 13 (5%) guardians over 3 focus group meetings. Phase Two will be conducted in July 2019 utilizing the aforementioned procedures to monitor participant satisfaction and determine the source of community-based concerns. Distance and other logistical barriers that could impede compliance with monthly research visits at the clinic have been mapped and will be used to evaluate attrition. Decreased satisfaction over the course of the study may warrant proactive community education to alleviate study-related concerns.

988

THE EFFECT OF ADDING AZITHROMYCIN TO THE ANTIMALARIALS (SULPHADOXINE/PYRIMETHAMINE AND AMODIAQUINE) USED FOR SEASONAL MALARIA CHEMOPREVENTION ON THE IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM*

Joshua M. Obiero[1], Matthew Cairns[2], Aarti Jain[1], Omid Taghavian[1], Andrew Sy[1], Rie Nakajima[1], Algis Jasinskas[1], Issaka Zongo[3], Issaka Sagara[4], Jean-Bosco Ouedraogo[3], Alassane Dicko[4], Daniel Chandramohan[2], Brian Greenwood[2], Philip L. Felgner[1]

[1]*University of California, Irvine, CA, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,* [4]*University of Science, Techniques and Technologies of Bamako, Bamako, Mali*

Seasonal malaria chemoprevention (SMC) provides a high level of protection against non-severe and severe malaria in young children. However, it is unclear to what extent the immune system of children protected by SMC is affected by a diminished exposure to malaria infection, and how this might be affected by improving the efficacy of SMC regimens. A proteome micro-array study was conducted to determine differences in the naturally acquired anti-malaria antibody responses among children who received SMC with azithromycin (AZ) or with a placebo, and children who did not receive SMC treatment. A household randomized trial was conducted in Mali and Burkina Faso from 2014 to 2016, starting in August each year, during which approximately 20,000 children aged 3-59 months at the beginning of each malaria transmission season received four, monthly cycles of SMC with sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ), together with either AZ or a matching placebo. Serum samples from 101 study children aged between 36 and 48 months who had received SMC with placebo or AZ for three transmission seasons were collected together with samples from 100 children who did not receive SMC, and 35 adults. Samples were probed on protein micro-arrays containing 228 unique *P. falciparum* 3D7 proteins to determine differences in antigen specific antibody levels between the groups. We identified 128 and 175 antigens recognized by more than 10% of the children and adults respectively. Children who did not receive SMC had significantly higher immune responses to 84 antigens compared to children who received SMC. These results show no demonstrable difference in the antibody response against malaria following the addition of AZ to SMC. In contrast, children who received SMC had significantly lower antibody levels and a narrower range of responses compared to children who did not receive SMC. This lower antibody response is an expected consequence of the lower parasite burden experienced by children in the SMC group. Whether these lower anti-parasite immune responses in the SMC group increases future susceptibility to malaria infection is not yet clear.

989

RECEPTOR TRANSPORTER PROTEIN 4 (RTP4) NEGATIVELY REGULATES IFN-I RESPONSE AND ANTI-MALARIAL IMMUNITY

Xiao He, Xinzhuan Su

National Institutes of Health, North Bethesda, MD, United States

Malaria infection stimulates complex and delicately regulated host immune responses. Recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) activates a series of signaling cascades, leading to production of cytokines including type-I and type II interferons (IFN-I and IFN-II) and chemokines that mediate migration of immune cells to affected tissues. Previously, we performed a trans-species expression quantitative *loci* analysis (Ts-eQTL) using progeny from a genetic cross involving parasite strains that stimulate differential IFN-I responses and identified many putative interferon-stimulated genes (ISGs). We have focused on an ISG gene called RTP4 (Receptor Transporter Protein 4) that is known as a probable chaperone protein playing a role in trafficking and cell surface expression of some G-protein coupled receptors (GPCRs). Here we show that RTP4 is also a negative regulator of malaria induced IFN-I response. RTP4's protein level is upregulated after malaria infection *in vivo* or malaria RNA/DNA transfection *in vitro*. Over expression of RTP4 *in vitro* can significantly reduce IFN-I production. Disruption of RTP4 *in vivo* enhances IFN-I production and slightly improves host survival after *Plasmodium* yoelii infection. RTP4 binds to many molecules in IFN-I signaling pathways including STING, TRAFs, TBK1, IRF3 and S6k1. RPT4 also interferes with TBK1 binding to STING, reduces phosphorylation of TBK1 and IRF3, and decreases ubiquitin levels of many adaptor proteins in the IFN-I signaling pathways. These results demonstrate that RTP4 can function as a negative regulator of IFN-I signaling in addition to its reported function in protein transport.

990

FUNCTIONAL CHARACTERIZATION OF ANTI-RH5 ANTIBODIES FROM A MALARIA ENDEMIC AREA FOR FUTURE VACCINE DEVELOPMENT

Alexandra C. Willcox[1], Kazutoyo Miura[1], Ababacar Diouf[1], Daniel G. Alanine[2], Rebecca A. Dabbs[2], Jing Jin[2], David J. Pattinson[2], Mahamadou Diakite[3], Simon J. Draper[2], Carole A. Long[1]

[1]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [2]*Jenner Institute, University of Oxford, Oxford, United Kingdom,* [3]*University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali*

The reticulocyte-binding protein homologue 5 (RH5) is a conserved protein on *Plasmodium falciparum* merozoites that is essential for parasite invasion of host erythrocytes. RH5 is currently the most promising *P. falciparum* blood-stage vaccine candidate, and a recent Phase I/IIa clinical trial demonstrated that vaccination with RH5 could reduce parasite growth rate in malaria-naïve volunteers in a blood-stage controlled human malaria infection (CHMI) model. Characterization of pre-existing anti-malarial immunity in endemic populations will support future RH5 vaccine development. Our group previously reported anti-RH5 IgG titers to be ~100-fold lower in RH5-immune Malians than the peak titers in RH5-vaccinated malaria-naïve UK volunteers. In this study, we affinity-purify RH5-specific IgGs from naïve, RH5-vaccinated UK volunteers and from naturally infected Malians in order to functionally characterize these antibodies *in vitro*. Using the growth inhibition assay (GIA), we find that some RH5-specific IgGs from Mali inhibit parasite growth at the same antibody titer as IgGs from the vaccinees, while others fail to inhibit parasite growth. Further experiments are underway to explain these differences. We also combine human anti-RH5 mAbs, which were generated from the vaccinees, with total IgGs from Malians. We identify a subset of Malian IgGs that work synergistically with the mAbs to inhibit parasite growth in GIA. Experiments will be performed to determine what distinguishes this subset of Malian IgGs from others. Our results will demonstrate how naturally-acquired anti-malarial immunity may interact with vaccine-induced anti-RH5 immunity.

991

SEROLOGIC MARKERS OF PREVIOUS MALARIA EXPOSURE AND FUNCTIONAL ANTIBODIES INHIBITING PARASITE GROWTH ARE ASSOCIATED WITH PARASITE KINETICS FOLLOWING A *PLASMODIUM FALCIPARUM* CONTROLLED HUMAN INFECTION

Isaie J. Reuling[1], Jane Achan[2], Zen Yap[1], Edgar Dabira[2], Abdullahi Ahmad[2], Momodou Cox[2], Davis Nwakanma[2], Kevin Tetteh[3], Lindsey Wu[3], Guido Bastiaens[1], Yonas Abebe[4], Anita Manoj[4], Kazutoyo Miura[5], Carole Long[5], Peter F. Billingsley[4], B. Kim Sim[4], Stephen L. Hoffman[4], Chris Drakeley[3], Teun Bousema[1], Umberto D'Alessandro[6]

[1]*Radboud University Medical Center, Nijmegen, Netherlands,* [2]*Medical Research Council Unit, Fajara, Gambia,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Sanaria Inc., Rockville, MD, United States,* [5]*National Institutes of Health, Rockville, MD, United States,* [6]*Medical Research Council Unit, Fajara, United Kingdom*

We assessed the impact of exposure to *P. falciparum* on parasite kinetics, clinical symptoms, and functional immunity after controlled human malaria infection (CHMI) in two cohorts with different levels of previous malarial exposure. Nine adult males with high (sero-high) and ten with low (sero-low) previous exposure received 3200 controlled human malaria infection by direct venous inoculation and were followed for 35 days for parasitemia by thick blood smear (TBS) and quantitative polymerase chain reaction (qPCR). End points were time to parasitemia, adverse events and immune responses. Ten of Ten (100%) volunteers in the sero-low and 7 of 9 (77.8%) in the sero-high group (89%) developed parasitemia detected by TBS in the first 28 days (p = 0.125). The median time to parasitemia was significantly shorter in the sero-low group [9 days (7.5-11.0) vs.11.3 days (7.5-18.0), log rank test, p=0.005]. Antibody recognition of sporozoites was significantly higher in the sero-high (median 17.93 AU, IQR 12.95-24) than the sero-low volunteers (median 10.54 AU, IQR 8.36-12.12); p=0.006. Presence of blood-stage antibodies was also significantly higher (p=0.0003) in the sero-high group (median 50.98 AU, IQR 22.46-65.07) than in the sero-low group (median 3.16 AU, IQR 2.43-8.71). Growth inhibitory activity (GIA) was significantly higher in the sero-high (median 21.8%, IQR 8.15-29.65) than in the sero low (median 8.3%, IQR 5.6-10.23) (p=0.025). CHMI was safe and well tolerated in this population. Individuals with serological evidence of higher malaria exposure were able to better control infection and had higher parasite growth inhibitory activity.

992

PROLONGED *PLASMODIUM FALCIPARUM* EXPOSURE LEADS TO THE DEVELOPMENT OF ABERRANT $CD56^{NEG}CD16^{POS}$ NATURAL KILLER CELLS

Catherine S. Forconi[1], Cliff Oduor[2], John M. Ong'echa[3], Jeff A. Bailey[2], Ann M. Moormann[1]

[1]*Department of Medicine, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, MA, United States,* [2]*Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI, United States,* [3]*Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya*

Natural Killer (NK) cells are essential in the clearance of red blood cells infected with *Plasmodium falciparum*, however previous studies have been limited to investigations of $CD56^{dim}CD16^{pos}$ NK cells. Chronic viral infections have been found to promote the expansion of a CD56 (negative) NK cell subset with impaired effector function. In our study of otherwise healthy Kenyan children, we found they had significantly more $CD56^{neg}CD16^{pos}$ NK cells after prolonged exposure to malaria compared to age-matched children from a low malaria transmission area (13.5% versus 6%, respectively, p=0.006). Moreover, elevated levels of $CD56^{neg}CD16^{pos}$ NK cells appear to persist into adulthood (median 10.6% [3.6 to 17.2%]). We characterized this $CD56^{neg}CD16^{pos}$ NK subset using histology staining, multicolor flow cytometry and RNA-sequencing. We found $CD56^{neg}CD16^{pos}$ NK cells to be morphologically similar to $CD56^{dim}CD16^{pos}$ NK cells but had higher expression of FCGR3B (CD16b) (p=0.000008) and MPEG1 (Perforin 2) (p=0.0000007), and lower expression of KLRF1 (NKp80), IL18RAP and IL18R1 (IL18 receptor) (p=0.000003, p=0.000003 and p=0.00005, respectively). Phenotypically, $CD56^{neg}CD16^{pos}$ NK cells share characteristics of adaptive NK cells, however, they differ from "memory-like" NK cells, with consistent expression of NKG2C/CD57, lower expression of NKp46 and CD160, and higher expression of KIR3DL1 compared to $CD56^{dim}CD16^{pos}$ NK cells. Finally, higher plasma levels of IL-12p70 and IL-2 (known to help NK cell activation and proliferation) and IL-6 and MIP1a were found in *P. falciparum* un-exposed compared to exposed children. Together, these findings suggest that the expansion of $CD56^{neg}CD16^{pos}$ NK cells could help explain diminished cytotoxicity capacity against blood-stage infections and is a mechanism by which malaria evades NK-cell mediated immunity.

993

ALTERED EXPRESSION OF METABOLIC SIGNALING PATHWAYS IN MONOCYTES DURING ACUTE MALARIA IN CHILDREN

Katherine R. Dobbs[1], Paula Embury[1], David Midem[2], Peter Sumba Odada[2], John Vulule[2], James W. Kazura[1], Arlene E. Dent[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Kenya Medical Research Institute, Kisian, Kenya*

Monocytes are innate immune cells that play a key role in host protection and pathogenesis during malaria. As immune cells respond to infection, metabolic pathways are regulated to support or direct functional changes. We sought to determine whether metabolic signaling pathways

were altered in monocytes during acute malaria in children. Negatively selected monocytes were obtained from cryopreserved peripheral blood mononuclear cell (PBMC) samples from 6 children in western Kenya at presentation with acute uncomplicated malaria and 6 weeks following treatment. RNA and protein were purified from monocyte cell lysates and hybridized to a NanoString CodeSet of genes and proteins important to regulation of several cellular processes such as differentiation, apoptosis, and metabolism (mRNA PanCancer Pathways Panel of 730 target genes and protein Solid Tumor Panel of 25 total and phospho-proteins). Of the 730 target genes, 100 were differentially expressed, including 69 decreased and 31 increased during acute malaria compared to recovery. The protein panel revealed significantly decreased levels of phospho-Akt (log_2 fold change -0.93, p=0.02) during acute malaria compared to recovery. Several genes up- and down-stream in the phosphoinositide 3-kinase (PI3K)/Akt pathway were differentially expressed, including increased expression of the Akt inhibitors *PTEN* (log_2 fold change 0.67, p=0.002) and *PPP2CB* (log_2 fold change 0.67, p=0.02) and increased expression of *CCND3* (log_2 fold change 1.62, p=0.0004), which encodes the protein cyclin D3 and is important for cell cycle regulation and glucose metabolism. In addition, protein levels of phospho-c-Raf were decreased during acute malaria (log_2 fold change -0.41, p=0.03) along with altered expression of several genes in the MAPK/ERK pathway (including *CSF1R, PRKCA, RASA4, RASGRP1, MAP2K1*, and the ERK1/2 inhibitors *DUSP2, DUSP5,* and *DUSP6*). Data analysis is ongoing and will be correlated with cellular metabolic and functional assays. These data show that acute uncomplicated malaria is associated with altered expression of pathways important to monocyte metabolism.

994

ENHANCING MALARIA ELIMINATION AND CONTROL EFFORTS IN HIGH AND LOW BURDEN AREAS OF ZAMBIA USING SPATIO-TEMPORAL MODELLING OF TRENDS IN INCIDENCE AND RISK

Jailos Lubinda[1], Yaxin Bi[2], Busiku Hamainza[3], Adrian J. Moore[1]

[1]*Ulster University, Coleraine, United Kingdom,* [2]*Ulster University, Newtown Abbey, United Kingdom,* [3]*National Malaria Elimination Center, Lusaka, Zambia*

Consistent progress and a decade of optimism has shaped the emphasis of many malaria programs, inspiring a shift from control to elimination. Zambia has made significant strides in reducing national malaria mortality and has seen declining incidence rates in several districts. This enthused the agenda to eliminate malaria by 2021. Tailored approaches target elimination in low-burden areas, while intensified control efforts will lead up to elimination in high-burden areas. In order to achieve this strategic approach to control and elimination, a co-ordinated, accurate method for the identification of priority high-burden and low-burden areas is required. However, no such method currently exists to accurately identify, map and help guide the deployment of appropriate intervention resources to such areas. We developed a multi-criterion spatio-temporal approach to map malaria risk, rates and trends at district level based on data from 2000 to 2015. Spatiotemporal concentration and variation of malaria burden was calculated using risk, rate, and trend indices, then ranked and mapped across a spectrum from high to low-burden. Low burden areas are suitable for elimination while high-burden areas, characterised by high incidence rates, elevated risk and increasing trend compared with their geographical neighbours are suited for intensified control leading to elimination. Our results confirmed that malaria morbidity increased in most areas since 2010, but mortality continued declining. Over 3 million people live in high mortality/incidence burden areas with increasing temporal trends, while 1.6 million are in high-incidence burden districts. Under 5 children carry the highest incidence and mortality burden. Identifying high-burden areas is fundamental to targeting intervention programmes for maximum impact. Our robust method provides a practical means to prioritize clusters of both high and low-burden malaria that can inform and support tailored programmes and tools for control or elimination. The incorporation of relevant age categories within such methods is highlighted as being important for control and elimination efforts.

995

MAPPING FOR MALARIA CONTROL IN GRAND ANSE, HAITI USING A MULTI-METRIC BAYESIAN APPROACH

Punam Amratia[1], Ewan Cameron[1], Alyssa Young[2], Katherine Twohig[1], Andre Python[1], Darlene Bhavnani[2], Emilie Pothin[3], Arnaud Le Menach[2], Justin Cohen[2], Samson Marseille[4], Jean-Frantz Lemoine[4], Ambre Dismer[5], Jean-Baptiste Mérilien[4], Karen Hamre[5], Eric Rogier[5], Michelle Chang[5], Peter Gething[1], Katherine Battle[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Clinton Health Access Initiative, Boston, MA, United States,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [4]*Programme National de Contrôle de la Malaria/MSPP, Port-au-Prince, Haiti,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

The island of Hispaniola is determined to eliminate malaria. Multiple metrics have been collected through surveillance programs in relatively high-risk areas such as Grand'Anse, Haiti. However, it becomes challenging to interpret transmission intensity when using multiple metrics, yet if we use a single metric, we may fail to capture aspects of the transmission landscape. Here we present an integrated multi-metric approach that jointly models serological prevalence, incidence and health facility catchments such that each metric can be leveraged to produce fine-scale spatio-temporal outputs for the Grand'Anse Department in Haiti. Data used for this model comes from monthly reported incidence for 2017, case tracing from health facilities done by the National Malaria Control Program for 2017 and serological markers from transmission assessment surveys (TAS) from 2012 to 2016. A Gibbs sampler containing a joint gaussian process model for serology and monthly incidence was designed with spatial and spatio-temporal structure respectively. The catchment modelling was done using travel time distances in a simplified gravity model. Non traced cases per health facility were predicted using the incidence and catchment surfaces for the most likely pixel location. Weakly informative priors were chosen for all parameters in the model. Fit and accuracy of the model was checked by spatial cross-validation and Bayesian probability integral transform test. A sensitivity analysis for incidence was done by removing serology, then catchments from the Gibbs sampler.The final outcomes produced an annual seroprevalence and monthly predicted case incidence surfaces for 2017. Incidence had a biphasic seasonal trend, with most cases concentrating closer to the coastal region. The use of travel times in the catchment model was able to capture the likeliest movement pathways for treatment seeking and predict most likely pixels for non-traced cases. These outcomes are intended to optimise the placement of community health workers in Grand'Anse department as well as focussed application of control interventions.

996

UTILIZING HIGH RESOLUTION MALARIA MAPS AND FUTURE FORECASTS TO OPTIMIZE SITE SELECTION FOR CLINICAL TRIALS IN MALARIA

Daniel J. Weiss[1], Samir Bhatt[2], Ivan Demin[3], David Hughes[3], Peter W. Gething[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Imperial College, London, United Kingdom,* [3]*Novartis Pharma AG, Basel, Switzerland*

To support site selection for future phase 2 and 3 clinical trials in malaria, the Malaria Atlas Project (MAP) created annual *Plasmodium falciparum* prevalence and incidence maps for Africa. This work extends the existing MAP approach by applying autoregressive integrated moving average (ARIMA) forecasting to predict the potential range of burden to 2023. Model uncertainty is propagated from both the geostatistical and the ARIMA models, and uncertainty expands through time in years following the most recently collected response data. The resulting burden estimates were used to derive the expected incidence and absolute number of malaria cases for a set of 54 candidate sites in 23 endemic countries in sub-Saharan Africa. The approach will help in identifying sites with higher likelihood of greater patient numbers for future clinical trials. Additionally, model predictions were derived for 8 sites currently involved

in an ongoing phase 2b clinical trial in malaria using maps for year 2018. The model predictions were compared to the observed recruitment rates, which indicated a positive correlation between both. This provides further confidence in using the malaria maps forecasts for site selection.

997

TRACKING PROGRESS TOWARDS MALARIA IN ELIMINATION IN CHINA: A MODELLING STUDY

Isobel Routledge[1], Shengjie Lai[2], Katherine E. Battle[3], Kyle Gustafson[4], Azra C. Ghani[1], Manuel Gomez-Rodriguez[5], Joshua Proctor[6], Swapnil Mishra[1], Zhongjie Li[7], Samir Bhatt[1]

[1]*Imperial College London, London, United Kingdom,* [2]*University of Southampton, Southampton, United Kingdom,* [3]*University of Oxford, Oxford, United Kingdom,* [4]*U.S. Navy, Washington, DC, United States,* [5]*Max Planck Institute for Software Systems, Saarbrücken, Germany,* [6]*Institute for Disease Modelling, Bellevue, WA, United States,* [7]*Chinese Centre for Disease Control, Beijing, China*

The People's Republic of China has achieved major reductions in malaria incidence in recent years and is on track to elimination, having reported zero locally acquired cases in 2017 and 2018. Understanding the spatio-temporal pattern underlying this decline, in particular the relationship between locally acquired and imported cases, can help inform efforts to maintain elimination and prevent re-introduction. This is particularly pertinent in Yunnan province in the Greater Mekong Subregion, where the potential for local transmission remains a concern. Using an individual level dataset of all confirmed and probable cases of *P. vivax* and *P. falciparum* malaria in Yunnan province recorded between 2011 and 2016 (N = 4287), we jointly estimate the case reproduction number, *Rc* and the number of unobserved source infections (caused by either missed cases or asymptomatic infections generating secondary case). We use these estimates within spatio-temporal geostatistical models to map how transmission varied over space and time and to provide estimates of the timeline to elimination and the risk of resurgence. We estimate the mean *Rc* between 2011 and 2016 to be 0.171 (95% CI = 0.165, 0.178) for *P. vivax* cases and 0.089 (95% CI = 0.076, 0.103) for *P. falciparum* cases. From 2014 onwards, no cases were estimated to have *Rc>1* . The proportion of cases with *Rc>0* also declined during this period, with this decline occurring earliest in the more accessible areas of the province. An unobserved source of infection was estimated to be moderately likely ($p<0.5$) for 19/ 611 cases and high ($p<0.8$) for 2 cases, suggesting high levels of case ascertainment. Our estimates suggest that, if current intervention efforts are sustained, Yunnan is unlikely to experience sustained local transmission up to 2020. However, even with a posterior mean *Rc* of 0.02 (95% CI: 0 - 0.32) projected for the year 2020, sporadic locally-acquired cases could occur due to the large number of imported cases. The declines in *Rc* estimated here coincide with the implementation and improved adherence to China's elimination policy and support China being on track to eliminate malaria by 2020.

998

PROSPECTIVELY ESTIMATING THE HEALTH IMPACT OF UPCOMING PRESIDENT'S MALARIA INITIATIVE IMPACT MALARIA PROJECT-SUPPORTED SEASONAL MALARIA CHEMOPREVENTION CAMPAIGNS IN 70 DISTRICTS ACROSS NIGER, MALI AND CAMEROON IN 2019

Keith Esch[1], Matt Hamilton[2], Eline Korenromp[2], Elizabeth Lacroix[3], Gladys Tetteh[1], Moussa Thior[1]

[1]*PMI Impact Malaria Project, Washington, DC, United States,* [2]*Avenir Health, Glastonbury, CT, United States,* [3]*Population Services International, Washington, DC, United States*

In 2019, the U.S. President's Malaria Initiative's Impact Malaria (IM) project supported National Malaria Control Programs to conduct seasonal malaria chemoprevention (SMC) campaigns in 70 high burden districts across 6 sub-national regions in Niger, Mali and Cameroon. There are 3,704,842 targeted children ages 3-59 months in these districts, representing 37% of eligible children in the 3 countries. IM prospectively estimated the deaths, episodes and disability-adjusted life years (DALYs) averted by these campaigns using epidemiological models. Avenir Health's Spectrum-Malaria (Sp-MA) impact model projected the number of children under 5 (CU5) deaths and episodes avertable by scaling up SMC to reach 90% of children ages 3-59 months with at least 3 treatment cycles during the SMC campaign in sub-national regions receiving IM-support. The model assumes constant coverage of other malaria interventions at 2016 levels, with SMC scale up from 2019 to 2020 and impact from scale up occurring in 2020. Each cycle consists of 1 SP tablets and 3 amodiaquine tablets. Results were then converted into CU5 deaths and episodes averted per targeted child. Finally, DALYs averted per targeted child were calculated by applying 2017 Global Burden of Disease disability weights to CU5 malaria deaths. An estimated 4,694 deaths; 1,153,118 cases; and 405,631 DALYs will be averted during the three IM-supported campaigns. These include: In Niger's 17 districts across 2 regions, an estimated 1,747 deaths; 456,364 cases; and 151,051 DALYs. In 9 districts in Mali, across 2 regions, 1,561 deaths; 292,864 cases; and 134,593 DALYs, and 1,386 deaths; 403,890 cases and 119,998 DALYs across 45 districts in 2 regions of Cameroon. Results show the significant health impact expected from ongoing scale-up of SMC across the Sahel. There are differences across countries and between their regions in the relative benefit of SMC per beneficiary. This modeling can be applied non-IM supported SMC campaigns as well. These results can be used by governments and other implementers to plan and target SMC campaigns geographically to maximize impact and may be useful in advocacy efforts.

999

DEVELOPING OPEN SOURCE SOFTWARE TO SUPPORT CLIMATE DATA INTEGRATION FOR OPERATIONAL MALARIA FORECASTS IN ETHIOPIA

Dawn M. Nekorchuk[1], Worku Awoke[2], Mastewal Worku[3], Zelalem Mehari Nigussie[2], Abere Mihretie[4], Aklilu Getinet[4], Justin K. Davis[1], Michael C. Wimberly[1]

[1]*University of Oklahoma, Norman, OK, United States,* [2]*Bahir Dar University, Bahir Dar, Ethiopia,* [3]*Amhara Public Health Institute, Bahir Dar, Ethiopia,* [4]*Health, Development, and Anti-Malaria Association, Addis Ababa, Ethiopia*

The goal of the Epidemic Prognosis Incorporating Disease and Environmental Monitoring for Integrated Assessment (EPIDEMIA) is to develop and implement malaria early warning systems that integrate public health surveillance with environmental monitoring of climate variations. Through a co-development process including research partners from the U.S. and Ethiopia, we identified the following major requirements for malaria forecasting software. The system must 1) automate the major steps required for operational data processing, modeling, and report generating, 2) work in concert with, but not replicate, existing surveillance programs and health information system, and 3) be implementable in LMICs with existing computational resources and personnel for long-term sustainability. To meet these requirements, we developed the R package epidemiar to provide a generalized set of functions for disease forecasting. R is a free software environment for statistical computing. In addition, we designed workflows and wrote customized code for disease forecasting, including a Google Earth Engine script to capture the necessary summaries of the environmental variables and formatting scripts to create distributable reports with maps and graphs of the results. Since the beginning of 2019, a local team based at Bahir Dar University in Ethiopia has been using EPIDEMIA to produce weekly malaria forecasting reports. The epidemiar package was written to be flexible for implementation in other locations and across a wide range of infectious diseases that are affected by environmental conditions. A companion R project epidemiar-demo contains a fully functional demonstration of the epidemiar forecasting workflow. Both projects are available publically on Github. These software tools facilitate the integration of earth science data with epidemiological data to support early detection and early warning of transmission events for infectious diseases with environmental drivers. The

results complement operational public health surveillance and can help to target interventions at the times and locations where outbreaks are most likely.

1000

MACHINE LEARNING APPROACHES TO BETTER UNDERSTANDING DRIVERS OF *PLASMODIUM FALCIPARUM* MALARIA AND ANEMIA CO-INCIDENCE IN SOUTHWESTERN MADAGASCAR

Akshaya V. Annapragada[1], Benjamin L. Rice[2], Hervet J. Randriamady[3], Christopher D. Golden[4]

[1]*Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, United States,* [2]*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, United States,* [3]*Madagascar Health and Environmental Research (MAHERY), Maroantsetra, Madagascar,* [4]*Department of Nutrition, Harvard TH Chan School of Public Health, Boston, MA, United States*

In Madagascar, there is geographic overlap of regions with high prevalence of malaria and anemia, and recently malaria incidence has been increasing in some regions of the country. This motivates study of the local drivers of malaria infection and co-incidence with anemia. Anemia prevalence is determined by a combination of factors, including nutritional and infectious disease status, but is biologically linked to malaria, as malaria parasites lyse red blood cells as a part of the infection cycle. For vulnerable, rural communities in Madagascar, such as those in the semi-arid southwest, a lack of health monitoring infrastructure and paired data on demographics and disease status has limited previous studies. Therefore, we analyzed newly available data from cross-sectional surveys performed in 2017 (n = 2690 individuals, all ages, both sexes) in Atsimo Andrefana province that recorded malaria infection status, anemia status, and over 200 socio-demographic variables. We present preliminary estimates of malaria and anemia prevalence, and their co-incidence. We observed an overall 16.9% malaria prevalence and 34.3% anemia prevalence among individuals sampled. Of those with anemia, 52.3%, 44.8% and 2.9% had mild, moderate and severe anemia, respectively. Among individuals testing positive for malaria, 50.7% also had anemia and 8.6% of all individuals had both malaria and anemia; a significant association (chi-squared test of independence, p < 1e-15). Further, we aimed to develop machine learning methods to determine key drivers of malaria, anemia, and co-incidence from the dataset, and predict individuals and communities susceptible to disproportionate disease burden. We employ matrix factorization methods for dimensionality reduction, such as Principle Component Analysis and k-means clustering, to identify the subset of survey questions significantly contributing to variation. In the future, we aim to train a Convolutional Neural Network to predict malaria, anemia, and co-incidence using the key socio-demographic variables we identify with the methods above.

1001

MODELLING HYPOTHESIZED INTERACTIONS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

Roslyn Hickson[1], Ricardo Aguas[2], Angela Devine[3], James McCaw[1], Lisa White[4]

[1]*University of Melbourne, Parkville, Australia,* [2]*University of Oxford, Nuffield Department of Medicine, United Kingdom,* [3]*Menzies School of Health Research, Darwin, Australia,* [4]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand*

Half of the global population are at risk of malaria, and there are over 200 million cases and 400,000 malaria deaths annually. The greatest threat is posed by two of the parasite species that infect humans, with 1.98 billion people at risk of both *Plasmodium falciparum* and *P. vivax*. *P. vivax* is posing a challenge to the elimination of malaria with the relative and absolute prevalence increasing in several countries, such as Papua New Guinea and India. There are several hypotheses about both the biological mechanisms for the interactions of these two species, and about the best intervention approach for *P. vivax* to enable elimination efforts to align with those for *P. falciparum*. We are developing mathematical models of population level transmission of *P. falciparum* and *P. vivax* to identify which of the hypothesised species interactions are supported by available data.

1002

THE INFLUENCE OF CHOICE OF METHOD, SEASONALITY AND MOVEMENT ON THE DETECTION OF MALARIA HOTSPOTS

Josephine K. Malinga, Yeromin P. Mlacha, Amanda Ross

Swiss Tropical and Public Health Institute, Basel, Switzerland

There has been increasing interest in variation in the risk of malaria at local scales such as within and between villages. As transmission has declined in many settings, targeted interventions for pockets of higher transmission have been trialled and the impact of hotspots of transmission on the surrounding areas debated. Methods to detect hotspots frequently rely on statistical significance and do not consider underlying processes such as the geographical distance between parent and offspring infections, season, the relationship between the malariological outcome and underlying transmission intensity, or the shape and gradient of the hotspot. We use an individual-based spatial simulation model to assess the influence of these features on the performance of measures of heterogeneity to identify underlying pockets of higher transmission intensity from a commonly used outcome, malaria prevalence. We calculate the sensitivity and specificity for households lying inside the pockets of higher transmission. The sensitivity, the proportion of households correctly identified as lying inside the pockets of higher transmission, was lower when there was a gentle decay in risk from the hotspot boundary, the hotspot was irregularly shaped or with a greater mean distance between parent and offspring infections. Both the gradient in risk and greater infection spread increased the size of the detected hotspot. The size of the detected hotspots also depended on the season. The Kulldorff's scan statistic with elliptical or circular windows and the Bayesian model both had greater sensitivity and specificity than Tango's flexible scan statistic in our tests. Only the Bayesian model gave estimates of how much higher the prevalence was in the pocket of higher transmission. The choice of outcome, method of detection and features affected the sensitivity and specificity of the measures and should be taken into account when interpreting analyses of heterogeneity. The characteristics of a pocket of higher transmission may partly determine how well it can be detected as a hotspot, and are also likely to affect the impact of the hotspot on the surrounding areas.

1003

MODELLING THE ROLE OF *ANOPHELES FUNESTUS* IN AN EAST-AFRICAN SETTING WHERE INSECTICIDE-TREATED NETS ARE ALREADY WIDELY USED BUT MALARIA TRANSMISSION PERSISTS

Manuela Runge[1], Halfan Ngowo[2], Tomas A. Smith[1], Nakul Chitnis[1], Fredros Okumu[2], Emilie Pothin[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Ifakara Health Institute, Ifakara, United Republic of Tanzania*

Major malaria vectors contributing to malaria transmission in Sub-Saharan Africa are members of the *Anopheles funestus* and *An.gambiae* complex. Effective use of long-lasting insecticidal nets has substantially reduced transmission by *An. gambiae s.s.* Continued effective control might contribute to diminishing the role of the highly anthropophilic *An. funestus*. In Kilombero, Tanzania, *An. funestus* and *An. arabiensis* are dominant malaria vectors with *An. funestus* now being the main contributor to transmission despite low abundance. We considered whether malaria elimination was possible if *An. funestus* were eliminated locally and if so, determined the required reduction of *An. funestus* mosquitoes to interrupt transmission, using the dynamic transmission model 'OpenMalaria'. Data from Kilombero informed the effective treatment coverage, the vector composition and the biting behaviour of mosquitoes. The impacts of reducing adult mosquitoes, larvae or both on vector abundance, entomological inoculation rates (EIR), and

malaria prevalence in humans were simulated using generic intervention deployments for ten years for settings with varying pre-intervention EIR values. In all settings, preventing emergence of *An. funestus* reduced malaria prevalence by 50% within six months, but prevalence decreased more slowly after that in higher transmission settings (mediated by *An. arabiensis*). Reducing *An. funestus* emergence by 10% to 50% was sufficient to achieve malaria prevalence of <1% within 5 years in low EIR settings, and by 80% to 100% in moderate EIR settings. Such reductions in prevalence were not possible in high EIR settings. Elimination was only achieved at very low EIR and >75% *An. funestus* emergence reduction. Targeting adults only could also lead to local elimination if outdoor biting mosquitoes could be targeted. In conclusion, the model suggested that the local elimination (or a substantial reduction in emergence) of *An. funestus* would interrupt malaria transmission at low EIR settings, but not in higher EIR settings. Additional larval control may be crucial in settings with a high proportion of outdoor biting.

1004

EFFECTIVE TREATMENT WITH ANTIMALARIALS AGAINST *PLASMODIUM FALCIPARUM* MALARIA 1992 - 2016

Susan F. Rumisha, Giulia Rathmes, Tim C. Lucas, Andre Python, Michele Nguyen, Anita Nandi, Peter W. Gething, Daniel J. Weiss

Malaria Atlas Project, Big Data Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Malaria incidence, prevalence and mortality are influenced by variations in treatment efficacy, treatment seeking behaviour, and the proportional use of available antimalarials. The combination of these factors is crucial for enumerated ineffectively (or untreated) cases, which constitute the cases at risk of resulting in severe disease and even death. As such, effective treatment with an antimalarial is a key input into our malarial mortality models, and high-resolution maps of this metric provide a guide to malaria elimination programs for improving antimalarial coverage. This project utilized data generated from therapeutic efficacy studies (TES), demographic health surveys and malaria indicator surveys (DHS-MIS), and ACTWatch project to estimate effective treatment in all MEC globally between 1992 - 2016. Data on 232 TES comprising 756 treatment arms, 89,713 individuals across 50 countries on Artemisinin Combination Therapies (ACTs), Sulfadoxine-Pyrimethamine (SP) and Chloroquine (CQ) covering a period between 1992 - 2016, were analysed using Bayesian geostatistical models, fitted separate for ACTs and non-ACTs to predict spatiotemporal patterns of efficacy for individual antimalarials in malaria endemic countries (MEC). Models were fitted using integrated nested Laplace approximations validated on predictive ability and accuracy. Efficacy level was set to over 90% treatment success. We summarized proportional usage for specific-antimalarials from DHS-MIS (2,365,905 children) and ACTWatch (2,153,507 cases) for the same period for each country. Treatment seeking patterns were estimated using DHS survey data. Synthesizing the three components, we calculated the population-adjusted proportion of individuals who received effective treatment in 110 MEC for the 25-year period.

1005

USING PREDICTIVE MODELING FOR THE PROACTIVE IDENTIFICATION OF MALARIA HOTSPOTS IN SENEGAL

Maya Fraser[1], Jean-Louis Lankia[2], Michael Betancourt[3], Michael Hainsworth[1], Yakou Dieye[2], Kammerle Schneider[1], Hana Bilak[1], Laurence Slutsker[1], Hannah Slater[1]

[1]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States,* [2]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Dakar, Senegal,* [3]*Freelance, New York, NY, United States*

Malaria continues as a major public health problem in Senegal. However, recent progress in program scale-up has resulted in substantial burden reductions and a national commitment to malaria elimination. As malaria incidence declines, transmission becomes more spatially heterogeneous, meaning that a large proportion of cases occur in fewer locations particularly in the north region. Currently, these localized clusters of malaria cases (outbreaks) are targeted reactively—when five cases occur within 15 days around 100m, a team is deployed to treat all individuals in the index case household and neighboring houses. However, this approach relies on the occurrence of an outbreak to trigger a response and likely is too late to prevent additional infections in individuals near the targeted neighborhood, or potentially even visitors to the area. We have developed a novel predictive statistical modeling framework to proactively identify "hotspots"—areas at high risk of experiencing outbreaks—for proactive targeting of measures (e.g., IRS, focal drug administration) to prevent outbreaks before they occur. Geolocated routine surveillance data (via the DHIS 2 national malaria reporting platform) from individual health facility catchment areas were regressed against a range of predictive covariates selected based on our understanding of malaria epidemiology and the drivers of transmission, including access to treatment, rainfall, temperature, and vector control coverage. A Bayesian generalized linear spatial model was used and implemented in Stan. Maps were developed identifying health facility catchment areas most at risk of outbreaks in the forthcoming transmission season and were integrated within a malaria visualization platform used in-country to inform intervention planning. The next step is to design a pilot study to evaluate the effectiveness of this approach to proactive malaria surveillance.

1006

SHARING THE KNOWLEDGE: THE PRESIDENT'S MALARIA INITIATIVE (PMI) HOSTS A SCIENTIFIC CONFERENCE IN HONOR OF WORLD MALARIA DAY, ANTANANARIVO, MADAGASCAR, 2018

Catherine M. Dentinger[1], Jocelyn Razafindrakoto[1], Laurent Kapesa[1], Jemima Andriamihamina[1], Andritiana Tsarafihavy[2], Eliane Razafimandimby[3], Henintsoa Rabarijaona[4], Sedera Mioramalala[5]

[1]*US President's Malaria Initiative, Malaria Branch, Centers for Disease Control and Prevention, Antananarivo, Madagascar,* [2]*USAID Mikolo Project, Antananarivo, Madagascar,* [3]*USAID Maternal Child Survival Program, Antananarivo, Madagascar,* [4]*Malaria Program, World Health Organization, Antananarivo, Madagascar,* [5]*National Malaria Control Program, Antananarivo, Madagascar*

Madagascar malaria researchers attend international meetings, but their presentations are rarely shared in-country in a forum that permits discussing implications, identifying applications, and refining research agendas. In honor of World Malaria Day 2018, PMI and the Roll Back Malaria Partnership to End Malaria held a conference to share findings. PMI and the *Institut Pasteur de Madagascar* hosted a half-day abstract writing workshop; PMI implementing partners leveraged resources to cover conference costs, an estimated $3,000. PMI and a medical faculty professor served as moderators; students registered participants and distributed evaluations. Evaluation data were entered into an Epi-Info database and analyzed using Microsoft Excel 2016. Fifteen researchers attended the abstract-writing workshop, 4 (27%) of whom submitted evaluations, which indicated that the workshop was beneficial but too short. For the conference, 19 submissions were chosen for 8 oral and 11 poster presentations. Topics included new approaches to prevention; findings from surveillance, parasitology and entomology studies; and improving community-based services. Of 194 attendees, 94 (48%) completed evaluations. Participants included clinicians, laboratorians, epidemiologists, program managers, public health practitioners, and faculty and students from medical and nursing schools; 92% indicated the event met their expectations and 97% stated they would like to attend again. One presenter noted that the conference allowed for feedback that enabled them to refine their work in advance of the ASTMH conference. Suggestions for future conferences included hosting a full-day event, finding a means to include regional and district public health personnel, including participants from other sectors such as education and environment, and sharing copies of posters and presentations with participants. This first PMI-supported conference in Madagascar to share

results of malaria prevention projects and studies was well-received. Plans to host a one-day event in 2019, using technologies to include field-based practitioners, are underway.

1007

ZAMBIA MALARIA INDICATOR SURVEY 2018: CONTINUED PROGRESS TOWARD NATIONAL COVERAGE AND BURDEN REDUCTION TARGETS

Busiku Hamainza[1], Maya Fraser[2], Elizabeth Chizema-Kawesha[1], Kafula Silumbe[3], Mercy Mwanza-Ingwe[1], Hawela Moonga[1], Anthony Yeta[1], Mutinta Mudenda[1], Fred Masaninga[4], John M Miller[3]

[1]*National Malaria Elimination Centre, Lusaka, Zambia,* [2]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States,* [3]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [4]*World Health Organization, Lusaka, Zambia*

The Zambia National Malaria Indicator Survey (MIS) 2018 was conducted by the Ministry of Health through the National Malaria Elimination Centre in April and May 2018 with the support of its partners. The MIS is a national household survey designed to assess the coverage of key malaria interventions and malaria prevalence in children under five years of age. The 2018 MIS was based on a nationally representative two-stage cluster sample of 4,475 households selected from 179 standard enumeration areas. A total of 4,177 households were interviewed in the survey, along with 3,680 women of reproductive age. A total of 2,883 children under the age of five provided finger prick blood samples for malaria testing. Consenting households and household members were asked about their household dwelling as well as standardized questions about insecticide-treated mosquito net (ITN) coverage and use, indoor residual spraying (IRS), prevention of malaria during recent pregnancies, and management of fever among children. Overall, national malaria parasite prevalence by microscopy was 9% among children under five years of age in 2018, a reduction compared to the previous MIS in 2015 (19%). Zambia achieved high coverage (80%) of at least one ITN per household, while 45% of households reported having at least one ITN for every two household members. Thirty-five percent of Zambian households reported IRS within the previous 12 months. Among women of reproductive age, 81% reported receiving at least two monthly doses of intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) during their most recent pregnancy, and 67% reported receiving three doses of SP. Additional results from key coverage indicators will be presented. The 2018 MIS represents significant progress across nearly all key indicators compared with previous similar surveys. This success is based on a foundation of solid programming and implementation efforts by a range of partners supporting the National Malaria Elimination Programme.

1008

TRAINING OUTCOME FOR MALARIA MICROSCOPY DURING AN ANTIMALARIAL THERAPEUTIC EFFICACY STUDY 2017

Safia M. Ali

Zanzibar Malaria Elimination Program, Zanzibar, United Republic of Tanzania

Antimalarial therapeutic efficacy studies (TES) should be linked with accurate malaria microscopy in order to monitor patient response to prescribed antimalarial medications based on WHO protocol. Our objectives were to conduct malaria microscopy training and assess laboratory technician improvements in microscopy. A 5-day malaria microscopy theory and practical training course was conducted in May 2017 to precede the biannual TES in Zanzibar. Eleven hospital laboratory technicians from the three TES recruitment health facilities received training on parasite detection, species identification, and parasite counting. Technicians completed pre-course and post-course written and practical tests including microscopy examination of blood slides with known *Plasmodium falciparum*, *P. malariae, P.* ovale, and mixed infections with densities ranging from 100 to 30,000 parasites/μL, as well as negative slides. We calculated sensitivity and specificity of the trainees microscopy readings of 128 samples collected during the TES using the readings by senior microscopists as the gold standard. The average test score before the training was 48.6%, while the average score after the training increased to 85.3% ($p<0.01$). Compared to the microscopy readings by the senior microscopists, the sensitivity and specificity of the trainee readings were 95% and 100%, respectively. Malaria microscopy training for hospital technicians is essential before starting antimalarial TES, and training improved performance in Zanzibar. Similar training might also improve the quality of microscopy for routine diagnosis.

1009

INFECTING HEALTHY VOLUNTEERS WITH MALARIA: CAN THIS BE DONE IN THE GAMBIA? A QUALITATIVE STUDY

Edgard Diniba Dabira, Jane Achan, Umberto D' Alessandro

MRCG at London School of Hygiene & Tropical Medicine, Banjul, Gambia

Controlled human malaria infection (CHMI) model, in which healthy volunteers are infected with *Plasmodium falciparum* to assess the efficacy of novel malaria vaccine and drugs has become a valuable tool to accelerate the development of vaccines and drugs. Perceptions and acceptability of the community is key for participation and retention of study participant in such studies and this is vital for the success of the trial. However, until now, little is known about communities' perceptions and acceptability regarding the CHMI model. As an ancillary study of the recently conducted CHMI study in The Gambia, we assess communities' perceptions and acceptability. We conducted an exit interview with the 19 CHMI trial participants and selected five participants for an in-depth interview; ten semi-structured interviews and three focus groups discussions were conducted at the community level. Respondents included students, ethic committees' members, researchers and civil servants and key local leaders. Participants acknowledged the risk involved in the CHMI model and appreciated the fact that the study is carried out in the country. Financial compensation was a major motivation for participation, but a fair compensation is required. Parents are key for decision-making process. Although the CHMI model presents a perceived risk, respondents acknowledged the importance and the necessity to conduct the CHMI study in The Gambia as it would ultimately benefit to the country.

1010

LESSONS LEARNED IN ENSURING EFFECTIVE IMPLEMENTATION OF INTEGRATED COMMUNITY CASE MANAGEMENT (ICCM): UGANDA'S EXPERIENCE

Maureen Amutuhaire, Damian Rutazaana, Allen Nabanooba, Jimmy Opigo, Jesca Nsungwa Sabiiti, Denis Rubahiika

Ministry of Health, Kampala, Uganda

The Ministry of Health (MoH) and development partners introduced the integrated community case management (ICCM) program due to the high under-five mortality rate attributed to malaria, diarrhea and Pneumonia. In 2016, the National Malaria control program scaled up efforts to optimize the implementation of iCCM. To evaluate the effect of the interventions, we reviewed data from January 2017 to December 2018. The data was analyzed from Health information records of village health teams and facilities, supervision reports from the district health team, partners and MoH and minutes of iCCM meetings held at national level, district and at facility level. The majority of the sick children (55%) were seen by Village Health Teams compared to 45% by the facility health workers. There was a threefold increase in the number of sick children seen by the VHTs from 675,982 cases seen in 2017 to 1,973,335 cases in 2018. 474,193 children diagnosed with pneumonia in 2017 compared with 188,698 children in 2018. 1,069,292 children diagnosed with Malaria seen by the VHTs in 2017 compared to 394,266 in 2018. Community reporting increased from 17.8% to 31.9% and stock out of iCCM commodities reduced by 45%. District MoH engagements, harmonization of program implementation

and reporting, stock management innovations, regular VHT support supervision , home visits and continuous sensitization of communities enabled these achievements in iCCM implementation

1011

IMPROVED PERFORMANCE OF THE MALARIA SURVEILLANCE, MONITORING AND EVALUATION SYSTEM FROM 2015 TO 2018 IN MADAGASCAR

Jean-Marie NGbichi[1], Maurice Ye[1], Alain Rakotoarisoa[2], Léa Bricette Andriamampionona[2], Solo Harimalala[3], Mauricette Andriamananjara[3], Laurent Kapesa[4], Yazoume Ye[1]

[1]*MEASURE Evaluation, ICF, Rockville, MD, United States,* [2]*Direction de la Veille Sanitaire, de la Surveillance Epidémiologique et Riposte, Ministry of Public Health, Antananarivo, Madagascar,* [3]*National Malaria Control Program, Ministry of Public Health, Antananarivo, Madagascar,* [4]*USAID PMI, Antananarivo, Madagascar*

MEASURE Evaluation has worked with the National Malaria Control Program (NMCP) since 2015 to strengthen malaria surveillance in Madagascar with support from the U.S. President's Malaria Initiative and partners. After a 2015 baseline survey of key malaria surveillance indicators in the national integrated disease surveillance and response (IDSR) system including completeness, timeliness of reporting and data accuracy we conducted a follow-up survey in 2018 and compared the results of the two surveys. *T-test* for means from independent samples were estimated and *p-values* calculated. Differences were considered significant if p<0.05. Completeness of routine malaria reporting by health facilities (*Centres de Santé de Base:* CSB) was 73.0% in 2018 compared with 65.3% in 2015 (*p=0.214*). Timeliness of CSB reporting was 68.4% in 2018 compared with 45.5% in 2015 (*p=0.0005*). Completeness of community-based reporting was 94.3% in 2018 compared to 8.6% in 2015 *(p<0.00001*) while timeliness was 55.0% in 2018 compared to 5.2% in 2015 *(p<0.00001).* Regarding data accuracy, the comparison of data from weekly reporting forms to weekly malaria data in registries showed 87.0 % accuracy in a randomized sample of CSB in 2018 compared to 73.3% in 2015 *(p<0.00001).* MEASURE Evaluation's support in strengthening malaria SME in Madagascar has likely contributed to these significant improvements in malaria and overall IDSR data reporting both at facility and community levels. These trends need to be maintained and improved, especially because Madagascar is considering malaria elimination in the coming years.

1012

EXPLORING BARRIERS AND FACILITATORS OF ACCESS AND ADHERENCE TO PEDIATRIC ARTEMISININ-BASED COMBINATION THERAPIES IN FREETOWN, SIERRA LEONE

Kristin Banek[1], Deborah D. DiLiberto[2], Emily L. Webb[1], Samuel Juana Smith[3], Daniel Chandramohan[1], Sarah G. Staedke[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*McMaster University, Hamilton, ON, Canada,* [3]*National Malaria Control Program, National Malaria Control Programme, Ministry of Health and Sanitation, Freetown, Sierra Leone*

Medication adherence is an important step in the treatment effectiveness pathway. However, medicine-taking behaviors do not occur in isolation, but are influenced by the environment in which the medicine is taken or administered. This qualitative study was embedded within a randomized controlled trial comparing adherence to artemether-lumefantrine and co-formulated amodiaquine-artesunate for the treatment of uncomplicated malaria in children under-five. The aim of the study was to explore contextual factors and identify barriers to and facilitators of access and adherence to malaria treatment at two public health facilities in Freetown, Sierra Leone. Caregivers of enrolled children were purposively selected based on their study arm and their primary adherence outcome, and were invited to participate in an in-depth interview. All interviews were conducted in Krio or English and were electronically recorded, transcribed, and translated. Interview transcripts were coded and aggregated into themes, applying a thematic content approach. Interviews with 49 caregivers were analyzed. Caregivers' responses highlighted three key aspects of the treatment effectiveness pathway that influenced access to medications and adherence to treatment: 1) health system related factors; 2) characteristics of the medications; and 3) caregivers' previous experience with malaria treatment. Caregivers reported confidence in the public health system and trust the health workers, which may have translated into better health behaviors. Ease of administration of the medication and perceived risk of side effects (namely weakness and vomiting) coupled with caregivers' prior experience with treating malaria influenced the way medications were administered to their children and whether they adhered to the prescribed treatment. In order to improve antimalarial adherence, the contextual factors in which medication-taking behaviors occur, such as must be considered. Moreover, further development and deployment of antimalarials that are easier to administer (i.e. dispersible or chewable formulations) may improve treatment adherence in children.

1013

SERVICE AVAILABILITY AND READINESS ASSESSMENT OF MALARIA CASE MANAGEMENT CAPACITIES IN HEALTH FACILITIES OF COMMUNES TARGETED FOR MALARIA ELIMINATION IN GRAND'ANSE, HAITI

Matt Worges[1], Vena Joseph[1], Thom Druetz[1], Jean Frantz Lemoine[2], Bernadette Fouche[3], Michelle Change[3], Prabhjot Singh[4], Eric Ndofor[4], Rainier Escalada[4], Joshua Yukich[1], Thomas Eisele[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti,* [3]*Malaria Branch, Division for Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Pan American Health Organization, Washington, DC, United States*

Haiti and the Dominican Republic are the only 2 countries in the Caribbean with endemic malaria transmission. Haiti accounts for over 95% of all documented malaria cases among the two nations. The Haiti Malaria National Strategic Plan calls for malaria elimination by 2025. Access to quality-assured malaria case management and diagnostics, as well as a high-quality surveillance and response system, are prerequisites for malaria elimination. In Haiti, chloroquine (CQ) plus single-dose primaquine (PQ) is the first-line treatment for uncomplicated malaria, which is predominantly due to *Plasmodium falciparum*. The Haiti national policy stipulates malaria diagnosis and case management are free. To assess malaria case management and diagnostic capacities and practices, a modified service availability and readiness assessment was conducted from November-December 2017 in 5 communes of Grand'Anse - the Department targeted for aggressive elimination strategies as part of the Malaria Zero Alliance. All 16 functioning health facilities were surveyed. Preliminary results show all facilities had malaria treatment guidelines available, but only 3 of 16 had the required fever case management algorithms. All facilities reported use of rapid diagnostic tests as the primary test for malaria with 4 also reporting use of malaria microscopy. 15 of 16 facilities had CQ tablets in stock on the day of the survey and 1 had CQ tablets expired by one month. Unexpired PQ tablets were observed in all facilities. 14 of 16 facilities reported charging general user fees to outpatients, which averaged 27.5 GDES (USD 0.33). However, 5 of 16 facilities reported user fees were either not charged or refunded to malaria test positive patients. No facilities reported charging clients specifically for malaria testing/ treatment. These results are encouraging and show that the majority of surveyed facilities have the basic capacities to perform malaria case management and diagnostic practices.

1014

SCALE UP OF INTEGRATED COMMUNITY CASE MANAGEMENT (ICCM) OF MALARIA FOR CHILDREN UNDER FIVE YEARS OLD IN LIBERIA, 2017-2018

Jannie M. Horace[1], Isaac Mwase[2], Eric Gaye[2], Barbara Jones[2], Jessica M. Kafuko[1], Mamadou O. Diallo[3]

[1]*U.S. President's Malaria Initiative, U.S. Agency for International Development (USAID), Monrovia, Liberia,* [2]*Partnership for Advancing Community-Based Services (PACS), Monrovia, Liberia,* [3]*U.S. President's Malaria Initiative, Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States*

Liberia's health care delivery system is organized into three tiers. The first level, primary health care, consists of clinics and the community health program. National malaria case management guidelines recommend that, as part of the Integrated Community Case Management (iCCM) program, Community health Assistants (CHA) should identify suspected malaria cases through routine household visits and confirm cases using malaria rapid diagnostic tests (RDT) prior to treatment. The overall goal is to rapidly manage malaria in children under-five and provide treatment within 24 hours of symptoms onset to prevent complications. Since 2015, the Partnership for Advancing Community-based Services (PACS) has collaborated with the Ministry of Health (MOH) and recruited, trained and supported over 640 CHAs to implement iCCM in communities at least 5 km away from clinics in Bong, Lofa and Nimba counties. PACS supported the MOH to develop and disseminate health communication strategy to promote health-seeking behavior and increase iCCM uptake in the community. Malaria awareness activities, including local radio call-in shows, have exposed hard-to-reach communities to information on malaria services. PACS iCCM maintains a database to monitor program implementation and generate reports. From October 2017 to September 2018, the CHAs provided malaria treatment with the first line artemisinin-based combination therapy (ACT) to 32,910 malaria cases in children under-five. Before initiating treatment, 76.9% (CI: 76.4% -77.3%) of malaria cases were confirmed with RDTs. Sixty-eight percent (CI: 67.5% - 68.5%) of these cases were treated within 24 hours. On average, the CHAs treated 2,743 malaria cases per month. The proportion of children treated within 24 hours has steadily increased, from 54.4% (CI: 51.2% - 57.6%) in October 2017 to 75.7% (CI: 74.3% - 77.1%) in September 2018 ($p<0.001$). Challenges for iCCM implementation include frequent RDT and ACTs stock outs. PACS successfully implemented iCCM to scale up early access to malaria treatment, but the success of this approach rests on a reliable stock of malaria commodities to test and treat cases.

1015

PRIORITIZING HEALTH FACILITIES FOR MALARIA CASE MANAGEMENT TRAINING IN GHANA IN THE ERA OF LIMITED RESOURCES

James Sarkodie[1], Amos Asiedu[1], Eric LaFary[1], Richard Dogoli[1], Raphael Ntumy[1], Lolade Oseni[2], Gladys Tetteh[2]

[1]*Impact Malaria Ghana, East Legon, Accra, Ghana,* [2]*Jhpiego Baltimore, Baltimore, MD, United States*

Ghana has made significant recent improvements in malaria control, reducing malaria deaths by 70% (1565 in 2015 to 468 in 2018) with a corresponding decline in under-5 malaria case fatality rate (CFR) from 0.51% to 0.19%. However, significant geographical variations in malaria morbidity and mortality persist. To achieve greater impact, a one-size fits all training approach may no longer be the most effective option. To prioritize facilities for refresher malaria case management training, the U.S. President's Malaria Initiative-funded Impact Malaria Project in collaboration with Ghana Health Service established systematic evidence-based criteria informed by quantitative and qualitative data. Using routine HMIS data from October 2017 to September 2018, total malaria admissions, malaria deaths, malaria case fatality rates were determined for all districts in respective regions. Districts with high burden malaria mortality and morbidity were ranked using a Pareto chart; districts with CFRs above the regional average were also identified. Stakeholder meetings discussed the findings using additional qualitative information including referral patterns, access and facility ownership to explain the findings. Regional health management teams triangulated the information to generate district lists prioritized for additional training focusing on assessment for complications, treatment, managing complications, monitoring and using quality improvement methods to identify change ideas to test to improve malaria management. The percentage of districts with an under-5 malaria CFR above the regional average was 31% Ashanti (AR), 28% Brong Ahafo (BAR), 31% Eastern (ER), 15% Upper East (UER) and 27% Upper West (UWR). In AR, 30% of districts accounted for 80% of all malaria deaths; in BAR, 34% of districts for 99% deaths; in ER, 38% of districts for 80% deaths; 23% of districts reported 99% deaths in UER; and 36% districts reporting 90% of deaths in UWR. Using routine HMIS data backed by qualitative information, a rational replicable basis for the prioritization of districts for intervention can be created based on evidence.

1016

ECONOMIC BURDEN OF MALARIA IN PREGNANT WOMEN IN RIVERS STATE, NIGERIA

Ifeyinwa N. Chijioke-Nwauche, Terhemen Kasso, Omosivie Maduka, Abimbola T. Awopeju, Ibinabo L. Oboro, Paul I. Nsirimobu, Lucy E. Yaguo Ide, Mark Ogoro, Godly Otto, Chijioke A. Nwauche

University of Port Harcourt, Port Harcourt, Nigeria

Malaria remains a public health concern among pregnant women and accounts for about 11% of maternal death in Nigeria. Treatment of malaria illnesses places a great financial burden on households. The study examined the economic burden of malaria among pregnant women in 12 communities in Rivers State, Nigeria. A pretested interviewer administered questionnaire was used to harvest relevant information from a sample population of 1008 participants. Obtained data was analysed using SPSS version 22 and results are presented in frequencies, percentages and tables. The socio-demographic characteristics showed a mean age of 30.56±4.93 years; with married and single constituting 988 (98.0%) and 17 (1.7%), others 3 (0.3) respectively. Educational attainment for the respondents were tertiary 656 (65.1); secondary 348 (34.5), primary 4 (0.4). Participants included traders/business women, teachers, civil servants, unemployed persons, artisans and others with average family income of 93,504.77 Naira (260 USD) per month. Out of 413 (41%) diagnosed with malaria, 382 (92.5%) were treated with an average cost of 3,241.42 Naira (9 USD) for one episode of malaria inclusive of laboratory test. The mean cost of treatment for hospitalised persons was 19,642.86 (54.6 USD) with a loss of 6.37 days of man hours and these costs were paid through out of pocket expenses by the spouses. 26 participants reported having negative effect of hospitalization on their finances. The association between malaria diagnosis and the socio-economic status of participants were all statistically significant with p-values <0.05 at 95% confidence interval. There was also a statistical significant relationship between malaria diagnosis and treatment, however the relationship between malaria diagnosis and preventive methods was insignificant (p-value >0.05). Cost of malaria treatment constitutes a great financial burden on households since this burden is completely born by the families. We recommend that the public health systems provide free treatment for pregnant mothers or reduce the cost of treatment in order to decrease the economic burden on the households.

1017

IMPROVEMENT IN DATA QUALITY USING FRONTLINE DATA VALIDATION OFFICERS IN SEVEN STATES IN NIGERIA

Chinedu Chukwu[1], Daniel Ofem[1], Isaac Adejo[1], Victoria Erinle[1], Faith Benebo[1], Thomas Hall[2], Mariah Boyd-Boffa[3], Bala Mohammed Audu[4], Perpetua Uhomoibhi[4], Ibrahim Maikore[4], Issa Kawu[4], Sonachi Ezeiru[5], Nnaemeka Onugu[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*Management Sciences for Health (MSH), Medford, MA, United States,* [4]*National Malaria Elimination Program, Abuja, Nigeria,* [5]*Catholic Relief Services, Abuja, Nigeria*

In Nigeria, inconsistencies between District Health Information System 2 (DHIS2) data, National Health Management Information System registers, and monthly summary forms are common. During the first quarter of 2018, a baseline assessment identified that 62% of 5,416 facilities in seven states (Adamawa, Delta, Kwara, Katsina, Ogun, Osun and Taraba) had incomplete data in the DHIS2 related to seven key malaria indicators. Without complete data, program performance is difficult to assess and planning to address gaps becomes a major challenge. Management Sciences for Health in collaboration with the Global Fund and in partnership with Catholic Relief Services and the National Malaria Elimination Program, developed a Data Validation Template, identified and trained 1,134 Validation Officers, and supported mentoring of health facility staff in 5,416 health facilities. Data Validation Officers download the data from the DHIS2 into the template and then take the data with them to health facilities to mentor staffs on any issues related to their respective data. Data validation meetings at the local government authority level were used to reinforce improvements. One year after implementation of this approach another assessment in the seven states determined that incomplete data in the DHIS2, in relation to the seven key malaria indicators, had reduced to only 13% of the 5,416 health facilities. Coaching and mentoring of health facility staffs responsible for data entry using Data Validation Templates by Validation Officers and reinforced during Data Validation Meetings improved the completeness of data in health facilities across seven states. Continuous mentoring, and identifying data issues, is necessary to ensure improvements are maintained.

1018

RETENTION OF MALARIA KNOWLEDGE AND SKILLS AND ADHERENCE TO NATIONAL TREATMENT GUIDELINES BY INTEGRATED COMMUNITY MALARIA VOLUNTEERS IN THREE STATES/REGIONS IN MYANMAR

Ni Ni Aye

Jhpiego, Yangon, Myanmar

The PMI-supported Defeat Malaria project aims to enhance technical and operational capacity of the National Malaria Control Program and providers at all levels of the health system in 3 States/Regions (S/R). In 2017, Myanmar introduced a new type of cadre, Integrated Community Malaria Volunteers (ICMV), as a foundation for integrated malaria control activities at village level. Defeat Malaria is developing their capacity to ensure malaria case management (MCM) according to National Treatment Guidelines (NTG). To date, Defeat Malaria has prepared 71 national and S/R level trainers to train and supervise 776 ICMVs caring for a population of nearly 600,000 people. The trainers offer a 5-day modular course to ICMVs, 2 days focusing on community-based prevention and MCM, including use of RDTs, ACTs, chloroquine and primaquine. ICMVs are coached using checklists as they diagnose and treat clients until they master the skills. From February-September 2018, 776 ICMVs were trained. On the pre-course test, 54% of 741 ICVMs achieved a passing score (≥80%) on the knowledge test, while 80% achieved a passing score on the post-course knowledge test. One-hundred percent achieved a passing score on use of RDTs using a standardized skills checklist during a simulation session. To date, 36 ICMVs have undergone post-training assessment of retention of malaria knowledge and RDT skills at one month after training. Ninety-four percent achieved passing scores on knowledge tests, and 83% conducted RDTs according to the skills checklist. During case simulations and per review of registers, 100% of the 36 ICMVs adhered to NTGs. ICMVs will receive regular supervision and will be followed up at 6 months after training to determine longer-term retention of knowledge and skills (data available by October 2019). While ICMVs demonstrate retention of knowledge and skills at one month after training, assessment at 6 - 12 months is necessary to ascertain long-term retention and persistent ability to care effectively. In the interim, we recommend that ICMVs receive regular supervision to ensure knowledge and skills retention associated with appropriate use of RDTs and NTGs.

1019

THE IMPACT OF IMPROVED AND COORDINATED BI-MONTHLY FACILITY REPORT ON UN-INTERRUPTED SUPPLY OF MALARIA HEALTH PRODUCTS FOR PATIENTS UNDER THE GLOBAL FUND MALARIA GRANT, (2018-2020) IN PUBLIC HEALTH FACILITIES IN ADAMAWA STATE: A CASE STUDY OF FEDERAL COLLEGE OF EDUCATION (FCE) STUDENTS' CLINIC, YOLA, ADAMAWA STATE, NORTHEASTERN NIGERIA

Melis Esi[1], James Audu[1], Thomas Hall[2], Isaac Adejo[1], Mariah Boyd-Boffa[3], Emmanuel Nfor[2], Olumide Elegbe[1], Bala Mohammed Audu[4], Kenji Goyit[4], Olukayode John[4], Sonachi Ezeiru[5], Chukwudi Uche[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*Management Sciences for Health (MSH), Medford, MA, United States,* [4]*National Malaria Elimination Program, Abuja, Nigeria,* [5]*Catholic Relief Services, Abuja, Nigeria*

The purpose of the study is to assess the impact of improved quality of national bimonthly facility stock reports (BFSR) on malaria health product management at a students' clinic, Federal College of Education (FCE), Yola, Adamawa State. In March 2018 the stock out rates of artemisinin-based combination therapy (ACT), rapid diagnostic tests (RDT) and long-lasting insecticidal nets (LLIN) were 50%, 16.9% and 3.9% respectively, due to poor quality BFSR and weak governance contributing to missed treatment and prevention opportunities of malaria infection in the clinic. Management Sciences for Health (MSH) in partnership with Catholic Relief Services, the National Malaria Elimination Program, and the Global Fund, is responsible for strengthening Supply Chain Management Systems in 13 states in Nigeria, to ensure continuous availability of RDTs, ACTs, and LLINs. MSH provided technical assistance to three health care workers at the FCE clinic, and five members of the state logistic management coordinating unit on quality BFSR generation, LLIN inventory management and timely requisition practices, including last mile distribution (LMD) planning from March 2018 to March 2019. MSH conducted analysis using LMD improvement tools to determine the variance between malaria products available for distribution and quantities ordered by the facility. After the intervention, stock outs of malaria health products declined at FCE clinic by March 2019, with ACTs down to 17.9%, and to 0% for RDTs and LLINs. The number of students and staff treated for uncomplicated malaria with ACTs after undergoing RDT and testing positive increased from 149 in March 2018 to 346 in March 2019. Generation of accurate BFSR improves supplies of malaria health commodities, reduces stock-out rates and improves service uptake among students and faculty in this tertiary, institutional health clinic. The Federal and State Ministries of Health should use these methods to improve BFSR accuracy and timeliness, which would reduce stock outs and facilitate malaria services delivery to those in need.

1020

EFFECTIVENESS OF SEASONAL MALARIA CHEMOPREVENTION IN AREAS OF INTENSE, SEASONAL MALARIA TRANSMISSION: SECONDARY ANALYSIS OF DATA FROM A HOUSEHOLD-RANDOMIZED CLINICAL TRIAL IN BURKINA FASO AND MALI

Matt Cairns[1], Issaka Sagara[2], Issaka Zongo[3], Irene Kuepfer[1], Frederic Nikiema[3], Serge Yerbanga[3], Modibo Diarra[2], Amadou Barry[2], Amadou Tapily[2], Ismaila Thera[2], Halidou Tinto[3], Paul Milligan[1], Jean Bosco Ouédraogo[3], Daniel Chandramohan[1], Adoulaye Djimde[2], Brian Greenwood[1], Alassane Dicko[2]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Malaria Research and Training Center, University of Science, Techniques, and Technologies of Bamako, Bamako, Mali,* [3]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso*

Seasonal malaria chemoprevention is now widely used to prevent malaria in young children in the Sahel sub-region of Africa, including in areas of extremely high malaria transmission. Such areas are a particular concern given their important contribution to the global malaria mortality burden. We explored the effectiveness of SMC in two areas of intense, seasonal malaria transmission in Burkina Faso and Mali. Between August 2014 and December 2016, approximately 20,000 children aged 3-59 months at the start of each rainy season received four monthly cycles of SMC with sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ), along with either azithromycin or placebo; this analysis focuses on the placebo group, who received the standard SMC regimen. Despite high coverage of SMC and provision of new long-lasting insecticidal nets to study children, approximately two-thirds of hospital admissions were caused by malaria. The overall incidence of hospitalisations for malaria was 12.5 per 1000 child-years at risk, with a clear peak each year in July, before SMC delivery began in August. Among children who had received SMC in the past 28 days, malaria incidence was approximately five-fold lower than among children without recent SMC (incidence rate ratio, 0.21 (95% CI 0.19, 0.22)). In all three years of the study, and in both countries, prevalence at the end of the rainy season exceeded 50% in school-age children who did not receive SMC, and was below 10% in study children. The effectiveness of SMC with SP-AQ remains very high in this setting, but malaria is far from being brought under control. Our results emphasise the need for additional monthly cycles of SMC to adequately cover the entire transmission season, and new interventions to further reduce the malaria burden.

1021

WHAT CAN DESCRIPTIVE NORMS TELL US ABOUT CARE-SEEKING FOR CHILDREN WITH FEVER IN AFRICA?: A MULTI-COUNTRY STUDY

Stella Babalola[1], **Angela Acosta**[1], Grace Awantang[1], Olamide Oyenubi[1], Mathew Okoh[2], Bolanle Olapeju[1], Ian Tweedie[1], Anna McCartney-Melstad[1], Michael Toso[1], Gabrielle Hunter[1], Abdul Dosso[3], Blaise Kouadio[4], Danielle Naugle[1], Mieko McKay[5]

[1]*Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [2]*Breakthrough ACTION-Nigeria, Abuja, Nigeria,* [3]*Johns Hopkins Center for Communication Programs, Abdijan, Côte D'Ivoire,* [4]*United States Agency for International Development - United States Agency for International Development, Abidjan, Côte D'Ivoire,* [5]*Johns Hopkins Center for Communication Programs, Abidjan, Côte D'Ivoire*

There is abundant evidence of the positive links between social norms and various health behaviors, including contraceptive use, antenatal care attendance, HIV care and support, immunization, and adolescent sexual behaviors. However, studies that focus on the role of social norms for malaria-related outcomes are rare. Using cross-sectional household survey data collected between 2015 and 2018 from four African countries, this study assesses the association of descriptive norms with care-seeking in a health facility for children aged less than five years with fever. The study countries include Côte d'Ivoire (n=723 children with fever), Liberia (n=726), Madagascar (n=769) and Nigeria (n=1174). Prior studies have shown that people who perceive specific behaviors to be the norm in their community are more likely to adopt the behavior. In this study, descriptive norm is defined as the perception that at least half of the people in one's community take the action of interest. According to survey respondents, the proportion of under-five children with fever that were taken to a health facility for care was 76.7% in Côte d'Ivoire, 78.8% in Liberia, 55.4% in Madagascar, and 42.6% in Nigeria. After controlling for socio-demographic, psychosocial, household and community variables in a logistic regression model, descriptive norm was a significant correlate of care-seeking for children with fever in a health facility in three of the four countries: Côte d'Ivoire, Madagascar and Nigeria. The odds ratio associated with descriptive norm was 2.73 (CI: 1.65-4.52) in Côte d'Ivoire, 1.38 (CI: 1.01-1.89) in Madagascar, and 1.72 (CI: 1.32-2.42) in Nigeria. Social and behavior change programs designed to promote facility-based treatment of fever in children may use social norm-modeling approaches to position the care-seeking behavior as a community norm. In addition, community mobilization approaches designed to foster positive community norms about care-seeking for children with fever are recommended.

1022

FACTORS ASSOCIATED WITH SEEKING CARE FOR FEVER IN CHILDREN UNDER FIVE YEARS OF AGE IN CÔTE D'IVOIRE

Diarra Kamara[1], Abdul Dosso[1], Monne Therese Bleu[2], Amadou Diabaté[3], Grace Awantang[4], Antoine Kouame[5], Mieko McKay[1], Antoine Mea Tanoh[2], Blaise Kouadio[6], Colette Yah Kokrasset[2], Stella Babalola[4]

[1]*Johns Hopkins Center for Communication Programs, Abidjan, Côte D'Ivoire,* [2]*Ministry of Health and Hygiene, Cote d'Ivoire National Malaria Prevention and Control Program, Abidjan, Côte D'Ivoire,* [3]*Johns Hopkins Center for Communication Programs Cote d'Ivoire, Abidjan, Côte D'Ivoire,* [4]*Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [5]*Save the Children Cote d'Ivoire, Abidjan, Côte D'Ivoire,* [6]*United States Agency for International Development, Abidjan, Côte D'Ivoire*

In Côte d'Ivoire, malaria is a major cause of child mortality and morbidity, with an estimated incidence of 286.9 per 1000 in children under 5 years of age, leading to a high proportion of school absenteeism. The national policy recommends seeking care at a health facility for a child with fever within 24 hours. According to the country's 2016 Multiple Indicator Cluster Survey, advice or treatment was sought from a facility or health provider for 45% of children under 5 years of age with fever in the past two weeks. Using data from a 2018 national survey on behavioral determinants of malaria, we conducted logistic regression to examine the association of contextual, household and individual variables on facility-based care seeking for a child under five with fever. The study sample included 723 women with a child with fever in the two weeks before the survey. Whereas the majority of the children (90%) were taken for care during their sickness, appropriate care (taking the child for care within 24 hours and in a health facility as a first recourse) was sought for only 63%. The caregiver's perception that prompt care seeking for fever is the norm (odds ratio = 1.90), that community health workers are good at treating malaria in children (1.85), and positive attitude towards prompt care seeking (1.80) were the strongest factors positively associated with seeking appropriate care for a child with fever. Other supporting factors included: if the caregiver and their partner discussed malaria; exposure to malaria messages; and the perception that malaria drugs are consistently available at the facility. Malaria programs would be advised to increase exposure to malaria messages by using a multimedia platform and community approaches. Social and behavior change activities would benefit from promoting 24-hour fever care-seeking for children as the norm, positive attitudes, dialogue between family members regarding care seeking within 24 hours for children with fever and creating platforms to foster trust in community health worker abilities to treat children. Programs should also consider ensuring consistent availability of antimalarial drugs in health centers.

1023

IMPROVING INTERMITTENT PREVENTIVE TREATMENT FOR PREGNANT WOMEN (IPTP) COVERAGE USING COMMUNITY-BASED OUTREACH STRATEGY (CBOS) IN 2 HEALTH ZONES IN BENIN: ADD (APLAHOUE-DOGBO -DJAKOTOMEY) AND DAGLA (DASSA GLAZOUE)

William Houndjo

National Malaria Control Program, Cotonou, Benin

Malaria is the leading cause of morbidity and mortality in Benin and accounts for >40% of hospital admissions. Malaria infection in pregnancy is a major public health problem; it was the main cause of death among pregnant women in 2017. To reduce malaria morbidity, the World Health Organization recommends that all pregnant women receive at least three doses of intermittent preventive treatment of malaria in pregnancy (IPTp1, 2, and 3) during routine antenatal care (ANC). IPTp has been available in Benin since 2013 but uptake is low; 28% of pregnant women nationwide receive IPTp3. To improve IPTp uptake in Aplahoue-Dogbo-Djakotomey (ADD) and Dassa Glazoue (DAGLA) health zones, the National Malaria Control Program adopted a community-based outreach strategy (CBOS) in 2018 where community health workers conducted community-based educational sessions on malaria in pregnancy and collaborated with health providers to identify pregnant women in the community who never had an ANC visit or who initiated IPTp but were lost-to-follow up, provide a dose of IPTp to all eligible pregnant women, and refer all pregnant women to the closest ANC provider. Proportions of pregnant women attending ANC who received IPTp1, 2, and 3 in each health zone were assessed before and after the CBOS intervention. During CBOS in DAGLA and ADD, 298 and 554 pregnant women who were lost-to-follow-up were identified and received subsequent IPTp doses, and 92 and 376 pregnant women who had never had ANC were enrolled, respectively. After CBOS implementation, the proportion of pregnant women attending ANC who received IPTp increased when compared to before CBOS. In DAGLA, the overall proportion of pregnant women attending ANC who received IPTp1 increased from 60% to 68%, IPTp2 from 29% to 49%, and IPTp3 from 21% to 31%. Similarly, in ADD, the overall proportion of pregnant women attending ANC who received IPTp1 increased from 67% to 78%, IPTp2 from 29% to 41%, and IPTp3 from 8% to 17%. IPTp uptake among women attending routine ANC increased after CBOS, likely because CBOS led to increased ANC attendance. CBOS should be scaled up in other health districts in Benin to improve IPTp uptake nationwide.

1024

THE RESULTS OF THE ROO*PF*S STUDY: RANDOMIZED CONTROLLED TRIAL TO ASSESS WHETHER IMPROVED HOUSING PROVIDES ADDITIONAL PROTECTION AGAINST CLINICAL MALARIA OVER CURRENT BEST PRACTICE IN THE GAMBIA

John Bradley[1], Margaret Pinder[2], David Jeffries[2], Jakob Knudson[3], Balla Kandeh[4], Musa Jawara[2], Umberto D'Alessandro[2], Steve W. Lindsay[5]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Medical Research Council Unit, Fajara, Gambia,* [3]*Schools of Architecture, Design and Conservation, Copenhagen, Denmark,* [4]*National Malaria Control Programme, Banjul, Gambia,* [5]*University of Durham, Durham, United Kingdom*

Is housing protective against malaria? Most sub-Saharan African malaria transmission occurs indoors at night, suggesting housing that impedes entry of mosquitoes may reduce the malaria burden. Observationally, those living in modern housing have a lower prevalence of malaria, but it is difficult to separate housing quality from other socio-economic factors associated with a lower malaria burden. Unfortunately, there is little high-quality evidence on the question and there have been no randomized trials showing an impact of housing on clinical malaria episodes. In the Roopfs trial, 800 traditional thatched-roofed houses in rural villages in the Gambia were randomized to either no intervention or to be fitted with metal roofs, closed eaves, screened windows, a metal-louvered screened door at the front and a metal-screened door at the back. A cohort of children living in study houses aged 6 months to 13 years was followed biweekly for two years. Those with fever were tested for clinical malaria with a rapid diagnostic test. In total 859 children were followed, and 393 malaria cases were recorded in 69,319 visits. Unexpectedly, incidence of clinical malaria was higher in the modified houses than in the traditional houses (rate ratio = 1.23 [0.99, 1.52] p=0.07) but this was not significant. CDC light traps were used to measure indoor mosquito density, which was also slightly higher in the modified houses (rate ratio = 1.28 [0.87,1.89] p=0.21). There are several plausible explanations for this result. Firstly, it is possible the screened doors and windows were insufficiently robust to prevent vector entry. Secondly, doors may have been left open since the modified houses led to higher indoor temperature and humidity. Thirdly, the higher temperature and humidity may have led to less bed net use. Fourthly, metal roofs may lead to higher concentration carbon dioxide, a mosquito attractant. While the failure of this particular intervention to reduce malaria transmission does not mean that other housing modifications will not be effective, it shows that reducing malaria through housing improvements will not be straightforward.

1025

EVALUATION OF MALARIA PARASITAEMIA AND ASSESSING THE KNOWLEDGE, ATTITUDE AND PRACTICE OF PREGNANT WOMEN ATTENDING HEALTH FACILITIES IN OWERRI METROPOLIS TOWARDS MALARIA PREVENTION AND TREATMENT

Ikechukwu Vincent Ejiogu[1], Chinyere I. Okoro[1], Chidimma M. Iyke-Ejiogu[2], Francis Ihenetu[3], Oluchi I. Okoro[4]

[1]*Federal Medical Center Owerri, Owerri, Nigeria,* [2]*Federal University of Technology, Owerri, Nigeria,* [3]*Department of Microbiology, Federal University of Technology, Owerri, Nigeria,* [4]*Beulah Medical Diagnostic Laboratory and Research, Owerri, Nigeria*

There are several studies on malaria in pregnancy but studies focusing on the perception and current practices of malaria prevention and treatment among pregnant women in the study area are sparse. This study was aimed at assessing the knowledge, perceptions about malaria prevention with emphasis on knowledge about placental malaria as well compliance to use of LLIN and IPTP among pregnant women in Owerri, South Eastern Nigeria. A hospital based cross sectional study. Data was obtained from 200 randomly selected consenting pregnant women using a pretested structured questionnaire. Recognition of malaria symptoms during pregnancy, knowledge about IPTP and placenta malaria were items used to assess the level of knowledge about malaria in pregnancy. Their blood samples were also examined for malaria parasitaemia. Malaria prevalence in the study population by microscopy was 13.5%. Also 62% of the respondents were knowledgeable about the possible detrimental effects of the presences of malaria during pregnancy. About 79.5% of the respondent positively associated mosquitoes to malaria infection while 31% and 14% associated cause of malaria infection to dirty surrounding and stagnant water respectively. Malaria Prevention attitude and Practice in this study was good (60%) while 18.5% had fair practice. More so and 15.5% of the respondents had been involved in poor practice. About 34% of the pregnant women slept under a mosquito net often .study observed statistical significance between age, occupation and malaria prevention practices (P=0.014, 0.0089).There was also statistically significant association between respondent's practice and respondent attitude (p=0.0034). Knowledge regarding the use of Sulphodaxine primethamine (SP) as drug of choice during pregnancy was limited (12.0%) as use of chloroquine was ranked highest (48%) The study demonstrated low peripheral malaria prevalence and also demonstrated that pregnant women's Knowledge, attitude and practice towards malaria prevention was considerably on the average. However, there was poor knowledge about intermittent preventive treatment of malaria (IPTP) as recommended

1026

KEY LEARNINGS OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANT WOMEN ATTENDING ANTENATAL CARE SERVICES IN SEGOU AND MOPTI REGIONS IN MALI

Idrissa Cisse[1], Boubacar Guindo[2], Noella Umulisa[2], Sanogo Vincent[1], Assitan Dembele Coulibaly[1], Saidou Kanambaye[2], Mariam Diatty Diallo[2], Moussa Thior[3], Pharath Lim[3], Renion Saye[2], Oumar Yattara[2], Moussa Sacko[4], Adboulaye Ouologuem[4], Kathryn Malhotra[3], Tabitha Kibuka[3], Jules Mihigo[5]

[1]*Mali PNLP, Bamako, Mali,* [2]*PMI Impact Malaria Project, Bamako, Mali,* [3]*PMI Impact Malaria Project, Washington, DC, United States,* [4]*INRSP, Bamako, Mali,* [5]*USAID/PMI, Bamako, Mali*

Malaria is the primary cause of morbidity and mortality in Mali. Use of intermittent preventive treatment (IPTp) is a proven cost-effective intervention for preventing malaria in pregnancy (MiP). Mali's MiP strategy is based on WHO's recommended three-prong approach, which includes the promotion and delivery of at least three doses of sulfadoxine pyrimethamine (SP) for IPTp as early as possible in the second trimester of pregnancy. In December 2018, the U.S. President's Malaria Initiative Impact Malaria project, together with the NMCP, conducted an assessment in 12 health facilities (HF) in Segou and Mopti Regions. One objective was to assess the uptake of IPTp-SP and the barriers to its use in pregnant women attending ANC services. Quantitative and qualitative methods were used for the assessment. Data from ANC registers, district reports, and in-depth interviews with MoH stakeholders at central and regional were collected and analyzed. A systematic sample of pregnant women's ANC register records for the three months prior to the assessment were collected and reviewed from the 12 HFs (N= 204). Overall, 5.4% (n=11), 59.8% (n=122), and 34.8% (n=71) received their first dose of IPTp at 13 weeks, 14-27 weeks, and 28-40 weeks of pregnancy, respectively. Although IPTp-SP has been provided since 2006, the indicators for three or more doses of IPTp during the last two years still remain low at only 21% (MIS 2015-2016). The sample of quantitative records reviewed indicated that many pregnant women are starting IPTp-1 much later than recommended by the MoH. A workshop is planned in April 2019 to discuss current barriers to IPTp and to identify solutions to increase the utilization and early attendance of ANC. In addition, a new outreach training and supportive supervision (OTSS) MiP module will be added to routine supervision visits starting later this year. This data will provide additional information on quality of IPTp services by health providers to supplement earlier findings.

1027

IMPACT OF SEASONAL MALARIA CHEMOPREVENTION AMONG CHILDREN FIVE TO TEN YEARS OF AGE IN KITA AND BAFOULABE DISTRICTS, MALI

Sory I. Diawara[1], Erin Eckert[2], Jules Mihigo[3], **Beh Kamate**[4], Drissa Ouattara[4], Diakalia Kone[5], Mariam Tall[5], Eric Swedberg[6], Samba Coumaré[4], Drissa Konate[1], Moctar Tounkara[1], Mahamadou Diakité[1], Seydou Doumbia[1], Nathalie Gamache[4], Protais Ndabamenye[4]

[1]*Malaria Research Training Center, Bamako, Mali,* [2]*USAID/US President's Malaria Initiative, Washington, DC, United States,* [3]*USAID/US President's Malaria Initiative, Bamako, Mali,* [4]*Save the Children, Bamako, Mali,* [5]*National Malaria Control Program, Bamako, Mali,* [6]*Save the Children, Fairfield, CT, United States*

Seasonal malaria chemoprevention (SMC) is the administration of complete therapeutic courses of antimalarial to all children 3–59 months old during the malaria transmission season. This study measured coverage, impact and cost of adding SMC in children aged 5-10 years. A non-randomized, pre-post design, with an intervention (Kita) and control (Bafoulabe) district implemented SMC for children 5-10 years old through the health system in 2017 and 2018. SMC implementation consisted of the administration of SP + AQ at monthly intervals in children 5-10 years in July, August, September and October each year. Baseline and endline household surveys were conducted in both districts. Separate surveys to measure adherence and tolerance to treatment occurred annually in the intervention district (200 households) following each of the four treatment rounds. Routine data on malaria cases tested and treated and information on SMC campaign and treatment costs were collected. SMC coverage was over 90% in both years. In four rounds, 89% and 81% of children received all three doses in 2017 and 2018 respectively. The most reported side effect by parents was weakness in 15% of cases. Parents reported positive opinions (96%) of SMC treatment. At baseline, malaria infection prevalence was similar in the two districts (23% in Kita vs. 27% in Bafoulabe p=0.28). At study end, the prevalence of malaria infection was 22% in the comparison district compared to 17% (p=0.01) in the intervention district with a 40% reduction in malaria infection prevalence. Routine data showed a 21% and 62% reduction of simple malaria and severe malaria prevalence in the intervention district versus the control district. Mild anemia and severe anemia were comparable in the two districts. The level of malaria molecular resistance rate remains below the threshold. Quintuple mutation (dhfr triple +dhps437+dhps540) remained <5% after intervention in both districts. The additional cost of prevention treatment of children 5-10 years old was US$1.22 per child in Kita. The SMC strategy contributed to malaria prevention in 5-10 year old children and should be integrated to SMC for children 3-59 months.

1028

AVAILABILITY AND COST OF ANTIMALARIAL CHEMOPROPHYLAXIS AND TREATMENT IN THE UNITED STATES FOR TRAVELERS AT HIGH RISK OF ACQUIRING MALARIA

Beth K. Thielen[1], Emily Walz[1], Hannah R. Volkman[1], Jonathan D. Alpern[2], William M. Stauffer[3], Danushka Wanduragala[4], Mackenzie L. Smith[5], Wilhelmina V. Tolbert Holder[6], Anne Frosch[7]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*HealthPartners, St. Paul, MN, United States,* [3]*University of Minnesota and HealthPartners, Minneapolis, MN, United States,* [4]*Minnesota Department of Health, St. Paul, MN, United States,* [5]*Carleton College, Northfield, MN, United States,* [6]*New Americans Alliance for Development, St. Paul, MN, United States,* [7]*HennepinHealthcare, Minneapolis, MN, United States*

Reported cases of imported malaria to the U.S. continue to increase and disproportionately occur among individuals traveling to endemic areas to visit friends and relatives (VFR). Among VFR travelers, use of malaria chemoprophylaxis is less common than among non-VFR travelers, and a recent study suggests that cost and availability of medications can be barriers. To quantify the availability and affordability of antimalarial drugs in the United States for both chemoprophylaxis and treatment, we conducted a survey of pharmacies in two cities with large populations of VFR travelers--New York City, NY and Minneapolis/Saint Paul, MN. Surveys were conducted in areas with either high and low likelihood of having VFR travelers to Sub-Saharan Africa. Pharmacies provided data on anti-malarial medication availability (in stock at time of call) and the cash price (price if no insurance billed). To estimate cost, we calculated cost per 30-day trip to account for differences for dosing intervals and required number of pre- and post-travel doses. 138 pharmacies were surveyed, 70 in Minnesota (40 high and 38 low likelihood areas) and 70 in New York (32 high and 38 low). Inter-pharmacy variability in pricing was high, with prices varying >10-fold for medications such as atovoquone-proguanil. We noted significant geographic differences in availability of drugs, with lower availability of atovoquone-proguanil and chloroquine in New York compared to Minnesota. There was significantly decreased availability of atovoquone-proguanil in communities with a high burden of malaria cases. In summary, there were marked differences in both price and availability across cities and neighborhoods, most notably for atovoquone-proguanil, a favored drug for prevention of malaria among African travelers. These findings reinforce observations that cost and availability are barriers to VFR travelers and that the market for antimalarial is not equitable and suggest that interventions to increase access to affordable prophylaxis, particularly in locations with high concentrations of VFR travelers, could be a useful strategy to decrease imported malaria cases.

1029

MALARIA PREVENTIVE PRACTICES AMONG UNDER-FIVES IN DELTA STATE, NIGERIA

Nsirimobu Ichendu Paul[1], NDDC Professional chair on malaria elimination[2]

[1]*University of Port Harcourt, Port Harcourt, Nigeria*

Malaria is a leading cause of morbidity and mortality in under 5 children. A holistic malaria preventive practice in endemic areas is key to reducing the mortality associated with malaria. The study assessed malaria preventive practices among under-five children in Delta State, Nigeria. This was a cross sectional study carried out in public and private health facilities in Delta state. A multi staged stratified sampling method was used to select the health facilities and to recruit the subjects for the study. Data was collected using a pretested interviewer administered questionnaire and analysed by descriptive statistics. A total of 1008 children participated in the study constituting of 542 (53.8%) male and 466(46.2%) female. Mean age of participants was 1.78±1.08 years. The modal informant in the study were the mothers, accounting for 964 (95.6%) of the total participants. Most of the participants had obtained secondary education; among fathers, there were 559 (56.6%) and 572 (57.1%) among mothers. Most of the informants had tertiary degree; 605 (53.4%) and 697 (61.8%) among mothers and fathers respectively. Among the occupations of fathers; trader/business man, self-employed, and public servants were more represented, constituting 332 (33.4%), 224 (22.5%) and 115 (11.6%) respectively. Traders/business women, the self-employed and teacher were most represented among other occupations of mothers in the study, these accounted for 500 (50.2%), 157 (15.8%) and 80 (8.0%) respectively. Malaria preventive practices included, protective window nets, use of bed net, indoor residual spraying, use of drugs, no method used and use of mosquito repellants which constituted 707 (70.1%), 569 (56.4%), 359 (35.6%), 205 (20.3%), 134 (13.3%) and 7 (0.7%) respectively. The study shows a low bed net use rate and this is worrisome considering efforts being made by the Nigerian and State Governments. It is also surprising that intentional bush clearing and disposal of cans that encourage breeding places for malaria vector, use of long protective wears in the evenings and larviciding are not yet being practiced. This calls for health education intervention.

1030

MALARIA PREVENTIVE PRACTICES AMONG PREGNANT WOMEN IN AKWA IBOM STATE, SOUTHERN NIGERIA

Terhemen Kasso, Ibinabo L. Oboro, Omosivie Maduka, Abimbola T. Awopeju, Ichendu P. Nsirimobu, Lucy E. Yaguo-Ide, Ifeyinwa Chijioke-Nwauche, Godly Otto, Carol Iyalla, Chijioke A. Nwauche

University of Port Harcourt, Port Harcourt, Nigeria

Malaria is a serious public health problem in developing countries with Africa bearing most of its burden. Pregnant women are more susceptible to malaria and it is one of the leading causes of maternal and perinatal mortality/morbidity. Use of effective malaria preventive methods during pregnancy reduces the disease burden with its complications. The aim of the study was to assess malaria preventive practices among pregnant women in Akwa Ibom state. It was a cross sectional study of pregnant women attending public and private health facilities in Akwa Ibom state. Ethical approval was obtained from the Research and Ethics Committees of Universities of Uyo and Port Harcourt Teaching Hospitals and the state ministry of Health. An informed written consent was obtained from the participants. Stratified sampling method was used in selecting the health facilities and the study participants. Information was obtained with pretested questionnaires by trained personnel with the aid of Open Data Kit (ODK) on android phones. Data was managed with SPSS 22.0 and P-value of <0.05 was considered statistically significant. There were 935 participants in the study. Their mean age was 28.52 ± 5.09 years with 879 (94.0%) being married. Most had secondary and tertiary degrees: 451(48.2%) and 440(47.1%). Majority of them were traders/business women and self-employed (58.2%) while 7% were unemployed. Most of the women 471(50.4%) were primigravidae. The mean gestational age of participants was 27.57 ± 7.40 weeks. Malaria preventive practices were use of window net, bed net and insecticides accounting for 659(71%), 447(48.2%) and 207(22.3%). There were 612 (65.5%) participants that received malaria drugs for prevention with 452(73.9%) receiving Intermittent Preventive Treatment in Pregnancy (IPTp) with Sufadoxine-Pyrimethamine (SP). Only 296 (31.7%) of them slept under bed nets the night before. There was statistically significant association between frequency of malaria diagnosis, treatment and non-usage of bed nets. Efforts should be intensified in creating awareness about the various preventive measures in order to curtail this menace.

1031

EFFECT OF MALARIA BEHAVIOR CHANGE COMMUNICATION PACKAGING ON ITN USE AND INCIDENCE OF MALARIA IN RURAL WESTERN KENYA: A RANDOMIZED THREE ARM CONTROLLED TRIAL

Judith Mangeni[1], Jane Namae[1], Lucy Abel[1], Stephen Karuru[1], Wendy Prudhomme O'Meara[2]

[1]*Moi University College of Health Sciences, Eldoret, Kenya,* [2]*Duke University, Durham, NC, United States*

Behavior change communication has been shown to improve utilization of ITNs. Appropriate utilization of ITNs remains low in Bungoma County despite high distribution of the same. In addition, a high prevalence of malaria has been reported in the same region. We sought to identify the best packaging of "ITN use" behavior change communication among pregnant mothers and their households at the Webuye Health and Demographic Surveillance Site located in Bungoma East Sub-County. The primary endpoint was "correct ITN use" measured at baseline, one month, three months and six months follow up. The secondary endpoint was the incidence of malaria infections. We conducted a three-arm randomized control trial. *Intervention A was a* standardized verbal health message delivered by a health worker at the clinic plus a poster with health information on ITN use and care. *Intervention B was* a standardized verbal health message that is delivered by the health workers at the clinic plus one 'hang up' visit and the control was the usual standard practice at the Ante-Natal Clinic. Pregnant mothers who met the eligibility criteria were individually randomized in equal proportions to either of the intervention arms or the control. ITN use information was collected as well as RDT testing done for the pregnant mother and her household members. A total of 303 pregnant mothers were recruited for this study (101 in each arm). The study is currently ongoing and we will be able to report the actual results in early September after completion of the study. This study will improve our understanding of how to maximize the return on investment of public health commodities like nets. The long follow up rather than just a few weeks post-exposure to the BCC is also a major strength for this study. Finally, another strength of the study is the fact that we used a biomarker of correct ITN use such as infection which can be compared between arms.

1032

BED NET USE AND KNOWLEDGE OF MALARIA PREVENTION IN SIERRA LEONE

Beah Joe Lebby[1], Haja Abie Manasaray[2], Ivan Macauley-Black[1], Julian Mattia[1]

[1]*Njala University, Bo, Sierra Leone,* [2]*Eastern Polytechnic (Njala University), Kenema, Sierra Leone*

Malaria is one of the most common diseases in Sierra Leone and is believed to be responsible for about 50% and 38% of outpatient visits and admissions respectively. It is the leading cause of premature death and overall death in Sierra Leone. Anopheles is the primary vector with *Plasmodium falciparum* being responsible for 100% of cases. The government introduced free distribution of bed net in 2002 as a primary means of prevention which was later expanded to all age groups in 2010. In June 2017, the Ministry of Health and Sanitation implemented universal

distribution of bed nets to households. The current report assesses the coverage of the distribution and knowledge of the importance of the use of bed net. A total of 59 chiefdoms in 7 districts were selected and questionnaires administered to a total of 383 individuals in the rural communities. Key questions included whether they had received bed nets during the campaigns, quantity of bed nets, previous use of bed nets, knowledge of causes and prevention of malaria. A total of 383 persons were interviewed with an average 6 persons per chiefdom. Overall, 98% of persons interviewed recalled receiving bed net for their household. However, 40% said it was not enough for the beds they had in the household. Also, 21% of those interviewed said they had never used bed net. On the knowledge of the primary cause of malaria, 98% knew mosquito was responsible for the transmission of malaria while 99% knew that the use of bed net can prevent malaria transmission. Also, 91% of the households received more than 1 bed net. Compared to a 2010 assessment which indicated that 87.6% of those interviewed recalled receiving bed nets during the campaign, the results show an increment in the coverage in 2017. Also, the proportion of households that received more than 1 bed net (91%) is a significant increment compared to 67% in 2010. The results show that there has been significant increase in bed net coverage but below the countries target of 100% coverage of households. It also shows that knowledge of the cause and prevention of malaria is high among individuals in the country.

1033

DETERMINANTS OF MALARIA TESTING AT HEALTH FACILITIES: THE CASE OF UGANDA

Ruth N. Kigozi[1], John Baptist Bwanika[1], Emily Goodwin[1], Peter E. Thomas[2], Patrick Bukoma[1], Persis Nabyonga[1], Fred Isabirye[1], Paul Oboth[1], Carol Kyozira[3], Mame Niang[4], Kassahun Belay[5], Gloria Ssebikaari[5], James K. Tibenderana[6], Sam S. Gudoi[1]

[1]*PMI Malaria Action Program for District Project, Uganda, Kampala, Uganda,* [2]*US President's Malaria Initiative, Malaria Branch, Centers for Diseases Control and Prevention, Atlanta, GA, United States,* [3]*Health Information Division, Ministry of Health, Uganda, Kampala, Uganda,* [4]*US President's Malaria Initiative, Malaria Branch, Centers for Diseases Control and Prevention, Kampala, Uganda,* [5]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda,* [6]*Malaria Consortium, London, United Kingdom*

The World Health Organization (WHO) recommends prompt malaria diagnosis with either microscopy or malaria rapid diagnostic tests (mRDTs) and treatment with an effective antimalarial, as key interventions to control malaria. However, in sub-Saharan Africa, malaria diagnosis remains influenced by clinical symptoms, with patients and care providers often interpreting all fevers as malaria. The Ministry of Health in Uganda defines malaria suspects as those with a fever of history of fever. A target of conducting testing for at least 75% of malaria suspects was established by the National Malaria Reduction Strategic Plan. We investigated factors that affect malaria testing at health facilities in Uganda using data collected in a cross sectional survey of health facilities from the 43 districts of Uganda that are supported by the US President's Malaria Initiative (PMI). We assessed health facility capacity to provide quality malaria care and treatment. Data were collected from all 1085 public and private health facilities in the 43 districts. Factors assessed included support supervision, availability of malaria management guidelines, laboratory infrastructure, and RDT training to health workers. Survey data were matched with routinely collected health facility malaria data obtained from District Health Information System (DHIS2). Associations between testing at least 75% of malaria suspects and various factors were examined using multivariate logistic regression. Key malaria commodities were widely available; 92% and 85% of the health facilities reporting availability of mRDTs and artemether-lumefantrine, respectively. Overall, 933 (86%) of the facilities tested over 75% of patients suspected to have malaria. Predictors of meeting the testing target were: supervision in the last six months (OR 1.72, 95% CI (1.04-2.85)) and a health facility having at least one health worker trained in the use of mRDTs (OR 1.62, 95% CI (1.04-2.55). Our findings underscore the need for malaria control programs to ensure that all facilities have trained health workers, have a reliable supply of commodities and importantly receive regular supervision.

1034

EFFECTIVENESS OF MALARIA BOOTCAMP TRAINING: EQUIPPING PEACE CORPS VOLUNTEERS AND THEIR COUNTERPARTS TO IMPLEMENT MALARIA PREVENTION ACTIVITIES IN RURAL MADAGASCAR, 2018

Belen Godinez-Santana[1], Jocelyn Razafindrakoto[2], Mamina Herizo[1]

[1]*Peace Corps, Antananarivo, Madagascar,* [2]*President's Malaria Initiative, Antananarivo, Madagascar*

Malaria causes substantial morbidity and mortality in Madagascar, particularly in rural areas where most Peace Corps Volunteers (PCV) serve. To train PCVs to support malaria prevention activities, Peace Corps Madagascar adapted the Senegal-based Malaria Boot Camp (MBC). All 150 Madagascar PCVs were welcome to apply to the five-day training along with their community counterparts. We describe the first Madagascar-based MBC in which PCVs and their community counterparts were included, including a follow-up survey, and discuss implications. Participants were selected based on their malaria prevention work, counterpart collaboration, willingness to train neighboring PCVs, and time left in service. Sessions on malaria biology, diagnosis, treatment and prevention, and information on evidence-based behavior change techniques were led by malaria specialists. Participants learned about project design and proposed a project for their communities. PCV participants completed telephone surveys immediately following training, and three months afterward. Of 20 (13%) PCVs who applied, 16 PCVs and their respective counterparts were selected and participated. Nine (56%) PCV participants responded to either the immediate or the three-month post-MBC survey; 100% had initiated their proposed project. Participants reported they used MBC tools to conduct focus group, one-on-one, and door-to-door surveys to identify community needs. PCVs and their counterparts implemented activities including: malaria committees; bike rides to promote insecticide-treated bed net (ITN) use; surveys to determine if families needed help with ITN hang-up, use or care after a mass distribution campaign; and analysis of malaria data at their local health clinic. At least 56% of PCV participants initiated prevention activities in their communities within five months of completing MBC training; a six-month follow-up is planned. These trainings may be an effective approach to promote evidence-based malaria prevention practices in rural Madagascar.

1035

QUANTIFYING THE POTENTIAL IMPACT OF MASS DRUG ADMINISTRATION ON THE PARASITE RESERVOIR IN AN AREA OF DECLINING MALARIA TRANSMISSION IN UGANDA

Joaniter I. Nankabirwa[1], Jessica Briggs[2], John Rek[3], Emmanuel Arinaitwe[3], Sarah G. Staedke[4], Philip J. Rosenthal[2], Moses R. Kamya[1], Grant Dorsey[2], Isabel Rodriguez-Barraquer[2], Bryan Greenhouse[2]

[1]*Makarere University Kampala, Kampala, Uganda,* [2]*Division of HIV, ID, and Global Medicine, University of California San Francisco, San Francisco, CA, United States,* [3]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Significant reduction in malaria burden has been achieved in areas where indoor residual spraying (IRS) has been rolled out in Uganda. However, a large proportion of the population in some of these regions remains parasitemic, providing a potential reservoir for onward transmission. Using detailed data from an intensely followed cohort study in Uganda, we quantified the potential effect of adding mass drug administration to IRS on the parasite reservoir in a formerly high endemic setting with declining malaria transmission following sustained IRS. Between October

2017 and October 2018, we followed 492 participants enrolled in a cohort in Tororo, Uganda. One round IRS with actellic was rolled out in June/July 2018. Parasitemia was identified by active surveillance every four weeks and continuous passive surveillance using qPCR. We used this detailed longitudinal dataset to quantify the potential impact of mass drug administration (MDA), by simulating a single round of MDA administered on October 30th 2017. We assumed that the drug used for MDA would be 100% effective and clear all active infections present on the day of its administration, as well as provide protection against new infections for the subsequent 28 days. The outcomes of interest were parasite prevalence at six months and one year of follow up, and the total number of parasitemic days over the same time periods. We used linear interpolation of parasite densities to fill in the data for the days between study visits. A single round of MDA was associated with a significant decline in parasite prevalence in participants aged 5 -15 years (from 17.8% to 2.9% at 6 months; p<0.001 and from 14.4% to 4.7% at one year; p=0.001) and in participants >15 years (from 14.2% to 2.6% at 6 months; p<0.001 and from 10.8% to 3.2% at one year; p=0.008) but not in children < 5 years. MDA also reduced the total number of parasitemic days irrespective of age-group and duration of follow-up (P<0.001). MDA may provide an effective strategy for targeting the residual reservoir of malaria parasites, especially in school age children and adults, in areas with declining malaria transmission following effective vector control.

1036

ROLLOUT OF SINGLE LOW-DOSE PRIMAQUINE IN TWO SOUTH AFRICAN DISTRICTS TARGETING MALARIA ELIMINATION: ASSESSING PROGRESS AND CHALLENGES

Jaishree Raman[1], Elizabeth Allen[2], Theresa Mwesigwa[1], Aaron Mabuza[2], Bheki Qwabe[3], Gillian Malatje[4], Frank M. Kagoro[2], Karen I. Barnes[2]

[1]*South African National Institute for Communicable Diseases, Johannesburg, South Africa,* [2]*Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa,* [3]*KwaZulu-Natal Provincial Malaria Control Programme, Jozini, KwaZulu-Natal, South Africa,* [4]*Mpumalanga Provincial Malaria Elimination Programme, Nelspruit, Mpumalanga, South Africa*

The addition of single low-dose (SLD) primaquine, a gametocytocide, to an artemisinin-based combination treatment of malaria is recommended to accelerate low transmission regions towards malaria elimination. South Africa included SLD primaquine among its suite of elimination strategies during the 2018/2019 malaria season. As primaquine is not currently registered in South Africa, its use required special approval from South African Health Products Regulatory Authority (SAHPRA), and is subject to regulatory requirements being met. Health facilities in Nkomazi District, Mpumalanga Province and uMkhanyakude District, KwaZulu-Natal Province who reported >10 malaria cases during the previous two malaria seasons, together with border malaria surveillance units were prioritised for SLD primaquine rollout. Beforehand, stakeholder meetings were held with all the relevant personnel and feedback received was used to streamline training materials and work aids. Each selected facility received on-site training on dosing with SLD primaquine and collecting SAHPRA-required information. Stock levels were monitored, with facilities replenished as needed. Patient demographic information was obtained from the provincial District Health Information System (DHIS), with response to treatment extracted from malaria case investigation forms. Over the first three months 486 tablets were dispensed, 337 in Mpumalanga and 149 in KwaZulu-Natal. The border surveillance units dispensed most tablets (300/486) during their active malaria case detection activities. The additional information required by SAHPRA was initially poorly documented at clinics and in the DHIS system, but improvements were seen over time after refresher training. Initial challenges identified were corrected with close supervision and regular refresher training session. However, to be sustainable it is imperative that primaquine is registered as soon as possible in South Africa. In addition, a cost-effective supply of primaquine is essential for achieving the high coverage rates required to impact on malaria transmission.

1037

FACTORS ASSOCIATED WITH ADHERENCE TO PRIMAQUINE 8 WEEK REGIMEN AMONG *PLASMDIUM VIVAX* CASES IN KAYIN STATE, MYANMAR

Myint Oo

ARC, Yangon, Myanmar

Treatment adherence to primaquine is crucial for radical cure and interruption of *Plasmodium vivax* transmission while progressing toward the goal of malaria elimination in Myanmar by 2030. *P. vivax* has become the dominant species in Kayin State, where 9,816 (65%) of the total 15,080 malaria cases reported in 2017 were *P.vivax* infections. A cross-sectional descriptive study was carried out in 4 Townships of Kayin State to assess the adherence to primaquine 8-week regimen (0.75mg/kg once weekly for 8 weeks), which, together with a 3-day course of chloroquine, is the regimen recommended by the national malaria treatment guidelines for uncomplicated *P.vivax* cases when treated by community-based village malaria workers (VMWs). Out of the total 342 *P.vivax* cases detected and reported in the 4 Townships from October 2017 to September 2018 by the 71 VMWs supported by the Defeat Malaria project, 140 could be interviewed by the study team with a semi-structured questionnaire in November-December 2018. The results of the interviews showed that 100 (71%) of them were male, 100 (71%) migrant, 89 (64%) forest workers, and 110 (79%) of Kayin ethnicity. Of the 140 cases who received primaquine treatment from VMWs, 84 (60%) were considered as probably adherent (full course of 8-week completed at the right dose and right interval) based on their self-reported behavior. Among the 56 cases (40%) considered as non-adherents, the non-adherence to primaquine treatment was found to be associated with being migrant vs non-migrant OR 3.84 (95% CI, 1.61-9.15) and being forest worker vs non-forest worker OR 2.14 (95% CI, 1.03-4.46), whereas no significant association was found with male vs female OR 0.52 (95% CI, 0.23-1.14) and Kayin vs non-Kayin ethnicity OR 1.76 (95% CI, 0.77-4.00). The main reasons reported for non-adherence were forgetfulness (29%), travelling (11%), busy work (13%), long treatment course (2%), and dizziness (1%). Migrant and forest-related work were the main factors associated with non-adherence to the primaquine 8-week regimen.

1038

USE OF STRATEGIC INFORMATION TO DRIVE IMPACT: OPERATIONALIZING THE 'HIGH BURDEN TO HIGH IMPACT' APPROACH IN UGANDA

Daniel J. Kyabayinze[1], Damian Rutaazana[1], Paul Mbaka[2], Catherine Maiteki-Sebuguzi[1], Peter Mbabazi[1], Julius Ssempiira[3], Jimmy Opigo[1], Charles Katureebe[2], Bayo Fatunmbi[2], Abdisalan Noor[4], Maru W. Aregawi[4]

[1]*National Malaria Control Programme, Kampala, Uganda,* [2]*World health Organisation, Kampala, Uganda,* [3]*Makerere University, School of Public Health, Kampala, Uganda,* [4]*Global Malaria Program, World Health Organisation, Geneva, Switzerland*

The 'High Burden High Impact' initiative was launched in Mozambique in 2018 coupled with AFRO operationalization meeting January 2019 at Mauritania as response to limited performance, slow progress of high burden countries that contribute over 70% of global burden. It is a call for action by Member States through WHO and RBM Partnership. HBHI aims are 1) to galvanize political attention to reduce malaria deaths, 2) to drive impact through the strategic use of information to identify the locally appropriate mix of interventions and best means of delivery, 3) to establish guidance, policies and strategies, 4) to coordinate response, demonstrating success in accelerating burden reduction. Uganda is the first country to launch and operationalize the High burden to high impact" a country-led response - catalysed by WHO and the RBM Partnership to reignite the pace of progress in the global malaria fight. WHO, US PMI, UK DFID, Action Against Malaria Foundation, TASO, UNICEF, ALMA, The Global Fund, Malaria Consortium, CHAI and others from international, national and sub-national levels supported the Malaria Programme in

designing and are supporting the operationalization of the approach. Current actions to support strategic use of information have so far included 1) strong commitment to enhance data sharing and analysis and use for action by partners and stakeholders, allowing real time decision making and action. These data will feed into national planning processes and allow the country to target resources to those most in need. 2) Setting up an analytical team of all partners led by NMCP and WHO. The team has 1) developed incidence maps by district and by sup-county, 2) developed a National Malaria Data Repository with work schedules assigned, 3) Identifying the locally appropriate mix of Interventions through mapping vectors, insecticide resistance and intervention, 4) engaging with Districts and Sub-counties for coordinated response 5) sharing information and 6) plans to conduct a malaria program review in 2019 that will result in subsequent updating of the National strategic plan, and eventually leading to 5-year implementation of a new strategy.

1039

SENEGAL CHARTERING THE PATH TO MALARIA ELIMINATION: A PROCESS DESCRIPTION

Moustapha Cisse

NMCP Senegal, Dakar, Senegal

Senegal's progress against malaria is a public health success story, and a source of potential lessons for other countries on the path to elimination. Malaria has historically been one of Senegal's major health challenges. Less than 20 years ago, it accounted for 1/3 of outpatient visits nationwide. However, strong political leadership, community engagement, sustained support from technical and financial partners, and a proactive approach to policy adoption and intervention scale-up have had a major impact on malaria disease burden. There are now several northern districts where elimination appears to be an achievable short-term goal. With a goal of nationwide elimination by 2030, numerous milestones have been set: - Strong advocacy and sensitization towards authorities and the population - SOP development for malaria case investigations and response SOP development for accelerated transmission reduction strategies in borderline areas - Realization of the joint Elimination Scenario Planning (ESP) in Senegal and Gambia - Launch of a nationwide weekly malaria reporting system - Introduction of single-low dose primaquine in pre-elimination areas - Updating of the national guidelines for malaria surveillance to address the needs for a pre/elimination context - Assessing the performance of national malaria SME Systems in the context of malaria elimination - Decentralization and updating of the national malariology and malaria SME training courses - Development of a national manual for malaria elimination - Introduction of genomics for parasite surveillance - Development of a guide for insecticide resistance management - Development and implementation of a joint action plan for cross-border management of malaria with The Gambia - Development of a roadmap for decentralizing malaria activities With the organizational reforms recommended in the national elimination manual, the continued support of current and new partners and a strong advocacy for the engagement of national authorities in mobilizing the necessary financial resources, Senegal can be at the rendezvous for malaria elimination by 2030

1040

COMBINED SEMI-FIELD STUDIES AND A VILLAGE-BASED TRIAL TO ASSESS THE IMPACT ON *ANOPHELINE* POPULATIONS OF ZOOPROPHYLAXIS-AIDED IVERMECTIN-BASED VECTOR ELIMINATION (ZAIVE) USING PERI-DOMESTIC CATTLE TREATMENT IN THE HIGHLANDS OF VIETNAM

Andrew Lover[1], Ian Mendenhall[2], Jeffrey Hertz[3], Nguyen X. Quang[4], Huỳnh H. Quang[4]

[1]*University of Massachusetts Amherst, Amherst, MA, United States,* [2]*Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore,* [3]*U.S. Naval Medical Research Unit Two, Singapore, Singapore,* [4]*Institute of Malariology, Parasitology, and Entomology (IMPE), Ministry of Health, Quy Nhon, Vietnam*

In line with regional goals in the Greater Mekong Subregion (GMS), Vietnam is committed to national malaria elimination by 2030. However, interventions targeting vulnerable populations in forested areas are lacking. Ivermectin, an endectocidal drug, has recently emerged as a potential new tool towards malaria elimination, as humans and animals retain blood concentrations that impact feeding anophelines (decreased fecundity, shortened lifespan, and lethality). Zooprophylaxis is a strategy that uses herd animals as bait to attract vectors; these alternative feedings provide passive protection to human populations. High cattle ownership in many rural communities suggests combining these strategies in an 'attract-and-kill' approach. This study utilizes semi-field testing and a community-scale randomized experimental design to assess if sufficient densities of ivermectin-treated cattle can drastically decrease vector populations and alter mosquito composition in village-based settings. In Quy Nhon, Vietnam, colonies of *Anopheles dirus* ss and *An. epiroticus* will be allowed to feed on ivermectin-treated and control cattle in a semi-field study to ensure adequate drug dosing, and to quantify impacts of ivermectin in local vectors. Thereafter, six villages in Krong Pa district (Gia Lai) are to be randomized as either control or intervention areas. In intervention villages, full herds of all consenting households will be treated with a standard veterinary dosing of 1% ivermectin by injection. At all village study sites (intervention and control), CDC light traps, cattle-baited trapping, and human-landing catches will be used to survey anopheline populations before and after the ivermectin dosing of cattle. The primary endpoint will be reduction in captures from cattle-baited traps; secondary endpoints will be changes in vector population composition. Data collection was initiated in April 2019 and will be completed in mid-2019. Trial results will be presented that quantify impacts of ivermectin-treated cattle on vector populations for development of a novel strategy targeting residual malaria transmission in the GMS.

1041

INITIATING CASE NOTIFICATION AND CASE INVESTIGATION FOR *PLASMODIUM FALCIPARUM* CASES IN CAMBODIA

Dr. Siv Sovannaroth[1], Peng By Ngor[1], Abishek Thiagaraj[2], Bunmeng Chhun[2], Pedro Pagalday-Olivares[2]

[1]*National Center for Parasitology, Entomology and Malaria Control in Cambodia, Phnom Penh, Cambodia,* [2]*Clinton Health Access Initiative, Phnom Penh, Cambodia*

In order to achieve the elimination of *Plasmodium falciparum* (Pf) in 21 endemic provinces in Cambodia, a strong malaria information system is needed to identify local cases/*foci*, improve timeliness of response, and determine origins of transmission. The Cambodia National Center for Parasitology, Entomology and Malaria Control (CNM) developed a phased approach to introduce surveillance for elimination activities. CNM designed a pilot project in two HCs and 21 VMWs in Kampong Chhnang province to introduce case notification and case investigation and observe operational feasibility. CNM, the Clinton Health Access Initiative (CHAI) and the University of Oslo (UiO), developed a customized, DHIS2-based Android Capture App to facilitate real-time reporting with tablets located at every Health Center (HC) and with smartphones at every Village Malaria Worker (VMW). Once the initial version of a DHIS2-based malaria information system (MIS) module was released, 3 phases of testing were conducted from May-November 2018. A web platform with dashboards was also developed that could be tailored to end users. With 360 cases reported from the 2 HCs and their 21 VMWs during the pilot period, CNM demonstrated operational feasibility. High rates of completeness (86%), timeliness (95%), and case investigation (100%) were achieved. Case investigation was also conducted for all reported Pf and mixed infection cases (n=34), with 100% of them being classified as local. Operational Districts (ODs) and CNM analysed key indicators through the visual dashboard each month to enable timely response. For the first time, case classification and investigation activities were conducted independently

by the public health facilities without NGO support. Results indicated that with targeted subnational support, it is feasible to operationalize surveillance for elimination in Cambodia. These activities were transitioned from DHIS2 to the CNM MIS in December 2018, and CNM continues to improve case investigation completeness and timeliness of data in the MIS. These activities will be expanded to 9 additional provinces in 2019.

1042

THE IMPACT OF MORBIDITY AND MORTALITY RATE REDUCTION CONTRIBUTED BY VILLAGE MALARIA WORKERS AND MOBILE MALARIA WORKERS IN CAMBODIA 2004 - 2018

Po Ly[1], Siv Sovannaroth[1], Huy Rekol[1], Khengthavrin Bou[1], Kimhong Gove[2], Josh Christenson[2]

[1]*National Center for Parasitology, Entomology and Malaria Control in Cambodia, Phnom Penh, Cambodia,* [2]*Clinton Health Access Initiative, Phnom Penh, Cambodia*

The goal in the Cambodia National Strategic Plan for Malaria Elimination is to eliminate *Plasmodium falciparum* and reach and sustain zero malaria deaths by 2020. In order to reach these targets the Cambodia National Center for Parasitology, Entomology and Malaria Control (CNM) invested in a Village Malaria Workers (VMWs) Program. The VMWs scheme was established in 2004 with 235 VMWs villages and has grown to 3,025 VMWs villages (6,050 VMWs) including Mobile Malaria Workers (MMWs) in 2019, providing both preventive and curative interventions to approximately 2 million at-risk populations in hard to reach communities. CNM conducted a retrospective analysis of malaria cases and deaths reported through the health information system (HIS) between 2004 and 2018 to assess the impact of VMWs on annual morbidity and mortality trends. VMWs' performance included an examination of core activities of case management, vector control, surveillance, and community education. Between 2004 and 2018, deaths declined from 396 to 0, and cases declined from 11,398 to 5,041. VMWs consistently reported an average of 50% of total malaria cases found in the public health sector. Of the top seven highest burden provinces, tests done by VMWs were on average triple the tests done in health facilities. Health facilities organizing regular monthly meetings (with 95% of VMWs in attendance), paid monthly VMW incentives in a timely manner through electronic payments, completed training on treatment guideline updates to 3025 VMWs villages, and equipped VMWs with sufficient RDTs and ACTs. Malaria case management services provided by VMWs/MMWs to high risk populations in Cambodia has been associated with a significant reduction in the mortality rate through a chain of linkages of increasing early diagnosis and prompt treatment, care quality, targeted behavior change communication and health outcomes. CNM is exploring the additional roles to existing VMWs/MMWs such as conducting surveillance for elimination activities (investigation, classification and reactive case detection) and outreach activities to provide testing and treatment services in the forested areas.

1043

ADHERENCE TO RADICAL CURE FOR *PLASMODIUM VIVAX* MALARIA IN PAPUA, INDONESIA

Annisa Rahmalia[1], Jeanne R. Poespoprodjo[1], Chandra U. Landuwulang[1], Maya Ronse[2], Enny Kenangalem[1], Faustina H. Burdam[1], Benedikt Ley[3], Ric N. Price[3], Kamala Thriemer[3], Koen Peeters Grietens[2], Charlotte Gryseels[2]

[1]*Papuan Health and Community Development Foundation, Timika, Indonesia,* [2]*Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium,* [3]*Global and Tropical Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Australia*

Adherence to radical cure for *Plasmodium vivax* is critical for achieving the timely elimination of malaria in endemic areas. The only widely available drug for killing the dormant liver stages of *P. vivax* is primaquine (PQ) which is usually administered as a 14-day regimen. The prolonged treatment course results in poor adherence and effectiveness. To develop suitable strategies to improve patient adherence, a mixed methods study was undertaken to explore people's perceptions and understanding of malaria and malaria treatment in southern Papua, Indonesia. For the qualitative strand, qualitative interviews (n=46) and observations (n=12) were done with purposively selected informants. The quantitative component consisted of a structured questionnaire with all trial participants (n=619). Results showed that the socially accepted and commonly known malaria medication dihydroartemisinin-piperaquine - "the blue pills" - evoked confidence in the relation between the type of malaria it targeted, its perceived side effects and efficacy, and optimal adherence to the 3-day regimen. The different colour and size of the PQ tablets - "the brown pills" - and the long treatment duration, however, prompted a re-interpretation of the perceived side effects and treatment effectiveness which aligned more to perceptions of vitamin supplements. Although 84% of trial participants reported that they had finished "all drugs", only 60% reported to have taken both "the blue" and "the brown" pills needed for radical cure. In conclusion, patients continually evaluate the characteristics of their medicines, in relation to the illness and the perceived benefits and risks of the treatment. These (re-)interpretations underpin patients' decisions to adhere to or to abandon PQ treatment. Even with partial supervision patients' adherence to a 14-day PQ regimen is suboptimal.

1044

MALARIA SURVEILLANCE IN ZANZIBAR PATTERNS OF CASE NOTIFICATION AND INVESTIGATION IN LINE WITH WHO'S 1-3-7 DAYS APPROACH

Shabbir Lalji[1], Humphrey Mkali[1], Abdul-wahid Al-mafazy[1], Ssanyu Nyinondi[1], Joseph Joseph[1], Mike McKay[2], Abdallah Ali[3], Wahida Hassan[3], Mohamed Kitwasi[3], Chonge Kitojo[4], Naomi Kaspar[4], George Greer[4], Eric Reaves[5], Richard Reithinger[2], Jeremiah Ngondi[2]

[1]*RTI International, Dar es Salaam, United Republic of Tanzania,* [2]*RTI International, Washington, DC, United States,* [3]*Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania,* [4]*US President's Malaria Initiative, United States Agency for International Development, Dar es Salaam, United Republic of Tanzania,* [5]*US President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania*

The World Health Organization (WHO) recommends a "1-3-7 days" approach for case notification, investigation, and focus investigation in areas of malaria elimination. The Zanzibar Malaria Elimination Programme's (ZAMEP) guidelines recommend case notification within one day of diagnosis, case investigation within two days, and focus investigation within 7 days. ZAMEP's Malaria Case Notification (MCN) system notifies district malaria surveillance officers (DMSOs) of confirmed cases by health workers from all the 243 health facilities in Zanzibar. We examined timeliness of case notification and investigation by all 20 DMSOs from 2013 to 2018. Overall, 21,386 (97%) confirmed cases were notified by all health facilities. Of these, 10,100 (47%) were notified in line with WHO's recommendation, i.e. within one day (24 hours) of diagnosis. During this period, 16,342 (76%) of the notified index cases were investigated further by DMSOs. Of these, 7,924 (37%) were investigated within two days following ZAMEP's current guidelines, 4,006 (19%) within 7 days, and 3,487 (44%) after 7 days. In Unguja, where the number of malaria cases was four times higher than in Pemba, only 35% of notified cases were investigated within two days by DMSOs, compared to 48% in Pemba. Overall, 42% of case investigations were done within the WHO-recommended 3 days (Unguja: 40%; Pemba: 53%). While overall case investigation rates were high, DMSOs were unable to reach all index cases within ZAMEP's or WHO's recommended timeframe of two and three days, respectively. This might reflect limitations in the number of DMSOs, especially during high transmission season. It will be important to support improvements in timeliness of both notification and case investigation if Zanzibar is to progress further on the path of malaria elimination.

1045

DO MALARIA INFECTIONS CLUSTER AT THE HOUSEHOLD LEVEL? A REVIEW OF THE EVIDENCE TO INFORM ACTIVE INFECTION DETECTION STRATEGIES FOR MALARIA CONTROL PROGRAMS

Gillian H. Stresman[1], Charlie Whittaker[2], Teun Bousema[3], Hannah Slater[4], Jackie Cook[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Imperial College London, London, United Kingdom,* [3]*Radboud University Medical Centre, Nijmegen, Netherlands,* [4]*PATH, Seattle, WA, United States*

As transmission reduces to pre-elimination levels, many malaria programmes engage in active detection to target asymptomatic infections. Three approaches have been considered: 1) re-active case detection (RACD), where the household (and in some settings, neighbouring households) of each passively detected case are tested and/or treated, 2) mass screening and treatment (MSAT) where symptomatic individuals in the community are tested and 3) mass testing and treatment (MTAT) where all individuals are tested regardless of symptoms, with treatment in the latter two approaches either targeted at individuals testing positive or all those residing in the same household. RACD is by far the most widely adopted by malaria control programs. However, formal evidence of the effectiveness of this intervention to target the parasite reservoir is lacking. Here we collate evidence from published studies on the degree of clustering of asymptomatic infections to estimate the odds ratio of infections detected by molecular methods in households with an infection detected following the RACD, MSAT, or MTAT models compared to households with no programmatically detectable infection. Results were categorized according to the three approaches. A pooled OR was calculated and a linear trend between magnitude of effect and malaria prevalence was tested. We obtained 36 data points from 30 of 44 eligible studies. The pooled odds of infections clustering in index households from RACD, MSAT and MTAT models was 4.08 (95% CI: 2.55-6.52), 3.22 (95% CI: 2.04-5.08) and 6.68 (95% CI: 4.04-11.04), respectively. There was a significant linear trend whereby the magnitude of clustering increased with decreasing transmission. A similar trend was observed when analysing data from demographic health surveys. Preliminary analysis suggests that infections cluster around programmatically detectable infections, and this clustering is greatest in low transmission settings. These results provide the first evidence of the effectiveness of active detection strategies to identify asymptomatic infections in a population.

1046

IMPLEMENTING 24-HOUR MALARIA CASE NOTIFICATION SYSTEMS FOR THE PRIVATE SECTOR IN ELIMINATION SETTINGS: LESSONS LEARNED FROM MYANMAR AND VIETNAM

Rebecca Potter[1], Ngo Thi Thuy Nga[2], Nguyen N. Loan[2], Pham Van Chau[2], Khaing Wai Wai Phyo[3], Phone Si Hein[3]

[1]*Population Services International, Washington, DC, United States,* [2]*PSI Vietnam, Hanoi, Vietnam,* [3]*PSI Myanmar, Yangon, Myanmar*

NMCPs in Myanmar and Vietnam are rolling out elimination surveillance guidelines with a focus on the public sector, with 24-hour case notification in targeted geographies designed to trigger timely case investigations. In Myanmar and Vietnam in 2018, private sector providers diagnosed and reported 14% and 13% of the national reported case load respectively. PSI's Greater Mekong Sub-region Elimination of Malaria through Surveillance (GEMS) program is partnering with the Myanmar and Vietnam NMCPs to ensure that private sector malaria cases are notified according to standard elimination surveillance protocols. Routine surveillance data were analyzed post-intervention and supported by key informant insights. In Myanmar, 614 private general practitioners were trained to notify confirmed cases via SMS to PSI and NMCP focal points beginning in June 2018. Between July 2018 and Jan. 2019, 73% (n=275) of confirmed cases were notified to focal points by SMS within 24 hours. In Vietnam, 175 clinics in four provinces were trained to complete a shorter version of the national case notification form and submit via e-mail or Zalo (a popular social media app) to the district focal point within 24 hours. Between Sept. 2018 and Feb. 2019, 82% (n=373) of positive cases detected by private providers were reported on time to the district. Most national malaria surveillance systems do not systematically capture data from the private sector. These results demonstrate that elimination surveillance systems can and should extend to the private sector, where increasingly focal high-risk populations in the GMS continue to seek fever care. In Vietnam, modifying the lengthy case notification forms to capture the most critical data points for private sector case notification was helpful to ensuring compliance. Both settings show that mobile devices and social media apps can be used as an effective tool for submitting malaria case notification data within 24 hours. These successes may be partly attributed to providers' engagement in an existing malaria program, where PSI supports private providers on case management, reporting, and stocking commodities.

1047

ASSESSING IMPACTS OF GOVERNMENTAL CONTROLS OF ILLEGAL LOGGING ON MALARIA TRANSMISSION IN SOUTHERN LAO PDR

Emily Dantzer[1], Andrew A. Lover[2], Bouasy Hongvanthong[3], Francois Rerolle[1], Sophia Hocini[4], Rattanaxay Phetsouvanh[5], Adam Bennett[1]

[1]*University of California San Francisco, Malaria Elimination Initiative, San Francisco, CA, United States,* [2]*Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA, United States,* [3]*Lao PDR Centre for Malariology, Parasitology, and Entomology (CMPE), Vientiane, Lao People's Democratic Republic,* [4]*University of California Los Angeles, Los Angeles, CA, United States,* [5]*Department of Communicable Disease Control (DCDC), Ministry of Health, Lao PDR, Vientiane, Lao People's Democratic Republic*

Lao PDR has made significant progress in the fight against malaria in recent years with reported cases falling from 23,047 in 2010 to 8,907 in 2018. Malaria transmission is highly heterogenous with 98% of reported cases occurring in the five southernmost provinces, which are characterized by hilly, forested terrain and a workforce engaged primarily in forest-based and agricultural activities. Aligned with the regional targets of the Greater Mekong Subregion, Lao PDR aims to eliminate *Plasmodium falciparum* nationally by 2025 and all human malaria species including *P. vivax* by 2030. In countries approaching malaria elimination including Lao PDR, populations at higher risk for infection generally share similar occupational, behavioral, and social characteristics that increase their exposure to outdoor-biting and/or forest-dwelling mosquitoes. In May 2016, the Lao government enacted a decree implementing stringent regulations on the sale and export of timber to curtail illegal logging operations and consequent deforestation. To document and assess any potential changes in forest-based activities stemming from this legislation, a series of formative studies and population-based surveys were conducted between mid-2015 and November 2018. After triangulating data, these studies found that both local residents and key malaria stakeholders attributed the recent decline in cases to the logging ban. Other findings suggest that enforcement of the decree has nearly eliminated large-scale logging efforts by both foreign workers and Lao nationals, resulting in the closure of local sawmills and other businesses catering to mobile and migrant clientele. This case study highlights the potential implications of national and international policy-level interventions on malaria transmission, and underscores the potential synergies arising from strong and coordinated intersectoral government initiatives.

1048

A TWO DOSE SPOROZOITE CHALLENGE MODEL IN MICE HIGHLIGHTS LIVER STAGE DURATION AS ONE OF THE MOST IMPORTANT DIFFERENCES BETWEEN MURINE AND HUMAN *PLASMODIUM* FOR VACCINE DEVELOPMENT

Chaitra Parthiban, Zachary P. Billman, Tayla M. Olsen, Brad C. Stone, Melanie J. Shears, Sean C. Murphy

University of Washington, Center for Emerging and Re-Emerging Infectious Diseases, Seattle, WA, United States

Pre-clinical malaria vaccine studies to evaluate protective antigens rely on *Plasmodium yoelii* (*Py*) and *P. berghei* (*Pb*) rodent models. However, *Py* and *Pb* complete their liver stage in two days compared to six days for the *P. falciparum* liver stage in humans. We hypothesized that the difference in duration of the liver stage has critical but overlooked implications for how protective outcomes of $CD8^+$ T cell-based vaccine research are considered. When a vaccinated mouse is challenged, cytotoxic T lymphocyte (CTL) activation occurs within 48 hours, but T cell clonal expansion does not occur until later. Sterile protection in rodent models therefore relies on antibodies and CTLs but derives no benefit from expanding T cells. This may lead us to dismiss protective antigens as non-protective. To evaluate this possibility, we developed a novel two-dose challenge model wherein vaccinated or naïve mice are challenged first with a low dose of genetically-attenuated sporozoites to initiate T cell activation (Dose 1). This is followed by a wild-type sporozoite challenge after 48 hours (Dose 2), just as activated T cells begin to rapidly expand. This extends the window of mouse liver stage challenge to four days, closer to that in humans from an immunological perspective. By *in vivo* imaging, we saw no difference in wild-type liver burden between one- and two-dose challenge in naïve mice, indicating that Dose 1 does not reduce uptake of Dose 2. Dose 1 does not induce a significant type I interferon response at 48 hours. However, the role of expanding T cells was apparent since 80-100% of BALB/cJ mice immunized with *Py* radiation-attenuated sporozoites were protected against a two-dose challenge whereas one-dose challenge protection was 0-40%. C57BL/6 mice have been reported to be difficult to protect against *Py* challenge, but our data showed that 1-2 doses of irradiated *Py* sporozoites protect such mice against a two-dose but not one-dose challenge. Thus, by allowing both activated and expanding CTLs to contribute, the two-dose challenge model is more sensitive for determining the maximum degree of protection that can be derived from pre-clinical vaccine candidates.

1049

SAFETY AND PROTECTIVE EFFICACY AGAINST CONTROLLED HUMAN MALARIA INFECTION OF MULTI-DOSE PRIMING REGIMENS OF PFSPZ VACCINE WITH AND WITHOUT BOOST IN EQUATORIAL GUINEAN ADULTS

Said A. Jongo[1], Thomas Richie[2], Kamaka Kassimu[1], Raul Chuquiyauri[3], Peter F. Billingsley[2], Elizabeth Nyakarungu[1], Maxmillian Mpina[4], Ali Mtoro[1], Ali Hamad[1], Jose Raso[3], Anna Deal[4], Tobias Schindler[4], Vicente Urbano[3], Maria Silvia A. Lopez[3], Beltran Pasialo[5], Marta Alene Owono Eyang[3], Escolastica Raquel Mansogo Maye[3], Gertrudis Owono Bidjimi[3], Martin Eka Ondo[3], Matilde Riloha Rivas[6], Gabriel Mba Abegue[3], Yolanda Rimoy Veri[3], Carlos Cortes Falla[3], Federico Comsil Chochi[3], Dolores Mbang Ondo Mandumbi[3], Guillermo Garcia[3], Manuel Francisco Nfumi Machimbo[3], Ines Toichoa Bela[3], Juan Carlos Momo[3], Carl Maas[7], B. Kim Lee Sim[2], Bonifacio Manguire[7], Preston Church[2], Marcel Tanner[4], Claudia A. Daubenberger[4], Salim Abdula[1], Stephen L. Hoffman[2]

[1]*Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania,* [2]*Sanaria Inc., Rockville, MD, United States,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [5]*Swiss Tropical and Public Health Institute, Malabo, Equatorial Guinea,* [6]*Ministry of Health and Social Welfare of Equatorial Guinea, Malabo, Equatorial Guinea,* [7]*Marathon Oil Corporation, Malabo, Equatorial Guinea*

PfSPZ Vaccine, composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ), has been well tolerated and safe when administered as single and multi-dose priming regimens in 5 month to 65 year olds in the US, Europe, and 6 African countries, including in HIV+ adults (NCT03420053). In a previous trial we reported that increasing the dose of PfSPZ in PfSPZ Vaccine from $9.0x10^5$ to $1.8x10^6$ (3 doses at 8 week intervals) in Tanzanian adults significantly reduced vaccine efficacy (VE) against controlled human malaria infection (CHMI) from 100% to 33% (NCT02613520). When $9.0x10^5$ PfSPZ were administered on days 1, 3, 5, 7 (priming) with a 16-week boost, VE against heterologous CHMI in U.S. adults at 12 weeks was higher than with any previous regimens. We administered the same priming regimen (1,3,5,7) following by a 4-week boost to healthy Tanzanian adults and showed 80% VE against CHMI (NCT03420053). A condensed administration schedule of PfSPZ Vaccine with high VE would facilitate its deployment. To identify a regimen for a Phase 3 clinical trial, we conducted a double-blind, placebo-controlled trial in 104 Equato guinean adults that assessed the safety, immunogenicity, and VE against CHMI at ~6 weeks after last dose of one 16-week and three condensed (<28 days) vaccination regimens. In three regimens, $9.0x10^5$ PfSPZ were administered four times on days 1, 3, 5 and 7 followed by a boost on day 113 (regimen 1), a boost on day 29 (regimen 2) or no boost (regimen 3). In regimen 4, $9.0x10^5$ PfSPZ were administered on days 1 and 8 with a boost on day 29, to see if a 2-dose multi-dose prime was as effective as a 4-dose multi-dose prime. The complete unblinded results of tolerability, safety, immunogenicity and VE of this spectrum of vaccination regimens will be presented.

1050

IMMUNE ACTIVATION AND MAGNITUDE AND BREADTH OF *PLASMODIUM FALCIPARUM* HUMORAL IMMUNITY IN MALARIA PRE-EXPOSED VOLUNTEERS WITH OR WITHOUT HIV INFECTION DURING PFSPZ VACCINATION AND CONTROLLED HUMAN MALARIA INFECTION

Anneth-Mwasi N. Tumbo[1], Freia-Raphaella Lorenz[2], Jean-Pierre Dangy[3], Maximillian Mpina[1], Tobias Schindler[3], Florence A. Milando[1], Gloria Nyaulingo[1], Matthieu Perreau[4], Kamaka Ramadhani[1], Said Jongo[1], Philip L. Felgner[5], Benjamin Mordmueller[2], Thomas Richie[6], B.Kim Lee Sim[6], Marcel Tanner[3], Salim Abdulla[1], Stephen L. Hoffman[6], Rolf Fendel[2], Claudia Daubenberger[3]

[1]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [2]*Institute of Tropical Medicine, University of Tubiengen, Tubiengen, Germany,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [4]*Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland,* [5]*University of California, Irvine, CA, United States,* [6]*Sanaria Inc., Rockville, MD, United States*

In 2018, we evaluated the safety, immunogenicity and efficacy of metabolically active, radiation attenuated, purified *Plasmodium falciparum* sporozoites in HIV negative and HIV positive Tanzanian adults (ClinicalTrials.gov Identifier: NCT03420053). Marked differences in protection against homologous controlled human malaria infection (CHMI) were observed between HIV positive and HIV negative volunteers that were under anti-retroviral treatment during the study. For licensed vaccines, it has been described that systemic inflammation and ongoing co-infections at time of vaccination can potentially contribute to reduced magnitude and duration of vaccine-induced immune responses. We investigated the impact of HIV infection status on systemic immune activation before PfSPZ Vaccination and after CHMI. Serum cytokine and chemokine concentrations before and during vaccination were quantified *ex vivo* using a validated 45 plex human bead array platform . HIV positive volunteers had significantly higher concentrations of Eotaxin, while in HIV negatives Tumor necrosis factor alpha (TNF-α), Growth regulated oncogene-alpha (GRO-alpha), Interleukin-8 (IL-8), Macrophage inflammatory protein-1beta (MIP-1beta), Brain derived neutrophic factor (BDNF) and Vascular endothelial growth

factor-alpha (VEGF-alpha) were elevated when compared to HIV positives at baseline. During the vaccination, most of these cytokines/chemokines remained elevated in the HIV negative cohort. In the HIV positive volunteers, Interleukin 12, p70 (IL-12p70) , Interleukin-2 (IL-2) and Eotaxin were increased 28 days post CHMI while the HIV negatives showed higher levels of BDNF and MIP1-beta only. IgG and IgM antibody profiles in both cohorts before and after vaccination, and after CHMI were investigated using a novel proteome microarray encompassing 262 *P. falciparum* (3D7) antigen data points. Collectively, we will present for the first time data on immune activation and antibody profiles in malaria pre-exposed HIV positive volunteers undergoing PfSPZ Vaccination and CHMI and correlate these to vaccine induced protection.

1051

CHEMOPROPHYLAXIS VACCINATION (PFSPZ-CVAC) WITH HIGH DOSE OF SANARIA® PFSPZ CHALLENGE NF54 UNDER PYRIMETHAMINE OR CHLOROQUINE LEADS TO PROTECTIVE EFFICACY AGAINST HETEROLOGOUS CONTROLLED HUMAN MALARIA INFECTION IN MALARIA NAÏVE ADULTS

Agnes Mwakingwe-Omari[1], Jacquelyn Lane[1], David M. Cook[1], Sara A. Healy[1], Susan Pfeiffer[1], Sahand Kalhori[1], Charles Wyatt[1], Omely Marte-Salcedo[1], Alemush Imeru[1], Martha Nason[2], Irfan Zaidi[1], Junhui Duan[1], Jillian Neal[1], Jake Raiten[1], Jen C.C. Hume[1], Esther J. Jeon[3], Gary Fahle[4], Tooba Murshedkar[5], Adam J. Ruben[5], Sumana Chakravarty[5], Anita Manoj[5], Anusha Gunasekera[5], B. Kim Lee Sim[5], Peter F. Billingsley[5], Eric R. James[5], Thomas L. Richie[5], Stephen L. Hoffman[5], Patrick E. Duffy[1]

[1]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [2]*Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Clinical Center Pharmacy Department, National Institutes of Health, Bethesda, MD, United States,* [4]*Department of Laboratory Medicine, Molecular Microbiology, National Institutes of Health, Bethesda, MD, United States,* [5]*Sanaria, Inc., Rockville, MD, United States*

Administration of 3 doses of $5.12x10^4$ *Plasmodium falciparum* (Pf) sporozoites (SPZ) (Sanaria® PfSPZ Challenge NF54) by direct venous inoculation (DVI) to subjects taking chemoprophylaxis (Sanaria® PfSPZ-CVac) with pyrimethamine (PYR) did not lead to significant vaccine efficacy (VE) against homologous (NF54) controlled human malaria infection (CHMI) 12 weeks post 3rd vaccination (1/8 protected). Therefore, we assessed a 4-fold higher dose of PfSPZ Challenge NF54 with either PYR or chloroquine (CQ) against homologous (NF54) and heterologous (7G8) CHMI. All participants received 3 doses of $2x10^5$ PfSPZ by DVI at 4 week intervals with either PYR (PfSPZ-CVac-PYR [n=19]) or CQ (PfSPZ-CVac-CQ [n=10]). Vaccinations were safe and well tolerated: 2 serious adverse events occurred, including one possibly related and one unrelated to study procedures. By qPCR assays, we confirmed that 50 mg of PYR given 2 and 3 days post injection with $2x10^5$ PfSPZ prevented development of asexual Pf parasitemia. CHMI with $3.2 x10^3$ PfSPZ Challenge (NF54 or 7G8) was performed in unvaccinated controls (NF54 controls; n=4 and 7G8 controls; n=8) and in vaccinated groups approximately 12 weeks post 3rd vaccination. All 12 unvaccinated controls were infected. In PfSPZ-CVac-PYR groups, 7/8 (87.5%) participants were protected from homologous and 7/9 (78%) from heterologous CHMI. In the PfSPZ-CVac-CQ group, 6/6 (100%) were protected from heterologous CHMI. Immunological assays to determine immune factors and parasite antigens contributing to this remarkable response are ongoing. With these unprecedented results we are initiating a field study to explore the effectiveness of these regimens in malaria-experienced populations.

1052

COMPLETE *PLASMODIUM FALCIPARUM* LIFE CYCLE USING FRG HUHEP MICE AS A MODEL FOR SPOROZOITE INFECTIVITY

Tao Li[1], Christiane Urgena[1], Sumana Chakravarty[1], Abraham G. Eappen[1], Asha Patil[1], Yonas Abebe[1], Adam Frock[1], Minglin Li[2], BKL Sim[2], Stephen L. Hoffman[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Protein Potential LLC, Rockville, MD, United States*

The immunocompromised and fumarylacetoacetate hydrolase-deficient mouse (Fah-/-, Rag2-/-, Il2rg-/-, termed the FRG mouse) engrafted with human hepatocytes (FRG huHep) has been shown to be an excellent model for *Plasmodium falciparum* (Pf) liver stage development. FRG huHep mice supports vigorous, quantifiable Pf liver stage development that culminates in complete maturation of liver stage at approximately 7 days after infection, providing a relevant model for liver stage development in humans. We report the complete Pf full life cycle using FRG huHep mice. FRG huHep mice were injected with either fresh PfSPZ or cryopreserved PfSPZ on day 0. Six and 7 days later the FRG mice received two consecutive transfusions of processed packed human erythrocytes, and 6 hours after the last transfusion, blood was drawn from the mice and put into continuous culture for further development of Pf human erythrocytic stage parasites. Full development and maturation of gametocytes were observed. In all experiments, Pf human erythrocytic stage parasites, both asexual and sexual stages, developed in the cultures from the FRG mice blood post either fresh PfSPZ or cryopreserved PfSPZ immunization. The mature Pf sexual stage parasites were then harvested from the cultures and used to prepare artificial infectious blood meals to infect non-aseptic mosquitoes and developed to PfSPZ in mosquitoes. The method is reproducible and can be used as a model for the infectivity of PfSPZ and other potentially novel antimalarial candidates against *Plasmodium falciparum*.

1053

COMPARATIVE REGULATORY PATHWAYS FOR CLINICAL DEVELOPMENT AND COMMERCIALIZATION OF PFSPZ VACCINE IN THE US, EUROPE AND AFRICA

Asqual Getachew, Anusha Gunasekera, Shachi Shah, BKL Sim, Thomas L. Richie, Stephen L. Hoffman, Tooba Murshedkar

Sanaria Inc., Rockville, MD, United States

PfSPZ Vaccine is Sanaria's leading malaria vaccine candidate and is currently in Phase 1 and Phase 2 clinical trials. The goal is to develop and commercialize this highly effective malaria vaccine such that it can be used to immunize entire populations to confer high-level protection against *Plasmodium falciparum* (Pf) (responsible for more than 95% of malaria associated severe illness and death world-wide). PfSPZ Vaccine is a whole sporozoite malaria vaccine that is radiation-attenuated, live, metabolically active, non-replicating, aseptic, purified, and cryopreserved in liquid nitrogen vapor phase (LNVP). With this goal of licensure in mind, a regulatory process was initiated in 2005 that commenced with a pre-IND meeting with the U.S. FDA, followed by a successful IND submission. Sanaria has progressed from conducting pre-clinical, IND-enabling studies to Phase 1 and subsequently Phase 2 clinical trials. We now anticipate initiating end-of-phase 2 discussions with FDA. In collaboration with various partners, Sanaria has conducted 18 clinical trials of PfSPZ Vaccine in the US, Europe and Africa. All Sanaria trials are conducted under U.S. FDA oversight (under an IND), with additional ethical and regulatory oversight from the host European (Germany) and African (Tanzania, Kenya, Mali, Gabon, and Equatorial Guinea) countries. The goal is to initiate a Phase 3 program next year (2020) for PfSPZ Vaccine. The regulatory pathways for clinical trials in the U.S., Europe and Africa will be compared and licensure / marketing approval processes for a malaria vaccine within the US, European and some African markets will be presented.

1054

THE REGULATORY PRODUCT DEVELOPMENT EXPECTATIONS AND REQUIREMENTS FOR INVESTIGATIONAL USE AND COMMERCIALIZATION OF A MALARIA VACCINE IN THE US, EUROPE AND AFRICA

Shachi Shah, Asqual Getachew, Anusha Gunasekera, Anita Manoj, BKL Sim, Stephen L. Hoffman, Tooba Murshedkar

Sanaria Inc., Rockville, MD, United States

Sanaria's most promising malaria vaccine candidate, PfSPZ Vaccine, is currently an investigational product with the eventual goal of licensure through the US FDA, EMA and other international regulatory bodies and subsequent commercialization. The regulatory expectations of the product through its development process (chemistry, manufacture and controls (CMC) information) evolve from the time of conducting the first pre-clinical studies to filing the first Investigational New Drug (IND) application to conducting Phase 1 and 2 studies, to conducting Phase 3 studies and subsequent licensure and commercialization. CGMPs are an integral part of the vaccine product development program at every stage. For Sanaria's investigational vaccine, ensuring its sterility, potency and stability have been key to ensuring product quality. We will review regulatory expectations for the CMC information at various stages of the development process. For example, at the pre-IND stage, safety of an investigational product is the primary concern. CMC information submitted to FDA in either an IND, Master File (MF) or Biologics License Application (BLA) fall under Module 3 (with summaries provided under Module 2) of the International Conference on Harmonisation (ICH) Common Technical Document (CTD) structure. We will concurrently review the submission components and phase-specific regulatory expectations of the product development process for our malaria vaccine within US FDA's, EMA's and certain African nations' regulatory framework.

1055

QUALIFICATION OF THE MICROBIAL GROWTH TEST FOR IN-PROCESS SAMPLES FOR PRODUCTION OF SANARIA® PFSPZ VACCINE

Minglin Li[1], Anita Manoj[1], Bing Jing[1], Lixin Gao[1], Abraham G. Eappen[2], Tao Li[2], BKL Sim[1], Stephen L. Hoffman[2]

[1]*Protein Potential LLC, Rockville, MD, United States,* [2]*Sanaria Inc., Rockville, MD, United States*

Title: Qualification of the microbial growth test for in-process samples for production of Sanaria® PfSPZ Vaccine Sanaria® PfSPZ Vaccine is composed of aseptic, purified, cryopreserved, radiation attenuated *Plasmodium falciparum* (Pf) sporozoites (SPZ). Subsequent to the promising vaccine efficacy results, Phase 3 clinical trials are planned for 2020 to support a Biologics License Application to the FDA in 2021. Other PfSPZ products are PfSPZ Challenge and PfSPZ GA (genetically attenuated) parasites that are also aseptically manufactured. The common challenge faced for all live organism products is the absence of terminal sterilization prior to or post-vialing. As such strict and clear methods have been established to ensure aseptic processing throughout manufacture. The manufacturing process involves the use of aseptic mosquitoes to produce PfSPZ. The entire life cycle of aseptic mosquitoes occurs in aseptically maintained vessels. Each stage is assessed including eggs, pupae, adult mosquitoes and infected blood meals, as well as other in process step materials. We report here the development and qualification of the microbiological test methods used to asses our aseptic processing.

1056

STATUS OF BIOLOGICAL CONDITIONS AND PATHOLOGIES EXCLUDING VOLUNTEERS FROM MALARIA VACCINE CLINICAL TRIALS (PFSPZ) IN 2014 AND 2016 IN DONEGUEBOUGOU, MALI

Amatigue Zeguime[1], M'Bouye Doucoure[1], Sidiki Perou[1], Boucary Ouologuem[1], Souleymane Traore[1], Abdoulaye Katile[1], Allaye Tolo[1], Baba Djiguiba[1], Mahamadou S. Sissoko[1], Boubacar Traore[1], Jordyn Manucci[2], Jen C.C. Hume[3], Patrick E. Duffy[3], Ogobara Doumbo[1]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Division of Intramural Research, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

In general, a clinical trial for the development of a new product requires healthy volunteers who do not suffer from acute and chronic pathology with normal biological parameters. Sufficient recruitment of such volunteers entails many tests (biological and other), and a majority of volunteers are excluded during screening. The MRTC, LMIV/NIAID/NIH, and Sanaria, Inc conducted PfSPZ vaccine clinical trials in 2014 and 2016, recruiting male and female volunteers aged 18 to 50. Eligibility for enrollment was determined by screening tests including EKG, biochemistry, as well as hematological, serological, urine, and pregnancy tests. The eligibility rate was 40% in 2014 and 29% in 2016. EKG abnormalities excluded 21% in 2014 versus 4% in 2016. Biochemistry abnormalities were represented by high serum creatinine levels in 30% of volunteers in 2014 and 32% in 2016, and elevations of ALT in 6% of volunteers in 2014 compared to 4% in 2016. Abnormal low hematological parameters included the following: WBC 3%; PNN 5%; PLT 2%; HGB 8% in 2014 vs. WBC 5%; PNN 9%; PLT 1%; HGB 13% in 2016. Serological tests were positive for hepatitis B (23% vs. 16%), hepatitis C (2% vs. 12%), HIV (3% vs. 1%) in 2014 and 2016, respectively. Urine tests such as proteinuria and hematuria were abnormal in 2% and 4% of volunteers in 2014 and 7% vs. 18% in 2016, respectively. Pregnancy tests were positive in 0% of volunteers in 2014 and 2% in 2016. The non-inclusion of 21% vs. 4% due to EKG abnormalities in 2014 and 2016, respectively, is probably related to interpretation criteria. For hepatitis B, the drop from 23% in 2014 to 16% in 2016 is likely due to spontaneous healing. For hepatitis C, the higher rate in 2016 can attributed to the fact that the same volunteers from 2014 returned in 2016 for re-screening. Differences in hematuria between 2014 and 2016 may be related to the fact that 2016 screening was done in December, a period of schistosomiasis transmission in Doneguebougou and intensive agricultural activities. These results present a trend regarding the frequency of some pathologies in our populations, and can serve not only as a warning system, but may also contribute to the design of future studies.

1057

THE EFFECT OF IMMUNIZATION WITH PFSPZ VACCINE ON EPSTEIN-BARR ANTIBODY LEVELS AS A MARKER OF VIRAL REACTIVATION AND AS A SURROGATE RISK MARKER OF ENDEMIC BURKITT LYMPHOMA

Ann Moormann[1], **LW Preston Church**[2], Catherine Forconi[1], Said Jongo[3], Ali Mtoro[3], Maximillian Mpina[3], Claudia Daubenberger[4], Thomas Richie[2], B. Kim Lee Sim[2], Salim Abdulla[3], Stephen L. Hoffman[2]

[1]*Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA, United States,* [2]*Sanaria Inc., Rockville, MD, United States,* [3]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Epstein-Barr virus (EBV) is a commonly occurring, asymptomatic infection of children. However, prolonged interactions between EBV and *Plasmodium falciparum* (Pf) asexual erythrocytic stages during childhood

is associated with an increased risk of endemic Burkitt lymphoma (eBL). The radiation attenuated Pf sporozoites (SPZ) in PfSPZ Vaccine never develop to the asexual erythrocytic stage. Nonetheless, in our first trial involving pediatric subjects in Africa (Tanzania), we measured EBV viral capsid antigen (VCA) IgG and IgM at baseline, 2 and 8 weeks after the last dose in subjects < 18, or 28 days after controlled human malaria infection (CHMI) in adults to look for evidence of increased viral expression as has been seen during acute Pf infection. IgG and IgM antibodies to EBV VCA (a marker for eBL risk) and EBNA1 (not associated with eBL risk) were measured in 89 subjects. Sera from EBV seronegative Kenyan infants were negative controls. Sixty of 68 subjects >12 months of age were EBV infected prior to enrollment. 14 of 21infants aged 6 to 12 months were not infected at study start. Over the study period of 24 weeks, 5 subjects (3 infants) seroconverted. In volunteers who were EBV seropositive at enrollment, there was no trend towards increased EBV-VCA IgG levels in vaccinees and no difference between vaccinees and controls. In the 5 volunteers who seroconverted during immunization, the final antibody levels were similar to the chronically EBV-infected population. Immunization with PfSPZ Vaccine was not associated with an increase in serum levels of EBV-VCA IgG in persistently EBV-infected volunteers and did not contribute to higher than expected antibody levels in recently infected volunteers. In this study of 89 subjects there was no indication that vaccination with PfSPZ Vaccine increased risk of progression to eBL.

1058

SAFETY OF TWO *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE (PFSPZ) SCHEDULES IN HEALTHY ADULTS IN OUELESSEBOUGOU, MALI

Halimatou Diawara[1], Agnes Mwakingwe-Omari[2], **Djibrilla Issiaka**[1], Jacquelyn Lane[2], Seydou Traore[1], Ibrahim Soumbounou[1], Mahamoudou Samassekou[1], Gaoussou Santara[1], Oumar Attaher[1], Almahamoudou Mahamar[1], Kailfa Diarra[1], Amadou Konate[1], Adama Dembele[1], Amatigue Zeguime[1], Zonghui Hu[3], Michal Fried[2], Amagana Dolo[1], Peter Billingsley[4], B. Kim Lee Sim[4], Thomas L. Richie[4], Stephen L. Hoffman[4], Alassane Dicko[1], Patrick E. Duffy[2]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [4]*Sanaria, Inc., Rockville, MD, United States*

In a previous study of Sanaria® *Plasmodium falciparum* (Pf) sporozoite (PfSPZ) Vaccine [NF54] ($1.8x10^6$ PfSPZ/dose) given at 0, 8 and 16 weeks in malaria experienced adults was safe and had a vaccine efficacy (VE) against Pf infection of 52% by time to 1st event during 24 weeks of follow up. More condensed regimens have resulted in increased VE in malaria naïve adults. In an ongoing double-blinded study, we are evaluating the safety and VE of two regimens of $9x10^5$ PfSPZ Vaccine. 210 participants were randomized into 4 arms between June and August 2018 to receive injections with PfSPZ Vaccine or normal saline. Arms 1 (vaccine, n=70) and 3a (control, n=35) received injections at 0, 8 and 16 weeks. Arms 2 (vaccine, n=70) and 3b (control, n=35) received injections at 0, 1 and 4 weeks. Participants received artemether/lumefantrine 2 weeks prior to 3rd vaccination. All participants received the 3rd vaccination at the same time and were followed for Pf infection every 2 weeks and/or at time of illness starting immediately after 3rd vaccination. Follow-up will be completed in April 2019.Vaccinations have been safe and well tolerated with no serious adverse events reported. Of 831 total adverse events (AEs), 16 (1.9%) were grade 3 (one, a decrease in platelets was possibly related). The other grade 3 AEs, clinical malaria and hypertension, were not related to the study. 8.5% of total AEs were grade 2, most were laboratory abnormalities that were unrelated to the study. Headache and injection site pain were the most frequent grade 1 AEs. A total of 164 episodes of Pf infection were documented by microscopy, 53% in Arm 1/3a and 47% in Arm 2/3b. PfSPZ Vaccine given at 0, 8, 16 weeks and 0, 1, 4 weeks appears to be safe and well-tolerated. The study is still blinded, with plans to evaluate the safety and protective efficacy of a booster dose during a second malaria transmission season.

1059

PREVALENCE OF HEPATITIS B AND HIV INFECTIONS AMONG HEALTHY VOLUNTEERS TO PARTICIPATE IN A PFSPZ VACCINE CLINICAL TRIAL IN EQUATORIAL GUINEA

Maria Silvia A. Lopez[1], Ali Hamad[2], Kamaka Ramadhani[2], Vicente Urbano[1], Gertrudis Owono[1], Fortunata Lobede[1], Ali Mtoro[2], Said Jongo[2], Jose Raso[1], Maximilian Mpina[2], Elizabeth Nyakarungu[2], Matilde Riloha Rivas[1], Carlos Cortes[3], Guillermo A. Garcia[4], Raul Chuquiyauri[5], L.W. Preston Church[6], Peter Billingsley[6], Claudia Daubenberger[7], Thomas Richie[6], Salim Abdulla[8], Stephen L. Hoffman[6]

[1]*Equatorial Guinea Malaria Vaccine Initiative, Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Equatorial Guinea Malaria Vaccine Initiative, Ifakara Health Institute, Malabo, Equatorial Guinea,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Medical Care Development International, Silver Spring, MD, United States,* [5]*Equatorial Guinea Malaria Vaccine Initiative, Medical Care Development International, Sanaria Inc., Malabo, Equatorial Guinea,* [6]*Sanaria Inc., Rockville, MD, United States,* [7]*Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [8]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania*

Hepatitis B and HIV are infections that are commonly spread through sexual contact and usually do not show symptoms in the early phase of infection. This results in a greater risk of passing the infection from one individual to others. Globally 1.34 million deaths were caused by viral hepatitis in 2015, and 940 000 deaths by HIV in 2017. The first strategic direction of the WHO Global Health Sector Strategy on Sexually Transmitted Infections 2016 - 2021 is to collect information on STI prevalence and incidence to help focused action for planning and implementation. Specifically the strategy on viral hepatitis set target to test 90% and treat 80% of people with HBV by 2030. However, at present very limited information is available on the prevalence of Hepatitis B and some information for HIV infections in Equatorial Guinea. This study primarily aimed to screen out healthy volunteers with Hepatitis B and HIV infections not to participate in the PfSPZ vaccine clinical trial in Equatorial Guinea for safety considerations. The trial's objective was to evaluate the safety, tolerability, immunogenicity, and protection that indicate the best regimen(s) of PfSPZ vaccine in Equatorial Guinea (ClinicalTrials.gov Identifier: NCT03590340). The PfSPZ vaccine study screened a total of 176 healthy adults male and female volunteers between the age of 18-45 years. Out of these 176, 16 were found to have hepatitis B infection (9.1%), 1 had hepatitis C infection (0.6%), and 4 were found to have HIV infection (2.3%). Among the volunteers excluded (72 volunteers) due to different reason, Hepatitis B &C and HIV constitute about 24% and 5.6% respectively. These infections were found in volunteers who deemed themselves to be healthy. The SDG set target to end the epidemics of AIDS ad combat hepatitis by 2030. In order to accomplish this target more effort need to be done in the improvement of the Hepatitis B vaccination coverage, prevention of HIV, diagnosis and treatment of these infections and increase the awareness of the community of these diseases in Equatorial Guinea.

1060

BASELINE MALARIA EPIDEMIOLOGY STUDY IN PREPARATION FOR A PHASE 3 MALARIA VACCINE TRIAL IN BIOKO ISLAND, EQUATORIAL GUINEA

Gertrudis Owono[1], Vicente Urbano[1], Fortunata Lobede[1], Maria L. Gozo[1], Ali Mtoro[2], Ali Hamad[2], Said Jongo[2], Kamaka Ramadhani[2], Jose Raso[1], Maximilian Mpina[2], Elizabeth Nyakarungu[2], Carlos Cortes[3], Guillermo A. Garcia[4], Matilde Riloha Rivas[1], Raul Chuquiyauri[5], L.W. Preston Church[6], Peter Billingsley[6], Claudia Daubenberger[7], Thomas Richie[6], Salim Abdulla[8], Stephen L. Hoffman[6]

[1]*Equatorial Guinea Malaria Vaccine Initiative, Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Equatorial Guinea Malaria Vaccine Initiative, Ifakara Health Institute, Malabo, Equatorial Guinea,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Medical Care Development International, Silver Spring, MD, United States,* [5]*Equatorial Guinea Malaria Vaccine Initiative, Medical Care Development International, Sanaria Inc., Malabo, Equatorial Guinea,* [6]*Sanaria Inc., Rockville, MD, United States,* [7]*Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [8]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania*

The Bioko Island Malaria Control Program (BIMCP) conducts annual malaria infection surveys using rapid diagnostic tests (RDTs). However malaria incidence for *Plasmodium falciparum (Pf)* infections in selected areas for phase 3 trial remains unknown. In addition, if a substantial number of residents harbor low parasite densities, these infections are likely not detected in these surveys, missing an important population that is believed to contribute on sustained malaria transmission. In prepraration for a phase 3 trial with PfSPZ Vaccine in Bioko island, EG , we conducted a baseline assessment of the incidence of *Pf* infections to guide selection of an appropriate sample size to measure the vaccine efficacy of PfSPZ vaccine against natural infection for this upcoming phase 3 trial. Our primary objective is to determine the incidence of Pf infection by RDTs and thick blood smear slides (TBS) over a 6-month period in 240 individuals equally categorized in 3 age groups (G1: 6-59 months, G2: 5-17 years, and G3: 18-45 years) and reside in selected areas of Bioko Island in close proximity to the research center where the estimated parasite prevalence by RDT has been consistently higher than 10% for surveys conducted from 2016 to 2018. After pre-treatment with 6 doses of artmether-lumefantrine, volunteers were actively followed every two weeks for 6 months. Infections were assessed by RDT, highly sensitive RDT, and TBS prepared in accordance with a WHO standard procedure and by qPCR. The malaria incidence data will be presented.

1061

SPZ VACCINE: FOUR BILLION+ ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES INJECTED WITH NO BREAKTHROUGH INFECTIONS

Eric Robert James, Steve Matheney, James Overby, B Kim Lee Sim, Abraham G. Eappen, Tao Li, Ming Lin Li, Anita Manoj, Peter F. Billingsley, Thomas L. Richie, LW Preston Church, Sumana Chakravarty, Anusha Gunasekera, Tooba Murshedkar, Stephen L. Hoffman

Sanaria, Rockville, MD, United States

Sanaria®PfSPZ Vaccine comprises aseptic radiation-attenuated sporozoites (SPZ) of *Plasmodium falciparum* (Pf). PfSPZ are irradiated in the mosquitoes' salivary glands using a ^{60}Co source and then are recovered by dissection and gland dissociation, purified, vialed, and cryopreserved. Mosquitoes are transferred for irradiation into infected mosquito transport containers (IMTC) and then irradiated. IMTCs were mapped using radiochromic film and alanine transfer dosimeters and the minimum and maximum dose locations determined. A reference dosimeter on the outside wall of the IMTC monitored each irradiation event. PfSPZ Vaccine production between 2010 and 2019 generated multiple lots released for use in phase 1 and 2 clinical trials and involved 458 separate irradiation events. Dosimeters were processed by the National Institute of Standards and Technology (NIST). A target minimum dose of 150 Gy was adopted as the attenuating PfSPZ Vaccine dose based on early studies using PfSPZ administered by bite of infected mosquitoes and our 6-day hepatocyte assay, in which breakthroughs occurred using 120 Gy, but not 150 Gy. Dosimeters exposed to doses of 120-170 Gy were used to calibrate the time required for a dose of 150 Gy including the lower 95% confidence interval at the minimum dose location inside the IMTC by regression analysis and the corresponding dose at the reference location was determined. A formula incorporating the half-life of ^{60}Co was used to construct tables of the irradiation times required to deliver 150 Gy on all days for each calendar year from 2010 to 2019. Analysis of the 458 reference dosimeters indicated a normal distribution with a mean of 153.4 Gy (range 150.0 to 157.8 Gy) delivered to the minimum dose location inside the IMTC. A plot of the trending data for all irradiation events recorded over this period was completely flat. As of April 2019, 18 clinical trials of PfSPZ Vaccine have been conducted, with 1,526 volunteers receiving 4,893 doses of vaccine for a total of 4.36 billion PfSPZ administered: there have been no breakthrough infections, confirming the consistency and robustness of the radiation attenuation process.

1062

CONTROLLED HUMAN MALARIA INFECTION PRODUCT TO PROBE HETEROLOGOUS PROTECTIVE EFFICACY, INFECTIONS AND STRAIN DIFFERENCES IN *PLASMODIUM FALCIPARUM*

B. Kim Lee Sim[1], Eric R. James[1], Yonas Abebe[1], Anita Manoj[1], Henry Huang[1], Peter F. Billingsley[1], Benjamin Mordmueller[2], Peter Kremsner[2], Patrick Duffy[3], Agnes Mwakingwe[3], Kirsten Lyke[4], Matthew B. Laurens[4], Thomas L. Richie[1], Stephen L. Hoffman[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany,* [3]*Laboratory of Malaria Immunology and Vaccinology, National Institutes of Health, Rockville, MD, United States,* [4]*Division of Malaria Research, Institute for Global Health, University of Maryland School of Medicine, Baltimore, MD, United States*

Sanaria® PfSPZ Challenge (Pf7G8) is composed of aseptic, purified, cryopreserved infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) that are used to conduct controlled human malaria infection (CHMI) studies to assess anti-malarial vaccines and drugs, and innate and acquired resistance to malaria. Pf7G8 was isolated in Brazil and has sinced been shown to possess unique and various genetic (SNPs) and predicted proteomic (T cell epitopes) diversity as compared to PfNF54 the strain used until recently for nearly all CHMIs. Thus far volunteers in 2 studies in the U.S., and 2 studies in Germany, have undergone CHMI with PfSPZ Challenge (Pf7G8). PfSPZ Challenge (Pf7G8) is stored in liquid nitrogen vapor phase (LNVP) at less than -150 degrees C, and the stability of PfSPZ Challenge is assessed at Sanaria by a sporozoite membrane integrity assay (viability assay) and *in vitro* growth and development in hepatocytes (potency assay). However, the most powerful indicator of the stability of PfSPZ Challenge over time has been the infectivity of PfSPZ Challenge in humans of product held in LNVP over time. For PfSPZ Challenge (NF54), 78/78 control subjects from the U.S. or Europe who received 3,200 PfSPZ up to 33 months after manufacture became infected. PfSPZ Challenge (7G8) was assessed in two dose escalation CHMIs in the U.S. and Germany ~25 months after manufacture (N=25 volunteers), and in two vaccine trials, one ~ 3 years and one more than four years after manufacture (N=14 non-immunized control volunteers). In these studies, 25/26 subjects who received 3,200 PfSPZ of PfSPZ Challenge (7G8) became infected, including 14/14 at 3 and 4 years. CHMI has been an enormously important tool to establish the lot to lot consistency and stability of infectivity of PfSPZ Challenge and by analogy of all of Sanaria's PfSPZ-based products.

1063

A HIGH CONTENT SCREENING PLATFORM FOR ABSOLUTE QUANTITATION OF *PLASMODIUM* SPOROZOITES

Urvashi Rai[1], Timothy Hackett[2], Richard Fan[1], Anita Manoj[1], B. Kim Lee Sim[1], Stephen L. Hoffman[1], Sumana Chakravarty[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*University of Nebraska, Omaha, NE, United States*

Plasmodium falciparum (Pf) sporozoite (SPZ) quantitation is one of the quality control (QC) assays used for characterizing Sanaria's multiple PfSPZ-based clinical products. The majority of quantitation is done during stability testing. More than 4,600 individual quantitation events (an operator adding a PfSPZ sample to a disposable hemocytometer and determining the absolute PfSPZ quantities by microscopy) of PfSPZ products have been done to generate stability profiles of released lots of PfSPZ products, including Sanaria® PfSPZ Vaccine, PfSPZ Challenge, and PfSPZ-GA1. In addition, quantitation is an integral part of the QC release of these PfSPZ products. Here we report on development of a robust automated quantitation protocol for PfSPZ, based on a high content image cytometry system, the CeLLInsight CX5 (ThermoFisher Scientific™). Using this platform, we have developed a highly specific PfSPZ quantitation procedure relying on a fluorescent antibody-based detection method. It features at least a 10-fold increase in the number of PfSPZ detected in the samples used to estimate the population counts as compared to microscopy The large sampling size reduces the potential for variability between samples from the same source. It also allows accurate quantitation of low PfSPZ concentrations. Following an initial staining step, the instrument scans a total area of 25.6 mm^2 with 100-4000 PfSPZ in about 3.5 minutes, while it takes an operator (microscopist) about 7 minutes to enumerate 360-400 total PfSPZ over an area of 4 mm^2. Both operations are performed on disposable hemocytometers and the images and data captured by CeLLInsight CX5 can be digitally archived for future reference. Moreover, we can accurately detect and enumerate PfSPZ concentrations in unpurified preparations containing mosquito salivary gland components.

1064

TRANSLATING A SEMI-AUTOMATED MOSQUITO MICRODISSECTION SYSTEM FOR MANUFACTURING PFSPZ VACCINES UNDER CGMPS

Hajar Hazime[1], Urvashi Rai[1], Mariah Schrum[2], B. Kim Lee Sim[1], Stephen L. Hoffman[1], Russell H. Taylor[2], Sumana Chakravarty[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Johns Hopkins University, Baltimore, MD, United States*

Plasmodium falciparum (Pf) Sporozoite (SPZ)-based vaccines are the only intervention in humans to have demonstrated >90% protective efficacy against controlled human malaria infection. They form the basis of Sanaria's unique technology platform of aseptic, purified, cryopreserved live *Plasmodium falciparum* sporozoites (PfSPZ). Manufacture of all PfSPZ-based products at Sanaria requires microdissection of mosquito salivary glands. Our goal was to semi-automate the key step of isolating PfSPZ from mosquito salivary glands based on integrating various biological, physical, chemical and engineering principles. Repeated sequential decapitation of mosquitoes and extrusion of glands over stereo-microscopic dimensions induces fatigue and requires extensive training. In a phased approach to automate mosquito microdissection we focused on converting the decapitation and gland extrusion steps from a sequential to a batch process. Prototypes were developed for mosquito alignment, decapitation and gland extraction, and integrated into a semi-automated mosquito microdissection system termed sAMMS. This system allows batch-processing of 30 to potentially hundreds of mosquitoes at once. Mosquito processing capacity using one unit of sAMMS compared to a single operator performing manual dissection has tripled per unit time, since inception of this project in 2014. The time to fully train and qualify individual operators for mosquito processing using sAMMS, is 15-20-fold less than manual dissection training time. We will report progress on all aspects of instituting sAMMS in cGMP operations that not only requires stringent materials compliance for clinical use, but also demands performance consistency with regard to PfSPZ yields and relative purity compared to manual dissection.

1065

INSECTICIDES SUSCEPTIBILITY AND PREVALENCE OF KDR AND ACE1 MUTATIONS AMONG WILD *ANOPHELES ARABIENSIS* AND *AN. MELAS* POPULATIONS IN THE COASTAL ZONE OF LOW MALARIA TRANSMISSION IN SENEGAL

Ousmane Sy[1], Elhadji Amadou Niang[1], Abdoulaye Kane Dia[1], Kevin Ochieng Opondo[2], Benoit S Assogba[2], Magatt Ndiaye[1], Mouhamed A Nourdine[1], Pape Cheikh Sarr[1], Lassana Konaté[1], Oumar Gaye[1], David Weetman[3], Martin Donnely[3], Ousmane Faye[1]

[1]*UCAD, Dakar, Senegal,* [2]*Medical Research Council unit the Gambia at the London School of Hygiene & Tropical Medicine, Fajara, Gambia,* [3]*Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Great progress has been made against malaria in the central western Senegal thanks to the successful implementation of IRS and SMC interventions. Nevertheless, the residual transmission maintained by *An. arabiensis* and *An. melas* in some hotspots may hamper the achievement of the elimination goal. This study was conducted to better characterize the vectors populations of this area by updating data on the two vectors susceptibility to insecticides and the prevalence of Kdr and Ace-1 mutations. Larvae were collected from different breeding sites of different level of salinity. Insecticide susceptibility tests were conducted on 3-5 days old unfed adult females. Aggressive and endophilic mosquitoes were caught respectively by human landing catches and pyrethrum spray catches. Overall, 667 specimens of *An. gambiae* s.l. were tested to 5 mains insecticides. Anopheles from the low salinity breeding sites consisting of 97.7% *An. arabiensis*, were susceptible to Deltamethrin but suspected to be resistant to Permethrin. While population collected from highly salty breeding sites, exclusively represented by *An. melas*, were susceptible to Bendiocarb, Pirimiphos-methyl and DDT. Molecular identification revealed that the host-seeking and resting populations in the study area consisted mainly of *An. arabiensis* which was found with significantly higher proportions (57.4%) than the *An. melas* (42.2%). *An. coluzzii* was very poorly represented (0.4%) and no *An. gambiae* was found. A very large distribution of the Kdr mutation was noted in the study area with a preponderance of Kdr-East over the Kdr-West in both endophilic and aggressive populations. The Kdr gene was mainly carried by the species *An. arabiensis* and less frequent in *An. melas* while the Ace-1 gene was only found in two individuals of *An. arabiensis*. This study demonstrated the susceptibility of *An. melas* to Bendiocarb, Pirimiphos-methyl and DDT and provided an update of the prevalences of the Kdr and Ace-1 mutations among the natural population of the two main malaria vectors in central-western Senegal.

1066

NEXT GENERATION VECTOR SURVEILLANCE TECHNIQUES TO GUIDE NATIONAL MALARIA CONTROL PROGRAMS

Robert Farlow[1], Thomas R. Burkot[2], Tanya L. Russell[3]

[1]*R Farlow Consulting LLC, Burkeville, TX, United States,* [2]*Australian Institute of Tropical Health and Medicine, Stratford, Australia,* [3]*Australian Institute of Tropical Health and Medicine, Cairns, Australia*

Malaria vector surveillance is being increasingly recognized as critical to the success of national malaria control programs. The World Health Organization recently published the objectives for malaria vector surveillance and provided guidance on core vector surveillance activities by transmission scenarios and vector control interventions deployed. Effective programmatic vector surveillance is limited in part by the methods used to quantify vector parameters critical to monitoring the effectiveness of interventions. This presentation is a follow-up to a survey of 35 national

malaria control programs that identified programmatic limitations to vector surveillance. In this study, 40 vector control experts were interviewed to gather expert assessments of the limitations of current vector control techniques and to clarify new ideas to improve vector control. This data was used to define target product profiles of next generation vector surveillance tools to address technical limitations of the presently deployed tools, as well as research requirements to develop and optimize NextGen surveillance tools including algorithms for representative sampling, translation of trap catches to population and epidemiologically relevant parameters and data requirements to improve sampling.

1067

ONE YEAR OF MONITORING PHYSICAL DURABILITY OF LONG LASTING INSECTICIDAL NETS IN MALI

Moussa Bm Cisse[1], Ibrahim Traore[1], Lansana Sangare[1], Abdourhamane Dicko[2], Yacouba Dansoko[1], Alice Dembele[1], Jean Marie Sanou[1], Jean Bedel Evi[3], Jules Mihigo[4], Aliou Diallo[4], Erin Eckert[5], Ousmane Koita[1]

[1]*Laboratoire de Biologie Moléculaire Appliquée/ Université des Sciences Techniques et des Technologies de Bamako, Bamako, Mali,* [2]*Programme National de Lutte contre le Paludisme, Bamako, Mali,* [3]*US Agency for International Development Global Health Supply Chain Program Procurement and Supply Management, Bamako, Mali,* [4]*President's Malaria Initiative US Agency for International Development, Bamako, Mali,* [5]*President's Malaria Initiative US Agency for International Development, Washington, DC, United States*

In 2018, 85% of households owned at least one long lasting insecticide nets (LLIN). In Mali, LLIN are distributed through mass distribution campaigns and through routine health services to pregnant women and infants. Both net durability and the "average useful life" of a net are critical factors for determining the frequency at which nets need to be replaced and the types of nets to be procured. The objectives were to 1) assess the physical durability of polyester LLINs in 2 sites; 2) compare their durability and 3) identify major determinants of field performance after one year of use. The study was carried out after the mass LLIN distribution campaign held in December 2017 in southern Mali. The study was conducted in Kenieba (Site 1) and Kita (Site 2) districts which have similar malaria epidemiology, climatic and socio-ecological profiles. Cohorts of 471 LLINs (75 denier polyester) in Site 1 and 514 (100 denier polyester) in Site 2 were identified for monitoring during the study. Physical durability monitoring was performed by taking into account the net loss rate and the physical condition of surviving nets which was measured by the proportionate hole index (pHI) after one year. After 12 months, 53% (245/465) of the LLIN in Site 1 and 60% (305/511) in Site 2 were present. In Site 1, 17% (77/465) nets were being used elsewhere and 2% (8/511) had been discarded. In Site 2, only 9% (48/514) nets were being used elsewhere. Approximately one quarter of the LLIN had unknown status (nets present but owner absent) 27% (124/461) in Site 1 and 31% (158/511) in Site 2. The all causes attrition rate was approximatively 30% in both sites. Attrition due to wear and tear was less than 2% in Site 1 and was 0% in Site 2 (100% of all the nets were intact). Similarly, the proportion of nets with pHI <64 was lower in Site 1 (66%) than in Site 2 (93%). Tears were the main type of damage, 73% in Site 1 and 34% in Site 2. The proportion of nets surviving in serviceable condition was lower in Site 1 (85%) than in Site 2 (95%; $p = 0.004$). Further analyses are being conducted to identify the reasons for the apparent lower performance of the LLINs in Site 1.

1068

CITIZEN SCIENCE FOR MOSQUITO MONITORING AND MALARIA VECTOR CONTROL IN RUHUHA, RWANDA

Marilyn Milumbu Murindahabi[1], Constantianus J. Koenraadt[2], Willem Takken[2], Leon Mutesa[3], Emmanuel Hakizimana[4], P. Marijn Poortvliet[5], Arnold J.H. van Vliet[6]

[1]*College of Science and Technology, University of Rwanda, Kigali, Rwanda,* [2]*Wageningen University and Research, Wageningen, Netherlands,* [3]*College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda,* [4]*Malaria and other Parasitic Diseases Division, Rwanda Biomedical Center, Kigali, Rwanda,* [5]*Strategic Communication Group, Wageningen University & Research, Wageningen, Netherlands,* [6]*Environmental Systems Analysis Group, Wageningen University & Research, Wageningen, Netherlands*

To support the Rwandese mosquito monitoring system, a citizen science approach for mosquito monitoring was implemented in five selected villages from Ruhuha sector in Bugesera district, the eastern province of Rwanda. Ruhuha is known as an agro-ecosystem, in which water irrigation schemes create potential areas for mosquito breeding, and the residents have elevated high risk of contracting malaria infection. In Ruhuha, regular mosquito monitoring programs are established due to limited budgets for mosquito surveillance. The 108 recruited volunteers in this citizen science study were asked to report experienced mosquito nuisance and confirmed malaria cases in their domestic environment. Additionally, the selected volunteers were asked to collect and submit mosquito specimens using passive traps. More than 2,000 mosquitoes were collected in a period of six months, from 2018. The main mosquito specimens submitted were *Culex* species (90%), while the remaining species were *Anopheles* species (10%). From the total *Anopheles* species collected, *Anopheles gambiae* sensu lato (43%) and *Anopheles ziemanni* (45%) specimens were the highest malaria vectors identified in the study area. Both the mosquito nuisance as the numbers of mosquitoes caught varied in space and time. The highest and lowest mosquito nuisance were reported in Kibaza and Kagasera respectively. The highest and the lowest *Anopheles gambiae* s.l. species collected were collected in Kibaza and Kagasera respectively. Ongoing work is validating the citizen science collections with traditional trap collections and aims to assess the links with the numbers of malaria cases reported in the area.

1069

IS FLUDORA FUSION A VIABLE ALTERNATIVE TO DDT?

Rajendra Maharaj[1], Bheki Qwabe[2], Moses Mkhabela[2], Nomfundo Mfeka[3], Melanie Holder[3], Vishan Lakan[1]

[1]*South African Medical Research Council, Durban, South Africa,* [2]*KwaZulu-Natal Department of Health, Jozini, South Africa,* [3]*Bayer, Johannesburg, South Africa*

Indoor residual spraying and insecticide impregnated nets are the main methods of preventing malaria transmission. Considering increasing insecticide resistance, new chemicals that are cost-effective are needed for elimination. Currently the gold standard is DDT, an insecticide that has a long residuality and is affordable to malaria control programmes in Africa. A new insecticide mixture Fludora Fusion, a combination of clothianidin and deltamethrin, has been registered for use in malaria vector control programmes. This combination of active ingredients, with unrelated modes of action, is intended to address the challenge of insecticide resistance in malaria-transmitting mosquitoes. The study was conducted in Othobothini in KwaZulu-Natal on houses with mud, cement and painted walls to determine the suitability of using this new insecticide compared to DDT. For each of the three wall types, the insecticides were evaluated in ten different structures. WHO Cone bioassays were carried out monthly following insecticide application using 3-5-day old, non-blood fed female *Anopheles arabiensis* mosquitoes. On mud surfaces, 100% mortality was achieved after 144 hours post exposure for Fludora Fusion for all months. DDT also produced 100% mortality at the 4-day post exposure evaluation. On cement surfaces, Fludora achieved an average of 97% at 48 hours evaluation for the 12 months of evaluation. An average of 100% mortality

was attained at 120 hours from months 10. DDT also produced 100% mortality at the 72-hour evaluation for all months. On painted surfaces, 100% mortality was reached by the insecticide combination by 120 hours post-exposure. DDT produced 100% mortality at the 96 hours evaluation for all months. Compared to DDT, the mortality achieved with the use of this combination insecticide is excellent. DDT resulted in 100% mortality within 96 hours on mud and 72 hours on cement and paint surfaces. Fludora Fusion also achieved 100 % mortality within 96 hours on mud but required 72 hours to achieve 100% mortality on cement and painted surfaces. Fludora Fusion is a viable alternative to DDT.

1070

VECTOR SURVEILLANCE FOR EVIDENCE-BASED DECISION MAKING IN MALARIA VECTOR CONTROL IN BURUNDI

Virgile Gnanguenon[1], Anatolie Ndashiyimiye[2], Jeanne d'Arc[2], Denis Sinzinkayo[2], Lievin Nsabiyumva[3], Jenny Carlson[4], Aklilu Seyoum[5]

[1]*Abt Associates/PMI AIRS, Bujumbura, Benin,* [2]*NMCP, Bujumbura, Burundi,* [3]*United States Agency for International Development, Bujumbura, Burundi,* [4]*United States Agency for International Development, Washington, DC, United States,* [5]*Abt Associates/PMI VectorLink, Washington, DC, United States*

Malaria is a major public health problem in Burundi with 23 districts experiencing high incidence of the disease. Since 2017, Cankuzo, Gihofi and Kiremba districts were selected for indoor residual spraying (IRS) due to the high malaria incidence. From December 2017 through September 2018, monthly entomological surveys were conducted in eight sentinel sites using human landing catches. Overall, the average human biting rate of *Anopheles gambiae* s.l. was similar indoors (6.61 bites/person/night) and outdoors (9.27 bites/person/night) (p=0.250). For *An. funestus* s.l., the biting activity was also similar indoors (1.88 bites/person/night) and outdoors (1.02 bites/person/night). The lowest indoor biting rate was observed from January to August 2018 in Kiremba, where IRS was implemented in December. The lowest parous rates of *An. gambiae* s.l. were observed at Mabayi (69.12%) and the IRS site of Gihofi (70.54%) while the lowest parous rate of *An. funestus* s.l. was observed at Gihofi (64.06%). The entomological inoculation rates (EIRs) were lower in 2018, compared with 2017 data. However, the highest EIR in 2018, 8.12 infective bites/person/month, was observed in January, whereas the highest EIR in 2017, 31.0 infective bites/person/month, was observed in March. Program data shows that the IRS intervention was implemented late, after the peak of EIRs in Cankuzo and Gihofi, at the end of May and July, respectively, when EIR was low (0-0.6 infective bites/person/month). Therefore, revising the timing of IRS to February could prove more effective. In IRS intervention sites, most of the transmission occurred outdoors. The average infective bites observed indoors was 0.46 infective bites/person/month versus 2.93 infective bites/person/month outdoors. In non-IRS sites, the transmission is more or less similar indoors and outdoors with an average of 0.46 infective bites/person/month versus 0.68 infective bites/person/month, respectively. Additional vector control tools may be required to further reduce the residual transmission occurring outdoors in IRS areas.

1071

DIAGNOSTIC DOSE DETERMINATION AND EFFICACY OF CHLORFENAPYR AND CLOTHIANIDIN INSECTICIDES AGAINST *ANOPHELES* MALARIA VECTOR POPULATIONS OF WESTERN KENYA

Silas Okoth Agumba

Maseno University, Kisumu, Kenya

Malaria vector control is dependent on chemical insecticides applied to walls by indoor residual spraying or to long-lasting insecticidal nets. However, the emergence and spread of insecticide resistance in the major malaria vectors may compromise malaria control and elimination efforts. To maintain gains made in the fight against malaria, it is important to evaluate alternative insecticides for mosquito control. The aim of this study was to estimate a diagnostic dose for chlorfenapyr (class: pyrrole) and clothianidin (class: neonicotinoid) and assess the baseline susceptibility of wild *Anopheles* malaria vectors of western Kenya. Centers for Disease Control and Prevention (CDC) bottle assays were used to determine the diagnostic doses of chlorfenapyr and clothianidin insecticides against a susceptible laboratory strain, *Anopheles gambiae,* Kisumu strain. Probit analysis was used to obtain the lethal doses at which 50% (LD50) and 99% (LD99) of the susceptible mosquitoes would be killed 24, 48 and 72 hours following exposure for 1 hour. Insecticidal efficacy was then evaluated against field collected female *Anopheles* mosquitos sampled from Nyando, Bumula and Ndhiwa sub-Counties in western Kenya. Members of *Anopheles gambiae* and *An. funestus* complexes were identified using polymerase chain reaction (PCR). The determined diagnostic doses of chlorfenapyr and clothianidin insecticides were 50 μg/bottle and 150 μg/bottle respectively for *An. gambiae,* Kisumu strain. When exposed to the diagnostic dose, *Anopheles* malaria vector populations in western Kenya were susceptible with 100% mortality observed after 72 hours. Mortality of mosquitoes exposed to deltamethrin increased over time but did not reach 100%. Mortality of mosquitoes from Nyando exposed to deltamethrin was 83% at 24h, 88% at 48h and 94.5% at 72 h while mortality of mosquitoes from Ndhiwa was 89% at 24h, 91.5% at 48h and 94.5% at 72h. Mosquitoes of western Kenya, despite being resistant to pyrethroids, are susceptible to chlorfenapyr and clothianidin but field evaluations of formulated product are needed.

1072

PROGRESS TOWARD FIELD APPLICATION OF TRANSGENIC MOSQUITOCIDAL ENTOMOPATHOGENIC FUNGI: A SEMI FIELD TRIAL TEST IN A MOSQUITO-SPHERE IN BURKINA FASO

Etienne Bilgo

IRSS/Centre Muraz, Bobo Dioulasso, Burkina Faso

Malaria control efforts require implementation of new technologies that manage insecticide resistance. The fungus, *Metarhizium pingshaense* provides an effective, mosquito-specific delivery system for potent insect selective toxins. Here we report on a semi field trial testing the efficacy of a mosquitocidal *Metarhizium pingshaense* strain (Mp-Hybrid) engineered to express an insect-specific spider neurotoxin called Hybrid (Ca++/K+ channel blocker) and a Green Fluorescent Protein (GFP). The experiments were conducted in a multi-chambered MosquitoSphere constructed for this purpose in Soumousso, a region of Burkina Faso where malaria is endemic. We used the sphere to test a variety of low technology treatment protocols that could be used routinely by householders and found that suspending Metarhizium in locally produced sesame oil and spreading that on netting or black sheets achieves a long term effect in the sphere. Within 2.5 days mosquitoes exposed to Mp-Hybrid were dying faster than those exposed to wild-type virulence expressing Red Fluorescent Protein (Mp-RFP). For infected mosquitoes, median lethal times (LT50) values for Mp-Hybrid and Mp-RFP were 5.19 ± 0.163 and 8.73 ± 0.295 days, respectively. The number of mosquitoes surviving from the uninfected control never dropped below 83.7%. We also demonstrated that Met-Hybrid reduced blood feeding and flight capacity by infected mosquitoes. In addition, Mp-Hybrid infected mosquitoes laid eggs earlier (5.42±0.727 days) compared with Mp-RFP and untreated mosquitoes (6.88±1.40 and 7.02±0.640 days respectively). This is the first evidence that transgene expression can induce an increased terminal investment response, and it could potentially undermine transgenic control approaches by affecting the evolution of infected mosquitoes. Finally, as Mp-Hybrid lasted longer at least 11 weeks than Mp-RFP (4 weeks). This study represents an important step in the progression of transgenic mosquito control technologies into field application.

1073

THE COST-EFFECTIVENESS OF ITN DISTRIBUTION STRATEGIES IN SUB-SAHARAN AFRICA

Sara Scates[1], Olivier Briët[2], Janna Wisniewski[1], Angela Acosta[3], Hannah Koenker[3], Joshua Yukich[1]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Johns Hopkins Center for Communications Programs, Baltimore, MD, United States*

Insecticide-treated nets (ITNs) are one of the most cost-effective interventions for preventing malaria. WHO recommends both large-scale mass distribution campaigns (MC) in conjunction with continuous distributions (CD) as part of a multifaceted approach to achieve and sustain universal access to and coverage of ITNs. A combination of these strategies has been effective for scaling up net coverage. In order for policy makers to make informed decisions on how to efficiently implement CD strategies, information on the cost effectiveness of these delivery systems is vital. The OpenMalaria platform was used to develop simulations of the health effects of various ITN distribution scenarios and these results were combined with cost results from a systematic review of the literature. Incremental Cost Effectiveness Ratios (ICER) and CE expansion paths were estimated under varying transmission contexts and intervention deployment histories. Initial results demonstrated that ANC and EPI distribution alone is likely to be the most efficient (i.e. lowest cost per case of malaria averted) use of resources for ITN distribution under the most severely resource limited situations. As resource levels increase, some CD systems are expected to perform at similar levels of efficiency to repeated mass campaigns. However, campaigns every three years, without CD, are similarly cost effective in some subset of transmission contexts and vector control histories. Estimates of cost-effectiveness, and the decision rules derived from these, should ideally incorporate uncertainty. We develop a bootstrapping method to estimate the uncertainty around the expansion path for net distribution systems, incorporating uncertainty in both costs and effects.

1074

DECREASING INSECTICIDE-TREATED MOSQUITO NET EFFECTIVENESS ASSOCIATED WITH INCREASING PYRETHROID PREVALENCE ACROSS SUB-SAHARAN AFRICA

David A. Larsen, Rachael Church

Syracuse University, Syracuse, NY, United States

Malaria prevention primarily relies on pyrethroid class of chemicals, through the insecticide-treated mosquito net (ITN). Increasing reports of pyrethroid-resistance in malaria vectors has worried malaria control programs. A few geographically-limited studies have found reduced efficacy of ITNs in areas with established pyrethroid resistance, however no large-scale evaluation of ITNs in the presence of pyrethroid resistance has to date been conducted. We utilized recently published Malaria Atlas Project estimates of pyrethroid resistance across sub-Saharan Africa and nationally-representative two-stage cluster surveys conducted in 2010 or later to estimate the association between ITN ownership and having a *Plasmodium falciparum*infection in the presence of varying levels of pyrethroid resistance. We first extracted levels of pyrethroid resistance to the survey cluster. We second matched households on education, wealth, and region and then utilized mixed effects logistic regression model with the matched group as a random intercept to estimate the association between ITN ownership and a *P. falciparum* infection. We finally tested the model for an interaction between the ITN ownership and estimated pyrethroid resistance. We found that in areas with zero estimated pyrethroid resistance ITN ownership was associated with a reduction in the odds of having a *P. falciparum* infection (odds ratio [OR] = 0.60, 95% confidence interval [CI] = 0.40 - 0.90). In areas with complete pyrethroid resistance, ITN ownership was not associated with the odds of having a *P. falciparum*infection (OR = 1.00, 95% CI = 0.63 - 1.68). These results indicate widespread reductions in the protection provided by ITNs due to pyrethroid resistance.

1075

INSECTICIDE SUSCEPTIBILITY STATUS OF *ANOPHELES GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. TO PUBLIC HEALTH INSECTICIDES FROM SENTINEL SITE SURVEYS IN UGANDA

Michael Okia

Abt Associates Inc., Kampala, Uganda

Insecticide resistance is an increasing threat to the two core vector control interventions in Uganda, indoor residual spraying (IRS) and long lasting insecticide treated nets (LLINs). Using the World Health Organization (WHO) susceptibility test method, we investigated the susceptibility level of *Anopheles gambiae* s.l. to three pyrethroids (alpha-cypermethrin, deltamethrin and permethrin), one organophosphate (pirimiphos-methyl), and one carbamate (bendiocarb) insecticide in 10 sentinel surveillance sites across Uganda in 2018. The susceptibility of the local vectors to clothianidin and chlorfenapyr was also assessed in 14 and 10 sentinel sites (districts), respectively. Oxidase resistance mechanisms were investigated with synergist assays using the World Health Organization (WHO) test protocol with to piperonyl butoxide (PBO). Intensity of resistance was assessed using both the Centers for Disease Control and Prevention (CDC) bottle and WHO tube assay methods. Three to five day-old female *Anopheles* mosquitoes reared from field-collected larvae or indoor-resting adults were morphologically identified and tested. *An. gambiae* s.l. was found generally susceptible (99-100% mortality) to organophosphates, clothianidin and chlorfenapyr. Possible resistance to carbamates was detected in one site, Hoima (97% mortality). Resistance to pyrethroids was widespread with varying exposure mortality ranging from 0% to 85%. *An. funestus* s.l. was found susceptible (99-100% mortality) to pirimiphos-methyl, bendiocarb, chlorfenapyr, and clothianidin in Katakwi and Soroti districts but was found resistant to pyrethroids in Katakwi and Soroti (2-52% mortality). Resistance intensity studies of *An. gambiae* s.l. using the CDC bottle and WHO tube assays provided differing resistance intensities. Pre-exposure of *An. gambiae* s.l. to PBO fully or partially restored susceptibility to pyrethroid insecticides in all test sites. The susceptibility of the two vectors to next-generation insecticides provide alternative insecticide choices for IRS, which can be rotated to help mitigate resistance.

1076

POSITIVE IMPACT OF PIRIMIPHOS-METHYL BASED IRS ON ENTOMOLOGICAL INOCULATION RATE (EIR) IN A CONTEXT OF MULTIPLE RESISTANCE MECHANISMS TO INSECTICIDES IN MALARIA VECTORS IN ALIBORI AND DONGA, TWO REGIONS OF NORTHERN BENIN

Razaki Osse

Cotonou Entomological Research Center, Cotonou, Benin

Widespread resistance to insecticides is a major concern for National Malaria Control Programs (NMCP) as this phenomenon remains a serious handicap for the success of insecticide-based control tools in some areas. In the current study, we investigated the impact of a pirimiphos-methyl based Indoor Residual Spraying (IRS) on EIR in two regions of Northern Benin where *An. gambiae* s.l. populations developed multiple insecticide resistance mechanisms. Entomological monitoring was conducted in five IRS districts in Benin including Kandi-Gogounou in the Alibori region and Djougou-Copargo-Ouake in the Donga region. Two unsprayed districts served as control. In all eight districts, frequencies of *kdr*-w and *Ace1R* and the expression of enzymatic activities compared to *Anopheles gambiae* Kisumu susceptible strain were evaluated. Mosquito collections were also organized before (2016) and after (2017) IRS implementation to assess the Entomological Inoculation Rate (EIR). Before IRS, the resistance frequencies in *An. gambiae* s.l. were between 0.67-0.88 for *kdr-w* and 0-0.06 for *Ace-1R* among sites. After IRS, the resistance frequencies in *An. gambiae*

s.l. were between 0.84-0.92 for *kdr-w* and 0.02-0.04 for *Ace-1R* among sites. Furthermore, the Gogounou district showed high oxidase activity while those of Ouake and Copargo displayed an elevated esterase activity. Both types of enzymatic activities were overexpressed in Djougou and Kandi. Despite these worrying indicators of resistance levels, a significant reduction in EIR was recorded after IRS [20.4 infected bites per person per night pre-IRS compared to 3.1 ib/p/n post-IRS (84.9% reduction, p<0.0001) and 2.3 ib/p/n in IRS districts compared to 13.6 ib/p/n in control districts (83.3% reduction, p<0.0001)]. Overall, our results show that the multiple insecticide resistance mechanisms were not a handicap for the efficacy of the pirimiphos methyl-based IRS on the EIR.

1077

PHYSICAL AND INSECTICIDAL DURABILITY OF PERMANET 2.0 AND DAWAPLUS 2.0 LLINS AFTER 36 MONTHS IN THE FIELD: FINDINGS FROM A COHORT STUDY IN MYANMAR

Si Thu Thein[1], Ye Kyaw Aung[1], Aye Aye Myint[2], Feliciano Monti[3], Than Htike Win[4], Phyu Khine Thet[4], Sean Blaufuss[5], Hannah Koenker[5], Albert Kilian[6], Aung Thi[7]

[1]*U.S. President's Malaria Initiative (PMI) VectorWorks Project, Population Services International Myanmar, Yangon, Myanmar,* [2]*Vector-Borne Disease Control Program, Ministry of Health and Sports, Yangon, Myanmar,* [3]*U.S. President's Malaria Initiative (PMI), United States Agency for International Development, Yangon, Myanmar,* [4]*Department of Food and Drug Administration, Ministry of Health and Sports, Nay Pyi Taw, Myanmar,* [5]*U.S. President's Malaria Initiative (PMI) VectorWorks Project, Johns Hopkins Bloomberg School of Public Health Center for Communication Programs, Baltimore, MD, United States,* [6]*U.S. President's Malaria Initiative (PMI) VectorWorks Project, Tropical Health LLP, Montagut, Spain,* [7]*National Malaria Control Program, Ministry of Health and Sports, Nay Pyi Taw, Myanmar*

The durability ("average useful life") of long-lasting insecticidal nets (LLIN) varies by brand and country context. This study assesses and compares the physical and insecticidal durability of two LLIN brands (PermaNet 2.0 and DawaPlus 2.0), which are made from polyester fibers treated with deltamethrin. This was a three-year prospective cohort study of LLINs distributed through a mass campaign in 32 villages of Tamu Township, Sagaing Region, in December 2015. Within six months following the mass campaign, a representative sample of campaign nets was identified through a cluster household survey approach to form the study cohort. The attrition and physical conditions of the cohort LLINs were assessed at 6-, 12-, 24-, and 36-month follow-up intervals. Sub-samples of the campaign LLINs were selected for insecticidal effectiveness testing after 12, 24, and 36 months, using cone bio-assays with deltamethrin-susceptible mosquitoes. Insecticide chemical residue was tested after 24 and 36 months. A total 739 campaign LLINs from 300 households were included in the cohort, comprising 398 PermaNet 2.0 and 341 DawaPlus 2.0. Overall attrition of nets during the follow up were 19.9%, 22.0%, 27.8% and 34.2% at 6-, 12-, 24-, and 36-month, respectively. At 36 months, 79.6% of the observed nets had any holes, a marked increase from 15.1% at 6-month, 45% at 12-month, and 63.7% at 24-month. The median proportional hole index (pHI) were 23, 19, 34 and 43 over 4 rounds. There was no significant difference in the nets surviving in serviceable condition between two brands, which was 84.6% (95% CI 76.9-90.1%) for PermaNet 2.0 and 78.2% (95% CI 69.5-84.9%) for DawaPlus 2.0. In cone bio-assays conducted at 36-months, 24-hour mortality was 66.2% (95% CI 60.8-71.6%) and 57.0% (95% CI 51.6-62.4%), resulting in 86.7% of PermaNet 2.0 and 76.7% of DawaPlus 2.0 meeting WHO minimal effectiveness criteria. Deltamethrin chemical residue testing results at 36 months will be available in May 2019. In summary, most of the cohort LLINs nets were still in physically serviceable conditions at 36 months although insecticidal effectiveness had declined.

1078

FIPRONIL AND IVERMECTIN TREATMENT OF CATTLE REDUCES SURVIVAL AND OVARIAN DEVELOPMENT OF FIELD-COLLECTED *ANOPHELES ALBIMANUS* IN NORTHERN BELIZE

Jefferson A. Vaughan[1], Staci M. Dreyer[1], Donovan Leiva[2], Marla Magaña[2], Marie Pott[2], John P. Grieco[3], Nicole L. Achee[3]

[1]*University of North Dakota, Grand Forks, ND, United States,* [2]*Belize Vector and Ecology Center, Orange Walk Town, Belize,* [3]*University of Notre Dame, South Bend, IN, United States*

Most malaria vector control programs rely on indoor residual spraying of insecticides and insecticide-treated bed nets. This is effective against vector species that feed indoors at night and rest inside the house afterwards. In Central America, malaria vectors have different behaviors and are typically exophagic (*i.e.,* bite outdoors), exophilic (*i.e.,* remain outdoors after feeding), and zoophagic (*i.e.,* as likely to feed on non-humans as humans). Thus malaria elimination in Central America may require additional tactics. This pilot study investigated whether commercially-available products used to treat livestock for ticks could also be used to against zoophagic malaria vectors that feed on treated cattle in Belize. Cattle were treated with either a pour-on formulation of 1% fipronil (3 heifers) or injection of 1% ivemectin (1 heifer). Control heifers (n=2) were left untreated. Field-collected *Anopheles albimanus* contained in screen-top cages were strapped onto cattle and allowed to feed for 15 minutes. Mosquito feedings were conducted prior to treatment and at 2, 5, 7, and 14 days after treatment. Mosquito mortality was monitored once a day for 4 successive days. Surviving mosquitoes were dissected and ovarian development assessed. A total of 1,078 female *A. albimanus* mosquitoes were fed and monitored for mortality. Both fipronil and ivermectin significantly reduced survivorship of *A. albimanus* for up to 7 days after treatment. By 14 days, the ivermectin treatment completely lost its effectiveness. Fipronil-treated cattle were still killing significantly more mosquitoes than were untreated cattle but the magnitude of mosquito killing had diminished. Throughout the 14 day trial, both ivermectin and fipronil-treated cattle significantly reduced ovarian development (i.e., fecundity) in those mosquitoes that survived feeding on treated cattle. These results suggest that efforts towards eliminating residual transmission of malaria by zoophagic vectors in Central America may benefit by targeted treatment of livestock with parasiticides, such as fipronil or ivermectin.

1079

ANOPHELES REPRODUCTIVE SWARM TRAPPING AS A POTENTIAL NEW MALARIA VECTOR CONTROL INITIATIVE IN THE GAMBIA

Benoît Sessinou Sessinou Assogba[1], Kevin O. Opondo[1], Musa Jawara[1], Jane Achan Achan[1], Charles Wondji[2], Abdoulaye Diabaté[3], Umberto D'Alessandro D'Alessandro[1]

[1]*MRC, Unit The Gambia at London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [2]*Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*Institut de Recherche en Science de la Santé/ Centre Muraz, Bobodioulasso, Burkina Faso*

Malaria remains a major health problem and its control is currently based mainly on Long Lasting Insecticidal Nets and indoor residual spraying. The contribution of these vector control tools to the observed decline in the malaria burden has been estimated at 79%. However, such gains are threatened by the emergence and spread of insecticide resistance. Alternative vector control tools are thus needed. Targeting Anopheles reproductive behavior and disrupting mating between male and females' mosquitoes may reduce vector density. We investigated how Mass Trapping of Anopheles reproductive swarms (MST) can be used as a community-based approach to reduce malaria transmission. In 2017, we characterized *Anopheles* reproductive swarm's behavior, and the ecological and environmental parameters associated with their formation and spatial distribution in six villages in eastern Gambia. We collected

data on female *Anopheles* density per house, the *Plasmodium* infection rate in *Anopheles* and humans to estimate entomological inoculation rate and malaria prevalence. During the 2018 transmission season, swarms were collected twice per week from previously identified locations. Twenty-three swarming sites of *Anopheles gambiae s.l.* close to human habitats were identified. We collected 36,327 *Anopheles* males from those swarms during the 5-month intervention of MST. Our preliminary results indicate that MST intervention resulted in 90% reduction in vector density, from 53 *Anopheles gambiae s.l.* per house to 3.5. Laboratory analyses to determine the malaria infection rates among vectors and humans are currently ongoing. In conclusion, from these results MST appear to reduce vector density however, its impact in malaria transmission is yet to be determined. If MST intervention proves successful, a larger cluster randomized trial aiming at determining the effect of the intervention over a large population would be needed before considering this intervention as a possible tool for malaria control.

1080

A FOUR-COUNTRY COMPARISON BETWEEN HUMAN-LANDING COLLECTION AND TWO NOVEL ADULT VECTOR MOSQUITO SURVEILLANCE METHODS FOR OUTDOOR-BITING ADULT ANOPHELINES THROUGH THE ASIA PACIFIC MALARIA ELIMINATION NETWORK

Ratchadawan Ngoen-klan[1], Jeffrey L. Hii[2], Thomas R. Burkot[3], Frances M. Hawkes[4], Michael J. Bangs[5], Perada W. Putit[6], Mihirini Hewavitharane[7], Vu Duc Chinh[8], Boonserm Aum-aung[9], Wannapa Suwonkerd[9], Theeraphap Chareonviriyaphap[1]

[1]*Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand,* [2]*Malaria Consortium Asia Regional Office, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [3]*Australian Institute of Tropical Health and Medicine, James Cook University, Queensland, Australia,* [4]*Department of Agriculture, Health and Environment, Natural Resources Institute, University of Greenwich, London, United Kingdom,* [5]*Public Health and Malaria Control Department, PT Freeport Indonesia, International SOS, Jl. Kertajasa, Kuala Kencana, Papua, Indonesia,* [6]*Vector Borne Disease Sector, Disease Control Division, MOH, Kuala Lumpur, Malaysia,* [7]*Anti Malaria Campaign, Ministry of Health, Colombo, Sri Lanka,* [8]*Department of Entomology, National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Vietnam,* [9]*Bureau of Vector Borne Disease, Department of Disease Control, MOPH, Bangkok, Thailand*

A knowledge of which vector species are present in a particular area, their abundance, feeding behavior, sporozoite infectivity, and insecticide susceptibility status are critical factors guiding malaria control programs in understanding transmission risk, implementing appropriately targeted vector control actions and monitoring impact of such interventions. A 'proof of concept' study was designed to evaluate the feasibility of replacing the outdoor human landing catch (HLC) with either the human double net trap (HDNT) or the host decoy trap (HDT) for outdoor biting mosquito populations in four Asian countries: Malaysia, Sri Lanka, Thailand and Vietnam. Two study sites, a rural village and a nearby forest settings, were selected in each country. The three outdoor trap methods were used concurrently each night at each study location, with each trap being rotated nightly between three set trapping positions, in a pre-assigned Latin Square Design. Each trap requires a human volunteer to serve as the primary attractant for host-seeking mosquitoes. Volunteers were rotated following the trap rotation to avoid bias. Six replicates, one replication comprising three consecutive nights of collections, were completed in Thailand, where a total of 1,555 mosquitoes were collected including 404 *Anopheles*, of which 396 (98%) *Anopheles* were captured from the forest site, whereas only 8 (2%) were collected from the village. Of forest *Anopheles*, 253 were collected from HLC, representing 63.8% of total forest collections, followed by HDT (73: 18.5%) and HDNT (70: 17.7%). A statistically significant differences in number of collected *Anopheles* was observed between the three trap methods ($P < 0.05$). We conclude that HLC is still the most productive and preferred method to collect outdoor *Anopheles* mosquitoes in Thailand. Species identifications and the outcomes from trials in Malaysia, Sri Lanka and Vietnam will be completed and summarized for presentation. Results will support national malaria programs in their efforts to improve entomological surveillance for evidence-based vector control strategies across APMEN.

1081

ENDEMIC TYPHOID INCIDENCE, KILIMANJARO REGION, TANZANIA, 2007-2018

Elena R. Cutting[1], Deng B. Madut[2], Michael J. Maze[3], Nathaniel H. Kalengo[4], Manuela Carugati[2], Blandina T. Mmbaga[4], Ronald M. Mbwasi[4], Kajiru G. Kilonzo[4], Annette Marandu[5], Calvin Mosha[5], Furaha S. Lyamuya[4], Grace D. Kinabo[4], Anne B. Morrissey[2], Venance P. Maro[4], Matthew P. Rubach[2], John A. Crump[3]

[1]*Duke University School of Medicine, Durham, NC, United States,* [2]*Division of Infectious Diseases and International Health, Department of Medicine, Duke University, Durham, NC, United States,* [3]*Centre for International Health, University of Otago, Dunedin, New Zealand,* [4]*Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania,* [5]*Mawenzi Regional Referral Hospital, Moshi, United Republic of Tanzania*

Recent prospective surveillance studies have demonstrated a moderate to high incidence of typhoid fever in some settings in sub-Saharan Africa. Growing evidence suggests considerable variation in endemic typhoid incidence over time, yet few settings have multiple year incidence estimates to inform investments in typhoid control. We sought to describe a decade of variation in typhoid fever incidence in the Kilimanjaro Region of Tanzania. A case of typhoid fever was defined as a blood culture positive for *Salmonella enterica* serovar Typhi in a febrile person. Cases were identified among febrile patients at two sentinel hospitals during three distinct periods: 2007-08, 2011-14, and 2016-18. To account for under-ascertainment at sentinel facilities, we derived adjustment multipliers from healthcare utilization surveys done in the catchment area of the sentinel hospitals in 2011 and 2018. Confidence intervals (CI) for incidence point estimates were derived using the 95% CI of our observed typhoid fever prevalence, of the healthcare seeking adjustment multiplier, and of published blood culture sensitivity. Of 3,556 participants, 50 typhoid fever cases were identified. Of typhoid cases, 26 (52%) were male and the median (range) age was 22 (<1-60) years; 4 (8%) were aged <5 years and 10 (20%) were aged 5 to 15 years. By time period, 32 (3.7%) of 870 cases enrolled in 2007-08, 15 (0.9%) of 1,753 in 2011-14, and 3 (0.3%) of 935 in 2016-18. Typhoid fever incidence was estimated as 60 (95% CI 15-283), 5 (95% CI 1-48), and 4 (95% CI 1-69) per 100,000 persons in 2007-08, 2011-14, and 2016-18, respectively. There were no deaths among cases. We identified a moderate typhoid incidence point estimate in 2007-08 and low incidence point estimates during later surveillance periods, but with wide and overlapping CIs across study periods. Our data are consistent with evidence that endemic typhoid may vary substantially over time. Multiple year surveillance provides a clearer picture of typhoid incidence than single-year studies and may be warranted in locations making decisions about typhoid conjugate vaccine introduction and other control measures.

1082

ANTIGEN-SPECIFIC GUT-HOMING B CELL RESPONSES IN HUMANS FOLLOWING CONTROLLED INFECTION WITH LYOPHILIZED *SHIGELLA SONNEI* 53G (CGMP LOT 1794)

Brielle A. Barnard[1], Kristen A. Clarkson[1], Robert W. Frenck, Jr.[2], Michelle Dickey[2], Akamol E. Suvarnapunya[1], Lakshmi Chandrasekaran[1], Kevin T. Lerner[1], Hailey P. Weerts[1], Monica McNeal[2], Katherine DeTizio[3], Susan Parker[2], Amy Hoeper[2], Chad K. Porter[3], Nicole Maier[4], Alan Fix[4], Lou Bourgeois[4], Malabi Venkatesan[1], Robert W. Kaminski[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States,* [3]*Naval Medical Research Center, Silver Spring, MD, United States,* [4]*PATH, Washington, DC, United States*

Shigella is one of the leading causes of diarrhea associated global morbidity and mortality. A lyophilized strain of *S. sonnei* 53G was produced under current good manufacturing processes (cGMP) and underwent a dose-finding assessment in a Phase 1 Controlled Human Infection Model (CHIM) to determine a dose that would safely induce shigellosis in ≥60% of volunteers. As *Shigella* is an enteric pathogen, characterizing the intestinal mucosal immune response post-infection is important in understanding the development of immunity against subsequent serotype-specific infection. Historically, mucosal immune responses have been measured directly via fecal IgA antibodies, however sample quality is a limiting factor. Antigen-specific IgA antibody responses determined in antibodies in lymphocyte supernatant (ALS) and antibody secreting cell (ASC) assays are also used, however both provide an indirect measurement of the mucosal response as homing patterns of these lymphocytes are unknown. A novel assay has been developed that separates lymphocytes based on the expression of the gut homing integrin, α4β7, allowing for a direct measurement of the mucosal immune response. Frozen peripheral blood mononuclear cells were thawed and magnetically separated into α4β7 positive (+) and negative (-) populations. Purity of cell populations post-separation was verified by flow cytometry and determined to be ≥90%. Both + and - cell populations were cultured *in vitro,* and ALS was collected and tested by ELISA to determine *S. sonnei* specific IgG and IgA endpoint titers. Oral inoculation with *S. sonnei* induced robust antigen-specific α4β7+ ALS IgG and IgA antibody titers, demonstrating that both IgA and IgG secreting B cells are homing to the gut and contributing to the mucosal immune response. A modest systemic immune response was induced post-infection, demonstrated by moderate antigen-specific α4β7- ALS IgG and IgA antibody titers. Finally, α4β7+ antibody titers correlated with multiple disease outcomes including disease severity score, dysentery, and diarrhea severity.

1083

CHILDHOOD STUNTING AND *CAMPYLOBACTER* COLONIZATION IN RURAL ETHIOPIA - FINDINGS FROM FORMATIVE RESEARCH OF THE *CAMPYLOBACTER* GENOMICS AND ENVIRONMENTAL ENTERIC DYSFUNCTION (CAGED) PROJECT

Dehao Chen[1], Sarah McKune[1], Nitya Singh[1], Jemal Y. Hassen[2], Wondwossen Gebreyes[3], Mark Manary[4], Kevin Bardosh[5], Yang Yang[1], Abdulmuen Mohammed[2], Yitagele Terefe[2], Kedir T. Roba[2], Mengistu Ketema[2], Negasi Ameha[2], Nega Assefa[2], Gireesh Rajashekara[3], Loic Deblais[3], Getnet Yimer[3], Isabel Ordiz[4], Nicholas Diaz[1], Arie H. Havelaar[1]

[1]*University of Florida, Gainesville, FL, United States,* [2]*Haramaya University, Dire Dawa, Ethiopia,* [3]*Ohio State University, Columbus, OH, United States,* [4]*Washington University, St. Louis, MO, United States,* [5]*University of Washington, Seattle, WA, United States*

Globally, 25% of children under 5 years of age are stunted, which has negative impacts throughout their life. Animal source foods (ASF) may reduce the risk of stunting. Poultry farming is one possibility for smallholder farmers to meet their household need for ASF. However, children's contact with poultry and their contaminated environments may increase *Campylobacter* exposure and colonization, which has been associated with Environmental Enteric Dysfunction (EED) and stunting. The formative research of the CAGED project includes an investigation of the burden of *Campylobacter* exposure in young children and other risk factors' association with stunting. This research will inform the design of a Cluster Randomized Controlled Trial to evaluate the effectiveness of a nutritional intervention using eggs combined with animal husbandry interventions in reducing exposure of children to animal feces. A cross-sectional study was conducted in Haramaya woreda in rural eastern Ethiopia. 102 children (age range: 360 - 498 days) were randomly selected for participation. Anthropometric measurements, *Campylobacter* colonization, EED biomarkers and questionnaire-based information on demographics, household wealth, hygiene and sanitation, nutrition, and women's empowerment were collected in late 2018. The prevalence of stunting and wasting was 42% (95% CI: 33% - 52%) and 5% (95% CI: 2% - 11%), respectively. A large majority (96%) of women are not empowered, according to the 5 domains of empowerment. Among children sampled, 88% were currently breastfed, and 56% consumed some ASF in the previous 24 hours. 47% and 50% of children had diarrhea and fever in the past 15-days, respectively. 54 of the 102 households kept chickens indoor overnight and only 26 of these households confined them. The prevalence of *Campylobacter* colonization of children by PCR was 78% (95% CI: 69% - 85%). No associations between stunting and potential explanatory variables were found. Despite adequate breastfed practices and some consumption of ASF, we find a high level of stunting, suggesting further investigation into the role of EED and *Campylobacter* infections are warranted.

1084

GENOME-WIDE ASSOCIATION STUDY OF ASTROVIRUS DIARRHEAL INFECTIONS IN BANGLADESHI INFANTS

Laura Chen[1], Rashidul Haque[2], Dylan Duchen[1], Genevieve Wojcik[3], Poonum Korpe[1], Beth Kirkpatrick[4], William A. Petri, Jr[5], Priya Duggal[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Stanford University, Stanford, CA, United States,* [4]*University of Vermont, Burlington, VT, United States,* [5]*University of Virginia, Charlottesville, VA, United States*

Astroviral infections are a common cause of acute nonbacterial gastroenteritis in children globally. However, there is no treatment available for Astrovirus, and huge Astroviridae strain diversity presents a challenge to potential vaccine development. We aimed to identify host genetic risk factors associated with astrovirus disease susceptibility that may explain disease mechanism and vaccine targets. We performed a genome-wide association study of children with and without astrovirus in the first year of life enrolled in PROVIDE or CRYPTO birth cohorts. Cases attributable to astrovirus were defined as diarrheal samples with qPCR Ct values >0 and <30, resulting in 119 cases and 314 controls in PROVIDE and 58 cases and 96 controls in CRYPTO. We independently analyzed each cohort under an additive genetic model in SNPTEST and then meta-analyzed the genome-wide results. A region on chromosome 1 near the *loricrin* gene (*LOR*) was associated with infection. Children with 1 copy of the A allele at SNP rs75437404 were 2x more likely to have an astrovirus diarrheal infection compared to children with the T allele, and this was consistent in both cohorts (meta-analysis p-value = 9.38×10^{-9}). We also identified an association with the *prolactin releasing hormone receptor* gene (*PRLHR*) on chromosome 10. Children with 1 copy of the G allele at SNP rs114810342 were about 3x more likely to have an astrovirus diarrheal infection compared to children with the A allele. We further stratified by WAZ and HAZ to account for any underlying malnutrition, and there were no differences. Loricrin is a major component of cornified cell envelopes. Previous studies suggest its expression in non-keritinizing epithelia represents a protective mechanism of the body. *PRLHR* encodes the receptor for prolactin-releasing peptide, which has been shown in mouse

models to influence feeding patterns and energy balance. This study identified 2 significant host genetic regions that may influence astrovirus infection susceptibility and should be considered in further studies.

1085

TYPHOID OUTBREAKS, 1989-2018: IMPLICATIONS FOR PREVENTION AND CONTROL

Grace D. Appiah[1], Alexandria Chung[2], Adwoa Bentsi-Enchill[3], Sunkyung Kim[1], John A. Crump[4], Vittal Mogasale[5], Rachel B. Slayton[1], Eric D. Mintz[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, United Kingdom,* [3]*Department of Immunization, Vaccines and Biologicals, World Health Organization, Geneva, Switzerland,* [4]*Division of Infectious Diseases and International Health, Duke University Medical Center; Centre for International Health, University of Otago, Dunedin, New Zealand,* [5]*Policy and Economic Research Department, Development and Delivery Unit, International Vaccine Institute, Seoul, Republic of Korea*

Typhoid fever remains an important public health problem in low- and middle-income countries, with large outbreaks reported from Asia and Africa. While WHO recommends typhoid vaccination for control of confirmed outbreaks, data on the epidemiologic characteristics of typhoid outbreaks have not been reviewed to inform vaccine use in outbreak settings. We conducted a literature review for typhoid outbreaks published since 1989. We recorded cases and case definitions as reported by the authors and used WHO Regions to categorize outbreak locations. We also abstracted data from unpublished field investigation reports to summarize outbreak interventions. We defined multi-drug resistant (MDR) *Salmonella* Typhi strains as resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole; extensively drug resistant (XDR) strains were defined as MDR and resistant to fluoroquinolones and third generation cephalosporins. Forty-eight published typhoid outbreaks were reviewed for key outbreak characteristics. There were 37,670 cases in 24 countries from 1989 through 2018. Outbreak characteristics varied considerably by region, with median outbreak size ranging from 12 to 1,101 cases, median duration from 19 to 140 days, and mean case fatality ratio ranging from 0 to 44%. The largest proportion of outbreaks, 12 (25%) each, occurred in the WHO South-East Asia and African Regions. Among 44 outbreaks reporting a mode of disease transmission, 25 (57%) outbreaks were waterborne, 17 (39%) foodborne, and 2 (4%) person-to-person transmission. Among the 35 outbreaks with antimicrobial resistance data, in 25 (71%) *Salmonella* Typhi was resistant to >1 antimicrobial, 17 (49%) had MDR strains and 1 had XDR strains. Our review showed a longer median duration of outbreaks caused by MDR strains (90 days vs 30 days for susceptible strains) although this difference was not statistically significance. Control strategies focused on water, sanitation, and food safety, with vaccine use described in 6 (13%) outbreaks. As typhoid conjugate vaccines become more available, their potential impact and role in outbreak control warrants further evaluation.

1086

REAL CONDITION SIMULATION STUDY ON THE THERMO-STABILITY FOR EUVICHOL-PLUS

Sunmi Han[1], Namseon Beck[1], Yun Chon[1], Yongsoo Chung[1], Julia Lynch[1], Jose Paolo M. Langa[2], Jucunu J. Chitio[2], Seukkeun Choi[3], Youngjin Lee[3]

[1]*International Vaccine Institute, Seoul, Republic of Korea,* [2]*INS Mozambique, Cuamba, Mozambique,* [3]*EuBiologics Co. Ltd., Seoul, Republic of Korea*

With over 30 million doses dispensed from the WHO/Gavi stockpile, Oral Cholera Vaccine (OCV) is a low cost, lifesaving intervention. Easy to administer in a mass vaccination campaign, OCV is recommended as a two dose regimen at 0 and 14 days. Growing evidence shows that OCVs can be safely kept outside the cold chain with considerable potential benefits including cost and manpower savings, prevention of vaccine damage caused by accidental freezing, and most importantly making it easier to reach people living in remote places who would otherwise remain unvaccinated. The study was conducted in Cuamba, Mozambique, as an effort to collect evidence facilitating 'delivery of the vaccine in resource limited settings' in parallel to a real preventive vaccination campaign using the same logistic resources. This 'Real Condition Simulation Study on Thermo-stability of Euvichol-Plus' aimed to verify vaccine potency after exposure to three different temperature conditions during the simulation period of vaccine delivery: 1) delivery in cold chain for the first and the second dose, 2) delivery in cold chain only for the first dose, and 3) outside cold chain for the first and second dose, assuming self-administration strategy for the second dose. The vaccines exposed to the three conditions were collected upon arrival at the beneficiaries and tested for potency using two different methods to assess the antigen content: On-site RDT tests (Crystal® VC Immunochromatographic One Step Rapid Visual Test for Vibrio cholera - Dipstick, ARKRAY Healthcare), and EuBiologics' ELISA potency test. While data from temperature loggers packaged with the vaccines showed the difficulty in temperature control in 'resource limited settings', neither RDT nor ELISA tests showed decrease of vaccine potency in any of simulated conditions. Percent changes of vaccine potency from the product release time were 12.6-14.1% for O1 Inaba, 0.9-4.8% for O1 Ogawa, and 15.2-19.6% for O139, respectively. These results are in line with the previous findings from various OCV stability studies and may support Euvichol-Plus' product quality in extended controlled temperature conditions.

1087

UPDATING OUR UNDERSTANDING OF SEVERE COMPLICATIONS OF TYPHOID FEVER: A CLINICAL STUDY IN BLANTYRE, MALAWI

Jillian S. Gauld[1], Franziska Olgemoeller[2], J.J. Waluza[3], Dalitso Zeka[3], Thomas Edwards[4], Steve Kamiza[3], Chisomo Msefula[3], Angeziwa Chirambo[5], Emma Thomson[3], Tiyamike Chilunjika[6], Jonathan Read[7], Eric Borgstein[3], Peter J. Diggle[7], Nicholas A. Feasey[4]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Malawi-Liverpool Wellcome Trust Clinical Research Programme, Blantyre, Malawi,* [3]*University of Malawi College of Medicine, Blantyre, Malawi,* [4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [5]*University of Liverpool, Liverpool, United Kingdom,* [6]*Queen Elizabeth Central Hospital, Blantyre, Malawi,* [7]*Lancaster University, Lancaster, United Kingdom*

Typhoid fever remains a major source of morbidity and mortality in low income settings. Its most feared complication is intestinal perforation. However, due to the paucity of diagnostic facilities in typhoid endemic settings, the etiology of intestinal perforation is frequently assumed, but rarely confirmed. This poses a challenge for accurately estimating mortality and severe complication rates. An epidemic of typhoid began in Blantyre, Malawi in 2011, increasing from approximately 14 cases per year (1998-2010), to more than 700 in 2013, accompanied by an increase in the number of intestinal perforations. We used a Poisson generalized linear model to estimate excess perforations attributed to the typhoid epidemic, using the temporal trend of S. Typhi throughout the epidemic as the predictor. We additionally recruited a longitudinal cohort of patients with confirmed intestinal perforation in 2016, in which enhanced microbiological investigations (blood and tissue culture, plus tissue PCR) were performed. Our model estimates that 1.50 [95% CI:1.15-1.84] intestinal perforations occur each month that are not attributed to typhoid fever, and that culture-confirmed cases of typhoid fever lead to 0.05 excess perforations per clinical typhoid fever case [95% CI:0.03-0.06]. The results from our surgical cohort are consistent with this model: 48% of the patients recruited to the cohort were culture or PCR positive for S. Typhi. For the same recruitment year, 2016, the model estimates that 42% of intestinal perforations were due to typhoid fever. Mortality from S. Typhi-attributed intestinal perforation remains high: 18% of the patients with culture-confirmed typhoid fever in the surgical cohort died. This study offers a valuable model for estimating perforation rates in Blantyre without

direct detection in the clinic, and provides an updated understanding of the rates of severe complications from typhoid fever. Our results help relate changes in typhoid incidence through control efforts to reductions in severe complications, and provide localized evidence to inform global mortality rates.

1088

PREDICTORS OF *SHIGELLA* SEVERITY AND DERIVATION OF A *SHIGELLA*-SEVERITY SCORE IN THE GLOBAL ENTERIC MULTICENTER STUDY

Patricia B. Pavlinac[1], James A. Platts-Mills[2], Jie Liu[2], Kirkby D. Tickell[1], Jane Juma[3], Furqan Kabir[4], Joesph Nkeze[5], Catherine Okoi[6], Darwin Operario[2], Jashim Uddin[7], Shahnawaz Ahmed[7], Pedro Alonso[8], Martin Antonio[6], Stephen M. Becker[2], William Blackwelder[5], Robert Breiman[9], Abu S. Faruque[7], Barry Fields[9], Jean Gratz[2], Rashidul Haque[7], Anowar Hossain[7], M Jahangir Hossain[6], Sheikh Jarju[6], Farah Qamar[10], Najeeha Talat Iqbal[10], Brenda Kwambana[6], Inacio Mandomando[8], Timothy McMurry[2], Caroline Ochieng[3], John Ochieng[3], Melvin Ochieng[3], Clayton Onyango[9], Sandra Panchalingam[5], Adil Kalam[4], Fatima Aziz[4], Shahida Qureshi[4], Thandavarayan Ramamurthy[11], James Roberts[2], Debasish Saha[6], Samba Sow[12], Suzanne Stroup[2], Dipika Sur[11], Boubou Tamboura[12], Mami Taniuchi[2], Sharon Tennant[5], Deanna Toema[5], Yukun Wu[5], Anita Zaidi[4], James Nataro[2], Myron Levine[5], Eric Houpt[2], Karen L. Kotloff[5]

[1]*University of Washington, Seattle, WA, United States,* [2]*University of Virginia, Charlottesville, VA, United States,* [3]*Kenya Medical Research Institute, Nairobi, Kenya,* [4]*Aga Khan University, Karachi, Pakistan,* [5]*University of Maryland, Baltimore, MD, United States,* [6]*Medical Research Council Unit, Banjul, Gambia,* [7]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh,* [8]*Centro de Investigação em Saúde da Manhiça, Maputo, Mozambique,* [9]*Global Disease Detection Division, Kenya Office of the US Centers for Disease Control and Prevention, Nairobi, Kenya,* [10]*Department of Paediatrics and Child Health, Aga Khan University, Karachi, Pakistan,* [11]*National Institute of Cholera and Enteric Diseases, Kolkata, India,* [12]*Centre pour le Développement des Vaccins, Bamako, Mali*

Shigella is a leading cause of childhood morbidity and mortality and an important vaccine development target. Vesikari score, designed to define severity of rotavirus diarrhea, may not predict severe *Shigella*. Moderate to severe diarrhea (MSD) cases from GEMS with *Shigella* infection identified by either microbiological culture or molecularly at a diarrhea-associated quantity (cycle threshold {CT} <27.9) with known vital status at follow-up were included in this analysis. We derived a severity score based on risk of dying in the 14-days after MSD presentation using forward stepwise Cox proportional hazards regression and Akaike Information Criteria for model-selection. Model performance was compared using the area under the curve (AUC) with bootstrapped standard errors and a chi-square statistic. Among 1,481 *Shigella* MSD cases, 59% were < 24 months of age, 57% had bloody stool, 40% had severe dehydration, and 33% were stunted at MSD presentation. Forty-two (2.8%) *Shigella* MSD cases died over the 60-day follow-up, 22 (52%) of which occurred in the first two weeks after presentation. In univariate models, age < 12 months (hazard ratio {HR}: 3.8, 95% confidence interval {CI}: 1.2-12.0); duration of diarrhea ≥3 days (HR: 5.8, 95% CI: 1.7-19.6); caregiver reported vomiting ≥ 3 times per day (HR: 3.8, 95%CI: 1.6-8.8); severe dehydration (HR: 17.7, 95%CI: 2.4-132.2); stunting (HR: 3.6, 95%CI: 1.5-8.7); and mid-upper arm circumference (MUAC) < 12.5cm (HR: 6.0, 95%CI: 2.4-15.1) were associated with deaths in the first 14-days. The newly derived score, comprised of age, dehydration, diarrhea duration, fever, vomiting, and stunting, performed similarly at predicting 14-day mortality (AUC=.85, 95%CI: 0.76-0.92) to the Vesikari score (AUC=0.79, 95%CI: 0.70-0.87, p= 0.24). When considering deaths after 14-days among 1,459 14-day survivors, the new score performed better (AUC= 0.81, 95%CI: 0.70-0.92) than Vesikari (AUC: 0.67, 95%CI: 0.53-0.81, p=0.0025). Among children with *Shigella*-attributed MSD, Vesikari scores identified those at immediate, but not extended, risk of death.

1089

DISTRIBUTION OF *E. COLI* PATHOTYPES AND THE STRUCTURE OF THE HUMAN GUT MICROBIOME ACROSS AN URBAN-RURAL GRADIENT IN ECUADOR

Karen Levy[1], Maria J. Soto-Girón[2], Lorena Montero[3], Shanon M. Smith[1], Angela Pena-Gonzalez[2], Maritza Paez[3], Estefania Ortega[3], Janet K. Hatt[2], Pablo Endara[3], William Cevallos[4], Gabriel Trueba[3], Konstantinos Konstantinidis[2]

[1]*Emory University, Atlanta, GA, United States,* [2]*Georgia Institute of Technology, Atlanta, GA, United States,* [3]*Universidad San Francisco de Quito, Quito, Ecuador,* [4]*Universidad Central del Ecuador, Quito, Ecuador*

Previous studies have reported lower fecal bacterial diversity in individuals from urban compared to rural settings, which can potentially reduce resistance to enteric pathogen invasion. However, most of these studies compared geographically distant populations. Previous studies of diarrhea etiology have also captured between- but not within-country heterogeneities in enteropathogen prevalence and severity. Focusing on how gut microbial communities and enteric infection rates differ within the same country avoids confounding factors such as different cultural context or diet. We conducted a case-control study of diarrhea to understand how gut microbial communities and enteropathogen infections vary across a rural-urban gradient in four sites in northern Ecuador. Metagenomic analyses revealed small but significant differences in the abundances of several taxa in rural versus urban populations, including higher abundance of *Prevotella* spp. and lower abundance of *Bacteroides* spp. and *Alistipes* spp. Individuals with diarrhea showed greater metagenomic shifts in urban compared to rural individuals in taxon abundance, functional pathways, and taxon-taxon interactions. We also saw variability by site in enteropathogen prevalence and outcomes of infection. Any *E. coli* infection, coinfections, diffuse adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC), and rotavirus were significantly associated with diarrhea. DAEC was more common, and more associated with disease, in urban areas. Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) were more common in rural areas. Phylogenetic analysis revealed that associations with disease were not driven by any single clonal complex. Higher levels of antibiotic resistance occurred in rural areas. Collectively, our data suggested that patterns of gut microbial communities, enteropathogen prevalence, virulence, and antibiotic resistance, and microbiota alterations due to diarrhea varied substantially by geography. Our results suggest that control of pathogens in urban areas could be a strategy to control downstream effects on disease in rural areas.

1090

IMMUNE RESPONSE TO ORAL CHOLERA VACCINE IN FORCIBLY DISPLACED MYANMAR NATIONALS IN BANGLADESH

Fahima Chowdhury

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

After the large influx of Rohingya nationals (termed Forcibly Displaced Myanmar National; FDMN) from Rakhine State of Myanmar to Cox's Bazar in Bangladesh, it was apparent that outbreaks of cholera was very likely in this setting where people were living under adverse water and sanitation conditions. Large campaigns of oral cholera vaccine (OCV) were carried out as a preemptive measure to control cholera epidemics. There was no evidence to demonstrate that the vaccine was immunogenic among the FDMN population. The aim of the study was to evaluate the immune responses of healthy adults and children after administration of two doses of OCV and compare with the response of Bangladeshi's vaccinated earlier. A cross-sectional immunogenicity study was conducted among FDMNs of three age cohort; 83 individual in the adult group (18+yrs), 63 in the older children group (6-17 yrs) and 80 in the younger children group (1-5 yrs). Two doses of OCV were given at 14 days interval with collection of capillary blood at three time points to measure the immune responses. We measured vibriocidal antibodies using either plasma or dried blood

spot (DBS) specimens. There was a significant increase of responder frequency of vibriocidal antibody titer at day 14 in all e groups for both *V. cholerae* O1, Ogawa and Inaba (Ogawa/Inaba: adults-64%/64%, older children-70%/89% and younger children-51%/75%). There was no overall difference of vibriocidal antibody titer between FDMN and Bangladeshi population at baseline (p =0.07- 0.08) and at day 14, day 28 in all age groups both for Ogawa and Inaba serotypes. The seroconversion rate and geometric mean titre (GMT) of either serotype were comparable using plasma and dried blood spot (DBS) methods where both specimens were tested. These results showed that oral cholera vaccine is capable of inducing robust immune responses in adults and children among the FDMN population which is comparable to that seen in Bangladeshi participants in different age groups or that reported from other cholera endemic countries and also suggest that the displaced population were exposed to *V. cholerae* prior to seeking shelter in Bangladesh

1091

CHARACTERIZATION OF CHOLERA ANTIGEN-SPECIFIC CELLS IN BLOOD AND MUCOSA

Md Taufiqur R. Bhuiyan

International Centre for Diarrhoeal Disease, Bangladesh, Dhaka, Bangladesh

We characterized the acute B cell responses in peripheral mononuclear cells and in lamina propria lymphocytes in *Vibrio cholerae* O1 infected participants by assessing the phenotypes of different subtypes of B cells. We sorted single plasmablasts, plasma cells and memory cells for generating cholera antigen specific monoclonal antibodies and for making clones of the specific cells. Earlier we have found that the cholera-induced responses were characterized by high levels of somatic hypermutation and large clonal expansions. We compared flow cytometry and nanowell technology first to validate data for this new technology. Comparable frequencies of $CD19^{+}$ B cells have been found by using this technology using epifluorescence microscopy. Then we moved forward with this nanowell technology for characterization and sorting of cholera antigen specific single cell. All the participants had a greater than 4-fold increase in their plasma vibriocidal antibody titer following cholera infection. We found that cholera induced a significant plasmablast ($CD27^{high}$ $CD38^{high}$) expansion in PBMCs at day 7 after cholera infection as compared to other days after cholera infection. However, for lamina propria lymphocytes, we did not observe any differences in the level of plasmablast and plasma cell responses. Meanwhile, we also noticed a significant increase of memory cell responses at convalescence in circulation (day 30) and LPLs (day 360). Using the microengraving technique, we also evaluated OSP and CT specific IgA and IgG isotype specific signals for sorting single antigen specific cells for production of human monoclonal antibodies. This technique appeared to be more advantageous than flow cytometry as we were able to sort both isotype and antigen specific single cell for exploring further details.

1092

ISOLATION OF *SALMONELLA TYPHI* AND *S. PARATYPHI* IN BLOOD AND STOOL FROM THE PATIENTS ENROLLED IN THE PASSIVE SURVEILLANCE IN DHAKA, BANGLADESH

Farhana Khanam

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Enteric fever causes over 21 million cases of febrile illness and 200,000 deaths annually worldwide. Predominantly due to infections caused by *Salmonella typhi* and *S. paratyphi* A. Rate of multidrug resistant (MDR) infections is increasing as well. Currently available data on enteric fever in Bangladesh are limited. As part of the Strategic Typhoid Alliance across Africa and Asia (STRATAA) consortium, a multicomponent study was designed to characterize the burden to generate evidence for implementation of typhoid vaccine in Bangladesh. Following an initial demographic census in Mirpur (wards 3 and 5), Dhaka, a passive surveillance system for typhoid was implemented. The census was updated biannually, with close out census conducted after two years. Passive surveillance was carried out from 21st August 2016 to 20th January 2019. Blood (N=6315) and stool (N=5829) specimens were collected for culture from enrolled patients. Blood was cultured using BacT/Alert automated system. Stool culturing was carried out to isolate *S*. Typhi and *S*. Paratyphi strains. Antimicrobial susceptibility profiles of isolated strains were assessed by disc diffusion method. Resistance pattern was determined as per the CLSI guideline. Baseline census was conducted from June, 2016 to August, 2016 in Mirpur and 110,731 individuals were enumerated in census. We found 385 *S*. Typhi, 100 *S*. Paratyphi A, 01 *S*. Paratyphi B bacteremic patients. Overall incidence of typhoid fever was 164 per 100,000 person-years and the highest incidence was found in children 5-9 years of age group. The incidence of paratyphoid fever is also high. Multi-drug resistance was observed in 38%; reduced susceptibility to ciprofloxacin and azithromycin was seen in 96% and 3% respectively for isolated *S*. Typhi strains from blood. In stool culture, 185 *S*. Typhi, 20 *S*. Paratyphi A and 5 *S*. Paratyphi B strains were isolated from the patients at acute stage of illness, among which 129 *S*. Typhi, 12 *S*. Paratyphi A, and 01 *S*. Paratyphi B were positive for both blood and stool. High disease burden and MDR typhoid fever in this population strongly support introduction of typhoid conjugate vaccine in efforts to control this disease.

1093

SENTINEL SURVEILLANCE FOR CHOLERA AND OTHER ENTERICS IN BANGLADESH: FINDINGS FROM NATIONWIDE HOSPITAL-BASED SURVEILLANCE

Ashraful I. Khan

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

The incidence of diarrhea remains high globally, although mortality has declined but significant morbidity still remains a concern. The objective of this study was to understand the epidemiology and disease burden of cholera and other common bacterial enteric diseases in Bangladesh. A nationwide enteric disease surveillance was initiated in Bangladesh in 2014 to conduct prospective surveillance in all divisions, initially at 10 sites for enteric pathogens (*Vibrio cholerae*, enterotoxigenic *Escherichia coli* (ETEC), *Shigella* and *Salmonella*) in collaboration with the Institute of Epidemiology, Disease Control and Research (IEDCR). From May 2016, surveillance was gradually expanded to an additional 12 health facilities focusing on cholera only. Stool specimens collected from diarrheal patients were transported and tested centrally at icddr,b using culture and PCR methods. Univariate and multivariate analyses were employed to identify risk factors. Of the cumulative 26,090 stool samples tested, 1606 (6.2%) were identified as *V. cholerae*. For ETEC, a total of 15,030 samples were tested and overall 3.1% (n = 463) were ETEC positive. Among 15,031 stool samples tested for *Salmonella* and *Shigella*, overall 2.7% (n=406) were positive for *Salmonella* and 236 (1.6%) were positive for *Shigella*. Along with the bi-annual cholera peak in each year, higher cholera positivity was observed during the post-monsoon season in the western regions, while cholera predominated during the pre-monsoon season in the eastern regions of Bangladesh. The risk of cholera was associated with age, occupation, and recent history of diarrhea inside households. Nationwide surveillance showed the presence of enteric pathogens including cholera in all geographical regions in Bangladesh. The information obtained from the surveillance can be used to plan future strategies for control of enteric diseases including cholera in Bangladesh. The best long-term public health control strategies will be to implement integrated comprehensive multi-sectoral approaches using both vaccination and appropriate water and sanitation interventions.

1094

PLASMA IGA AND IGG RESPONSES AGAINST TWO *SALMONELLA* ANTIGENS IDENTIFY PATIENTS WITH PARATYPHOID A FEVER

Jason Andrews[1], Farhana Khanam[2], Ariana Nodoushani[3], Nazia Rahman[2], Motaher Hossain[2], Isaac Bogoch[4], Krista Vaidya[5], Meagan Kelly[3], Stephen Calderwood[3], Taufiqur Rahman Bhuiyan[2], Edward T. Ryan[3], Firdausi Qadri[2], **Richelle C. Charles**[3]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Massachusetts General Hospital, Boston, MA, United States,* [4]*University of Toronto, Toronto, ON, Canada,* [5]*Dhulikhel Hospital, Dhulikhel, Nepal*

There is a need for a reliable and simple diagnostic assay for enteric fever. Development of improved diagnostics for enteric fever have been primarily focused on typhoid fever. There are currently no available serologic assays for paratyphoid fever; and development and evaluation of new diagnostic assays for paratyphoid fever have been limited. We have previously demonstrated that assessing plasma IgA responses to HlyE, LPS, and MP are important predictors of acute typhoid infection, even in highly typhoid-endemic areas. Since these antigens are also present in *S*. Paratyphi A (*S*. PTA), we assessed IgA and IgG responses against HlyE, *S.* Typhi and *S*. PTA LPS, and *S.* Typhi MP (membrane preparation) via standard ELISA in plasma drawn on patients presenting with a febrile illness in Bangladesh and Nepal (day 0) who subsequently were determined to have *S*. PTA bacteremia (n=35). We compared these responses to those detected in healthy endemic zone controls in Bangladesh (n=10). Overall, anti- *S*. PTA LPS IgG could distinguish paratyphoid fever from endemic controls (AUC 0.9943; sensitivity of 100%, specificity of 90%). Anti- *S.* Typhi LPS IgG (Typhi shares an O12 antigen with PTA) had an AUC of 0.9886 (sensitivity 97%, specificity 90%). The next best predictors were HlyE IgA and *S*. PTA LPS IgA with an AUC of 0.9286 (sensitivity 94% and 91% respectively; specificity 80%). Our results suggest that development of a diagnostic assay focused on antibody responses against HlyE and LPS would identify patients with acute paratyphoid fever.

1095

PROVIDER ATTITUDES TOWARDS AN ELECTRONIC CLINICAL DECISION SUPPORT TOOL FOR PEDIATRIC DIARRHEA

Joel I. Howard[1], Ben Brintz[1], Adrew Pavia[1], Eric Nelson[2], Adam Aluisio[3], Adam C. Levine[3], Karen Kotloff[4], Daniel Leung[1]

[1]*University of Utah, Salt Lake City, UT, United States,* [2]*University of Florida, Gainesville, FL, United States,* [3]*Brown University, Providence, RI, United States,* [4]*University of Marlyand, Baltimore, MD, United States*

Diarrheal illness is a leading cause of mortality in children under 5 years old in Low and Middle Income Countries (LMICs). While some causes of infectious diarrhea can benefit from specific antimicrobials, in many resource limited settings, testing of stool to determine etiology is not feasible. Clinical decision support tools (CDSTs) are one potential solution to help in the management of diarrhea. These tools can have varying degrees of complexity, and the adoption may vary significantly. There are few data regarding the overall use of CDSTs in LMICs. This study was designed to identify the ability and willingness of healthcare providers to use an electronic CDST for management of diarrhea. We administered a web-based survey through various networks targeted at clinicians in healthcare institutions in LMICs. 39 Healthcare providers from 23 unique institutions representing 9 countries (5 in Africa, 3 in Asia) were identified and data was collected through a brief, anonymous one-time online survey in REDCap. The median age of respondents was 35 years (IQR 32.3-41) and 24 (61.5%) were male. 37 (94.9%) respondents endorsed having access to a smart phone or tablet at work, and 28 (71.8%) use an EMR at their main institution. There was a wide range in testing practices, with a large proportion (18 [46.2%]) reporting testing <4% of the time, while 5 (12.8%) endorsed testing >20% of stools. 19 (48.7%) said they almost never treat, and 30 (76.9%) say they treat <9% of cases. However, 3 respondents endorsed treating more than 50% of cases. Use of CDSTs was high, with 37 (94.8%) endorsing at least some use of a clinical calculator, and 18 (46.2%) using a clinical calculator at least daily. A majority of CDST use was done through a phone (33 [89.2%)]. Importantly, a large majority of respondents (37 [87.2%]) stated that they would be willing to enter 3 or more symptoms into an App and 27 (69.2%) respondents would use an app that predicted the etiology of diarrhea. This study is ongoing. Data from a larger number of respondents, as well as a results from a qualitative survey will be presented.

1096

GEOGRAPHIC INEQUALITY IN CHILDHOOD DIARRHEAL MORBIDITY AND MORTALITY IN LOW-INCOME AND MIDDLE-INCOME COUNTRIES, 2000-2017

Robert C. Reiner, Kirsten Wiens, Aniruddha Deshpande, Paulina Lindstedt, Brigette Blacker, Simon Hay

University of Washington, Seattle, WA, United States

Across low-income and middle-income countries (LMICs), one in ten deaths in children under the age of 5 are attributable to diarrhea, resulting in the loss of more than 530 000 young lives annually. Previous studies have documented substantial country-to-country variation in both diarrhea incidence and mortality. Identifying regions with the highest burden and corresponding risk factors is necessary to reduce the preventable burden of childhood diarrhea. We used Bayesian model-based geostatistics and an extensive geolocated dataset in combination with established methods from the GBD 2017 study to estimate posterior distributions of continuous continent-wide surfaces of diarrhea prevalence, incidence, and mortality. Further, we investigated the drivers of the observed subnational patterns of burden by synthesizing our estimates and similarly created aggregated risk factor estimates. Regions in south and southeast Asia, and South America saw the greatest declines in diarrhea burden. While many children in Sahelian Africa are still at extremely high risk of death due to diarrhea, regions with the most deaths were outside Africa. Indonesia exhibited the greatest relative geographic inequality globally, with some regions nearly four times the country average mortality rate. Some of the highest reductions in mortality were driven by similar reductions in low oral rehydration solution coverage, poor sanitation, or childhood stunting, while most high-risk areas still had substantial mortality attributable to at least one of those three risk factors. By co-analyzing geospatial trends in both diarrheal burden and its key risk factors from 2000 to 2017, we are able to understand the drivers of death reduction in regions that improved. Similarly, by overlaying remaining burdens with these same risk factors, we can identify optimal intervention strategies for vulnerable populations. Given the competing demands and limited resources in many LMICs, accurate quantification of both the burden of diarrhea and its drivers are critical for precision public health.

1097

GEOGRAPHICAL DISTRIBUTION OF HUMAN LEPTOSPIROSIS INCIDENCE IN THE UPPER YANGTZE AND PEARL RIVER BASIN, CHINA: TOOLS TO SUPPORT SURVEILLANCE AND FOCUSED INTERVENTION

Pandji W. Dhewantara[1], Abdullah A. Mamun[2], Wenyi Zhang[3], Danhuai Guo[4], Wenbiao Hu[5], Wenwu Yin[6], Fan Ding[6], Ricardo J. Magalhaes[1]

[1]*School of Veterinary Science, The University of Queensland, Gatton, Australia,* [2]*Institute for Social Science Research, The University of Queensland, Brisbane, Australia,* [3]*Center for Disease Surveillance and Research, Institute of Disease Control and Prevention of PLA, Beijing, China,* [4]*Scientific Data Center, Computer Network Information Center, Chinese Academy of Sciences, Beijing, China,* [5]*School of Public Health and Social Work, Queensland University of Technology, Brisbane, Australia,* [6]*Chinese Center for Disease Control and Prevention, Beijing, China*

The incidence of leptospirosis in China remained steady at low-level since 2011 nearing elimination. Residual hotspots of leptospirosis are

still exist mainly in southwest along Upper Yangtze and south China along Pearl River basin. To help guide health authorities to identify areas where interventions should be improved, this study was carried out to quantify the effect of environment and socioeconomic factors on the spatial distribution of leptospirosis incidence and to develop predictive maps of leptospirosis incidence for the Upper Yangtze and Pearl River basin. Data on human leptospirosis reported during 2005-2016 across the two regions were geolocated at the county-level and included in the analysis. Bayesian zero-inflated Poisson conditional autoregression (CAR) models were developed incorporating environmental and socioeconomic data such as precipitation, normalized difference vegetation index (NDVI), normalized difference water index (NDWI), land surface temperature (LST), elevation, slope, land cover, crop production, livestock density, gross domestic product (GDP) and population density. The effect of environmental and socioeconomic variables was heterogeneous between regions. After adjusting the effect of environment and socioeconomic factors, the distribution of the high incidence areas is more widespread both in the Upper Yangtze and Pearl River region. In the Upper Yangtze, the highest predicted incidence was found along the border of Chongqing and Guizhou towards Sichuan basin and northwest Yunnan. The highest predicted incidence was also identified in counties in the middle and lower reaches of the Pearl River. This study demonstrated significant geographical heterogeneity in leptospirosis incidence within the Upper Yangtze and Pearl River basin. The surveillance and control strategies should be improved and extended towards counties adjacent to the high-incidence counties. In addition, public health interventions should be targeted on the population at-risk on the areas where incidence is predicted to be high.

1098

A STUDY ON THE SPLEEN MICROBIOME OF WILD RODENTS AND SHREWS CAUGHT IN MARIGAT COUNTY, KENYA

Rehema M. Liyai[1], Clement Masakhwe[2], Gathii Kimita[2], David O. Miruka[1], John N. Waitumbi[2]

[1]*Maseno University, kisumu, Kenya,* [2]*United States Army Medical Research Directorate, Kenya, kisumu, Kenya*

There is a global increase in emerging infectious diseases, with 13% of over 1400 known human pathogens being classified as emerging or re-emerging. Majority of the emerging pathogens cause zoonoses and wild mammals are reservoirs. This study used 16S rRNA metagenomics to evaluate the diversity of bacteria pathogens hosted by wild caught rodents and shrews. 54 small mammals were trapped from different sites in Marigat, Baringo County, Kenya. Taxonomy of the small mammals was confirmed by amplification of cytochrome B (*Cytb*) and cytochrome oxidase subunit 1 (*COI*) genes. Genomic DNA was extracted from the spleen biopsy samples and the V3-V4 regions of the 16S rRNA amplified by PCR. The generated amplicon were sequenced on the Illumina MiSeq to identify the diversity of bacteria community. Sequence data was analyzed with Mothur v1.35, queried against the Silver database and visualized on R. By molecular taxonomy, the small mammals were classified as 41 rodents and 13 shrews. In total, 175,629 sequences passed the$^{i\ ise}$t quality controls and classed into 987 (N) operational taxonomic units (OTUs) that mapped to 18 bacteria phyla, with *Proteobacteria* accounting for 55% (538/N), *Actinobacteria* 22% (220/N), *Firmicutes* 12% (114/N) and *bacteriodates* 5% (54/N). These 4 phyla accounted for 94% of the total OTUs. Of the remaining 6%, the phyla *Acidobacteria* and *Verrucomicrobia* were the most dominant. We identified 213 bacterial genera. On a heat map, *Bartonella* sequences predominated and accounted for 38% of the reads. There was no other dominant bacteria in the remaining 62% of the reads and were contributed by *Anaplasma*, *Methylobacterium, Coxiella*, *Salmonella, acinetobacter, Elrichia*, *Delftia*, *Rickettsia,* and *Brucella* among others. In conclusion, this study identified about 1000 distinct bacteria OTUs in the spleen of wild small mammals. Future studies will need to perform advanced characterization to identify the bacteria species.

1099

EXPRESSION OF VIRULENCE GENES ASSOCIATED WITH PHYLOGROUPS IN VAGINAL STRAINS OF *ESCHERICHIA COLI*

Eric Monroy[1], Gloria Luz Paniagua Contreras[1], Areli Bautista Cerón[1], Nancy Nolasco Alonso[2], Susana González Almazán[1], Ma. Patricia Sánchez Yáñez[1]

[1]*Universidad Nacional Autónoma de México, Estado de México, Mexico,* [2]*Instituto Mexicano del Seguro Social, Estado de México, Mexico*

The pathogenicity of *Escherichia coli* strains that cause cervico-vaginal infections (CVI) is due to the presence of several virulence genes. The objective of this study is to determine the expression patterns of virulence genes associated with antibiotic resistance genes and phylogenetic groups in cervico-vaginal *E. coli* (CVEC) strains. A total of 200 *E. coli* strains isolated from Mexican women with CVI from two medical units of the Mexican Institute of Social Security were analysed. *E. coli* strains and antibiotic resistance genes were identified using conventional polymerase chain reaction (PCR), and phylogenetic groups were identified using multiplex PCR. Virulence gene expression was measured through real-time PCR after infection of the vaginal cell line A431.The most common antibiotic resistance genes among the CVEC strains were *aac(3)II*, *TEM*, *dfrA1*, *sul1*, and *qnrA*. The predominant phylogroup was B2. The genes most frequently expressed in these strains were *fimH*, *papC*, *irp2*, *iroN*, *kpsMTII*, *cnf1*, and *ompT*. A total of 134 expression patterns of virulence genes were associated with antibiotic resistance genes and phylogenetic groups in CVEC strains. The genetic diversity of CVEC strains was high. These strains have a large number of expression patterns of virulence genes, and one-third of them carry three to seven antibiotic resistance genes.

1100

MOLECULAR CHARACTERIZATION OF PLASMID MEDIATED PENICILLIN AND TETRACYCLINE RESISTANCE IN *NEISSERIA GONORRHOEAE* ISOLATES RECOVERED FROM KENYA BETWEEN 2013 AND 2018

Mary Wandia Kivata[1], Margaret Mbuchi Mbuchi[2], Fredrick Lunyagi Eyase[2], Wallace Dimbuson Bulimo[2], Cecilia Katunge Kyanya[2], Valerie Oundo[2], Willy Sang[2], Wilton Mwema Mbinda[1], Ben Andagalu[2], Olusegun O. Soge[3], R. Scott McClelland[3], John Distelhorst[2]

[1]*Karatina University, Karatina, Kenya,* [2]*US Army Medical Research Directorate-Africa, Nairobi, Kenya,* [3]*University of Washington, Departments of Global Health and Medicine, Washington, WA, United States*

Emergence of high level gonococcal penicillin and tetracycline resistance is mediated by plasmid-borne genes. Knowledge of plasmid types and plasmid-borne resistance genes in gonococci is useful in monitoring spread of antibiotic resistance. To our knowledge, no report exists on plasmid types and genes mediating penicillin and tetracycline resistance in Kenyan gonococci. We investigated plasmid mediated resistance in *Neisseria gonorrhoeae* (GC) isolates recovered from four locations in Kenya. Plasmid and genomic DNA were extracted from 37 sub-cultured multidrug resistant GC isolates. Whole genomes were sequenced using Illumina platform. Reads were assembled *de novo* using CLC Genomics Workbench. Assembled sequences were mapped to FA 1090 reference and unmapped contigs searched against the NCBI database. Genome annotation was performed using Rapid Annotation using Subsystem Technology and Geneious Prime 2019. Susceptibility results were interpreted with reference to European Committee on Antimicrobial Susceptibility Testing standards. Twenty four (64.9%) isolates had both β-lactamase (TEM) and class M tetracycline resistance determinant (TetM) encoding plasmids whereas 8.1% isolates lacked either of the plasmids. TEM encoding plasmids were identified in 27 isolates, of which 26 had a backbone corresponding to that of African plasmid (pDJ5) while only 1 had a backbone corresponding to that of Asian plasmid (pDJ4). Of the 37 isolates, 31 (83.8%) had TetM encoding plasmids, 30 of which had American TetM determinants, whereas 1 had a Dutch TetM determinant. 88.9% isolates harboring

TEM encoding plasmids were penicillin resistant. All isolates with TetM determinants were tetracycline resistant and had increased doxycycline MICs. All analyzed isolates had non mosaic PenA mutations and S10 V57M mutation. High penicillin and tetracycline resistance in the analyzed GC isolates is mediated by plasmid-borne *blaTEM,* and *tetM* genes. An Asian TEM plasmid type and a Dutch TetM determinant which are less described in African gonococci were detected in Kenyan GC. The African TEM plasmid type, TEM1 and American TetM were most prevalent.

1101

A COMPARISON BETWEEN IGM-ELISA AND IGM RAPID IMMUNOCHROMATOGRAPHY TEST IN THE DIAGNOSIS OF LEPTOSPIROSIS DURING THE ACUTE PHASE OF ILLNESS IN SOUTHERN SRI LANKA

Weerasinghe M. D. G. B. Wijayaratne[1], Champika K. Bodinayake[1], L. Gayani Tillekeratne[2], Wasantha KodikaraArachchi[3], Vasantha Devasiri[1], Truls Ostbye[2], Ruvini P. Kurukulasooriya[1], Nishantha Gunasekara[1], Megen Reller[2], Chris W. Woods[2], Ajith Nagahawatte[1]

[1]*University of Ruhuna, Galle, Sri Lanka,* [2]*Duke University, Durham, NC, United States,* [3]*Teaching Hospital Karapitiya, Galle, Sri Lanka*

Leptospirosis is diagnosed on clinical grounds and confirmed by the immunological gold standard, microscopic agglutination testing (MAT). Leptospira IgM enzyme-linked immunosorbent assay (ELISA) has been shown to be more sensitive than MAT when performed during the acute phase of illness. This study assesses the usefulness of two immunodiagnostic assays, the IgM-ELISA (Serion-Virion) and rapid immunochromatography test (Leptocheck-WB), in the diagnosis of leptospirosis in the acute phase of illness in a cohort of clinically diagnosed leptospirosis patients. Both tests were performed on acute phase serum of 170 patients with clinically diagnosed leptospirosis (per modified WHO definition) admitted to the Teaching Hospital Karapitiya, Sri Lanka. Positive, indeterminate and negative anti-leptospiral IgM cutoff titers of IgM-ELISA were>20 IU/ml, 15-20 IU/ml and <15 IU/ml, respectively. Appearance of bands in both control and test windows were considered as positive for Leptocheck-WB test. The test positivity of IgM-ELISA and Leptocheck-WB during the acute phase of the illness were 51.18% (87/170) and 47.65% (81/170), respectively. When compared to IgM-ELISA, the sensitivity, specificity, positive predictive value and negative predictive value of Leptocheck-WB were 86.21%, 93.24%, 93.75% and 85.19%, respectively. Acute-phase IgM-ELISA and Leptocheck-WB had a Kappa coefficient of 0.789 indicating a good agreement between the two test results. Only 54.7% of clinically diagnosed patients were confirmed by any of the two methods during the acute stage. The results conclude that both IgM-ELISA and Leptocheck-WB were effective in the diagnosis of leptospirosis during the acute phase of the illness. Leptocheck-WB is a rapid and easy-to-use alternative that is as effective as IgM-ELISA for the diagnosis of acute leptospirosis.

1102

DIARRHEAL ENTERIC PATHOGENS ASSOCIATED WITH PEDIATRIC LINEAR GROWTH FALTERING: A SYSTEMATIC REVIEW OF THE LITERATURE

Caroline Zellmer, Shrish Budree, Majdi Osman, Pratik Panchal

OpenBiome, Cambridge, MA, United States

Acute diarrhea is a leading global cause of death in children under five. However, we are only scratching the surface on the true morbidity of diarrheal disease. Recent studies like GEMS and MAL-ED identified six of the most common causes of acute pediatric diarrhea: *Norovirus*, *Rotavirus*, *Campylobacter*, *Shigella*, *Cryptosporidium*, and *Astrovirus*. Linear growth faltering has been correlated to diarrhea, and we wanted to collate the available evidence to assess how this understanding has evolved with modern diagnostic and genomic capabilities. We conducted a systematic review to determine the association of these specific enteric pathogen diarrhea with linear growth outcomes in children. This query identified 404 unique papers from nine databases (Medline, Embase, Cochrane database of systematic reviews, Web of Science and regional databases including IMSEAR, AIM, LILACS, IMEMR, WPRIM). These databases were searched from inception to 05/18/2018 and were not limited by English language requirement. Papers were included if they described a pediatric population, mentioned the diarrhea etiology and were conducted in a low or middle income country. Data was extracted from 46 papers. Study quality was assessed as per PRISMA guidelines using the QUADAS II score. 36.9% (17/46) papers were located in Asia, 23.9% (11/46) were conducted in Africa and 19.6% were completed in South America, with six conducted across multiple, cross continental sites. All but eight (17.4%) studies took place in at least one rural setting. Study sample sizes ranged from 20 to 83,073 children. The most commonly evaluated pathogen considered within the context of linear growth faltering was *Cryptosporidium* (with 22/46 studies including it in the analysis). *Shigella* (45.7% of studies) and *Rotavirus* (26.1% of studies) were the next most frequent. Asymptomatic carriers were noted in 43.4% of papers. This review is unique as it collates all current evidence of key pathogen linkage to stunting. Next steps are to meta-analyze the effects and the strength of the association.

1103

NATIONWIDE SEROPREVALENCE OF LEPTOSPIROSIS AMONG YOUNG THAI MEN, 2012

Siriphan Gonwong, Thippawan Chuenchitra, Patchariya Khantapura, Dilara Islam, Nattaya Ruamsap, John M. Crawford, James W. Jones

Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

Leptospirosis, a global neglected zoonotic disease, is an important public health problem in Thailand. Leptospirosis is caused by a Gram-negative spirochete belonging to the genus Leptospira. Humans and animals are infected with Leptospira through direct contact with the urine of an infected animal or indirectly through environmental contamination such as soil and water. Due to its nonspecific symptoms, lack of laboratory confirmation, and underreporting contribute Leptospirosis' status as a neglected disease. To better understand the distribution of leptospirosis exposure in Thailand, a retrospective leptospirosis seroprevalence study was conducted on repository serum specimens obtained from young Thai men entering the Royal Thai Army during 2012 (n = 6,627) by using ELISA. The overall nationwide leptospirosis IgG seroprevalence among these young Thai men was 18% (95% confidence interval = 17-19%) and the range by region was 15-25% confirming leptospirosis as an endemic disease throughout Thailand. Group of individual with the lowest education, living in rural areas and working in agriculture had the highest leptospirosis seroprevalence. The highest seroprevalence was in the South region contrary to current morbidity reports supporting poor clinical diagnosis of leptospirosis in Thailand especially in the South region as has been reported. This nationwide distributed seroprevalence data of leptospirosis raise concerns regarding implementing public health interventions such as improved reporting and surveillance, better access to diagnostic tools for enhanced disease awareness, and effective public health education and awareness to prevent this disease.

1104

EVALUATION OF DIAGNOSTIC TESTS FOR HUMAN LEPTOSPIROSIS IN DIFFERENT STAGES OF THE DISEASE

Virginia C. Rodríguez, Ronald Soto, Ana M. Castro, Alfonso Calderón, Maria F. Yasnot

Universidad de Córdoba, Monteria, Colombia

Leptospirosis is the world's most important zoonotic disease, although there is limited knowledge due to the lack of data in the clinical identification, diagnosis, treatment and surveillance processes. A prospective, descriptive study was conducted to evaluate four diagnostic tests on humans with leptospirosis in different stages of the disease. By a convenience sampling were evalauted 118 patients with clinical

symptoms of leptospirosis. These patients signed a consent form and were requested to fill out an epidemiological record. Serum, blood and urine samples were obtained from these patients in the acute and convalescent phase. Microagglutination (MAT), IgM antibody by ELISA screening, immunochromatographies (Leptocheck WB) and polymerase chain reaction (PCR) were conducted in the acute phase. MAT, IgM antibody by ELISA screening, and immunochromatographies were performed in the convalescence phase. Positive and negative predictive values, Kappa coefficient, sensitivity and specificity were calculated taking into account the final MAT as a reference point. During the acute phase, the MAT had 58,8% and 100% of respectively. When comparing with the MAT, ELISA and Leptocheck WB had identical sensitivities (64.7%) and similar specificities (95, 8% -95%), respectively. The predictive values of the tests were variable. The majority of the evaluated tests increased their sensitivity and specificity during the convalescence phase. The consistency between ELISA and MAT was considerable in both phases. A positive PCR in blood or urine increased the probability that the patient was ill. The increase of positive results in the convalescence phase was demonstrated in a similar way for the ELISA and Leptocheck WB serological tests. Paired samples of MAT will behave as the best confirmatory test for leptospirosis. The PCR allowed the diagnosis of acute phase leptospirosis. IgM ELISA is a valid option for diagnosis in the acute phase of the disease, but its results should be confirmed by MAT. The Leptocheck WB can serve as a screening option in places with difficult geographic access.

1105

BORDERLINE METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS,* AN UNDERAPPRECIATED AND EMERGING PUBLIC HEALTH THREAT

Rebecca S. Fischer[1], Naledi Mannathoko[2], Heather T. Essigmann[3], Oluwakemi D. Alonge[1], Eric L. Brown[3]

[1]*Texas A&M University Health Science Center, College Station, TX, United States,* [2]*University of Botswana, Gaborone, Botswana,* [3]*The University of Texas Health Science Center, Houston, TX, United States*

Asymptomatic carriage of *Staphylococcus aureus*, an opportunistic pathogen of major medical importance worldwide, is a primary risk factor for infection and an important source of transmission. Understanding characteristics of *S. aureus* circulating in a community can guide clinical awareness (e.g. most likely disease manifestations) and management (e.g. effective antibiotics). The global spread of methicillin-resistance (MRSA) has received important attention; rare but increasing reports of borderline-resistant (BORSA) strains, which hyperproduce β-lactamase and respond incompletely to standard therapy, highlight it as an emerging public health threat. As part of a carriage study in Botswana, nasal swabs from HIV+ and HIV- outpatients in 2013 revealed a 39.3% carriage prevalence. We further characterized 499 *S. aureus* isolates, recovered from the 225 carriers (90.2% adult; 80.2% HIV+), by oxacillin E-test for MRSA, 18 antibiotics by disc diffusion, and *spa* sequence typing (Ridom) for strain lineage. MRSA was rare (4.6%; 23 isolates), but highly-resistant (4 isolates), MDR (15 isolates), and 25 (5.0%) isolates of BORSA were observed; only 90.4% of *S. aureus* was MSSA. Cefoxitin disk had low sensitivity to detect MRSA and failed to detect BORSA. Resistance was low to TMP-SXT (11%) but high to cloxacillin (87%). Isolates were from 77 *spa* types and 20 clonal complexes [mainly t891 (8%) and CC45 (20%)], as well as 15 novel *spa* types and 55 isolates that were 'non-typable' by the standard method. Several clinically important findings emerged:(1) both MRSA and BORSA are circulating in the community; (2) cloxacillin as empiric therapy requires reconsideration; and (3) Cefoxitin for MRSA detection was inadequate. This investigation of molecular and epidemiologic features of *S. aureus* in Botswana documents BORSA for the first time in this part of the world, as well as novel and nontypable *S. aureus*. Local understanding of epidemiologic and other characteristics of *S. aureus* can inform clinical guidelines and practices, judicious antibiotic prescribing, and interventions to prevent spread.

1106

A RARE COMPLICATED CASE OF PRIMARY PARIETAL PLEURAL HYDATID CYST RUPTURE; INVASIVE IN MULTIPLE ORGAN SITE

Giovanna F. Ramirez-Barbieri[1], Yurydia Jorge[2], Marcel C. Casasola Medrano[3], Jesus Garcia-Pinzas[4]

[1]*Beth Israel Deaconess Medical Center, Boston, MA, United States,* [2]*MedSpan Associates Inc. New York University Langone Hospital, Brooklyn, NY, United States,* [3]*Mount Auburn Hospital, Cambridge, MA, United States,* [4]*National Hospital Cayetano Heredia, Lima, Peru*

Echinococcosis Cyst an endemic parasitic infestation caused by *Echinococcus granulosus*. In humans can develop one or more hydatid cysts; common location is liver and lungs. The incubation period can last many years until hydatid cysts grow to the extent that triggers clinical signs. Primary pleural is a rare variant, and its complication of rupture consequently invade other organs. We will report this untypical case. A 55-year-old, male, comes to the hospital in Lima, Peru, with one year of intermittent left side dull chest pain with non-radiation and persistent chronic dry cough. Patient refers diagnostic of empyema with thoracentesis four years ago. On current evaluation emergency chest x-ray and CT-scan was performed; showing multi-cystic masses in both hemithorax with significant predominance on the left hemithorax and abdominal organs. Surgical Intervention was indicated and during resection, confirmed multiple hydatid cyst invasion on the interior surface of the parietal pleura chest wall and other abdomen organs. There were no complications in the postoperative period and before patient discharge a chest x-ray was performed with complete eradication of hydatid cyst. Albendazole was started 400mg/day oral to continue postoperative prophylactic measure to prevent recurrence. Early diagnosis of the hydatid cysts is difficult because of the long latency period between exposure and manifestation of the disease. We consider chest x-ray and CT-scan are essential tools for efficacy and follow-up treatment management. The involvement of hydatid disease could be seen as a uniloculated or multiloculated cyst with a thin or thick wall as we see in this patient; however, may see a solid mass which is difficult to differentiate from other tumors or diseases. The primary treatment is surgical excision and the use of albendazole in the preoperative and postoperative. Pleural echinococcosis is rare. A high index of clinical suspicion is necessary for an accurate diagnostic to avoid complications. Surgery is the primary intervention with adjuvant treatment of albendazole.

1107

QUALITY OF LIFE IN PATIENTS WITH SYMPTOMATIC EPILEPSY DUE TO NEUROCYSTICERCOSIS

Willy R. Zapata[1], **Javier A. Bustos**[2], Isidro Gonzales[1], Herbert Saavedra[1], Hector H. Garcia[2], for the Cysticercosis Working Group in Peru .[2]

[1]*Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru*

Neurocysticercosis (NCC) is the most common cause of late-onset epilepsy in the world, but there is minimal information on its impact in patient's quality of life. The present study evaluated quality of life using the QOLIE (Quality of Life in Epilepsy)-31 scale in a series of patients with symptomatic epilepsy due to neurocysticercosis (NCC). Cross-sectional study of 155 patients 16-70 years of age with symptomatic epilepsy due to intraparenchymal NCC with viable cysts treated at the National Institute of Neurological Sciences (INCN) during the period 2006-2011 were applied the QOLIE-31 quality of life scale before receiving antiparasitic treatment. The number of epileptic seizures was recorded and grouped into categories. Bivariate analysis was performed using the Kruskal-Wallis test and multivariate analysis using a generalized linear model (GLM). The average quality of life score was 55.8 (SD ± 7.6), with 119 individuals (76.8%) qualifying as having poor quality of life. On bivariate analysis, a greater number of GTC or secondarily generalized epileptic seizures showed statistically significant association with a lower score in the quality

of life. The level of education showed a value of p=0.05. Other covariates such as sex, age, antiepileptic medication, number of parasitic cysts, and number of compromised brain regions did not show a significant association with quality of life. On multivariate analysis, a greater number of generalized epileptic seizures (GTC or secondarily generalized) maintained a statistically significant association with a detrimental quality of life score. Neurocysticercosis carries a significant impact in the quality of life of affected individuals. The number of prior generalized epileptic seizures was associated with deterioration in the quality of life in patients with symptomatic epilepsy due to intraparenchymal NCC.

1108

SEIZURE RELAPSE IN CALCIFIED NEUROCYSTICERCOSIS AFTER ANTIEPILEPTIC TREATMENT WITHDRAWAL

Javier A. Bustos[1], Gianfranco Arroyo[1], Isidro Gonzales[2], Herbert Saavedra[2], Armando Gonzalez[1], Robert H. Gilman[3], Hector H. Garcia[1], for the Cysticercosis Working Group in Peru .[4]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*Universidad Peruana cayetano Heredia, Lima, Peru*

Neurocysticercosis is the infection of the human central nervous system by the cystic larval stage of the tapeworm *Taenia solium.* As part of their natural life cycle or subsequent to antiparasitic treatment, brain cysts degenerate and resolve, either disappearing or leaving a small calcified lesion. Factors that increase the risk of seizure relapse after antiepileptic dug (AED) withdrawal in calcified NCC are poorly known. This study involves the participation of patients who withdrew AED treatment. In the parent cohort, participants had a detailed history and clinical examination. During the follow-up, the study team carefully recorded dates and characteristics of seizure episodes, periods seizure-free, and day and reason of AED discontinuation. Patients were monitored by telephone every two weeks and also asked to attend a clinical visit at the study site every three months and instructed to immediately report to the study team any event compatible with a seizure. The study physician and neurologist confirmed the information and classified seizures according to the ILAE guidelines. Sixty three participants who withdrew AED were included in this sub-study. Their mean age was 38 (SD: 13.1) years and there were similar proportions of males (49%) and females (51%). Along a median follow up of 18.5 months, 18 (28.6%) patients presented seizure relapses. The incidence rate was 15 events per 1,000 patient-months with a cumulative survival incidence of 57.0% [95% IC 41.4%-78.4%] along a maximum of 53 months of follow-up. A cox regression model shows that having more than two years free of seizures is the main protective factor (HR 0.13 95% IC 0.03-0.57 p= 0.007), and having a history of 10 or more seizures (HR 3.6 95% IC 1.22-10.63 p= 0.02) represents an important risk factor for seizure relapse. Patients with more than two years without epilepsy and a history of 10 or more seizure are in a higher risk of having a seizure relapse when AED are withdrawn and should be kept under AED.

1109

HEPATITIS E AS AN UNRECOGNIZED CAUSE OF MISCATALOGED DRUG-INDUCED LIVER INJURY IN NEUROCYSTICERCOSIS PATIENTS DURING TREATMENT

Jesus T. Abanto[1], Arantxa Sanchez[1], Javier A. Bustos[1], Isidro Gonzales[2], Yesenia Castillo[1], Richard G. Madden[3], Harry R. Dalton[3], Hector H. Garcia[1], for the Cysticercosis Working Group in Peru Lima - Peru[1]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [3]*Royal Cornwall Hospital and European Centre for Environment and Human Health, University of Exeter Medical School, Truro, United Kingdom*

Hepatitis E virus (HEV) is the most common agent of liver infection worldwide and a growing cause of liver enzyme elevation initially miscataloged as drug induced liver injury (DILI). Although this infection is usually subclinical, some factors such as immunosuppression, age and pregnancy may condition to a more severe presentation. It has multiple transmission pathways that includes but are not limited to fecal-oral transmission and pork consumption which are shared with *Taenia solium* infection. This leads us to believe that there could be a relationship between these two infections with emphasis in those patients that presented with DILI during NCC treatment. In this study we determined the HEV serological status of neurocysticercosis (NCC) patients that received antiparasitic treatment compared to non-NCC neurological patients and healthy rural villagers by ELISA. We found that 23.8% of NCC patients had positive IgG serology for HEV. Both non-NCC neurological patients and healthy rural villagers showed similar and lower frequencies compared to the NCC patients (14.6%- p=0.046 and 14.4% - p=0.040 respectively). We also found difference in IgG ELISA ratios were higher in patients who already had liver enzyme elevation before starting NCC treatment compared to those that elevated after starting treatment. Healthy rural villagers also showed higher IgG ELISA ratios compared to non-NCC neurological patients. There was no difference in liver enzyme values according to HEV serological status in NCC patients. These findings suggest that HEV is a common entity that should be studied in cases of DILI in Peru and that the common transmission pathways with *Taenia solium* may play a role in its association with NCC. Both infections are closely related to sanitation and are endemic in poor settings. High IgG ELISA ratios in healthy rural villagers may also suggest a higher exposure in settings with poor access to sanitation.

1110

EVALUATION OF MICROGLIAL ACTIVATION AND NEURONAL DAMAGE IN THE CALCIFICATION PROCESS IN PIGS WITH NEUROCYSTICERCOSIS

Laura E. Baquedano Santana[1], Javier A. Bustos[2], Gianfranco Arroyo[1], Noemi Miranda[1], Armando E. Gonzalez[3], Robert Gilman[4], Hector H. Garcia[2]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto Nacional de Ciencias Neurologicas, Lima, Peru,* [3]*Universidad Nacional Mayor de San Marcos, Lima, Peru,* [4]*Johns Hopkins University, Baltimore, MD, United States*

Neurocysticercosis NCC is a parasitic disease caused by the *Taenia solium* larval stage (cyst) in the central nervous system. It is the main cause of acquired epilepsy in endemic countries. Calcifications produced by this disease are associated with seizure in 20% of patients. The aim of this study was to evaluate the neuropathological findings of calcifications in the brain of pigs with NCC. We used 15 pigs naturally infected with *T. solium*and they received antiparasitic treatment. Then, were sacrificed 5 pigs at 4, 8 and 12 months post-treatment. Brains were perfused, removed and fixated in paraformaldehide. Samples were included in paraffin, sectioned and stained. Hematoxylin-Eosinand Masson's Trichrome were used to determinate the response inflammatory. Also, we use Von Kossa and Alizarin red stain to describe the presence of calcium deposits. Immunohistochemistry studies were evaluated with Anti-Iba1 (AB5076 polyclonal antibody; 1:400) and Anti-Neurofilament (NF) (monoclonal antibody, 2F11 clone; Dako). The pigs with NCC had a response inflammatory significant at different times after treatment. We found differences in collagen scarring, microglial reactivity, calcium accumulation and axonal damage in the classic morphological appearance of traumatic axonal injury in the three experimental groups. Histological examination showed in the group of 8 months a significant agglutination of calcium and the immunoreactivity was detected with high intensity around the parenchymal cyst. Our data provide novel insight into relationship between neuronal damage, microglial reactivity and the progression of calcification of the neurocysticercosis on the research of antiparasitic treatments.

1111

A CASE SERIES OF AN EXTENDED COURSE OF IRON TREATMENT FOR PRESUMPTIVE IRON-DEFICIENCY ANEMIA IN CHILDREN

John D. McLennan[1], Maria Mosquea[2]

[1]*Children's Hospital of Eastern Ontario - Research Institute, Ottawa, ON, Canada,* [2]*Servicio Nacional de Salud, Santo Domingo, Dominican Republic*

Childhood anemia is prevalent worldwide, particularly in low- and middle-income countries. Whereas iron-deficiency is known to be the most common etiological factor, optimal duration of iron treatment for iron-deficiency anemia in children is unknown. Whether greater resolution of anemia would be attained by extending the duration of iron treatment for those who do not fully recover with a standard 12-week course of iron treatment is unknown. This study examined the extent of hemoglobin (Hb) change in a cohort of anemic children who received a second course of iron treatment for persistent anemia in a low-income setting in the Dominican Republic. Data were available from a cohort of young children identified with anemia (Hb<11.0g/dl) within a well-baby clinic who had been offered two courses of iron treatment (using liquid ferrous sulfate) as they demonstrated persistent anemia after the first course. Analysis of data from 42 children (52.4% male; mean age at first Hb: 15.5 [SD 6.4] months) found minimal Hb increases after both the first and second 12-week courses of ≥3mg/kg/day of elemental iron, with mean Hb increases of 0.14 (SD 1.06) and 0.13 (SD 1.14) g/dl, respectively. A "probable iron deficient anemia" subgroup (n=22) defined as Hb≤10.0g/dl and Red Cell Distribution Width >15% had larger, though still modest, mean Hb increases, 0.40 (SD 1.16) and 0.50 (SD 1.36) g/dl, respectively. Extended iron treatment may have a role for those with "probable iron deficiency anemia" who have had an inadequate response to a standard course of treatment, however, other factors that may have accounted for the improvement over time in this subgroup in this study cohort cannot be ruled out. Controlled studies assessing different durations of iron treatment with more definitely defined iron-deficiency anemia samples are required.

1112

MORE THAN JUST A SIGN: THOMAS WINTERBOTTOM IN WEST AFRICA AT THE TURN OF THE 19TH CENTURY

David Adams, Valerie Adams

Point University, Midway, GA, United States

Thomas Winterbottom is perhaps best known for the eponymous Winterbottom's Sign. His travels in West Africa in the late 18th and early 19th century, however, are important for more than the bump that now bears his name. Indeed, his accounts of his journeys suggest an astute observer of cultures that bore little resemblance to his own. This presentation, based on Winterbottom's first-hand accounts and contemporary publications, will examine two aspects of his work. First, it will discuss its important medical aspect and, second, it will discuss his appreciation of foreign mores that he could have easily classified as alien and, perhaps, savage. Indeed, Thomas Winterbottom, the physician-anthropologist, was indeed a man ahead of his time.

1113

OXYGEN AVAILABILITY ON PEDIATRIC INPATIENT WARDS IN UGANDA AND EASTERN DEMOCRATIC REPUBLIC OF THE CONGO

Christopher E. Clarke[1], Robert O. Opoka[2], Namasopo Sophie[3], Kasereka Masumbuko Claude[4], Yiming Huang[1], Qaasim Mian[1], Michael Hawkes[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Makerere University, Kampala, Uganda,* [3]*Kabale Regional Referral Hospital, Kabale, Uganda,* [4]*Université Catholique du Graben, Butembo, Democratic Republic of the Congo*

Pneumonia is the leading cause of child mortality globally. Oxygen therapy is a vital component of treatment of pediatric pneumonia but is not available in many resource-limited settings. Previous studies have demonstrated that oxygen concentrators powered by solar panels are an effective alternative to oxygen cylinders in reducing mortality and recovery time among children with pneumonia. To determine if a gap in oxygen therapy exists, and the potential scalability of solar powered oxygen delivery in Uganda and Eastern Democratic Republic of Congo (DRC), we conducted a cross-sectional survey of oxygen delivery capabilities of pediatric wards at 55 district hospitals and health centers (39 in Uganda and 16 in the DRC). Between July 2017 and July 2018, we visited 55 health facilities in Uganda and Eastern DRC with inpatient pediatric services. While cylinder oxygen was available at 24/55 (44%) of sites, dedicated cylinders were not available on the pediatric wards of any health facility. Oxygen concentrators were present at 48 (87%) of sites. Of these, 37 concentrators (86%) were operational. Of these, only 18 (58%) produced oxygen with ≥ 80% purity. Additionally, only 23% of sites had oxygen concentrators available on pediatric wards. 39/54 (72%) of sites reported that power outages were frequent. While 49/55 (89%) of sites had generators available, only 9/49(18%) turned them on when children needed oxygen. Because electricity is necessary to run oxygen concentrators, this suggested that access to a reliable power source is a major obstacle in delivering oxygen therapy. Overall, only 28/55 (51%) of sites had oxygen available for pediatric use. These findings suggest that a high proportion of health facilities that treat children do not have reliable access to therapeutic oxygen. Solar powered oxygen delivery, generated autonomously on-site, may be a viable alternative.

1114

THE BURDEN OF SCABIES AND BACTERIAL SKIN INFECTIONS IN FIJI

Li Jun Thean[1], Lucia Romani[2], Aalisha Sahukhan[3], Mike Kama[4], Meciusela Tuicakau[4], Rachel Devi[5], Joseph Kado[6], Daniel Engelman[1], Natalie Carvalho[7], Adam Jenney[8], Handan Wand[9], Margot Whitfeld[9], Ross Andrews[10], John Kaldor[2], Andrew Steer[1]

[1]*Murdoch Children's Research Institute, Melbourne, Australia,* [2]*Kirby Institute, Sydney, Australia,* [3]*Fiji Centre of Communicable Disease Control, Suva, Fiji,* [4]*Twomey Hospital, Suva, Fiji,* [5]*Ministry of Health and Medical Services, Suva, Fiji,* [6]*Fiji National University, Suva, Fiji,* [7]*University of Melbourne, Melbourne, Australia,* [8]*Alfred Health, Melbourne, Australia,* [9]*University of New South Wales, Sydney, Australia,* [10]*Menzies School of Health Research, Brisbane, Australia*

Scabies in Fiji is highly endemic with a national prevalence of 23.6%. Scabies predisposes its host to impetigo which can progress to cellulitis, abscesses and more severe skin infections. Control of these complications and systemic bacterial diseases is a key motivation for scabies control, but there are few population-based data on their incidence in scabies endemic settings. In the context of a planned scabies intervention program, we conducted surveillance for hospital admissions and primary care presentations for scabies and bacterial skin infections in the Northern Division of Fiji (population 131,918). Surveillance for hospital admissions with skin or soft tissue infection was conducted at Labasa Hospital, the divisional referral centre. In addition, a monthly reporting system for scabies and bacterial skin infections was established at all primary health

facilities in the Northern Division. During the first 24 weeks of surveillance, which commenced in July 2018, there were 364 hospital admissions for skin and soft tissue infections (crude annual incidence 598 per 100,000 population). Incidence was highest in the 0-4 year age group (923 per 100,000). Of all admissions, 53.8% were male and median age was 31.6 years. The mean length of stay was 7.2 days and 65.9% required surgery. In the same period primary health facilities treated 5048 individual presentations for scabies or skin infections (annual incidence 8654 per 100,000), including 2736 (54.1%) for abscess, 1542 (29.2%) scabies, 1147 (22.7%) impetigo, 131 (2.6%) cellulitis and 46 (0.9%) severe skin infections. Of the impetigo cases, 47.3% also had scabies. Together, the hospital and primary care data demonstrate the huge inpatient and primary health burden of scabies and bacterial skin infections in this low resource setting. The annual incidence of hospital admissions for skin and soft tissue infection is demonstrably higher than that of neighbouring high-income countries (Australia has a crude incidence of 62 per 100,000). The proportion of these infections that are attributable to scabies, and therefore likely to be responsive to scabies control, remains to be established.

1115

THE EPIDEMIOLOGY AND CLINICAL FEATURES OF MELIOIDOSIS IN LAO PDR: A 17-YEAR PROSPECTIVE HOSPITAL BASED STUDY

Manophab Luangraj

Mahosot Hospital, Vientiane, Lao People's Democratic Republic

Burkholderia pseudomallei (*Bps*), an environmental Gram negative bacillus, is known to be present in soil and water in Lao PDR (Laos). The first case of melioidosis in Laos was diagnosed in 1999 by the Microbiology Laboratory of Mahosot Hospital. Since then, the number of cases diagnosed there has increased year by year. Tha aim was to review the epidemiology and clinical aspects of cases of culture-positive melioidosis diagnosed by the Microbiology Laboratory of Mahosot Hospital between 1999 and 2016. 1088 cases of melioidosis were identified during the study period. As elsewhere, the majority of the cases presented in the rainy season. Cases originated from every province in Laos except Luang Namtha, although the majority were from Vientiane Capital and Vientiane Province, the main catchment area for Mahosot Hospital. Ages ranged from 1 to 95 years (median 43 years). 639 (59%) were male. 314 (39.1% of adult cases) were rice farmers. The majority (987; 90.7%) presented with acute illness. 432 (39.7%) of patients were known to have underlying diabetes and a further 84 (7.7%) were diagnosed as diabetic during admission. Other underlying conditions included chronic kidney disease (67; 6.2%), kidney stones (62; 5.7%), steroid use (32; 2.9%), blood diseases (26; 2.4%) and joint diseases (16; 1.5%). More than half of the cases (602; 55.3%) had disseminated infection: 550 (50.5%) were bacteraemic, 401 (36.8%) had pulmonary involvement, 302 (27.8%) had cutaneous infection, and parotitis was present in 149 (52.5% of children). Liver and splenic abscesses were detected in 53 (4.8%) and 80 (7.4%) respectively. Joint involvement occurred 84 (7.7%), lymphadenitis in 66 (6%) and prostatic abscesses were only detected 8 (1.6% of adult male patients). There were also single cases of brain abscess and pericardial infection. The overall mortality was surprisingly low (288; 26.4%). In conclusion. this study confirms that Laos is highly endemic for Melioidosis. It is likely that the diagnosis is still being missed in much of the country.

1116

THE ETIOLOGY, NEUROIMAGING AND NEURODEVELOPMENTAL OUTCOME OF SEVERE FEBRILE ENCEPHALOPATHY IN MALAWIAN CHILDREN

Stephen TJ Ray[1], Charlotte Fuller[1], Elisabeth Stockdale[1], Reena Dwivedi[2], Karen Chetcuti[1], Christopher Moxon[3], Terrie Taylor[4], Yamikani Chimalizeni[4], Michael J. Griffiths[5], Karl B. Seydel[4]

[1]*Malawi-Liverpool-Wellcome Trust, Blantyre, Malawi,* [2]*Department of Neuroradiology, Salford Royal NHS Foundation Trust, Salford, United Kingdom,* [3]*Wellcome Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom,* [4]*Blantyre Malaria Project, Blantyre, Malawi,* [5]*Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom*

Febrile coma in children is a common presentation in sub-Saharan Africa. Historically, the majority of cases were attributed to cerebral malaria (CM), but with the recent drastic reduction in malaria incidence, non-malarial coma is a larger proportion, and determining the etiologies is often diagnostically challenging, particularly in resource-limited settings. Our aims were to (1) determine the etiology of febrile coma in Malawian children (2) determine if non-malarial coma has a higher mortality and/or morbidity than CM. This prospective case-cohort study, in Blantyre, Malawi, has been recruiting for 13 months. Subjects are febrile children aged 3 months to 14 years in deep coma (Blantyre coma score ≤2). We are investigating etiology via routine culture, PCR and metagenomic NGS on blood & CSF, assessing host response (RNA signatures/proteomics) and performing acute MRI and EEG. Detailed neuro-developmental outcomes are assessed 1 and 6 months post-discharge using locally validated tools. To date, we have recruited 128 participants (75 CM controls (59%) and 53 non-malarial cases (41%)). Non-malarial etiologies include 17 (13.2%) cases of acute bacterial meningitis, 7 (5.4%) encephalitides, 5 (3.9%) TB meningitis, 2 (1.6%) acute viral meningitis, 9 with an immediate post malaria neurological syndrome (7.0%) and 10 (7.8%) unknown encephalopathies. Of the 35 non-malarial cases who underwent MRI, 27 (77%) had informative CNS abnormalities, including rare diagnoses e.g. diffuse neurocysticercosis and ADEM secondary to *Salmonella typhi*. Mortality is higher in the non-malarial group (30% vs 12%), as are neurodisabilities ([median] moderate vs mild neurodisability), while developmental delays are comparable (46.8% vs 45.2%). Malaria remains the most common cause of coma in our setting, however non-malarial coma contributed more to the burden of mortality and morbidity. By using molecular diagnostics and neuroimaging, we were able to increase the syndromic classification of non-malarial coma from 7% to 64%. More detailed diagnostic characterization (NGS, transcriptomic, proteomic) of these cases and controls is underway.

1117

A NOVEL METHOD FOR ASSESSING THE CASE DEFINITION SENSITIVITY, SPECIFICITY AND OVERLAP BETWEEN CO-CIRCULATING INFECTIOUS DISEASES APPLIED TO DENGUE, CHIKUNGUNYA AND ZIKA

Laurel M. MacMillan[1], Corey B. Meyer[1], Mikaela A. Finnegan[1], Gautham Venugopalan[1], Landy T. Sun[1], Yaritbel Torres-Mendoza[2], Dianet Giraldo[1], Emily Billings[1], Shawn S. Jackson[1], Ana B. Gorini da Veiga[3], Gerardo Chowell[4], Neeraj Mistry[5], Margaret A. Rush[1]

[1]*Gryphon Scientific, Takoma Park, MD, United States,* [2]*University of Georgia College of Veterinary Medicine, Athens, GA, United States,* [3]*Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil,* [4]*School of Public Health, Georgia State University, Atlanta, GA, United States,* [5]*Department of International Health, Georgetown University, Washington, DC, United States*

Disease case definitions are used by public health professionals and medical practitioners to classify and diagnose illness in patients. Traditional methods for evaluating case definitions, which assess accuracy in differentiating between diseased and healthy populations, may not reflect real-world performance when multiple diseases with similar clinical presentations are co-circulating. To address this gap, we developed a novel method and metrics for evaluating multiple disease case definitions in tandem and piloted these methods by evaluating the performance of surveillance case definitions for dengue (DEN), chikungunya (CHIKV), and Zika (ZIK) used in the Americas. We estimated the prevalence of symptoms in each disease from clinical data, then used those data to simulate large patient populations for each disease. We classified each simulated patient by comparing their symptom profiles to DEN, CHIKV, and ZIK case definitions developed by WHO, the US, and several countries in the

Americas. Using these classified populations, we estimated the sensitivity and specificity of each case definition, as well as the overlap between case definitions developed by the same source (i.e., fraction of patients classified as having more than one disease). We found that DEN case definitions showed the highest sensitivity (range: 80%-97%), while CHIKV case definitions were less sensitive and more variable (range: 38%-84%). ZIK definitions showed the most variability in sensitivity (range: 14%-98%). Case definition specificity was low in all cases (DEN, 1%-18%; ZIK, 0.3%-64%; and CHIKV, 10%-50%). The percentage of patients classified as having more than one disease ranged from 67%-97% (DEN), 38%-86% (CHIKV), and 12%-84% (ZIK) indicating significant overlap between case definitions that may reduce the accuracy of surveillance. These methods and metrics can be applied to other sets of similar, co-circulating diseases such as tick-borne diseases or respiratory illnesses to better understand case definition performance and develop a foundation for using machine learning optimization methods to improve case definitions.

1118

EPIDEMIOLOGY AND OUTCOMES ASSOCIATED WITH FEBRILE ILLNESS DURING DEPLOYMENT AND TRAVEL

Tahaniyat Lalani[1], Kalyani Telu[1], Gregory Utz[2], Anjali Kunz[3], Drake H. Tilley[2], Heather Yun[4], Charla Geist[5], Christa Eickhoff[6], Jamie Fraser[1], Indrani Mitra[1], David Tribble[1]

[1]*Infectious Disease Clinical Research Program, Rockville, MD, United States,* [2]*Naval Medical Center San Diego, San Diego, CA, United States,* [3]*Madigan Army Medical Center, Tachoma, WA, United States,* [4]*Joint Base San Antonio, San Antonio, TX, United States,* [5]*Landstuhl Regional Medical Center, Landstuhl, Germany,* [6]*Naval Medical Center Portsmouth, Portsmouth, VA, United States*

The epidemiology of deployment and travel related febrile illness is relevant to travel medicine, global health and Force Health Protection. Several cohorts have evaluated febrile illnesses in travelers seeking medical care, but these studies underestimate the incidence and impact of common and/or self-limited febrile illnesses during travel. We evaluated the risk factors, syndromic profiles and outcomes of illnesses associated with a subjective fever in a prospective cohort of travelers and deployed military personnel, enrolled between 2010 and 2018 (TravMil). Subjects traveled to regions outside of the continental United States, Western or Northern Europe, Canada or New Zealand and completed a survey upon return. Poisson regression models with robust error variance were used to estimate the relative risk (RR) of a febrile illness. Of the 3938 adults enrolled pre-travel, 3089 (78%) completed a survey: 1660 (54%) travelers and 1429 (46%) deployed personnel. The incidence of febrile illness was 7.7% (n=239; 5.93 per 100 person-months; 95% CI: 5.18-6.68) with the following syndromes: febrile respiratory illness (35%), febrile diarrheal illness (32%) undifferentiated febrile illness (i.e. not associated with diarrhea or a respiratory infection - 19%). 14% reported more than 1 febrile illness. Travel duration > 10 days (RR: 1.68 [95% CI: 1.03-2.74]), partial or non-compliance with malaria chemoprophylaxis (RR: 1.38 [95% CI: 1.02-1.87]), fresh water exposure (RR: 1.47 [95% CI: 1.08-2.00]) and consuming food from street vendors (RR: 1.26 [95% CI: 0.92-1.70]) were associated with a febrile illness. The median duration of incapacitation was 2 days (IQR 1-4 days), and 24% (57/239) reported seeking medical care. Risk factors and incidence of febrile illness syndromes varied by region of travel. There was no significant difference in the duration of incapacitation or proportion of subjects seeking care by febrile syndrome or geographic region. The syndromic profile of febrile illness varies by geographic region and other risk factors and can inform strategies to reduce the burden of deployment and travel-related febrile illness.

1119

DETERMINANTS OF INAPPROPRIATE ANTIBIOTICS USE IN RURAL CENTRAL GHANA: A MIXED METHODS APPROACH

Samuel Afari-Asiedu[1], Ellen Boamah-Kaali[1], Martha Ali Abdulai[1], Stephaney Gyaase[1], Felix Boakye Oppong[1], Alma Tostmann[2], Marlies Hulscher[2], Kwaku Poku Asante[1], Heiman Wertheim[2]

[1]*Kintampo Health Research Centre/Ghana Health Service, Kintampo, Ghana,* [2]*Radboud University Medical Center, Nijmegen, Netherlands*

The consequences of antibiotic resistance is projected to be high due to inappropriate use in developing countries. This study examined determinants of inappropriate antibiotic use at the community level in rural Ghana. Inappropriate antibiotic use include taking antibiotic without prescription, not completing treatment and noncompliance with instructions for use. A mixed methods study was conducted among community members in Kintampo Districts of Ghana from July 2016 to September, 2018. Initial 16 In-depth Interviews (IDI) and 6 Focus Group Discussions (FGD) were conducted. Results from the IDI and FGD were used to design surveys, involving two contacts (6 month apart) with 1,100 randomly selected households over one year. The survey focused on antibiotic use in the past 1 month before the interview. Additional 4 IDI and 2 FGD were performed to explain the survey results. Qualitative data were coded with Nvivo 10 and thematically analyzed. Determinants of inappropriate antibiotic use were determined by chi-square test and multivariate logistic regression analysis. A total of 1,100 households were contacted and antibiotic use were identified in 585 (53.18%) households in the one year period. From these 585 households, 676/3193 (21.17%) members used antibiotics for 761 disease episodes. Of the 761 antibiotic use episodes, 659 (86.60 %) were used inappropriately. Overall, 489 (64.26 %) used antibiotics without prescription, 345 (45.34%) did not complete treatment and 336 (44.15%) did not follow instruction for use. Socioeconomic status, place of residence, paying for healthcare with national health insurance (OR: 0.26, 95% CI: 0.10-0.68), obtaining medicines from the pharmacy (OR: 0.24, 95% CI: 0.10-1.60) and drug peddlers and cost of antibiotics were predictors of inappropriate antibiotic use. The high inappropriate antibiotic use was influenced by socioeconomic status, place of residence, acquiring antibiotics from pharmacy and drug peddlers, paying for healthcare with national health insurance and cost of antibiotics. Continuous public health education on appropriate antibiotic access and use in rural settings is required.

1120

SCALING UP THE USE OF HEAD START IN NIGER: EVALUATION AND RE-TRAINING OF 133 TRICHIASIS SURGEONS

Chano Hamiden[1], Mahamane Abdou[1], **Hadiara Adamou**[2], Kadri Boubacar[1], Tchouloum Toudja[2], Youssouf Yayé[2], Josette Vignon[2], Abdou Amza[1], Amy Veinoglou[3], Yaobi Zhang[4]

[1]*National Eye Health Program, Ministry of Health, Niamey, Niger,* [2]*Helen Keller International, Niamey, Niger,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Providing quality surgical services to trichiasis (TT) patients is one of the critical components in achieving trachoma elimination. Niger adopted the use of the HEAD START surgical mannequin in 2016 to improve TT surgeons' operating skills and ultimately increase the quality of TT surgeries. An evaluation conducted in 2016 in the Maradi region highlighted the advantages of using HEAD START for training of both new and experienced TT surgeons who were already trained in the TRABUT method. Between January and July 2018, the majority of surgeons in five regions of Niger (Diffa, Dosso, Tahoua, Maradi and Zinder) were re-trained using HEAD START and their skills evaluated by a master trainer. Each surgeon first conducted surgery on a human eyelid under the observation of the master trainer during a regularly scheduled TT surgery campaign. The HEAD START mannequin was then used to correct any deficiencies noted during the surgery and before the next operation on a human

eyelid. Following capacity building with HEAD START, the master trainer ranked surgeons' skills, assigning a score varying from 1 to 5 to each trainee according to a surgical skills evaluation sheet. Eleven skills were evaluated, ranging from the use of sterile gloves to the correctness of the incision and suturing. Each surgeons' final score was the average combined score for all 11 skills, with a score of 3-5 considered acceptable. A total of 133 surgeons were evaluated (6 in Diffa, 8 in Dosso, 6 in Tahoua, 41 in Maradi and 72 in Zinder region). Of these, 108 (81%) had a score greater than or equal to 3 (acceptable), and 25 (19%) had a score less than 3 (unacceptable); these 25 surgeons are targeted for further re-training. These results validate the use of HEAD START as a training tool to improve the skills of experienced TT surgeons and ultimately enhance the acceptability of surgery by TT patients and communities.

1121

RISK FACTORS ASSOCIATED WITH INCREASED MORTALITY FROM INTUSSUSCEPTION IN AFRICAN INFANTS

Talia Pindyck, Umesh Parashar, Jason Mwenda, Jacqueline Tate

Centers for Disease Control and Prevention, Atlanta, GA, United States

Intussusception, or the invagination of one segment of the intestine within another segment, has been associated with rotavirus vaccination in high- and middle- income countries. Because mortality from intussusception is higher in Africa than in other regions of the world, we examined potential risk factors to help inform the implementation of rotavirus vaccines in the region. Infants with intussusception from 7 sub-Saharan African countries (Ethiopia, Ghana, Kenya, Malawi, Tanzania, Zambia, and Zimbabwe) were enrolled through active, hospital-based surveillance from February 2012 to December 2016. We examined demographic, clinical, and socioeconomic factors associated with death or intestinal resection following intussusception, using multivariable logistic regression. A total of 1020 infants <1 year of age with intussusception were enrolled. Overall, 13% of children (133/1020) died during the hospitalization, and 48% (467/969) required intestinal resection. The median duration of symptoms prior to hospitalization at the sentinel facility was 3 days (interquartile range: 1-4), and the median duration of symptoms prior to hospitalization at any hospital was 2 days (interquartile range: 0-3). In multivariable analyses, female sex (OR 1.8, 95% CI 1.2-3), longer duration of symptoms prior to presentation (OR 1.1; 95% CI 1.0-1.2), and undergoing intestinal resection (OR 3.3; 95% CI 1.9-5.9) predicted death after intussusception. Radiologic diagnosis (OR 0.4; 95% CI 0.3-0.7), presence of electricity at home (OR 0.5, 95% CI 0.3-0.7), and employment by a household member (OR 0.6; 95% CI 0.4-0.9) were associated with a reduced likelihood of undergoing intestinal resection. Delays in hospital presentation and female sex were significantly associated with death, whereas higher socioeconomic status and availability of radiologic diagnosis reduced requirement for intestinal resection. Efforts are needed to improve the awareness, diagnosis, and management of intussusception in sub-Saharan Africa, especially in resource poor settings.

1122

CHARACTERIZATION OF DENV-2 INFECTIONS OCCURRING IN A DENGUE ENDEMIC AREA AND PRESENTING WITH AN INCREASED NUMBER OF SEVERE CASES

Benedito A. Fonseca, Marcus Vinicius G. Silva, Mayara R. Agostinho, Luiza A. Castro-Jorge, Taline M. Klein, Flavia M. Moraes, Marcio J. Siconelli, Vitor G. Floriano, Beatriz S. Ribeiro, Daniel M. Jorge, Daniel C. Araujo, Luzia M. Passos, Danielle C. Gentil

School of Medicine of Ribeirão Preto, Ribeirão Preto, S.P., Brazil

Dengue viruses (DENV) constitute a significant public health problem to tropical and subtropical regions of the world. Ribeirão Preto, a city in the northeast region of São Paulo state, Brazil, is endemic for dengue virus infections and serotype circulation has changed over the years. In the last decade, intense dengue serotype circulation has been as follows: DENV-3 (2008-2010), DENV-1 (2010-2012; 2014-2018), and DENV-4 (2012-2014). DENV-2 circulated at very low levels in 1998, 2001, and 2009, and this has implicated in a large number of dengue-experimented persons susceptible to DENV-2 infections. In 2019, an ongoing clinical and virologic surveillance detected an increased number of severe dengue cases associated to a new introduction of DENV-2. From January to March, acute-phase serum samples from dengue patients with positive NS1 antigen detection were assayed for serotype identification by RT-qPCR. All samples were positive for DENV-2. Men and women were equally distributed (23/46; 50%) among the patients. The most frequent symptoms were fever (95%), headache (89%) and myalgia (78%), nausea (57%), back pain (54%), retro orbital pain (35%), rash (32%), arthralgia (32%), vomit (30%) and arthritis (19%). Seven (15%) patients were hospitalized either to alarm signs (67%) or severe dengue (33%). All hospitalized patients had thrombocytopenia and the most frequent alarm sign was severe abdominal pain. Patients with severe dengue had dengue hemorrhagic fever (grade I or II). Secondary dengue infection was detected in 59% of the hospitalized patients. Additionally, molecular studies show that the DENV-2 genotype responsible for the current outbreak belongs to the Asian/American genotype, the same as the 2009 outbreak. However, the current DENV-2 genotype has 26 synonymous and 1 non-synonymous mutation in the prM-E nucleotide sequence compared to the virus that circulated in 2009. Increased severity of dengue cases observed in the current outbreak is probably due to a combination of higher prevalence of dengue secondary infections and an increased virulence of DENV-2 circulating genotype.

1123

ELEVATED ACTIVATION OF NEUTROPHIL TOLL-LIKE RECEPTORS IN PATIENTS WITH ACUTE SEVERE LEPTOSPIROSIS: AN OBSERVATIONAL STUDY

Janet C. Lindow[1], **Annie J. Tsay**[1], Ruth R. Montgomery[2], Eliana AG Reis[3], Elsio A. Wunder Jr[1], Guilherme Araújo[3], Nivison RR Nery Jr[3], Subhasis Mohanty[2], Albert C. Shaw[2], Patty J. Lee[2], Mitermayer G. Reis[3], Albert I. Ko[1]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*Yale School of Medicine, New Haven, CT, United States,* [3]*Oswaldo Cruz Foundation, Salvador-Bahia, Brazil*

Leptospirosis is the leading cause of zoonotic morbidity and mortality globally, yet little is known about immune mechanisms contributing to pathogenesis and severe disease. Our pilot study examined immunopathologic role of neutrophils in severe leptospirosis by quantifying neutrophil activation markers during acute infection and convalescence in patients with different disease severities. Between July 2013 and August 2014, we identified 19 patients through active surveillance at a state-run infectious disease hospital in Salvador, Brazil. Among our cohort, 15 (79%) of 19 had laboratory-confirmed leptospirosis and 4 had febrile illnesses presenting similarly. We recruited 4 healthy Brazilian adults as controls. We collected baseline clinical characteristics and blood samples for the 19 patients 72-96h of hospital admission (acute samples) and 4 paired convalescent samples (32-57d post-admission). We categorized leptospirosis patients into 2 groups using severity of renal and/or pulmonary dysfunction. We quantified neutrophil activation markers, CD11b (β2 integrin) and CD62L (L-selectin), and assessed activation capacity (neutrophil marker levels following stimulation with Toll-like receptor agonists) for all samples using multi-parameter flow cytometry. Additionally, we assessed the levels of TLR2, TLR4 on neutrophils. We analyzed data from 50,000 $CD15^+$ cells and report percent of $CD15^+$ cells expressing activation markers or the fold-change in median fluorescent intensity (MFI). We observed no significant differences in neutrophil baseline activation or activation capacity regardless of disease severity (acute: 78.5% (75.5-84.3); severe: 86.0% (80.0-87.5)). However, patients with severe organ dysfunction had significantly higher TLR2, TLR4 expression than patients without organ dysfunction. Higher bacterial loads in whole blood positively correlated with higher expression of TLR2, TLR4 (TLR2: r=0.81 [0.47-0.94], TLR4: r=0.64 [0.13-0.88]). Our findings indicate higher initial bacterial loads or delayed neutrophil responses rather than TLR-driven inflammation, may drive severe disease outcomes.

1124

SOCIO-ECONOMIC BEHAVIORAL INDICATORS OF FALCIPARUM MALARIA PARASITEMIA AND MODERATE TO SEVERE ANEMIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN LAGOS, SOUTHWEST NIGERIA

Adeola Y. Olukosi[1], Oluwagbemiga O. Aina[1], Abiodun kanmi Olakiigbe[1], Olusola S. Ajibaye[1], Bassey A. Orok[1], Samuel Akindele[1], Adebayo T. Onajole[2], Samson T. Awolola[1], Tolulope Moji Arowolo[1], Bamgboye M. Afolabi[3]

[1]*Nigerian Institute of Medical Research, Lagos, Nigeria,* [2]*College of Medicine University of Lagos, Lagos, Nigeria,* [3]*Health, Environment and Development Foundation, Lagos, Nigeria*

Incidence of malaria and anemia are of public health importance especially in pregnant women in endemic regions, due to the negative health consequences to mother and fetus. The aim of this study was to assess the pattern of falciparum malaria infection and anemia, based on malaria prevention methods practiced by participants. A semi-structured tool was used to capture information on demographic, socio-economic and malaria prevention practices from 113 pregnant women attending antenatal clinics in 2 peri-urban health facilities in Lagos, Southwest Nigeria. Malaria microscopy was conducted and hematocrit measured. Logistic regression analysis was performed on the data collated from the survey. The prevalence of anemia among the pregnant women was 87.2%. The mean (±sd) Packed Cell Volume (%) of the 22 (19.5%) infected subjects (26.8±6.6), was significantly lower (t=-2.60, P-value=0.007) than that of the 91 (80.5%) uninfected subjects (30.8±6.0). The prevalence of infection was highest in the 3rd trimester (n=40, 35.4%) at 27.5% (11/40) and among those in their first pregnancy (n=32, 28.3%) at 25.0% (8/32). There was a significant difference (t=-2.23, P-value=0.01) in the mean PCV% of pregnant women who consumed herbal teas in pregnancy (28.2±5.2) compared to those who did not (30.8±6.6). Regression analysis showed that first pregnancy, "antimalarial" use and "Insecticide-treated nets" use night before study had increased odds of malaria infection in participants (OR=1.35, P=0.006, 95% CI=0.52-2.49; OR=2.3, P=0.005, 95% CI=0.14-0.41; OR=1.92, P=0.001, 95% CI=0.62-5.98) while IPT participation and formal education were strongly and significantly associated with lower risk of parasitemia (OR = 0.95, P= 0.025, 95% CI= 0.41-2.26; OR = 0.44 P= 0.005, 95% CI= 0.34-10.50). Interventions that will reduce malaria and moderate to severe anemia, especially in first pregnancy, should include education on correct use of LLIN, IPT and avoiding the dangers of herbal medication in pregnancy.

1125

PUTTING THE THREE DELAYS MODEL TO WORK: A PRAGMATIC 12-MONTH COMMUNITY-BASED COHORT STUDY TO ASSESS ACCESS TO EMERGENCY OBSTETRICAL AND NEONATAL CARE IN A REMOTE ISLAND COMMUNITY IN WESTERN KENYA

Nicholas DesLauriers[1], Evance Ogola[2], Gor Ouma[3], Brian Mattah[3], Louisa Ndunyu[2], Lily Muldoon[4], Richard Magerenge[3], Peres Okinyi[3], Marcus Salmen[3], Kelsi Hines[3], Robinson Okeyo[3], Ben Pedersen[5], John Ssenkusu[6], Shailey Prasad[1], Molly McCoy[1], Walter Opiyo[3], Hanna Nedrud[1], Kelsey Finn[1], Charles Salmen[1]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Maseno University, Maseno, Kenya,* [3]*Ekialo Kiona Centre, Kitawi Beach, Kenya,* [4]*University of California San Francisco, San Francisco, CA, United States,* [5]*Oregon Health and Sciences University, Portland, OR, United States,* [6]*Makerere University, Kampala, Uganda*

Mfangano Island is a remote fishing community in Lake Victoria, Kenya. Due to a fragmented health system, its residents experience critical delays in accessing emergency care as well as some of the highest maternal mortality rates in Kenya. In response, the Ekialo Kiona community-based organization developed the Health Navigation Program (HNP): a network of Community Health Volunteers (CHVs) who promote timely referral to health facilities and coordinate transfers to the mainland via a 24/7 on-call emergency boat. By applying the "Three Delays" framework, this rolling cohort study recruits all maternal and neonatal emergency cases referred by clinical staff to the mainland for higher level care or which resulted in a death on the island. A participatory case "audit" is completed with each participant after hospital discharge, capturing a narrative from symptom onset to definitive care, and measuring the three time delays (i.e. recognizing need, reaching care, receiving care) with a novel visual analog scale of events. The scale uses frames of reference such as sunrise and sunset to hone accuracy of recollection, and its validity will be assessed by comparing responses to real-time SMS timestamps sent by CHVs during cases. Delays will be compared between cases assisted by the HNP in participating communities and those proceeding through the pre-existing referral system alone. Preliminary data shows average time delay from symptom onset to reaching a facility is 11.7 hours (*N*=10, range 2-31.5), with delay from symptom onset to deciding to seek care averaging 10.2 hours. The delay from reaching a facility to initiation of definitive care on the mainland averages 14.9 hours (*N*=9, 6-25), with the delay from reaching a facility to departing the island after referral averaging 9 hours. Total delays from symptom onset to initiation of definitive care average 26.8 hours (*N*=9, 14.25-47). Developing practical ways to measure the "Three Delays", understanding barriers contributing to pre- and inter-facility delays, as well as the impact of the HNP, will inform interventions to improve access to emergency care in remote communities.

1126

CARDIAC INVOLVEMENT IN SCRUB TYPHUS IN A NORTHWESTERN INDIAN HOSPITAL

Navneet Sharma, Manisha Biswal, Manoj Kumar Debnath, Jyotdeep Kaur, Ashish Bhalla, Rajesh Vijayvergiya

PGIMER, Chandigarh, India

Scrub typhus (ST) is a disease caused by the bacterium *Orientia tsutsugamushi* transmitted to humans by the bites trombiculid mites. Cardiac involvement though less reported is being increasingly seen. A prospective study was carried out in 70 cases of ST (ELISA IgM antibodies against *O. tsutsugamushi)*. Measurements carried out in all cases were, left ventricular ejection fraction (LVEF) by 2D-Echocardiography and levels of troponin-T and serum creatine kinase-MB. The mean age was 34 ± 15 years and the male female ratio was 4:3. The mean duration of fever before presentation was 7.6±3.7 days. The most common symptoms were dyspnea 70%, altered sensorium 30%, abdominal pain 28.6%, decreased urine output 22.9%, jaundice 20% and headache 18.6%. Other clinical observations were tachycardia in 95.7%, tachypnea in 78.5%, splenomegaly in 77.1%, hepatomegaly in 67.1%, eschar in 12.8% and hypotension in 7.1%. On investigations, anemia occurred in 86%, leukocytosis in 47%, thrombocytopenia in 88% and elevated serum transaminase levels in 93% cases. On abdominal ultrasound, hepatomegaly was detected in 67.1%, splenomegaly in 77.1% and ascites in 22.9%. Complications seen were; acute respiratory distress syndrome in 87.1% and acute kidney injury in 38.5%. Although 2D Echocardiography showed a reduced LVEF in 42.8%, yet, clinical congestive heart failure was evident in 4.2% cases. The mortality rate was 7.1% (5 cases). On statistical analysis, a significant correlation was observed between the age and reduced left ventricular ejection fraction. The troponin-T level elevation in 72.8% and creatine kinase-MB elevation in 41.4% correlated with a decrease in the LVEF. Furthermore, patients with an elevated creatine kinase-MB levels also had an increased length of hospital stay. In conclusion, cardiac involvement occurred in 42.8% patients of ST, affected the younger age group and was easily recognisable with 2D Echocardiography showing a reduced ejection fraction and elevated levels of troponin-T and creatine kinase-MB.

1127

EFFICACY OF *IPOMEA PES-CAPRAE* OINTMENT AS AN ADD-ON THERAPY FOR JELLYFISH DERMATITIS: FINAL RESULT OF A SELF-CONTROLLED CLINICAL TRIAL

Watcharapong Piyaphanee[1], Vorada Choovichian[1], Keawmala Palakul[1], Jutarmas Olanwijitvong[1], Thitiya Ponam[1], Wasin Matsee[1], Thanasawat Chaiyakul[2]

[1]*Faculty Of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [2]*Naval Medical Department, Royal Thai Navy, Bangkok, Thailand*

Jellyfish sting is an important risk for among travelers who expose to seawater. In tropical countries, *Ipomea pes-caprae,* a medicinal plant, has been known as an effective treatment for jellyfish dermatitis. However, no clinical trial has been done to prove its efficacy. This was an open label, self-controlled clinical trial of *Ipomea pes-caprae* ointment in patient with jellyfish dermatitis. Adult patients with the onset of jellyfish dermatitis less than 7 days were invited to join. The investigator divided the dermatitis area of each patient into two parts (Test and Control area). Each patient received standard treatment depended on the severity of dermatitis in both areas. *Ipomea pes-caprae* ointment was applied only to the "test area" as an add-on therapy. Participants were re-evaluated 6 times in the 28-days study period. Primary outcome was the recovery time (non-active skin lesion) of both areas. The secondary outcomes were the duration of pain and itching after the treatment. Sixty patients with jellyfish dermatitis were enrolled. Their median age was 32 years, the majority of participants exposed to jellyfish on the day of enrollment. In total, 90% received topical steroid, 50% received oral antihistamine while 18% received oral prednisolone as standard treatment. The *Ipomea pes-caprae* ointment was applied to all patients but only on the test area. Time to non-active skin lesion in test area was shorter than control area (5.65 days VS 6.17 days, p=0.009). The duration of itching in test area was significantly less than the control area (3.33 days VS 3.73 days, p=0.044). However, there was no difference in duration of pain between test area and control area. Overall skin outcome (healing with/without scar) was the same in test and control area. We can conclude that *Ipomea pes-caprae* ointment was an effective add-on therapy in the treatment of jellyfish dermatitis. The recovery time and duration of itching in area that applied with the ointment were less than the control area. However, the overall skin outcome was the same in both areas.

1128

PROTEIN-ENERGY MALNUTRITION IN COMMUNITY-BASED EDUCATION AND SERVICE (COBES) CENTERS IN WESTERN KENYA DURING THE PERIOD 2017-2018

Arthur M. Kwena

Moi University, Eldoret, Kenya

COBES programme enables students to participate in various community projects during the period of their study at the College of Health Sciences of Moi University. In their second year of study, they are attached to 20 health Centres for a period of six weeks where they participate in community entry and Diagnosis. They determine the nutritional status of the community in which they are attached. The results reported here are for the latest data collected during the period 2017-18. The Broad objective of the study was to determine the current nutritional status in COBES centres in Western part of Kenya. Cross-sectional studies were carried out in March and May 2017 and 2018 in 20 COBES Health Centres in Western Kenya. Cluster sampling technique was used with each Health centre as the sampling unit. Anthropometric measurements were performed on all children aged 5-59 months within the households sampled. The nutritional status of the children was determined using the WHO recommended Z- score values as well as the Kenya Government Ministry of health recommended charts based on anthropometric measurements. Analysis of nutritional data was carried out using Epi-info 2000 program to determine the Z- score values from anthropometric data. A total of approximately 700 children were sampled in the seven Health Centers: (Stunting- HAZ<-2, Wasting-WHZ <-2, underweight -WAZ<-2 and MUAC, < 12.5mm). Results showed anthropometric measurements taken to fall within the WHO recommended reference values for ' normal' nutrition as exemplified by Kabuchai (WHZ>-1, WAZ>-1, HAZ >-1 and MUAC >-1) and Mosoriot Health Centres (WHZ> -2, WAZ=0, HAZ >-1, MUAC >-1). Preliminary published results indicated that Meteitei had the highest malnutrition prevalence (53% HAZ, 15% WHZ, 27% WAZ and 18.1 MUAC) whereas Chulaimbo showed the lowest prevalence (7% HAZ, 3% WAZ). The other centres showed mixed prevalence. Most COBES Health Centres showed improved nutrition status compared to previous results that showed one or two Centres to have extremely high malnutrition status.

1129

OCCURRENCE OF TYPHOID FEVER COMPLICATIONS AND ASSOCIATED RISK FACTORS: A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS

Ligia Maria Cruz Espinoza[1], Ellen McCreedy[2], Marianne Holm[1], Justin Im[1], **Ondari Mogeni**[1], Prerana Parajulee[1], Ursula Panzner[1], Se Eun Park[1], Trevor Toy[1], Andrea Haselbeck[1], Hye Jin Seo[1], Hyon Jin Jeon[1], Jong Hoon Kim[1], Soo Young Kwon[1], Jerome H. Kim[1], Christopher M. Parry[3], Florian Marks[1]

[1]*International Vaccine Institute, Seoul, Republic of Korea,* [2]*Center for Gerontology and Healthcare Research, School of Public Health, Brown University, Providence, RI, United States,* [3]*Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom*

Complications from typhoid fever (TF) disease have been estimated to occur in 10 to 15% of hospitalized patients with evidence of a higher risk in children and when there is a delay in implementing effective antimicrobial treatment. This study estimated the prevalence of complications in hospitalized, culture-confirmed TF patients, and the effects of symptom duration and age on the prevalence of complications. A systematic review and meta-analysis were performed using studies in the PubMed database. Any unfavourable evolution of the disease reported as a complication in a TF patient was considered a complication. We rated risk-of-bias of relevant publications and conducted random-effects meta-analyses. Analyses were stratified by symptom duration at hospital admission (SDA, <10 and ≥10 mean or median days of disease) and by age groups (children and adults). Differences in risk between SDA and age groups was assessed using odds ratios (OR) and 95% confidence intervals (CI). Heterogeneity and publication bias were evaluated. Twelve studies were included in the meta-analysis. The pooled prevalence of complications estimated among hospitalized TF patients was 25% (95%CI:20–29%, I^2= 88.2%, p=0.000). The stratified analysis revealed a higher prevalence of TF complications among cases reporting SDA≥10 (36%, 95%CI:29–43%) compared to cases with SDA<10 (16%, 95%CI:13–18%), translating into a three times greater risk of complications for those with prolonged symptoms duration at hospital admission (OR=3.00, 95%CI:2.14–4.17, p<0.0001). Similar higher risks were observed in specific complications. Likewise, a higher prevalence of complications was observed in children (27%, 95%CI:19–35%) compared to adults (17%, 95%CI:9–25%), however, the increased risk was not significant (OR=1.15, 95%CI:0.89–1.49, p=0.247). This review found limited quality evidence concerning TF complications. The meta-analysis identified a higher overall prevalence of complications than previously reported and a strong association between duration of symptoms prior to hospitalization and risk of serious complications.

1130

CLINICAL MANIFESTATIONS AMONG INDIVIDUALS BITTEN BY TRIATOMINES (KISSING BUGS) IN SOUTHERN ARIZONA

Norman L. Beatty[1], Nicole Behrens-Bradley[2], Shannon Smith[1], Maria Love[2], Justin O. Schmidt[3], Patricia L. Dorn[4], Nafees Ahmad[2], Stephen A. Klotz[1]

[1]*University of Arizona College of Medicine, Department of Medicine, Division of Infectious Diseases, Tucson, AZ, United States,* [2]*University of*

Arizona College of Medicine, Department of Immunobiology, Tucson, AZ, United States, [3]The Southwestern Biological Institute, Tucson, AZ, United States, [4]Loyola University New Orleans, Department of Biological Sciences, New Orleans, LA, United States

We surveyed residents (N=105) who were ≥18 years old from southern Arizona who had been bitten by triatomines (kissing bugs) to gather information regarding their experience. This included knowledge of the insect, behavioral changes after being bitten, and clinical manifestations of the bite. Median age was 58 years and 64% were female. Estimated total number of bites was >2,200 among the group (16% bitten once; 23% bitten between 2-4 times; 22% bitten between 5-10 times; 34% being bitten between 11-50 times; 5% bitten > 50 times). Most had been bitten while sleeping (N=90/105), with 62% sustaining more than one bite in the same night. The most common bite locations were in the extremities (legs, 54.3%; arms, 49.5%; back, 35%; face, 15%). Approximately one-third changed their sleeping habits as a result of a triatomine bite, and 14 individuals (13%) report now sleeping under mosquito nets. A large majority (N=91/105) experienced at least one localized cutaneous symptom (75% itchiness at bite site; 74% developed an erythematous welt; 55% experienced regional swelling), but only 19% described pain after being bitten. Systemic symptoms included a feeling of unease (N=23/105), diffuse pruritus (N=22/105), rash (N=20/105), shortness of breath (N=13/105), dizziness (N=12/105), and anaphylaxis (N=11/105). Seven individuals had one episode of anaphylaxis, one person had two episodes, one had three episodes, and two had greater than ten episodes in their lifetime. Those who had previously experienced anaphylaxis were now sleeping with an epinephrine autoinjector by their bedside. Thirty seven people (35%) took an over the counter medication in an attempt to alleviate the symptoms after being bitten. When asked to identify the three most common triatomines in Arizona amongst a panel of similar insects from the region, 93%, 91%, and 78% were able to correctly identify *Triatoma rubida, T. recurva,* and *T. protracta*. Bite exposure to triatomines in southern Arizona is common. Unlike the name "kissing bug" suggests, most individuals were bitten on the body. Bites can cause serious allergic reactions, including anaphylaxis, which can be fatal.

1131

A SINGLE CENTER, OPEN LABEL PILOT STUDY TO EVALUATE THE SAFETY AND EFFICACY OF CC-11050, A NOVEL PHOSPHODIESTERASE 4 INHIBITOR, IN NEPALESE PATIENTS WITH *ERYTHEMA NODOSUM LEPROSUM*

Mahesh Shah[1], Divya RSJB Rana[2], Kapil D. Neupane[2], Ken Arakawa[3], David M. Scollard[4], Preeti Maharjan[2], Vikram Khetani[3], Indra B. Napit[1], Deanna A. Hagge[2]

[1]*The Leprosy Mission Nepal, Anandaban Hospital, Kathmandu, Nepal,* [2]*The Leprosy Mission Nepal, Mycobacterial Research Laboratories, Kathmandu, Nepal,* [3]*Celgene Global Health, Summit, NJ, United States,* [4]*Department of Health and Human Services, Health Resources and Services Administration, Health Systems Bureau, National Hansen's Disease Programs (Retired Director), Baton Rouge, LA, United States*

Erythema Nodosum Leprosum (ENL) is an immunological complication that can occur before, during or even years after curative antibiotics in those affected by borderline lepromatous (BL) or lepromatous leprosy (LL). While prednisolone and thalidomide are recognized treatments for ENL, most patients require extended treatment, both drugs have significant side effects, and thalidomide is restricted or prohibited in some countries. Phosphodiesterase 4 inhibitors (PDE4i) are a class of compounds that affect cytokine production, neutrophil function, and antigen processing and presentation in pathways potentially relevant to ENL. Acting as a PDE4i, CC-11050 is a non-steroid, anti-inflammatory drug with few side effects that has been previously trialed and shown to be safe in humans. This study is a single center, phase 2, open-label trial, to be performed in two steps. Step 1 will evaluate immediate effect in safety and efficacy of CC-11050 treatment over 10-28 days in 10 males with a new or new recurrent episode of ENL. Upon review of Step 1 safety analyses and efficacy data by the Data Safety and Monitoring Committee (DSMB), a decision will be made whether to proceed to Step 2: an additional 40 ENL patients enrolled for up to 52 weeks of CC-11050 treatment. To date (April 10, 2019), 5 male subjects have been enrolled. According to preliminary observations, CC-11050 has been found safe and clinical improvements were observed in the first five subjects. Based on consensus between the study principal investigator and DSMB, the medication of the first five patients was extended from 10 to 28 days. No serious adverse events have occurred. The last therapeutic agent for ENL treatment was developed about 50 years ago; and there is an imperative need for new therapies with fewer side effects to be identified. DSMB review of CC-11050 Step 1 (10 patients) will determine progression to Step 2 (40 patients) of the study. Data analyses from ongoing enrolment is in process.

1132

ANTIBIOTICS AND ANTIMALARIAL DRUGS FOR CHILDREN AGED <5 YEARS HOSPITALIZED WITH ACUTE FEBRILE ILLNESS IN KENYA

Nailah Smith[1], Eric Ng'eno[2], Eric Osoro[2], Peninah Munyua[3], Doris Marwanga[4], George Agogo[3], Godfrey Bigogo[4], Victor Bandika[5], Paul Etau[6], John Wagacha Burton[7], John Kiogora[8], Terrence Lo[1], Lynda Makayotto[9], Joel M. Montgomery[1], Marc-Alain Widdowson[3], Jennifer R. Verani[3]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Washington State University, Nairobi, Kenya,* [3]*Centers for Disease Control and Prevention, Nairobi, Kenya,* [4]*Kenya Medical Research Institute, Nairobi, Kenya,* [5]*Coast Provincial General Hospital, Mombasa, Kenya,* [6]*Kenyatta National Hospital, Nairobi, Kenya,* [7]*United Nations High Commissioner for Refugees Kenya, Kakuma, Kenya,* [8]*International Rescue Committee, Kakuma, Kenya,* [9]*Kenya Ministry of Health, Nairobi, Kenya*

Acute febrile illness (AFI) is a common cause for pediatric hospitalizations in low- and middle-income countries and a major driver of antibiotic and antimalarial drug use; yet data on inpatient management of AFI in Africa are limited. We aimed to characterize antibiotic and antimalarial therapies used for patients admitted with AFI in Kenya. We analyzed data from children aged <5 years enrolled in AFI surveillance conducted at four sites: Kenyatta National Hospital (KNH) in Nairobi, Kakuma Refugee Camp General Hospital (KRCGH) in Turkana, Coast Provincial General Hospital (CPGH) in Mombasa and Kakamega County Referral Hospital (KCRH) in western Kenya. Patients admitted with temperature ≥38.0°C of <14 days duration had data collected through chart review. From 06/2017 to 07/2018, 1175 children aged <5 years were enrolled: 633 (54%) at KNH, 188 (16%) at KRCGH, 238 (20%) at CPGH, and 116 (10%) at KCRH. Median age was 11 months (interquartile range 5-21) and 56% were male. The most common clinical diagnosis overall was pneumonia (38%), though at KCRH malaria was most common (67%). Overall, 1063 (90%) received antibiotics and 230 (20%) received antimalarial drugs; 176 (15%) received both. Antibiotic use was >90% among cases at all sites except KCRH (67%). Among the sites, use of a penicillin class antibiotic ranged from 36-81%, cephalosporin 25-56%, and aminoglycosides 27-60%. Use of antimalarials was 77% at KCRH, 27% at KRCGH, 13% at KNH, and 5% at CPGH. Among the sites, use of artesunate ranged from 5-78%, artemeter-lumefantrine 1-23%, and quinine 0-18%. Use of both antibiotics and antimalarials was most common at KCRH (46%) and least common at CPGH (4%). Among cases treated with antimalarials, 33% (75/230) had a rapid malaria test, and 56% of those were positive. Most children admitted with AFI receive antibiotics; however, the frequency and type vary by location, as does the use of antimalarials, which were often prescribed despite negative rapid diagnostic testing. Further research is needed to assess appropriateness of antibiotics and antimalarials used and drivers of differences in clinical practice among sites.

1133

NEUROLOGICAL SYNDROMES IN THE PEDIATRIC POPULATION DURING THE ZIKA VIRUS EPIDEMIC IN COLOMBIA 2015 TO 2016

Diana Marcela Walteros[1], Marcela Daza[2], Ana Cristina Suarez[1], Marcela Mercado[1], Franklyn Prieto[1], Angelica Rico[1], Maritza Gonzalez[1], Martha Opsina[1]

[1]*Instituto Nacional de Salud, Bogota, Colombia,* [2]*Zika research division, Vysnova Partners, Bogota, Colombia*

Background: During the Zika virus (ZIKV) epidemic, the association between viral infection and neurological involvement was identified. In Colombia, the Instituto Nacional de Salud (INS) established as mandatory the individual report of neurological syndromes (NS) in this period, allowing thorough analysis at a population basis. Methods: Cases compatible with ZIKV-related NS, in population under 18 years of age, reported to the surveillance system (Sivigila) from October 2015 to June 2016, were selected. Medical records were reviewed and clinical information was collected. The Brighton Collaboration criteria were used to classify the NS. Results: During the outbreak, 419 ZIKV-related NS were reported to Sivigila, 14 % (59/419) occurred in the pediatric population. Within these cases, 64% were male (38/59) and the median age was 10 years (IQR 6-14). The NS were classified as GBS (Guillain Barré syndrome) 68% (40/59), encephalitis 14% (8/59), myelitis 8% (5/59) and ADEM (acute demyelinating encephalomyelitis) 7% (4/59). Accounting for ZIKV-related symptoms, 92% (54/59) presented with fever, rash and myalgia before the onset of the NS. The average of days between these symptoms and neurological manifestations were: GBS: 10 days, encephalitis and myelitis: 7 days and ADEM: 2 days. The most frequent neurological manifestations for each NS were: GBS: hyporeflexia 98% and limb weakness 95%; encephalitis: seizures and changes in behavior 75%; myelitis: lower limb weakness 100%, hyporeflexia and respiratory distress: 60%, and ADEM: lower limb weakness 100%, dysarthria and hyporeflexia 50%. Mortality rate was calculated in 5.1%, being two GBS and one myelitis case. Conclusions: ZIKV infection accounts as a relevant etiological factor for NS, GBS was the most frequent condition in this cohort. The predominance of motor compromise and the different time frames between ZIKV-related symptoms and each NS should considered when caring for children with these pathologies. The higher mortality rate identified in this population, than reported by other authors warrants further analysis.

1134

PUBLIC HEALTH AND COST-EFFECTIVENESS IMPACTS OF "SCREEN-AND-VACCINATE" APPROACH WITH CYD-TDV IN PUERTO RICO

Edward W. Thommes[1], Laurent Coudeville[2], Riyadh Muhammad[1], Maria P. Martin[1], Christopher B. Nelson[1], Ayman Chit[1]

[1]*Sanofi Pasteur, Swiftwater, PA, United States,* [2]*Sanofi Pasteur, Lyon, France*

Dengue, a major public health problem in the tropics, is endemic in Puerto Rico and has been a reportable condition for several decades. A cost-effectiveness analysis is presented for a vaccination program using Sanofi Pasteur's CYD-TDV dengue vaccine in Puerto Rico. The analysis is based on a previously developed age-structured, host-vector compartmental model that reproduces the transmission dynamics of the four dengue serotypes. Parameters were estimated with 5-year data from two large-scale phase 3 efficacy trials conducted in 10 countries and territories including Puerto Rico. Following WHO guidance for the use of CYD-TDV, a "screen-and-vaccinate" approach was assumed: individuals without a documented prior dengue infection are first serologically tested for prior infection, and only those testing positive are vaccinated. We considered the use of two tests, a current enzyme-linked immunosorbent assay (ELISA) test, and a future rapid screening test with improved sensitivity and high specificity. Our base case scenario consisted of a CYD-TDV screen-and-vaccinate program in which 30% of 11-year-olds were tested via ELISA and those testing positive (50% of the screened individuals) were then vaccinated. Over a ten-year time horizon, such a program was predicted to prevent 24,781 [95% CI; 16,660; 33,164] cases of dengue, 2,000 [1,406; 2,623] dengue-associated hospitalizations, 558 [394; 721] severe cases of dengue, and 14 [7; 24] dengue-associated deaths. A vaccine price per dose of $255 [168; 572] or less was predicted to yield an incremental cost-effectiveness ratio (ICER) of $50,000 or less per disability-adjusted life year (DALY) averted from a payer perspective. From a societal perspective, the threshold price was predicted as $442 [315; 785]. A hypothetical future scenario, wherein serostatus is assessed via rapid screening test at the point of care, was predicted to have a substantially higher impact, driven primarily by the assumption that such a test would allow the screening rate to be more than doubled.

1135

APPLICATION OF TAQMAN ARRAY CARD IN THE PROJECT TO UNDERSTAND AND RESEARCH PRETERM PREGNANCY OUTCOMES AND STILLBIRTHS IN SOUTH ASIA (PURPOSE)

Jean Kim[1], Anna Aceituno[1], Elizabeth McClure[1], Robert Goldenberg[2], PURPOSe study investigators[3]

[1]*RTI International, Durham, NC, United States,* [2]*Columbia University, New York, NY, United States,* [3]*India, Pakistan*

Expanding our application of molecular tools to global health studies has afforded us the ability to better understand the causes of potential adverse outcomes specifically in the area of infectious disease. The Center for Disease Control and Prevention (CDC) has developed several robust quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays allowing for multiplex identification using an array card platform (TaqMan Array Cards (TAC); Life Technologies). TAC has become a useful surveillance tool for the identification of pathogens and toxins in epidemiological studies such as the Aetiology of Neonatal Infection in South Asia (ANISA) study. Our study, Project to Understand and Research Preterm Pregnancy Outcomes and Stillbirths in South Asia (PURPOSe), has two objectives: (1) the primary objective is to determine the primary causes of death for preterm infants and stillbirths in South Asia (Pakistan and India), and (2) the secondary objective is to determine the proportion of stillbirths and neonatal deaths caused by infections. To accomplish these objectives, we implemented the TAC assays to identify 79 unique microorganisms and toxins from various tissues including the placenta and cord as well as fetal tissues using the minimally invasive tissue sampling (MITS) process, a novel and less invasive approach to autopsies relying on needle sampling. Additionally, histology performed on the various tissues will complement the TAC findings. In collaboration with the CDC, we helped establish and train the local scientists in Pakistan and India to perform the protocols for tissue processing, the TAC assay, and assay analysis. Here we describe this process and the challenges that we faced.

1136

VALIDATING A TOOL TO MEASURE THE CLINICAL IMPACT OF MERCURY TOXICITY AMONG INDIGENOUS MACHIGUENGA PEOPLE OF THE PERUVIAN AMAZON

George W. Hafzalla[1], Raveena Chhabria[1], Carlos Culquichicón[2], Stephanie M. Trujillo[2], Alycia Silman[3], Luis E. Fernandez[3], Andres G. Lescano[2], Claudia M. Vega[3], John W. Sanders[1]

[1]*Wake Forest School of Medicine, Winston-Salem, NC, United States,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Wake Forest University, Winston-Salem, NC, United States*

Organic mercury can be found in contaminated food and water, especially fish, and can cross the blood brain barrier and placenta, leading to neurological and cognitive deficits. In 2016, hair mercury levels in Madre de Dios, Peru were over tenfold the recommended level of 6 μg Hg/g. Unfortunately, current clinical assessment tools are inadequate, lacking regional cultural specificity. Our aim is to validate an adaptable neurocognitive battery sensitive to variable factors of language, numbering scale, environment, and culture, to assess neurophysiological status and impact of chronic mercury exposure, specifically short-term memory,

working memory, executive function, and attention. A cross-sectional study including physical exam, hair/urine/blood collection, and diet questionnaire was conducted on 114 Machiguenga volunteers living in the indigenous communities of Yomibato, Cacaotal, and Maizal. A subset (n=52) met criteria (age>10, and no pre-existing cognitive disorders) to undergo neurological and neurocognitive testing. Thus far, hair mercury levels have been analyzed for 39 participants. No physical findings of mercury toxicity were seen, but deficits were noted in neurocognitive tasks. 50% of our volunteers were underweight and 75% of adults presented with anemia (females <12 g/dL; males <13.5 g/dL). Initial data analyses suggest that increasing levels of mercury are correlated with declining performance on 6-sequence block tapping (testing visual short-term memory) and trail making (testing executive function and attention), but not 6-sequence word recall (testing auditory short-term memory) tasks. These findings need to be confirmed with higher level data analyses accounting for community variables. Malnutrition and lack of formal education could affect performance on neurocognitive assessments and will need to be considered in analyses. Further validation of this tool will require continued testing involving a broader population.

1137

CORRELATES OF FREQUENT HOSPITALIZATIONS IN CHILDREN DISCHARGED FROM HOSPITAL IN WESTERN KENYA (TOTO BORA TRIAL)

Rebecca Brander[1], Benson Singa[2], Kirkby Tickell[1], Christine McGrath[1], Hannah Atlas[1], Lucy Bunyige[2], Bertha Odhiambo[2], Grace John-Stewart[1], Barbra Richardson[1], Patricia Pavlinac[1], Judd Walson[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Kenya Medical Research Institute, Nairobi, Kenya*

Repeated hospitalizations are a significant burden to the families of children and to health systems in sub-Saharan Africa. Using baseline data from children under age 5 enrolled in a clinical trial of azithromycin given at hospital discharge at 3 hospitals in western Kenya (Toto Bora Trial, clinicaltrials.gov identifier NCT02414399), we evaluated frequency, admission diagnosis, and correlates of self-reported prior hospitalizations. Correlates were determined using log-binomial regression to estimate prevalence ratios adjusted for age and site (aPR) and 95% confidence intervals (CIs). Among 991 children enrolled to date, the most common reasons for hospitalization were pneumonia (29.5%), malaria (27.7%), diarrhea (17.5%), and sickle cell disease (SCD) (6.5%). One in 5 of these children (219, 22.1%) had at least 1 additional hospitalization in the past year, with 55.7% reporting 1 prior admission, 23.4% reporting 2, and 17.8% reporting 3 or more. Approximately half (110, 50.5%) were hospitalized in the prior year for the same condition as the index hospitalization. Of these prior hospitalizations that were due to the same condition as the index hospitalization, 13.3% were due to malaria, 11.9% to SCD, 11.1% to pneumonia, 8.3% to anemia, and 5.1% to convulsions. Children with SCD were more than twice as likely to report a previous hospitalization (aPR: 2.52 [95% CI: 1.74, 3.65]). Children diagnosed with convulsive disorder at the index hospitalization were also more likely to have been hospitalized previously (aPR: 2.29 [95% CI: 1.28, 4.06]). Neither HIV exposure nor infection were associated with prior hospitalization, nor were child's nutritional status, child's sex, breastfeeding history, or socioeconomic factors. Hospitalization presents an opportunity to identify children at high risk of subsequent re-hospitalization who are accessible for preventive interventions. In regions with high prevalence of malaria, pneumonia, and SCD, provision of tailored preventive interventions at discharge may have important public health impact in reducing hospitalizations.

1138

IN VITRO SUSCEPTIBILITY TESTING OF TEBIPENEM AGAINST EXTENSIVELY DRUG RESISTANT (XDR) *SALMONELLA TYPHI* ISOLATES

Sonia Qureshi[1], Farah Naz Qamar[1], Bushra Jamil[1], Elena Fernandez Alvaro[2], Nosheen Nasir[1], Aneeta Hotwani[1], Stephen Baker[3]

[1]*Aga Khan University Hospital, Karachi, Pakistan,* [2]*GSK, Madrid, Spain,* [3]*OUCRU, Ho Chi Minh City, Vietnam*

The XDR typhoid outbreak in Pakistan is the first of its kind, with resistance to 5 different antimicrobial classes. This isolate is susceptible to azithromycin and carbapenem only. Consequently, treatment options for XDR are limited with no existing clinical data for its treatment. The currently used carbapenems have significant cost implications as these drugs are expensive and require administration in hospital. Azithromycin resistance, although not reported from Pakistan, it is a very commonly used drug and resistance is inevitable and likely to spread rapidly. Hence it is imperative to find alternative treatment options that can offer an advantage to patients in improving clinical outcomes whilst also reducing treatment costs. Tebipenem (Orapenem), a novel oral carbapenem has been found to be effective *in vitro* against *E.coli* and *klebsiella*. Successful use for the treatment of community acquired pneumonia, and otitis media is reported. We propose the use of oral Tebipenem for the treatment of XDR-Typhoid in phase 1; we plan to demonstrate *in vitro* susceptibility of archived XDR *S.typhi* isolates from the outbreak Hyderabad to tebipenem. The MIC (Minimum Inhibitory Concentration) for respiratory pathogens is reported as: 1 ug/ml, 2 ug/ml and 4 ug/ml respectively for susceptible, intermediate and resistant isolates. We will test 85 *S. typhi* isolates for sensitivity against carbapenem using the same cut offs. *Klebsiella pneumoniae* ATCC was used as positive control by inoculating 5x 10^5 CFU/ml by Broth Micro dilution method. We have to date completed testing of 15 isolates and all were found to be sensitive to tebipenem with very low MICs. 4 (27%) have MIC of 0.25 ug/ml, 1(6.7%) has MIC value of 0.5 ug/ml and remaining 10 isolates have MIC of 0.12 ug/ml. Remaining 70 *S.typhi* isolates are being tested and results will be available by the time of presentation at ASTMH .We have screened tebipenem against MDR and XDR *S*. Typhi from Pakistan and found it to have excellent *in vitro* activity, making it a realistic oral treatment option for cases of XDR typhoid fever. However the results have to be validated in a randomized clinical trial.

1139

EFFECT OF LYMPHATIC FILARIASIS AND HOOKWORM INFECTION ON PREGNANCY COURSE AND OUTCOME IN WOMEN OF REPRODUCTIVE AGE IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Jérémy Campillo[1], Cédric B. Chesnais[1], Jean Paul Tambwe[2], Naomi P. Awaca-Uvon[2], Michel Boussinesq[1], Sebastien Pion[1]

[1]*Institut de recherche pour le Développement, Montpellier, France,* [2]*Programme National de Lutte contre l'Onchocercose, Ministère de la Santé, Kinshasa, Democratic Republic of the Congo*

We evaluated the associations between (i) *Wuchereria bancrofti* and hookworm infections and (ii) pregnancy course and outcome in a group of 82 women of reproductive age (WRA) living in a rural area of the Democratic Republic of the Congo. Standardized questionnaires were used to collect demographics and information on past pregnancies. *W. bancrofti* and hookworm infections were diagnosed using a filarial antigen-detection test and the Kato-Katz method, respectively. Analyses consisted of multivariable logistic regressions adjusting on age, number of deliveries and history of anthelmintic treatment (AHT). Median age of WRA was 35 [interquartile range IQR: 30-44], and their median number of deliveries was 5 [IQR: 3-7]. *W. bancrofti* and hookworm infection rates were 42% and 43%, respectively. Filarial antigenemia and hookworm infection were not associated with the number of deliveries (p=0.161 and p=0.141, respectively). The proportions of WRA with a history of pregnancy resulting in neonatal death, miscarriage, premature birth, and postpartum

hemorrhage were 56%, 44%, 23% and 36%, respectively. History of pregnancy associated with neonatal death was less frequent in WRA who had taken AHT (adjusted odds-ratio (aOR) (95% confidence interval): 0.19 (0.04-0.82), p=0.026), tended to be more frequent in WRA with filarial antigenemia (aOR=2.68 (0.65-11.1), p=0.172), and was not associated with hookworm infection (aOR=1.36 (0.33-5.58), p=0.668). None of the three other pregnancy or birth events studied were associated with filarial antigenemia or hookworm infection (miscarriage: aOR=0.77 (0.19-3.28), p=0.729 and aOR=0.74 (0.14-3.86), p=0.726, respectively; premature birth: aOR=0.70 (0.09-5.71), p=0.741 and aOR=1.62 (0.12-21.40), p=0.712; post-partum hemorrhage: aOR=0.38 (0.09-1.53), p=0.174 and aOR=0.40 (0.08-2.03), p=0.269). The positive association found between history of AHT and lower risk of neonatal death warrants investigation in larger groups of WRA. Should it be confirmed, further studies should be conducted to determine whether it is due to biological causes (eg, anemia) or other (eg, sociological) determinants.

1140

EXPOSURE TO HOUSEHOLD AIR POLLUTION FROM BIOMASS COOKSTOVES AND BIOMARKERS OF SYSTEMIC INFLAMMATION FROM DRIED BLOOD SPOTS AMONG WOMEN IN RURAL HONDURAS

Megan L. Benka-Coker[1], Maggie L. Clark[2], Sarah Rajkumar[2], Bonie N. Young[2], Annette M. Bachand[2], David Diaz-Sanchez[3], Lucas M. Neas[3], Robert Brook[4], Tray Nelson[5], John Volckens[6], Steve J. Reynolds[2], Ander Wilson[7], Christian L'Orange[6], Nicholas Good[2], Casey Quinn[6], Kiersten Koehler[8], Sebastian Africano[9], Anibal Osorto Pinel[10], Jennifer L. Peel[2]

[1]*Department of Health Sciences, Gettysburg College, Gettysburg, PA, United States,* [2]*Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, United States,* [3]*United States Environmental Protection Agency, Research Triangle Park, NC, United States,* [4]*University of Michigan Medical School, Ann Arbor, MI, United States,* [5]*Department of Health and Exercise Science, Colorado State University, Fort Collins, CO, United States,* [6]*Department of Mechanical Engineering, Fort Collins, CO, United States,* [7]*Department of Statistics, Colorado State University, Fort Collins, CO, United States,* [8]*Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States,* [9]*Trees, Water, People, Fort Collins, CO, United States,* [10]*Asociación Hondureña para el Desarrollo, Tegucigalpa, Honduras*

Household air pollution from the burning of solid fuels is estimated to cause 1.6 million premature deaths worldwide each year. Cardiovascular-related disease contributes substantially to the burden, although evidence is limited. In a community-engaged project in rural villages of La Esperanza, Honduras, we measured 24-hour gravimetric kitchen and personal fine particulate matter ($PM_{2.5}$) and black carbon concentrations for 106 female primary cooks using wood-burning traditional and *Justa* (engineered combustion chamber and chimney) stoves. As indicators of cardiovascular disease risk, markers of systemic inflammation (C-reactive protein [CRP], Serum Amyloid A [SAA], Interleukin 1-β [IL-1 β], IL-8, Tumor Necrosis Factor-α [TNF- α], Intercellular Adhesion Molecule 1 [ICAM-1], and Vascular Cell Adhesion Molecule [VCAM-1]) were measured from dried blood spots collected via finger-stick. We used linear regression, adjusting for age, body mass index, education, and household assets, to evaluate the cross-sectional associations between the pollutants and inflammatory markers. The 24-hour median personal $PM_{2.5}$ concentration was 80 μg/m^3, IQR: 51-137 μg/m^3 (traditional stoves: 115 μg/m^3, IQR: 65-154 μg/m^3; *Justa* stoves: 52 μg/m^3; IQR: 39-81 μg/m^3). Pollution concentrations were higher in kitchens (vs. personal) and among traditional stoves versus *Justa* stoves. In adjusted models, increased concentrations of pollutants were associated with increased levels of CRP (e.g., a 25% higher personal $PM_{2.5}$ concentration was associated with a 10.5% increase in CRP levels [95% CI (confidence interval): 1.2-20.6]). We observed similar results for SAA. We observed a positive association between kitchen black carbon and IL-8, IL-1 β, and TNF- α. Associations with ICAM-1 and VCAM-1 with all pollutants were consistent with the null association. The results are consistent with the ambient air pollution literature and support the hypothesis that exposure to household air pollution is associated with increased systemic inflammation, which has been linked to cardiovascular disease.

1141

ACUTE FEBRILE ILLNESS SURVEILLANCE AT FOUR MILITARY SITES IN GHANA

Janice A. Tagoe[1], Clara Yeboah[1], Selassie Kumordzie[1], Shirley Nimo-Painstil[2], Naiki Attram[2], Eric Behene[1], David Wolfe[2], Anne Fox[2], Andrew Letizia[2]

[1]*Noguchi Memorial Institute for Medical Research, Greater Accra, Ghana,* [2]*Naval Medical Research Unit-3 Ghana Lab, Greater Accra, Ghana*

In malaria endemic regions, such as Ghana, acute febrile illnesses (AFIs) are often assumed to be due to malaria and presumptively treated with antimalarial drugs. The nonspecific clinical presentation of AFIs limit pathogen identification and epidemiology especially in resource-limited settings. AFIs require laboratory confirmed diagnoses which are cost-prohibitive and require special training which contributes to a lack of epidemiological knowledge in Ghana. Between July 2015 and December 2018, 284 patients were enrolled at 4 Ghanaian military treatment facilities. Inpatients or outpatients, 30 days to ≤65 years of age, with documented or reported fever (> 38°C) were eligible for the study. In addition to clinical and demographic information, blood specimens were collected for culture, serology and molecular testing. Among the 284 enrolled patients, 155 have been tested for *Coxiella burnetii* and *Leptospira* IgM and IgG with 8.4%, 4.9% and 0.7% sero-positive, respectively. Of 103 that were tested for chikungunya, 11.7% were positive for IgM and 7.8% for IgG. Of the 74 samples tested for West Nile Virus, 5.6% were seroreactivity for IgG and none for IgM. Out of 173 that were tested for dengue virus, 4% were positive for IgM and 39.6% for IgG. IgG reactive antibodies to spotted fever and scrub typhus *rickettsiae* was detected in 6% and 1% of the samples, respectively. 2.5% of blood cultures were positive for *Salmonella typhi*. This preliminary information demonstrates the requirement for expanded laboratory and diagnostic capabilities in Ghana to elucidate and understand the many potential etiologies of AFIs. Understanding the burden posed by both malarial and non-malarial agents in Ghana is essential for patient care and public health.

1142

ANTIPYRETIC USE AMONG FEBRILE PATIENTS ATTENDING EMERGENCY DEPARTMENTS IN RIO DE JANEIRO, BRAZIL: A CROSS-SECTIONAL, OBSERVATIONAL STUDY

José Moreira, Roxana Mamani, Patricia Brasil, Andre Siqueira

Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil

Fever is a common reason for seeking care in Emergency Departments (ED). Data about the prevalence of antipyretic use at arrival at the ED and how it may relate to outcomes is sparse. We did a cross-sectional analysis of patients presenting to two urban ED in Rio de Janeiro between October 2018 and March 2019. Eligible subjects had either a history of fever before arrival at the ED or axillary temperature ≥ 37.5 °C at arrival at the ED. Information about recent use of antipyretics, measured temperature at the ED, tests performed at the ED were collected. The outcomes were to describe the use of antipyretics preceding the ED visit, the measured temperature at triage, the rate of diagnostic testing, and the treatment administrated between subjects who had or had not received recent antipyretic prior to ED. Categorical variables were compared with the Fischer exact test, while continuous variables with the T-test or Mann-Whitney. We triaged 1551 subjects, and 374 [24.1% (95% CI 22-26)] had a history of fever at home or a measured fever at arrival at the ED. The mean age was 30.6 [0-84] years, adults (82.5%) and females (54.8%) predominated. 198 febrile patients had a suspicion of infection, and upper respiratory infection (42.4%) followed by undifferentiated febrile illness (15.7%) and urinary infection (14.6%) were the source of infection. 249 [87.6% (95% CI 83.1-91.1)] subjects reported taking an antipyretic

before ED visit. Dipyrone (72.2%) was the antipyretic most frequently administered followed by acetaminophen (14.3%), and ibuprofen (6.3%). The mean temperature at arrival at the ED (37.08 ± 0.99 *vs*. 36.85 ± 0.86, p=0.26), and the rate of diagnostic testing (31.1% *vs*. 32.1%, p=0.91) did not differ between subjects treated with any antipyretics and untreated subjects, respectively. Antipyretic users had a higher proportion of antibiotic prescription compared to untreated ones (56.3% *vs*. 21.4%, p<0.001). The use of antipyretics among febrile patients attending EDs in Rio de Janeiro is common, but their effects on ED temperatures are minimal. These findings might have implications in the designing of fever etiology studies.

1143

URINARY FINDINGS AT HEALTHY COMMUNITY SCREENINGS IN A REGION OF NICARAGUA WITH A HIGH BURDEN OF UNEXPLAINED KIDNEY DISEASE

Anna Strasma[1], Hannah Worrall[2], Sreedhar Mandayam[1], Reyna Silva[3], **Rebecca S. Fischer**[4]

[1]*Baylor College of Medicine, Nephrology, Houston, TX, United States,* [2]*Baylor College of Medicine, Tropical Medicine, Houston, TX, United States,* [3]*Amigos for Christ, Chinandega, Nicaragua,* [4]*Texas A&M University Health Science Center, College Station, TX, United States*

A large and ongoing epidemic of kidney disease of unknown etiology affects the rural poor from Mexico to Panama and has resulted in greater than 50,000 deaths. Mesoamerican nephropathy (MeN) is a devastating and rapidly progressing disease that affects primarily young agriculture workers who are otherwise healthy and lack traditional risk factors for kidney disease. Very little is known about renal function in the community-at-large, especially among children. Urine specimens were collected from individuals of all ages at health fairs in 4 rural, agricultural communities in the Pacific lowland areas of Nicaragua, a region heavily affected by MeN and where morbidity and mortality due to the epidemic has more than quadrupled since its emergence. Semi-quantitative dipstick and microscopic analysis were performed on fresh specimens. We generated descriptive statistics and tested for differences by age and community by Chi-squared and ANOVA in Stata 15. Urine from 471 community residents, ages 3 months to 89 years (median 21 years) were analyzed. Almost all individuals (99%) were shedding leukocytes, many (21%) with >5 per field. Renal cell shedding (11%), hematuria (13.4%) were also noted. Proteinuria was rare (3.2%). Hematuria and leukocytosis varied by locale (p<0.05). In this community-based sample, clinical urine specimens indicate an underlying prevalence of markers of impaired renal function. Further investigations into MeN should target populations other than agricultural workers and should specifically look at renal function in children. Geographic differences in clinical indicators may also point to the highest risk communities.

1144

FINE SCALE LYMPHATIC FILARIASIS MICROFILARIAE AND RISK FACTOR MAPPING IN A HIGHLY ENDEMIC VILLAGE IN THE MADANG PROVINCE OF PAPUA NEW GUINEA

Melinda Susapu[1], Leo Makita[1], Winter Deikore[1], Hannah Betts[2], Louise Kelly-Hope[2]

[1]*National Department of Health, Port Moresby, Papua New Guinea,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Papua New Guinea (PNG) has an estimated 4 million people at risk of lymphatic filariasis (LF). LF is a debilitating disease caused by nocturnal periodic nematodes *Wuchereria bancrofti* and transmitted by *Anopheles* mosquitoes, similar to malaria. Although national data exists, the burden of disease and associated risk factors of LF within a smaller endemic unit (village level) are currently not well defined. This study investigated microfilariae (MF) prevalence and potential risk factors of LF in an endemic village, comprising 3 hamlets within 5kms^2 in a rural area of Madang Province. MF infection (night bloods), and a short demographic, housing infrastructure and intervention risk factor survey was administered to 301 individuals. In total 90 individuals (29.9%) were found to be MF positive, which varied significantly across the hamlets by prevalence as well as average MF count; Korona (24.6%; 16.6/µl; n=46/187), Koinduna (31.9%; 21.6/µl; n=15/47) and Tongona (43.3%; 17.3/µl; n=29/67). Males (34.5%) were found to have a significantly higher prevalence than females (23.4%). Overall there was an increasing trend in prevalence with age with significant differences found between certain age groups. No individuals living in permanent houses were positive, compared with 12.1% and 35.3% recorded for the semi-permanent and bush material houses respectively. People who used mosquito coils/mortein sprays in their house (n=6, 12.2%) had a significantly lower prevalence than those who didn't (n=73, 33.2%). Interpolation maps of Mf positives per house in each hamlet with the house/ roof type and mosquito coil/mortein spray data overlaid showed that more semi-permanent houses and use of house spray was spatially associated with low risk areas. The finer scale mapping of MF and different risk factors provides key information to the National LF Programme which will help to target public health intervention campaigns. It will also provide important sentinel site information to monitor the impact of any intervention scaled up in this area over time.

1145

COMPARISON OF HEMATOLOGICAL INDICES BETWEEN MICROFILAREMIC AND IVERMECTIN TREATED AMICROFILAREMIC ONCHOCERCIASIS PATIENTS IN THE UPPER PART OF THE VOLTA REGION OF GHANA

Joshua Labadah, Kwadwo A. Kusi, Michael D. Wilson

West African Centre for Cell Biology of Infectious Pathogens, Accra, Ghana

Onchocerciasis is a neglected tropical disease of the skin and eye caused by *Onchocerca volvulus*, a helminth nematode parasite that is transmitted by blackflies. The pathology of the disease is largely associated with the microfilaria. Full blood counts are almost always requested by physicians in the diagnosis and management of diseases. Full blood count results from a total of 80 participants aged 18-70 in the Nkwanta North District in Ghana; 33 (18 male, 15 female) individuals having microfilaria (mf) diagnosed by skin snips and 47 (25male, 22 female) individuals who are positive by serology, negative for skin microfilaria and have taken ivermectin 3 months prior were compared by Mann-Whitney test. p values at 0.05 α for the median and interquartile ranges was reported. Total white blood cell (10/uL) for microfilaremic (mf) individuals 445 (367.50-590) was lower (p=0.0455) than of amicrofilaremic (amf) individuals 547 (446-634). Basophil count (10/uL) was lower (p<0.0001) for the mf 3 (2-11.50) compared to amf individuals 16 (11-20). Lymphocyte count (10/uL) was lower (p=0.0060) for the mf 191 (161.50-233) compared with amf individuals 230 (195-288). Eosinophil count (10/uL) was not different (p=0.5207) between mf 43.00 (22.50-67.50) and amf 39 (17-61). Neutrophil of mf individuals 136.50 (52-234) was not different (p=0.1236) from amf 162.5 (121-251). Monocytes of the mf individuals 52 (34.50-69) was not different (p=0.6920) from amf 50 (40.00-66.00). Red Blood Cell (10^4/uL) was lower (p=0.0235) for mf 449 (409.00-511.50) compared to amf 491 (438-532). The Hemoglobin concentration (g/L) was not different (p=0.6599) between mf 136.00 (127.00-147.00) and amf 134 (122-148). Hematocrit, HCT (%) was lower (p=0.0003) for mf 38.05 (34.88-40.63) compared to amf 41.80 (38.10-45.20). Platelet count, PLT (10^3/uL) was not different (p=0.2941) between mf 175 (121.50-231.50) and amf 197.00 (143-276). The geometric mean for microfilaria for the mf group is 25.7. *Onchocerca volvulus* microfilaria has an impact on some hematological parameters of its human host and these parameters could be used in the understanding and management of the disease.

1146

DRUG DISCOVERY AND DEVELOPMENT APPROACHES FOR THE TREATMENT OF HELMINTHIASIS

Natalie A. Hawryluk

Celgene Global Health, San Diego, CA, United States

Helminths are the most infectious agents of humans in developing countries. The most common helminthiases are those caused by infection from soil-transmitted nematodes (STH), platyhelminth trematodes (schistosomiasis), and the filarial nematodes. Helmianthiases have been targeted for elimination under the London Declaration on Neglected Tropical Diseases, therefore, Celgene Global Health (CGH), in collaboration with DNDi, academia, and Zoetis, is focused on finding novel chemical entities to treat these diseases. Using a distributed research model, CGH has on-going screening and dedicated medicinal chemistry efforts focused on human and animal filarial and soil transmitted nematodes and trematodes. Celgene Global Health has identified novel macrofilaricidal onchocerciasis compounds which will be discussed

1147

HOW PHARMACOKINETICS IMPACT DRUG OPTIMIZATION IN NEGLECTED DISEASES

Julius Lim Apuy, Geraldine Hernandez

Celgene Corporation, San Diego, CA, United States

Pharmacokinetics had been used in the pharmaceutical industry to study the absorption, distribution, and elimination of drugs in different model species including humans. The pharmacokinetic parameters and profiles generated by different drug-like molecules, various formulations, specific dose levels, and different dosing routes inform PK scientists concerning systemic exposure, oral bioavailability, linearity of response, and half-life of the molecule. These parameters help assess whether a compound had sufficient exposure to enable pharmacological efficacy. The general PK workflows in the pharmaceutical industry are customized to the unique scientific challenges of neglected diseases that are host/vector specific, or target species other than human. In neglected disease, new animal models and new disease specific *in-vitro* and *in-vivo* assays have to be developed to complement the PK workflow. Herein, we present how we modified the PK workflow to address the unique challenges in specific neglected disease such as filariasis and cow wasting disease.

1148

ACCEPTABILITY AND EFFECTIVENESS OF IVERMECTIN MASS DRUG ADMINISTRATION AND DOXYCYCLINE TEST AND TREAT IN SEMI-NOMADIC COMMUNITIES IN MASSAGAM HEALTH DISTRICT, CAMEROON

Rogers Nditanchou[1], Laura Senyonjo[2], Kareen Atekem[1], Ruth Dixon[2], Benjamin Biholong[3], Joseph Oye[1], Joseph Kamgno[4], Joseph Okeibunor[5], Daniel Boakye[6], Elena Schmidt[2]

[1]*Sightsavers, Yaounde, Cameroon,* [2]*Sightsavers, Haywards Heath, United Kingdom,* [3]*Ministry of Health, Yaounde, Cameroon,* [4]*University of Yaounde* [1] *and CRFilMT, Yaounde, Cameroon,* [5]*University of Nigeria, Nsukka, Nigeria,* [6]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana*

After 20 years of ivermectin mass drug administration (MDA), there is an on-going high level of transmission of onchocerciasis in Massangam health area (HA) in the West Region of Cameroon. An alternative treatment strategy (ATS) has been implemented to accelerate progress, which includes using doxycycline, which target the adult *Onchocerca* parasite through a test and treat strategy. A large number of nomadic herdsmen has been identified as a potential reservoir of infection supporting transmission in the area. This qualitative study explored opportunities and barriers for the MDA and ATS in the Massangam HA with a particular focus on the nomadic populations, through 15 focus group discussions and 31 In-depth interviews. Specifically, the study focused on 1) the experiences of reaching the nomadic communities with annual ivermectin MDA and 2) the acceptability of the doxycycline test and treat approach. The nomadic communities welcomed the MDA but there were issues with the delivery and distribution of the drug. Remote camp settlements were usually missed, as they are not systematically considered during census and MDA planning. Even when the Community-directed Distributors (CDDs) managed to reach the camp, a large proportion of the population, particularly men, were away and could not receive the drug. There were also linguistic and cultural barriers with the CDDs coming from outside the community, which inhibited the awareness and the acceptability of the campaign. The doxycycline test and treat strategy was generally acceptable and welcomed but there were fears and suspicion of the testing process, including the concern over the skin taken for biopsy. In conclusion, social, cultural and environmental barriers need to be taken into account in the MDA and ATS campaigns targeting hard-to-reach nomadic communities in the West Region of Cameroon. Interventions need to be adapted to accommodate the nomad routine, make efforts to reach them in the remote locations and ensure culturally appropriate messages and practices to improve their awareness, treatment uptake and equity.

1149

LONG-TERM IMPACT OF ALBENDAZOLE PLUS IVERMECTINE DOUBLE DOSE, TWICE-YEARLY ON THE SUPPRESSION OF *MANSONELLA PERSTANS* MICROFILARIAL LOAD

Yaya I. Coulibaly[1], Housseini Dolo[1], Tounko Fayinke[1], Abdoul F. Diabate[1], Siaka Y. Coulibaly[1], Moussa B. Sangare[1], Lamine Soumaoro[1], Michel E. Coulibaly[1], Salif S. Doumbia[1], Abdallah A. Diallo[1], Benoit Dembele[2], Dramane Sanogo[1], Siaka Konate[3], Sekou F. Traore[1], Adama D. Keita[4]

[1]*ICER-Mali, Bamako, Mali,* [2]*Helen Keller International, Bamako, Mali,* [3]*International Committee of the Red Cross - ICRC, Geneva, Switzerland,* [4]*Université des Sciences, des Techniques et des Technologies, Bamako, Mali*

Mansonella perstans is asymptomatic in most individuals. A variety of symptoms have been described including angioedema, pruritus, fever and eosinophilia. To date, effective treatment options are limited to relatively long course such as doxycycline 200 mg daily for 6 weeks and diethylcarbamazine plus mebendazole administered for 21 days. Less constraining microfilaria suppression could potentially offer better treatment option for *M. perstans.* To determine the effect of increased dose and frequency of albendazole-ivermectin treatment on microfilarial clearance, 34 *M. perstans* microfilaremic residents of a *W. bancrofti* endemic area in Mali were randomized to receive 2 doses of annual, standard-dose albendazole-ivermectin therapy (400 mg and 150 µg/kg; n = 18) or 4 doses of twice-yearly, increased-dose albendazole-ivermectin therapy (800 mg and 400 µg/kg; n = 16). This was a secondary analysis of a *W. bancrofti* targeted albendazole and ivermectin trial. After baseline, the follow-up periods were at M6, M12, M18, M24, M30 and M36. Although microfilarial levels decreased significantly after therapy in both groups as compared to baseline, levels were significantly lower in the high-dose, twice-yearly group at 12, 18, 24, 30, 36 months with all p< .05, Wilcoxon matched pairs rank test at any time points. In the group of annual, standard-dose albendazole-ivermectin, a significant difference was observed at 24 and 30 months (p<.05, Wilcoxon matched pairs rank test) as.compared to baseline. Furthermore, clearance of detectable microfilariae at 36 months was more common within the 11 patients in the twice-yearly therapy group (100%) than the annual therapy group with 4 out of 16 patients (66.7%) (p=0.04, Fisher›s exact test). These findings suggest that increasing the dosage and frequency of albendazole-ivermectin treatment enhances suppression of *M. perstans* microfilaria even though the standard treatment on the long run may also get rid of this filarial infection. These observations need to be assessed with a more powered study.

1150

BIOACCUMULATION OF COMPOUNDS IN VARIOUS FILARIAL PARASITES

Geraldine Hernandez[1], Julius L. Apuy[2], Natalie A. Hawryluk[1], Tamara Kreiss[3], John Siekierka[3]

[1]*Celgene Global Health, San Diego, CA, United States,* [2]*Celgene, San Diego, CA, United States,* [3]*Sokol Institute of Pharmaceutical Life Sciences, Montclair, NJ, United States*

Filarial diseases caused by parasitic nematodes are responsible for significant morbidity and life-long disease in humans. There is an unmet medical need for drugs that can treat filarial diseases. In drug discovery, host pharmacokinetic studies provide a theoretical volume of distribution, the distribution of compound into body tissues. One can theoretically utilize the volume of distribution to correlate to how much compound is distribution into the parasitic nematode; however a direct measure of the amount of compound accumulated into the parasite is needed. In developing novel anti-filarial agents there is a need to understand the accumulation and distribution of compounds into the parasite. The objective in this study is to develop ex-vivo methods for determining compound bioaccumulation in a variety of filarial parasites and correlating compound levels with parasite motility and viability.

1151

EVALUATION OF THREE DIAGNOSTIC TESTS FOR MONITORING OF LYMPHATIC FILARIASIS ELIMINATION IN AHANTA WEST, NZEMA EAST AND ELLEMBELLE DISTRICTS, WESTERN REGION, GHANA

Frances Amonoo McCarthy[1], Dziedzom K. de Souza[1], Francis Anto[2], Michael D. Wilson[1], Irene O. Owusu[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*University of Ghana School of Public Health, Accra, Ghana*

Wuchereria bancrofti is a filarial parasite which causes one of the most important neglected tropical diseases; Lymphatic filariasis. The disease is known to be responsible for much morbidity and deformity which brings about social and economic burdens on affected persons. The disease requires diagnostic tools that show high specificity and sensitivity in addition to their feasibility in terms of field implementation and efficiency. This study was a community based cross-sectional survey involving persons aged 5 years and above living in Ahanta West, Ellembelle and Nzema East districts which was aimed at evaluating the performance of three diagnostic tools; filarial test strip (FTS), microscopy and Wb123 ELISA for monitoring of LF elimination in the three districts. Day-time finger prick blood samples were obtained from 1371 participants recruited from households in 18 selected communities and tested for *W. bancrofti* antigen using FTS. Antigenemia positive individuals were followed up for collection of night blood samples to investigate the presence of microfilariae by microscopy. Filter blood spots were obtained for participants aged between 5 to 10 years and antigenemia positive individuals. These samples were tested for the presence of IgG4 antibodies specific for stage L3 of *W. bancrofti* using Wb123 ELISA. One hundred and thirteen (8.2%) of the tested individuals were antigenemia positive, 12 (12.4%) were mf positive and 14 (14.4%) were positive for IgG4 antibodies for *W. bancrofti.* The performance of FTS, Wb123 ELISA and microscopy were compared. FTS had 9.0% score over Wb123 ELISA (1.1%) and microscopy (1.0%). FTS was found to be more reliable in terms of diagnosis of LF compared to the other two diagnostics and could therefore be employed for use in areas with low endemicity rates. This study demonstrated the performance of filarial test strip, Wb123 ELISA test and microscopy for point of care monitoring following mass drug administration to reduce time spent in diagnosing in areas where bancroftian filariasis is endemic.

1152

IN VITRO MAINTENANCE OF *MANSONELLA PERSTANS* MICROFILARIAE AND ITS RELEVANCE FOR DRUGS SCREENING

Abdel Jelil Njoeundou[1], Chi Anizette Kien[1], Manuel Ritter[2], Mathias Eyong Esum[1], Patrick W. Ndongmo[1], Fanny Fri Fombad[1], Narcisse Victor Gandjui[1], Flobert Njiokou[1], Peter Enyong[1], Kenneth Pfarr[2], Joseph Turner[3], Laura E. Layland[4], Achim Hoerauf[4], Samuel Wanji[1]

[1]*University of Buea, Buea, Cameroon,* [2]*Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany,* [3]*Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [4]*German Centre for Infection Research (DZIF), Bonn - Cologne partner site, Bonn, Germany, Bonn, Germany*

Mansonellosis affects millions of people of the tropics. In addition to the lack of suitable safe drug recommended for its control, the *in vitro* maintenance of *Mansonella perstans* stages with regards to drug development is not well documented. This study evaluated the survival of *M. perstans* microfilariae (mf) under *in vitro* conditions that have been shown to promote survival of *Loa loa,* a close related filarial nematode with blood dwelling microfilariae. Further, the *in vitro* microfilaricidal effect of 17 antiparasitic agents was assessed on these parasites. The performance of the two basic culture media Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI-1640) supplemented with 10% Fetal Bovine Serum and Monkey Kidney epithelial Cell line on the survival of *M. perstans* microfilariae was investigated. Then, anthelminthics (6), anti-malarials (5), anti-wolbachia (3), trypanocidal (2) and anti-cancer (1) agents were tested *in vitro* against these parasite stages. The suitability of the culture media as well as the effect of the anti - infective agents on the mf survival was assessed by scoring their motility. Serum supplement and LLC-MK_2 co-cultures significantly improved the survival of microfilariae in DMEM and RPMI-1640. RPMI-1640 supplemented with 10 % FBS and co - cultured with LLC-MK_2 sustained the maintenance of microfilariae for at least 20 days (100.00 ± 0.00 % survival). In co-cultures with LLC-MK_2 cells and in absence of serum, *M. perstans* microfilariae were maintained in DMEM and RPMI-1640 without serum (FBS) by day 5, with motility above 99 % respectively. Mefloquine displayed the highest microfilaricidal effect *in vitro* followed by Artesunate. Both RPMI and DMEM in the presence of Monkey kidney epithelial cells are suitable for the maintenance of *M. perstans* microfilariae *in vitro*. In absence of the feeder cells, the addition of 10 % FBS to RPMI improved the parasite survival rate and motility. The microfilaricidal activity of mefloquine and artesunate on *M. perstans* microfilariae can be considered as reference for further screening of agents against this parasite stage

1153

OPTIMIZATION AND EVALUATION OF THE ESPERANZA WINDOW TRAP TO REDUCE BITING RATES OF *SIMULIUM DAMNOSUM* SENSU LATO IN NORTHERN UGANDA

Denis Loum[1], Devon Cozart[2], Thomson Lakwo[3], Peace Habomugisha[4], Benjamin Jacob[2], Eddie W. Cupp[2], **Thomas R. Unnasch**[2]

[1]*Nwoya District Local Government, Gulu, Uganda,* [2]*University of South Florida, Tampa, FL, United States,* [3]*Vector Control Division, Kampala, Uganda,* [4]*The Carter Center Uganda Office, Kampala, Uganda*

The Esperanza Window trap (EWT), a simple trap originally developed to replace human landing collections for entomological surveillance of *O. volvulus* transmission was optimized, resulting in a 17-fold improvement in trap performance. The optimized trap was tested in trials in schools and in agricultural fields to determine if it could reduce vector biting locally. The traps resulted in a 90% reduction in biting in the school setting. In the field setting, results varied. In one location, the traps reduced biting by roughly 50%, while in a separate trial, the traps did not significantly reduce the biting rate. Examination of the two settings suggested that trap

placement may be critical to their success. These results suggest that the optimized EWT might be capable of reducing local vector black fly biting in areas commonly frequented by residents. Together with other recently developed methods of community directed vector control, the traps may augment ivermectin MDA, bringing the goal of onchocerciasis elimination within reach in much of Africa.

1154

TESTING STRATEGIES TO BETTER INFORM PARTICIPANTS OF AN ANTHELMINTHIC CLINICAL TRIAL

Marta S. Palmeirim[1], Shaali M. Ame[2], Said M. Ali[2], Ulfat A. Mohammed[2], Jan Hattendorf[1], Brigit Obrist[1], Jennifer Keiser[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Public Health Laboratory Ivo de Carneri, Chake Chake, United Republic of Tanzania*

Obtaining informed consent from participants is an ethical and legal requirement in clinical research. This step commonly relies on the informed consent form alone, yet this may not guarantee the participant's true understanding. Therefore, new methods of conveying information should be tested. We aimed at comparing the impact of different methods of conveying this information on the knowledge of caregivers of participants of a double-blind, randomized clinical trial on Pemba Island, Tanzania. This trial compared the safety and efficacy of two different regimens of mebendazole (3 days 100 mg bid *vs* 500 mg) against hookworm infections in school-aged children. A total of 252 caregivers were assigned to receiving (i) a pamphlet (n=63), (ii) an oral information session (n=61), or (iii) both the pamphlet and the oral information session (n=63) all covering the clinical trial procedures, their rights, benefits and potential risks. Before consenting to their child's participation in this trial, 252 caregivers responded to a knowledge assessment questionnaire about the study. One group of caregivers (n=65) did not receive any information before they responded to the questionnaire, serving as control. Cure rates of mebendazole against hookworm were significantly higher in the multiple dose arm (98%) than in the single dose arm (13%). Although the pamphlet did not have any effect on caregivers' understanding, attending an information session significantly increased caregivers' knowledge for some questions, mostly concerning the parasite and trial procedures. However, because this method did not properly convey all the important messages, we will explore new methods such as a theatre and slideshow during the information sessions of an upcoming clinical trial evaluating the efficacy, safety and acceptance of chewable mebendazole. The results of the latter will also be discussed in this presentation.

1155

FORECASTING THE IMPACT OF MASS DRUG ADMINISTRATION IN A HETEROGENEOUS ENVIRONMENT WITHIN THE DEWORM3 TRAIL IN BENIN, MALAWI AND INDIA

James Truscott[1], Robert J. Hardwick[1], Marleen Werkman[1], Judd Walson[2], Roy M. Anderson[1], DeWorm Trial Team[2]

[1]*Imperial College London, London, United Kingdom,* [2]*University of Washington, Seattle, WA, United States*

Soil-transmitted helminths (STH) affect 1.45 billion people worldwide, and high intensity infections are associated with morbidity especially in children. Annual mass-drug administration targeting children has some impact on morbidity but fails to break the parasite transmission cycle. The DeWorm3 project is a cluster-randomized trial to test the feasibility of interrupting the transmission of soil-transmitted helminths through twice-yearly community-wide MDA at a high coverage level in arms that treat just children or the whole community. The project has study sites in India, Benin and Malawi. We fit epidemiological parameters to the study baseline data using Bayesian techniques and a dynamic model of parasite transmission and MDA impact. We then use the fitted parameters to forecast by forward projection the impact of the MDA coverage achieved in the first round on parasite burdens at the study end point and beyond, when interventions have ceased or returned to standard annual school-based MDA programmes. We examine various assumptions concerning MDA coverage at rounds post the first round, and individual compliance to treatment on these projections. The ability to predict whether the project's goals have been met depends on the uncertainty in the values of the fitted parameters which reflect the diagnostic techniques used to collect the data. We examine the effect of more sensitive diagnostic techniques (e.g. qPCR) on the degree of confidence in the forecasts. The considerable variability in the prevalence and intensity of parasite burden between study sites and across the clusters in a given site means that the impact of MDA will vary between clusters by the end of the project. We investigate the relationship between MDA coverage, prevalence heterogeneity at baseline and the site level effect on the likelihood of interrupting transmission and the achievement of the WHO elimination of morbidity goals. We discuss how baseline information on heterogeneity can be used to optimize MDA public health interventions to achieve a given target.

1156

STATUS OF SOIL TRANSMITTED HELMINTH INFECTIONS IN SEMARANG, CENTRAL JAVA, INDONESIA

Johanna M. Kurscheid[1], Budi Laksono[2], Archie Clements[3], James McCarthy[4], Susana V. Nery[5], Donald Stewart[6], Darren Gray[1]

[1]*Australian National University, Acton, Australia,* [2]*Yayasan Wahana Bakti Sejatera Foundation (YWBS), Semarang, Indonesia,* [3]*Curtin University, Curtin, Australia,* [4]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [5]*University of New South Wales, Kensington, Australia,* [6]*Griffith University, South Brisbane, Australia*

Indonesia carries the heaviest burden of soil-transmitted helminth (STH) infection in Southeast Asia with more than 62 million children requiring preventative chemotherapy in 2017 alone. Prevalence data for many parts of the country are out-dated and knowledge of the risk factors involved in transmission are not clearly understood. The aim of this study was to determine human STH prevalence and knowledge and practices relating to hygiene behaviour in rural communities in Central Java. A cross-sectional survey of 16 villages was conducted in Semarang, Central Java in 2015. Data on demographic, household and knowledge and practices were elicited through face-to-face interviews. Stool samples were collected and examined using the flotation method. Children (2-12 years) also had their haemoglobin (Hb) levels, height and weight data collected, and BMI computed. A total of 6466 individuals from 2195 households were interviewed. One-third of the cohort were infected with at least one species of STH, with differing burdens of the four species identified. Risk of infection was significantly associated with several demographic and household factors. Infection with STH was not associated with negative health impacts (e.g. diarrhoea, low BMI or Hb levels); however rates of anaemia among surveyed 2-12 year olds were high (33%) especially in school-age children. Knowledge of and behaviour related to hygiene and gastrointestinal diseases varied widely and were generally not associated with STH infection. The limited number of associations identified in this study suggests other undetermined risk factors may play a role in STH infection. The study also revealed that STH infection still persists in Central Java despite ongoing deworming programs. Therefore, current control efforts would benefit from being re-evaluated to determine the best way forward.

1157

COMPLIANCE TO TREATMENT IN THE GESHIYARO PROJECT: TESTING THE FEASIBILITY OF INTERRUPTING TRANSMISSION

Alison K. Ower[1], Nebiyu Nigussu[2], Fikreselasie Seife[2], Kalkidan Mekete[3], James Truscott[1], Robert Hardwick[1], Heven Sime[3], Gemechu Tadesse[3], Julia Dunn[1], Obiora Eneanya[1], Emily McNaughton[1], Ebba Abate[3], Anna Phillips[1], Roy Anderson[1]

[1]*Imperial College London, London, United Kingdom,* [2]*Federal Ministry of Health, Addis Ababa, Ethiopia,* [3]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia*

The Geshiyaro project is a 4-year programme which seeks to test the feasibility of interrupting the transmission of STH and SCH in the Wolayita zone of south-western Ethiopia through two interventions: (i) expanded MDA through biannual community-wide treatment and (ii) provision of water sanitation and hygiene (WaSH) with complementary behaviour change communication (BCC). Achieving high coverage and individual compliance to treatment across treatment rounds is imperative, with systematic non-compliance having the potential to result in reservoirs of infection allowing for continued transmission. We seek to determine the likelihood of achieving the project aims based on baseline prevalence of infection and compliance to treatment in our first round of mass drug administration (MDA). Using baseline parasitological data from 13,700 individuals across 137 villages (kebeles) in the Wolayita zone, we estimate the variation in the basic reproductive number, R_0, and the magnitude of parasite aggregation parameter, k, across study sites using a simple parasite transmission model and Gibbs-sampling MCMC techniques. A stochastic model of STH transmission is used to forecast the effect of biannual MDA rounds. Individuals residing in the pilot district were registered with two identifiers at census, (i) biometric and (ii) study ID card. These two identifiers were used at MDA to register individual treatment records which could subsequently be linked to household census and parasitology data and allow for the future longitudinal analysis of individual compliance across the project. The results of this study will outline the utility of individual identifiers, study ID card compared to biometric, for the capture of treatment compliance data and subsequent treatment coverage estimates. The results of this study will influence the recommendations for expanded community wide MDA in areas of low STH prevalence, depending on the local transmission patterns and conditions, to reach transmission elimination.

1158

SOIL-TRANSMITTED HELMINTH INFECTIONS IN PRE-SCHOOL AND SCHOOL-AGED CHILDREN AND THE SCHOOL ENVIRONMENT IN SOUTHERN INDIA: BASELINE SURVEY RESULTS FROM THE DEWORM3 STUDY

Saravanakumar Puthupalayam Kaliappan[1], Katherine E. Halliday[2], Gokila Palanisamy[1], Janarthanan Maniyarasu[1], Jasmine Farzana[1], Rajeshkumar Rajendiran[1], Chinnadurai Pandi[1], David Kennedy[2], William Oswald[2], Kristjana Ásbjörnsdóttir[3], Gagandeep Kang[1], Judd L. Walson[3], Sitara S R Ajjampur[1]

[1]*Christian Medical College Vellore, Vellore, India,* [2]*The London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*University of Washington, Seattle, WA, United States*

Soil-transmitted helminths (STH) affect ~1.5 billion people worldwide and the current WHO strategy of targeted deworming of pre-school and school-aged children (PSAC and SAC) is aimed at controlling morbidity. School environments in endemic communities potentially have an important role to play in the transmission of STH within these age-groups. The DeWorm3 study is a series of cluster randomized trials underway in three countries (Benin, India, and Malawi) to test the feasibility of interrupting STH transmission with bi-annual community-wide MDA compared to the standard-of-care (school based bi-annual deworming in India). The study is being carried out in two sub-sites in India, Timiri (rural plains) and Jawadhu Hills (tribal area) in Tamil Nadu in a censused population of 140,932 demarcated into 40 clusters. During the baseline survey (Dec 2017 - Feb 2018), 2484 stool samples (1179 PSAC and 1305 SAC) were collected and the unweighted prevalence among PSAC and SAC for any STH infections was 3.1% and 7.1% and 2.7% and 6.7% for hookworm alone. In July 2018, a school survey was conducted to assess school environment including the presence of water and sanitation facilities and performance of school-based deworming. We plan to analyse prevalence of STH in PSAC and SAC alongside school environment characteristics to determine any associations between the two. A total of 445 schools in Timiri (307) and Jawadhu Hills (138) were enumerated of which 93% were public schools. The majority of schools had water available during the survey (97%), and 90% had a protected water source (well/bore well or piped water). Handwashing facilities were available in 72% of schools but only 50% had soap and water available. Toilets were present in 81% of schools. There were a total of 1090 toilets (98% of which were flush or pour-flush type) for 36,931 children of which, 830 (81%) were found to be usable (functional with sufficient privacy) equating to one toilet per 44 children. Water and sanitation facilities were comparable between the two sub-sites. School based deworming on the last National Deworming Day was recorded as having been conducted in 97% of the schools.

1159

CLINICAL CASE OF INFANTILE HOOKWORM IN THE PERUVIAN AMAZON AND LITERATURE REVIEW OF EFFICACY OF CURRENT ANTI-HELMINTHIC THERAPIES

Brian J. Medernach[1], Steven Goicoechea[2], Ravi Durvasula[3], Prakasha Kempaiah[3]

[1]*Center for Community and Global Health, Loyola University Chicago Stritch School of Medicine; Department of Medicine, Loyola University Medical Center, Maywood, IL, United States,* [2]*Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States,* [3]*Loyola University Chicago Stritch School of Medicine and Department of Medicine, Loyola University Medical Center, Maywood, IL, United States*

A 5-week-old female infant presented to a rural hospital in the Peruvian Amazon as a referral from the vaccine team for poor feeding and pallor. The child was born in her community at home on a dirt floor. Mother did not receive pre-natal care. Upon initial examination in the ER the child appeared ill and found to be pale and lethargic. The infant was afebrile with tachypnea and tachycardia. Laboratory findings were significant for anemia with hematocrit of 6 and creatinine of 1.0. Child was admitted for stabilization and further examinations. Stool study was positive showing eggs of intestinal hookworm infection (Necator americanus or Ancylostoma duodenale). She received blood transfusion and albendazole 200mg orally for 3 days and was discharged after recovery. Parents also tested positive for intestinal hookworm and were treated with albendazole 400mg orally for 3 days. Soil transmitted intestinal helminths (STH), belonging to phylum Nematoda (roundworms), infect ~1.5 billion people worldwide annually. These include Ascaris lumbricoides, Trichuris trichiura and hookworm morbidity is related to infection intensity and can cause many issues including dietary deficiencies like iron deficiency anemia, delayed cognitive and physical development. STH are most common in places without modern sanitation and infection occurs through contaminated soil, food and water with eggs. The primary anti-helminthic drug benzimidazoles (albendazole and mebendazole) have low efficacy against T. trichiura (whipworm) with inadequate egg reduction and cure rates. Current treatment and prophylaxis programs rely on single dosing of anti-helminthic agents which pose a risk for development of drug resistance. Recent studies have focused on combination drug therapy and identified several drug combinations with higher efficacy than single-dose albendazole for T. trichiura including albendazole-ivermectin, albendazole-oxantel pamoate, mebendazole-ivermectin, and tribendimidine-oxantel pamoate. However, there is a need for further research and development of anti-helminthic drugs.

1160

IDENTIFICATION OF PARASITES WITH METAGENOMIC BARCODING CONFIRMS MICROSCOPY FINDINGS AND DETECTS ADDITIONAL ORGANISMS

Leah A. Owens[1], Sagan L. Friant[2], Sarah Phillips-Garcia[3], Melissa Emery-Thompson[3], Tony L. Goldberg[1]

[1]*University of Wisconsin-Madison, Madison, WI, United States,* [2]*Pennsylvania State University, University Park, PA, United States,* [3]*University of New Mexico, Albuquerque, NM, United States*

Although microbiome research has led to an explosion of knowledge about prokaryotic communities and their influence on host health, a parallel revolution for eukaryotic communities has yet to be realized. The field of parasitology in particular would benefit from a metagenomic method for characterizing eukaryotes. We used a metagenomic barcoding approach to detect eukaryotic parasites in fecal samples using published pan-eukaryotic primers. After creating a validation set of parasites, we extracted genomic DNA, amplified a common barcode region (18S ribosomal RNA gene), and deep sequenced the amplicon. This approach identified 100% (n=6 helminths, n=6 protozoans) of organisms in the validation sample, 75% (9/12) to the species level and 25% (3/12) to the genus level. We then repeated the process with samples from wild nonhuman primates where matched fecal samples had been previously characterized by microscopy in published studies, adding steps to enrich for parasite DNA. Results were analyzed with a combination of mothur, qiime, and custom python scripts using the SILVA132 database. The percentage of host reads ranged from 2.77% to 5.1% with a mean= 3.79% and median= 3.45%. In one representative sample, 5.24% of filtered reads mapped to a ciliate species found via microscopy and 4.42% mapped to a protozoan not identified by microscopy but expected to be present based on PCR-based diagnostics. This work demonstrates the potential of a metagenomic barcoding approach for identifying eukaryotic gastrointestinal parasites.

1161

COMPARISON OF REAL-TIME POLYMERASE CHAIN REACTION AND PARASITOLOGICAL METHODS FOR DETECTION AND POSTTREATMENT FOLLOW-UP OF *STRONGYLOIDES STERCORALIS* IN FECAL SPECIMENS OF PATIENTS COINFECTED WITH HTLV-1

Sapha Barkati[1], Maria Gabriela Gastanadui Gonzalez[2], Milli B. Nath-Chowdhury[3], Rohan Mahimkar[3], Michael D. Libman[1], Cedric P. Yansouni[1], Momar Ndao[3], Martin Montes[2]

[1]*J.D. MacLean Centre for Tropical Diseases, McGill University Health Centre, Montreal, QC, Canada,* [2]*Instituto De Medicina Tropical "Alexander Von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*National Reference Centre for Parasitology, Research Institute of the McGill University Health Centre, Montreal, QC, Canada*

Strongyloides stercoralis (*SS*) is an intestinal nematode infecting over 100 million people worldwide, primarily in tropical and subtropical regions. The infection can remain quiescent indefinitely, but when associated to iatrogenic immunosuppression and/or HTLV-1 coinfection, parasites can rapidly replicate and lead to hyperinfection and death. Coinfection has also been associated with lower cure rates that lead to persistent low parasite burden. Parasitological methods such as Baermann (BM) and agar-plate culture (APC) are cumbersome and may have lower sensitivity when trying to define larvae eradication in coinfected patients due to periods of scant intermittent larvae output. Recent PCR methods have shown better performance for earlier detection of low parasite-load infections. We aimed to compare the performance of parasitological methods and PCR in detection and posttreatement follow-up of patients coinfected with *SS* and HTLV-1. Seven HTLV-1 positive patients with active *SS* coinfection were enrolled and followed-up at 2, 4 and 12 weeks after treatment, which consisted of 2 doses of oral Ivermectin 200μg/kg/day repeated at the 2-week visit. At initial and each subsequent visits, BM and APC were performed. Frozen stool samples were kept for real-time PCR targeting the 18s small subunit following a modified protocol for multiplexing qPCR. Twenty-seven specimens were tested with an overall agreement of 81.5% (22/27). A total of 9 specimens (33.3%) were positive by at least one of the methods. Amongst the positive specimen, 5 (55.6%) were positive by qPCR and at least by one other method. At follow up, 1 patient showed treatment failure, with a prolonged excretion of larvae only detected by qPCR. The sensitivity of qPCR, BM and APC for the detection of *SS* compared to a combination of all diagnostic techniques was 66.7%, 77.8% and 75.0% respectively. In patients coinfected with HTLV-1, combination of parasitological methods and qPCR may increase the sensitivity of detection of *SS* larvae and may be a major tool for early detection of treatment failure. A larger patient cohort will allow us to define the best diagnostic and follow-up strategy.

1162

APPLICATION OF STH QPCR DIAGNOSTIC ASSAYS TO SCREENING ENVIRONMENTAL SAMPLES

Brian Abrams[1], Nils Pilotte[1], Gretchen Walch[1], Maya Nadimpalli[2], Amy Pickering[2], Steven Williams[1]

[1]*Smith College, Northampton, MA, United States,* [2]*Tufts University, Medford, MA, United States*

Worldwide, approximately 1.5 billion people are infected with at least one species of soil-transmitted helminth (STH). Yet despite soil and drinking water serving as important environmental reservoirs for STH transmission, current molecular methods for monitoring STH infection rely solely on the sampling and testing of human stool. While effective, human sampling is cumbersome and frequently associated with significant stigma. Recognizing these limitations, previous work has demonstrated the potential utility of environmental sampling for monitoring STH rates. However, all such work has utilized microscopy-based techniques. In response, we developed and optimized methods for the extraction and detection of DNA from STH eggs existing in soil and drinking water. By spiking *Necator americanus* and *Ascaris suum* eggs into known quantities of both environmental materials, we conducted a controlled comparison evaluating a variety of commercially available DNA extraction kits for this purpose. Following DNA extraction, samples were analyzed using our established STH qPCR assays. Testing revealed that environmental detection of STH was possible at concentrations as low as 5 eggs/20 grams of soil and 50 eggs /4 liters of water. These results provide proof-of-concept for the recovery and detection of STH DNA from both soil and drinking water samples. As such, the potential to use soil and water sampling for detecting STH may be feasible. Future work will focus on correlating environmental STH rates, as determined by qPCR, with infection rates in the human population.

1163

INDOOR EXPOSURE OF INTESTINAL PARASITES AND RELATION TO INFECTION IN ECUADORIAN CHILDREN

Victor Seco Hidalgo[1], Diana Garcia Ramon[1], Evelyn Calderón Espinosa[1], Andrea Lopez Rodas[1], Philip Cooper[1], Rojelio Mejia[2]

[1]*Universidad Internacional De Ecuador, Quito, Ecuador,* [2]*National School of Tropical Medicine, Baylor College of Medicine, Houston, TX, United States*

Soil-transmitted helminths and protozoa with a fecal-oral route of infection all have an outdoor environmental stage of their life cycle. In extreme poverty settings, many houses have dirt floors, outdoor latrines, and multiple children sleeping in the same bed. A parasite prevalence study in rural Ecuador with stools and dust were collected by aspiration at the doorway inside the house and the children's bed. Stools were examined by Kato-Katz microscopy. Dust was processed using a novel DNA extraction method and analyzed by multi-parallel quantitative real-time PCR (qPCR). All samples were analyzed in Ecuador. A total of 87 dust samples from each house were positive for *Ascaris lumbricoides* (38.7%), *Ancylostoma duodenale* (9.3%), *Necator americanus* (1.3%), *Strongyloides stercoralis* (52%), *Toxocara canis/cati* (22.7% and 4.0%), *Trichuris trichiura* (4.0%), *Blastocystis* (79.7%), and *Giardia lamblia* (24.3%). Of the children living

in these homes, stool microscopy detected positives for *Ascaris* (17.4%), hookworm (2.9%), *Strongyloides* (0%), and *Trichuris* (20.3%). There was a positive correlation of having *Ascaris* on Kato-Katz stool microscopy and having *Ascaris* contamination in bed dust (p = 0.0019). This correlation was also seen with a mean *Ascaris* burden in the stools with the bed dust (Low 602 epg, p = 0.0412; Medium 4643 epg, p = 0.0076; High 57,451 epg, p = 0.023, interquartile levels, respectively). For both the bed and doorway being qPCR positive, there was an odds ratio of 6.944 (1.246 - 36.21), p = 0.0140 for *Ascaris*; and 16.69 (4.752 - 49.10), p = < 0.0001 for *Strongyloides*. This study showed the risk for parasite contamination also occurs inside the households. Extreme poverty leads to poor sanitary conditions. This may be another route of parasite infection. Future work will be to determine if dust has viable parasites by searching for mRNA using molecular methods.

1164

A CLUSTER-RANDOMIZED CONTROLLED TRIAL COMPARING SCHOOL AND COMMUNITY-BASED DEWORMING FOR SOIL TRANSMITTED HELMINTH CONTROL IN SCHOOL-AGE CHILDREN: THE CODE-STH TRIAL PROTOCOL

Naomi E. Clarke[1], Dinh Ng-Nguyen[2], Rebecca J. Traub[3], Archie CA Clements[4], Kate Halton[5], Roy M. Anderson[6], Darren J. Gray[7], Luc E. Coffeng[8], **Susana Vaz Nery**[1]

[1]*University of New South Wales, Sydney, Australia,* [2]*Tay Nguyen University, Buon Ma Thuot, Vietnam,* [3]*University of Melbourne, Melbourne, Australia,* [4]*Curtin University, Perth, Australia,* [5]*Queensland University of Technology, Brisbane, Australia,* [6]*Imperial College, London, United Kingdom,* [7]*Australian National University, Canberra, Australia,* [8]*University Medical Center, Rotterdam, Netherlands*

Current control guidelines and targets for soil-transmitted helminths (STH) focus on deworming school-aged children, given the high risk of associated morbidity in this age group. STH control programs are therefore often founded on a school-based deworming approach. However, expanding deworming programs to all age groups may achieve improved STH control among not only the community in general, but also the high-risk group of school-aged children, by reducing transmission in the total population. The CoDe-STH trial is a cluster-randomized controlled trial (RCT), aiming to compare the impact of school-based targeted deworming and community-wide mass deworming on STH infections among school-aged children. The trial is being conducted in 64 primary schools in Dak Lak province, Vietnam. Schools in the control arm receive one round of school-based targeted deworming with albendazole. In the intervention arm, community-wide mass deworming with albendazole is additionally implemented through house-to-house visits. STH infections will be measured in school-aged children using quantitative PCR, at baseline and 12 months following deworming. The primary outcome is the comparison of hookworm prevalence in school-aged children 12 months after deworming. Analysis will be intention-to-treat, with outcomes compared between study arms using generalised linear and non-linear mixed models. Additionally, cost-effectiveness of mass and targeted deworming will be calculated and compared; feasibility and acceptability of deworming approaches will be examined; and individual based stochastic models will be used to predict the impact of mass and targeted deworming strategies beyond the RCT timeframe. The CoDe-STH trial is the first large-scale trial comparing mass and targeted deworming for STH control in Southeast Asia. This study will provide key information for policymakers regarding optimising the design of STH control programs at a time when the future of STH control is being vigorously discussed. We will present results from a rapid prevalence assessment and implementation of baseline surveys.

1165

MALARIA CARE-SEEKING BEHAVIOR AMONG HIV-INFECTED PATIENTS RECEIVING ANTIRETROVIRAL TREATMENT IN SOUTHEASTERN NIGERIA: A CROSS-SECTIONAL STUDY

Uchechukwu Chukwuocha[1], Gregory Iwuoha[2], Geoffrey Nwakwuo[3], Chidinma Ezeihekaibe[4], Christopher Ekiyor[3], Ikechukwu Dozie[2], Sahai Burrowes[4]

[1]*University of Massachusetts, Amherst, MA, United States,* [2]*Federal University of Technology, Owerri, Nigeria,* [3]*RAHI Medical Outreach, Port Harcourt, Nigeria,* [4]*Touro University, Vallejo, CA, United States*

This study assesses malaria prevention and treatment behaviour among people living with HIV/AIDS (PLWHA) in Owerri, South Eastern Nigeria. Although Nigeria bears one of the world's largest burdens of both malaria and HIV, there is almost no research studying how co-infected patients manage their care. We systematically sampled 398 PLWHA receiving care at Imo State Specialist Hospital and the Federal Medical Centre in Owerri to complete a structured, pre-tested questionnaire on malaria care-seeking behaviour. Descriptive statistics were reported and chi-square tests and multivariate logistic regressions were also used. The majority of HIV-infected patients (78.9%) reported having had an episode of suspected malaria quarterly or more often. There was a large variation in care-seeking patterns: on suspicion of malaria, 29.1% of participants engaged in self-medication; 39.2% went to drug shops, and only 22.6% visited HIV/AIDS care centres. Almost 40% waited more than 24 hours before initiating treatment. Most (60.3%), reported taking recommended artemisinin-based combination treatments (ACT) but a significant minority took only paracetamol (25.6%) or herbal remedies (3.5%). Most (80%) finished their chosen course of treatment; and completion of treatment was significantly associated with the frequency of suspected malaria occurrence (p=0.03). Most (62.8%) did not take anti-malaria medication while taking antiretroviral treatment (ART) and almost all (87.6%) reported taking an ACT regimen that could potentially interact with Nigeria's first-line ART regimen. Our findings suggest the need to pay more attention to malaria prevention and control as a crucial element in HIV/AIDS management in this part of Nigeria and other areas where malaria and HIV/AIDS are co-endemic. Also, more research on ART-ACT interactions, better outreach to community-level drug shops and other private sector stakeholders,and clearer guidelines for clinicians and patients on preventing and managing co-infection may be needed. This will require improved collaboration between programmes for both diseases.

1166

HIGH PREVALENCE OF HIV AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN GABON

Saskia Dede Davi[1], Ghyslain Mombo-Ngoma[1], Johannes Mischlinger[2], Marylyn Addo[2], Michael Ramharter[2]

[1]*Centre de Recherches médicales de Lambaréné and Department of Tropical Medicine Bernhard Nocht Institute for Tropical Medicine, Lambaréné, Gabon,* [2]*Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine and I Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

The National HIV/AIDS Control Programme of Gabon has established the screening of HIV among antenatal clinics (ANCs) attendees as one of the methods for collecting data on HIV prevalence for pregnant women to strengthen the prevention of mother to child transmission (PMTCT) of HIV. This study provides trends on HIV prevalence for 21 ANCs during the year 2018 in seven towns of Gabon. The surveillance population included all pregnant women attending the ANCs of selected centres in Libreville, the capital city, and Lambarene, Fougamou, Tchibanga, Mayumba, Oyem, and Bitam. Testing of HIV from blood samples after counselling was done anonymously according to the national guidelines for diagnosis of HIV/AIDS. Prevalence trends were calculated as the number of positive cases by the number of women tested during the period evaluated. Overall, 16417 pregnant women had been screened for HIV in 2018 in the 21 evaluated ANCs. The overall prevalence was 4% (646/16417). There was a marked

variability between ANCs within Libreville, from 2% to 10% of prevalence. Between provinces, there was also an observed variability in prevalence as the northern province, Woleu-Ntem (Oyem and Bitam) had 6% prevalence while the Moyen-Ogooue province (Lambarene) in the centre, and the Ngounie and Nyanga provinces in the south had respectively 5%, 4%, and 2% prevalence of HIV among pregnant women attending the ANCs. The findings show a high prevalence of HIV among pregnant women in the evaluated settings in Gabon suggesting a high risk of mother to child transmission of HIV that should be actively prevented by existing interventions. The variability of HIV prevalence between the different geographical locations may encourage integrated approaches taking into account specificities of each localities.

1167

HOMOZYGOUS DELETION OF BOTH GSTM1 AND GSTT1 GENES IS ASSOCIATED WITH HIGHER CD4+ T CELL COUNTS IN GHANAIAN HIV PATIENTS

Joshua A. Kuleape[1], Emmanuel A. Tagoe[2], Peter Puplampu[3], Evelyn Y. Bonney[4], Osbourne Quaye[5]

[1]*Tokyo Medical and Dental University, Tokyo, Japan,* [2]*West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, Accra, Ghana,* [3]*Department of Medicine, Korle Bu Teaching Hospital, Accra, Ghana,* [4]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana,* [5]*West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Accra, Ghana*

Glutathione S-transferase (GST) family of enzymes are involved in a two-stage detoxification process of a wide range of environmental toxins, carcinogens and xenobiotics. The GST enzymes play important roles in oxidative stress pathways, and polymorphisms in the GSTM1 and GSTT1 genes mediate susceptibility and outcome in different diseases. Human immunodeficiency virus (HIV) infection is associated with oxidative stress, but there is limited data on the frequency of deleted GSTM1 and GSTT1 genes in HIV/AIDS patients and their effect on progression among Ghanaians. This study sought to investigate the association between homozygous deletion of GSTM1 and GSTT1 genes (both null deletion) with HIV/AIDS disease progression in Ghanaian patients. HIV-infected individuals on antiretroviral therapy (ART), ART-naïve HIV patients, and HIV seronegative individuals were recruited for the study. HIV/AIDS disease progression was assessed by measuring CD4+ cell count and viral load of the patients, and GST polymorphism was determined by amplifying the GSTT1 and GSTM1 genes using multiplex PCR, with CYP1A1 gene as an internal control. The mean CD4+ count of patients that were naïve to ART (298 ± 243 cells/mm^3) was significantly lower than that of patients on ART (604 ± 294 cells/mm^3), and viral load was significantly lower in the ART-experienced group (30379 ± 15073 copies/mm^3) compared to the ART-naïve group (209882 ± 75045 copies/mm^3). Frequencies of GSTM1 and GSTT1 deletions were shown to be 21.9% and 19.8%, respectively, in the HIV patients, and patients with homozygous deletion of both GSTM1 and GSTT1 were more likely to have their CD4+ count rising above 350 cells/mm^3 (OR = 6.44, 95% CI = 0.81-51.49, p = 0.039) suggesting that patients with homozygous deletion of GSTM1 and GSTT1 genes have slower disease progression. The findings of this study show that double deletion of glutathione S-transferases M1 and T1 is statistically associated with normal CD4+ count in patients diagnosed with HIV/AIDS. Further study is required to investigate the clinical importance of the both null deletion in HIV patients.

1168

FEMALE GARMENT WORKERS' UNDERSTANDINGS OF HIV/AIDS IN BANGLADESH

Shakeel Ahmed Ibne Mahmood

The University of Newcastle, Newcastle, Australia

The proposed objective is to examine female garment workers' (FGWs) personal understandings and experiences of their human immunodeficiency virus (HIV) in Bangladesh. A systematic and comprehensive literature search has been conducted, focusing on FGWs HIV health in Bangladesh, published between 1989 and 2018. Relevant information from selected articles extracted, presented and attempted to contribute to existing literature in the form of new findings and critically interpret existing findings. The corresponding review of the literature continues to form inherent components of the debate. According to one study (Hasan, et al., 2013), FGWs are an at risk group, who are regularly engaged in illegal and unsafe sex. Major causes of HIV vulnerability of FGWs are gender inequality, multiple sex partners, drug abuse and rape violence. These poor FGWs are not informed about contraceptive methods, safe sex, menstruation, hygiene and HIV infection due to their low literacy rate. In another study (Mondal, et al., 2012), FGWs revealed that 43% do not use condoms and 95% believe that HIV is treatable. Thus, HIV related risk behaviors of FGWs demonstrate a potentially substantial impact on the future course of HIV epidemic in Bangladesh. This research contributes to broader disciplinary knowledge and/or policy practice on HIV prevention within FGWs of Dhaka City. Empowering FGWs through formal health education on HIV is essential, including prevention of work place violence and intimate partner violence related training. Community leaders, private sector involvement and NGOs focusing on HIV need to be encouraged to aware the FGWs regarding safe sex. Counselling and additional information are critical components to support women in making sexual intercourse decisions and carrying them out both voluntarily and safely at the same time. To this point in time, surveillance has only been conducted in key population. Therefore, the results of the study recommend a large-scale study of the general female population in Bangladesh to guide policymakers and researchers on how to prevent HIV and improve FGWs health over time.

1169

THE MISSING 90 IN THE HIV CASCADE OF CARE: LATE PRESENTATION IN CARE IN THE DOMINICAN REPUBLIC

Leandro Tapia[1], Rosa M. Rodriguez-Lauzurique[1], Merelin Muñoz[2], Robert Paulino-Ramirez[1]

[1]*Instituto de Medicina Tropical y Salud Global UNIBE, Santo Domingo, Dominican Republic,* [2]*Centro de Orientación e Investigación Integral, Santo Domingo, Dominican Republic*

Current biomedical interventions are aimed to reduce the impact of HIV in the immune system, and diagnosis of opportunistic infections. The objective of this study was to evaluate patient engagement in the continuum of HIV care for HIV, and to evaluate factors associated with late diagnosis and attrition from care. We included all patients attending a primary care unit in Santo Domingo (Capital city) for HIV care during two years, data collected included age, gender identity, year of first positive HIV test, CD4 cell count (cells/uL), drug use, and HIV viral load. Late presentation at the time of entering into care was defined as a CD4 cell count <200 cells/uL or an AIDS-defining event (based on CDC stages) within 3 months of the first positive test. To evaluate the factors associated with late presentation. Significant factors ($p<0.10$) in the univariate model were included in the multivariate model. Descriptive statistics for the categorical variables were analysed using the Pearson test to evaluate correlation between late presentation and other variables. A total of 257 HIV (+) patients were enrolled in care during a period of 24 months. The mean age was 35.7 years old, with 55% being males. The mean of CD4 count on the first visit was 370 cells/uL, and viral load (SD: 701,626 copies/mL). Analysis of correlation between variables (Pearson Test) was -0.155 significant for late presentation and the use of drugs. No correlation was found on age, nationality or gender. This study reveals the importance of intensive case finding among key populations to reduce the impact on expenditure and clinical management of complications related to opportunistic infections, and transmission pockets among drug users and other populations.

1170

EPIDEMIOLOGY OF TUBERCULOSIS AND HIV COINFECTION AND ITS COLLABORATIVE SERVICES TOWARDS ENDING THE TB EPIDEMIC IN ETHIOPIA

Yalemzewod Assefa Gelaw[1], Ricardo J. Soares Magalhães[2], Yibeltal Assefa[1], Gail Williams[1]

[1]*School of Public Health, The University of Queensland, Brisbane, Australia,* [2]*UQ Spatial Epidemiology Laboratory, School of Veterinary Science, Faculty of Science, The University of Queensland, Gatton, Australia*

The co-epidemics of HIV and tuberculosis (TB) is an important public health challenge in Ethiopia. This study aimed to describe the burden, distribution and trends of HIV and TB co-infection in the adult population (aged 15-years and above) in Ethiopia. This is an ecological descriptive study based on the national sentinel TB and HIV co-infection surveillance data from the Ethiopian Public Health Institute from 2010 to 2015. The annual proportion of co-infection rate for each specific year in the region/city administration was reported using notification rate ratios and 95% CI. GIS was used for visualization. A TB and HIV collaborative activities have increased by 31.5 % in 2015 compared from 11.6 % in 2010 with considerable variations within regions/city administrations. TB notification among HIV positives during the study period included 4,230 cases (range, 237 – 1095), with the annual notification rates of 7.5 % (7.2, 7.7) range between 4.5% (3.8, 5.1) in Tigray and 10.8 % (9.3, 12.5) in Afar region. The estimated annual notifications of HIV infection among all and new TB patient was 18.11% (17.8, 18.4) and 18.1% (17.7, 18.5), respectively. The rate notifications of HIV infection in TB was higher in Addis Ababa city 31.0% (29.5, 32.5) and lower in Harari region 4.4 % (4.0, 5.0). The TB-HIV collaborative activities have found increased and the notification rates of co-infection decreased. Exploring the burden of HIV and TB co-infection and its trend at the sub-national level could assist the needs of TB and HIV collaborative activities in finding the missed cases of both TB and HIV at the delivery of health care services.

1171

HIGH RESISTANCE TO NEVIRAPINE AND EFAVIRENZ IN HIV-1 SUBTYPE *CRF02_AG* AND DUAL *CRF02_AG/G* -INFECTED PATIENTS IN GHANA

Selase D. Deletsu[1], Edward K. Maina[2], Osbourne Quaye[3], William K. Ampofo[4], Gordon A. Awandare[3], Evelyn Y. Bonney[4]

[1]*Tokyo Medical and Dental University, Tokyo, Japan,* [2]*Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya,* [3]*West African Centre for Cell Biology of Infectious Pathogens, Department of Biochemistry, Cell and Molecular Biology, Accra, Ghana,* [4]*Department of Virology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana*

The increasing prevalence of HIV drug resistance (HIVDR) is a global health issue and an obstacle to successful antiretroviral therapy (ART). Information on HIVDR is important for initiating ART, and deciding on choice of drugs for subsequent regimens. We evaluated HIV-1 subtypes and drug resistance mutations among 51 ART exposed and 12 ART naïve patients in two public health centres in Ghana. Blood samples were collected and processed into plasma and PBMC fractions. HIV RNA was extracted from plasma and the *protease* and *reverse transcriptase* genes were amplified and sequenced.Of the sixteen patient` samples successfully sequenced, we identified a predominance of HIV-1 subtype *CRF02_AG* (11/16, 68%). Subtypes *G* (2/16, 13%), dual *CRF02_AG/G* (2/16, 13%) and *CRF01_AE* (1/16, 6%) were also observed. Major nucleoside reverse transcriptase inhibitor (NRTI)-associated drug resistance mutations (DRMs) (*M184I/V*, *D67N*, *T215F* and *K70R/E*), non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated DRMs (*K103N*, *Y181C*, *V90I*, *F227L* and *V106A*), and protease inhibitors (PI)-associated mutations (*M46I*, *I54V*, *V82A*, *L90M* and *I471V*) were present in the *CRF02_AG* and subtype *G* sequences from ART-exposed individuals. Two NRTI-associated DRMs (*D67N* and *T69N*) were present in the *CRF02_AG* sequences from one ART-naive individual. No DRM was present in the *CRF01_AE* sequences.The detection of these DRMs in individuals on NRTI/NNRTI-containing first-line regimen is an evidence of prolonged drug exposure without viral load monitoring This is a real global health problem giving the importance of effective treatment in achieving UNAIDs 90/90/90 goal. Routine viral load monitoring and drug resistance testing would be respectively useful for early detection of virologic failure and informed choice of drugs in the subsequent regimens of such patients.

1172

RETROSPECTIVE ANALYSIS OF HIV-TB CO-INFECTION IN GOVERNMENT MEDICAL COLLEGE AURANGABAD

Jyoti Anil Iravane, Maitrik Dave, Shaikh Ambreen Fatema Hafiz

Government Medical College, Aurangabad, Aurangabad, India

According to UNAIDS data 2018, India has the third largest HIV epidemic in the world. In 2016, 12% of people newly enrolled in HIV care in India had active TB. Of the 410,000 people who died of TB in 2017, 11,000 were HIV positive. A retrospective study was conducted among HIV/TB co-infected patients attending ICTC centre in the Department of Microbiology, Government Medical College & Hospital Aurangabad for a period of 3 years from 2016 to 2018. A total of 901 HIV positive cases were screened for TB in our TB Culture & DST laboratory. 216 (23.97%) cases were found to be TB positive either by CBNAAT (Cartridge Based Nucleic Acid Amplification) or LPA (Line Probe Assay). Pulmonary & extra pulmonary samples were screened by microscopy. The smear negative samples were tested by GeneXpert and the smear positive samples were tested by line probe assay for first line drugs. Out of the 216 cases, 199 (92.12%), were sensitive to Rifampicin & INH. Rifampicin resistance was detected in 10 cases (4.62%) and Rifampicin & INH resistance was found in 4cases (1.85%). A total of 62 Extra pulmonary TB samples (EPTB) were screened, amongst which 16(25.80%) were positive for MTB and one (6.25%) was an MDR case. In our study, the major determinants of HIV TB confection were identified to be low CD4 counts and ART (Anti-retroviral therapy). We also studied the correlation of low CD4 counts and presence of MDR cases. HIV TB co infection constitutes a serious diagnostic and therapeutic challenge particularly in resource limited countries and it is important to screen MDR-TB cases to identify early resistance and also to prevent the spread of MDR-TB.

1173

PREDICTORS OF MORTALITY IN HIV PATIENTS WITH SEVERE *PNEUMOCYSTIS CARINII* PNEUMONIA ADMITTED TO INTENSIVE CARE UNIT: A SYSTEMATIC REVIEW

Amanuel Lomencho[1], Helena Fantaye[2]

[1]*Emerald Medical, AMC Infectious Diseases Center, Addis Ababa, Ethiopia,* [2]*Federal Ministry of Health, Addis Ababa, Ethiopia*

Despite current developments in HIV medicine and wide scale use of antiretroviral therapy (ART), Pneumocystis Carinii Pneumonia (PCP) still remains to be an important cause of respiratory failure requiring ICU (Intensive Care Unit) care in developing countries. To date, there have been published studies on mortality predictors in PCP patients in ICU but to the best of our knowledge there are no systematic reviews.We aimed to look in to mortality predictors in HIV patients with severe PCP receiving ICU care.A systematic review was done to look for predictors of mortality in HIV patients with confirmed severe PCP admitted to ICU. Eligible studies were cohort and case control study designs that report predictors of ICU mortality. Studies that reported separate outcome for ICU patients with HIV/PCP were included. PubMed, Embase and Medline search was made from 2005-2018. Two authors independently screened titles and abstracts of all citations identified in the search. Quality assessment of relevant articles was made using CASP tool for cohort study appraisal. Initial search resulted 257 articles, out of which a final 9 were included in the synthesis. Most studies were in the pre-HAART era. All studies had a cohort design. Trimethoprim/Sulfamethoxazole, Pentamidine and steroids were used for treatment. Overall mortality ranged from 53%-81% in the pre-HAART which reduced to 25% following introduction of HARRT. In

the high quality studies, need of mechanical ventilator, development of pneumothorax, and duration of medical therapy prior to ICU admission were significantly associated with mortality. Higher quality studies reporting separate mortality predictors for patients put on respiratory support showed statistical significance for acidosis and high PEEP(Positive End Expiratory Pressure) requirement but failed to do so for APACHE II, Acute Lung Injury Score, indices of oxygenation (PaO2, alveolar arterial oxygen gradient), serum lactate dehydrogenase and severity of chest X-ray. ART improved ICU mortality among HIV patients with severe PCP. Mortality predictors generally were comparable in pre and post HAART era.

1174

DETERMINANTS OF ADHERENCE TO ANTIRETROVIRAL THERAPY AMONG PERSONS LIVING WITH HUMAN IMMUNE DEFICIENCY VIRUS IN THE UPPER EAST REGION

Gifty Apiung Aninanya, Gilbert A. Abiiro, Michael W. Wombeogo

University for Development Studies, Tamale, Ghana

Antiretroviral therapy (ART) suppresses HIV replication and decreases progression to Acquired Immune Deficiency Syndrome (AIDS). High levels of adherence to ART are required to improve the quality of life of persons living with HIV and AIDS (PLHIV). But, little evidence exists on determinants of ART adherence in the Upper East Region of Ghana.This qualitative study examined determinants of ART adherence among PLHIV in the Upper East Region. Using descriptive phenomenology approach to qualitative enquiry, we conducted five focus group discussions (n=30) and twenty (20) in-depth interviews with persons living with HIV. In addition, twelve in-depth interviews were conducted with health staff. Purposive sampling technique was used to select study participants. Colaizzi's descriptive phenomenology approach was adopted and used to code the data with the aid of Nvivo 11 software before thematic content analysis. Barriers that affected adherence to antiretroviral medicines were lack of nutritional support, side effects of ART, occasional travels, inadequate social support, lack of health insurance, access to transportation, economic problems, lack of confidentiality, negative attitudes of some health staff, queuing up for antiretroviral, non-disclosure of HIV status and stigma and discrimination. Perceived facilitators to ART adherence were appropriate counselling and education, provision of nutritional support, improved health status due to ART, the use of reminder aids, pregnancy and stigma-reduction policies. Several factors have been found to have a negative effect on PLHIV adherence to antiretroviral therapy. Nonetheless, it is recommended that effective and appropriate counselling techniques, provision of food supplements, stigma-reduction policies and regular training programmes for health staff on HIV case management could help to improve adherence to antiretroviral therapy by PLHIV. If all these measures are executed, Ghana will achieve its aim of having zero AIDS-related deaths by 2030.

1175

INTESTINAL PARASITES INFECTIONS AMONG HIV INFECTED CHILDREN UNDER ANTIRETROVIRALS TREATMENT IN YAOUNDE, CAMEROON

Celine N. Nkenfou[1], William B. Abange[2], Hortense G. Kamga[3], Clement N. Assob[2], William Estrin[4]

[1]*CIRCB, Yaounde, Cameroon,* [2]*University of Buea, Buea, Cameroon,* [3]*University of Yaounde I, Yaounde, Cameroon,* [4]*California Pacific Medical Center, San Franscisco, CA, United States*

Intestinal parasitic infections are among the most common communicable diseases worldwide, particularly in developing countries. HIV causes dysregulation of the immune system through the depletion of CD^4+ T lymphocytes which gives rise to opportunistic infections. A cross-sectional study was conducted from January to October 2018. Stool and blood samples were collected from participants aged 1 to 19. Stool samples were analysed for intestinal parasites using microscopy techniques. Blood samples were analysed for HIV and CD^4 + T cell counts. Out of 214 children enrolled, 119 (55.6%) were HIV infected and 95 (44.4%) were HIV non-infected. All infected children were on antiretroviral treatment (ART). The prevalence of intestinal parasites was 20.2% in HIV infected and 15.8% in non-infected children. Among the 119 HIV infected children, 33 (27.7%) of them had a CD^4+ T cell count less than 500 cells/mm^3, and 7 children (5.9%) had a CD^4+ T cell count less than 200 cells/mm^3. Among HIV infected children, Cryptosporidium spp. was most frequently detected, 7/119 (5.9%), followed by Giardia lamblia 5/119 (4.2%) then Blastocystis hominis 3/119 (2.5%) and Entamoeba coli 3/119 (2.5%). Participants on ART and prophylactic co-trimoxazole for >10 years had little or no parasite infestation. In conclusion, although ART treatment in combination with prophylactic co-trimoxazole reduces the risk of parasitic infection, 20.2% of HIV infected children harbored intestinal parasites including Cryptosporidium spp. Stool analysis should be routinely performed on HIV+ children to detect Giardia lambia, Blastocystis hominis, and Entamoeba species in order to initiate treatment and thereby improve the quality of life in these children.

1176

EPIDEMIOLOGY OF CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS IN NICARAGUA

Santiago Ernesto Hernandez[1], Gerardo Blass[2], Manuel Gomez[2]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Universidad Nacional Autonoma de Nicaragua-Managua, Managua, Nicaragua*

Leishmaniasis continues to be a major public health burden in the world. The disease is present in five continents and 97 countries and occurs mostly in the rural sylvatic areas with favorable conditions for the reproduction of vectors and intermediate hosts. It is considered a major Neglected Tropical Disease (NTD) which puts a huge economic burden on already meager financial resources. The World Health Organization (WHO) has estimated an annual incidence of 700,000-1,000,000 cases and about 20,000-30,000 deaths per year. This number might be underestimated due to misdiagnosis of cases in rural areas of endemic countries and inconsistent reporting guidelines. Cutaneous leishmaniasis and mucocutaneous leishmaniasis are endemic in 18 countries of the Americas. Approximately 66,941 cases of CL are reported annually in the Americas. In Central America, approximately 39,000 cases are reported annually. Most of the cases are reported in Nicaragua, Panama and Costa Rica. In recent years, Nicaragua has presented alarmingly high numbers of cases and elevated incidence rates. In 2016, the incidence rate was 84.30 per 100,000 habitants. Five-thousand and ninety-three cases of leishmaniasis were reported in the ¨Sistemas Locales de Atención Integral en Salud¨ (SILAIS) of Jinotega (2,378), Matagalpa (1,544), Las Minas (651) and Nueva Segovia (415). The municipalities which presented the highest incidence were El Cuá (965), San José de Bocay (867), Rancho Grande (384) and Wiwilí (383). In 2017, Nicaragua had a 157% increase in its incidence

rate. This rise in the number of cases was recorded as the highest rate in the Americas due to an outbreak in El Cua, which is a town located in Jinotega Department

1177

SEROPREVALENCE OF CHAGAS DISEASE AMONG BLOOD DONORS IN THE STATE OF BAHIA, BRAZIL

Diego L. Miranda[1], Gilmar R. Júnior[2], Fernanda C. Lanza[2], Fred Luciano Santos[2], Renato B. Reis[2], Deborah B. Fraga[2], Luciano K. Silva[2], Marinho M. Neto[3], Iraildes J. Santana[3], **Mitermayer G. Reis**[2]

[1]*Faculty of Medicine of the Federal University of Bahia, Salvador, Brazil,* [2]*Oswaldo Cruz Foundation, Salvador, Brazil,* [3]*Foundation of Hematology and Hemotherapy of Bahia, Salvador, Brazil*

Chagas disease affects 5.7 to 7 million individuals in the world. In the state of Bahia, Brazil, its prevalence has reached 25.1%. There is an association between CD and the social profile of the population and a risk of re-emergence of the disease also due to non-vectorial transmission, such as blood transfusion. The aim of this study was to describe the seroprevalence of *T. cruzi* infection among blood donors in the state of Bahia, Northeastern Brazil, and their epidemiological profile in the last 10 years. It was conducted as a descriptive, cross-sectional study, through database review. Data was gathered from patients who had a non-negative result for *T. cruzi* infection over the last 10 years. 3,084 (0.62%) non-negative samples were identified in a first sample, and 810 (0.16%) were again defined as non-negative for *T. cruzi* infection in a second sample. Correlation between infection and age (30 years and older) and infection and lower educational level (12 years or less) in both samples was statistically significant. Males presented a higher risk for T. cruzi infection only in the first sample. 99,52% of the municipalities of Bahia were identified as cities of residence among all blood donors. Livramento de Nossa Senhora was the most prevalent city in both samples and the capital, Salvador, presented the greatest recurrence of cases. The seroprevalence found in these populations is lower compared to other similar studies conducted in Brazil, but some condensed areas with higher prevalence were found. In general, the epidemiological profile was in agreement with previous studies.

1178

INTEGRATION OF PHLEBOTOMINE ECOLOGICAL NICHE MODELLING, AND MAPPING OF CUTANEOUS LEISHMANIASIS SURVEILLANCE DATA TO IDENTIFY AREAS AT RISK OF UNDER-REPORTING

Clara B. Ocampo[1], Lina Guzmán-Rodríguez[1], Mabel Soraya Moreno[2], Carlos Valderrama Ardila[3], Neal Alexander[1]

[1]*Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM), Cali, Colombia,* [2]*Secretaria de Salud Pública Municipal, Cali, Colombia,* [3]*Universidad Icesi, Cali, Colombia*

Passive surveillance systems are thought to under-estimate the true incidence of cutaneous leishmaniasis (CL) by two- to five-fold, although this factor is intrinsically difficult to estimate. Here we report an integrated approach to identifying areas at risk of under-estimation. The project is taking place in one municipality of each of three departments (states) of Colombia: Risaralda, Tolima (Andean region) and Nariño (Pacific coast). The objective is to identify one or more townships (*veredas*) with high unreported incidence. The rationale is that a competent vector must be present in such townships, and that they are likely to be located nearby sites where the surveillance data indicate disease incidence. Results of ecological niche mapping, are used as risk factors in multivariable spatial analysis, more specifically, conditional autoregressive (CAR) Poisson regression. Maximum entropy niche models for the species of *Pintomyia* (*Pifanomyia*) *longiflocosa* and *Psychodopygus panamensis* have been developed from existing published and unpublished researcher data, using the MaxEnt software, Digital Elevation Models, and WorldClim layers of temperature, precipitation and Normalized Difference Vegetation Index. *Pi. longiflocosa* is the main vector in the township of Rovira, Tolima, and the niche modelling shows highest probability of presence midway up the eastern slope of the central range of the Andes. *Pi. panamensis* is the main vector in Pueblo Rico, Risaralda, and the model shows highest probability towards the higher elevations of the western range. Surveillance data on disease incidence, and field collections of sand flies have substantiated the risk of transmission in the Risaralda study site. Here, 11 phlebotomine species, with *Ps. panamensis* predominating, were found in 5 townships suggested by the niche model. The incidence of CL will be estimated in these and other areas identified at being at high risk of under-estimation, using a combination of methods, including chain referral and community-based active case detection, in order to quantify the extent of under-estimation.

1179

HOTSPOTS OF TRANSMISSION OF LEISHMANIASIS IN SRI LANKA

Nadira Karunaweera[1], **Guofa Zhou**[2], Samitha Ginige[3], Sanath Senanayake[1], Hermali Silva[1], Nuwani Manamperi[4], Nilakshi Samaranayake[1], Yamuna Siriwardana[1], Deepa Gamage[3], Upul Senerath[1]

[1]*Faculty of Medicine, University of Colombo, Colombo* [8]*, Sri Lanka,* [2]*University of California, Irvine, CA, United States,* [3]*Ministry of Health, Colombo* [10]*, Sri Lanka,* [4]*Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka*

Leishmaniasis outbreak due to *L.donovani* in Sri Lanka is perceived as a serious public health issue. A retrospective analysis of data from human leishmaniasis cases conducted from 2001 to 2018 demonstrated continuous increase in case incidence, steady expansion in risk areas and 2 persistent hotspots of disease in the country. A prominent rapid rise in the case incidence during the year 2018 was also evident. The disease hot spot in the north-central region showed a location shift and shrinkage in size over time, whereas the one in the southern coastal region remained at the same location with expansion in size over the years. There was significant difference in age and sex distribution of cases between the two regions. In the northern region males 15-49 yrs were mainly affected during 2001-03, but the pattern has changed significantly with ≥50 yrs of both sexes affected during 2015-18. There was no such change noted in the south. Enhanced surveillance, public awareness, and appropriate interventions are urgently needed to halt the outbreak.

1180

LEISHMANIASIS IN ABANCAY (PERU) DURING THE LAST DECADES. RETROSPECTIVE ANALYSIS AND STUDY OF THERAPEUTIC RESISTANCE

Natalia Cánovas García[1], **Roque Diaz Diaz**[1], Jesus Lopez Fidalgo[2], Bartolome Ribas[3], Jose Juarez[4], Paul Nguewa[1]

[1]*University of Navarra, Istun Institute of Tropical Health, Pamplona, Spain,* [2]*University of Navarra, Institute for Culture and Society (ICS), Pamplona, Spain,* [3]*Real Academia Nacional de Farmacia, Madrid, Spain,* [4]*Facultad de Farmacia y Bioquimica-Universidad Nacional Mayor de San Marcos (UNMSM), Lima, Peru*

The estimated incidence of cutaneous leishmaniasis is 0.7-1 million cases worldwide, 90% of which occurs in Afghanistan, Pakistan, Syria, Saudi Arabia, Algeria, Iran, Brazil but also in Peru. Treatments include paromomycin, liposomal amphotericin B and pentavalent antimonials…. However, the incidence of resistant forms is not uncommon, and a better description and understanding of these is critical for the development of more effective therapeutic regimens. We performed the data analysis (age, sex, clinical form, occupation, environment…) from patients affected by cutaneous leishmaniasis in Abancay (Peru) during the last decades. We shed some light on strategies applied to dramatically reduce the infection. Finally, we also studied the relapsing and resistant cases after treatment administration.

1181

IMPACT OF EXPERIMENTAL INFECTION OF DOGS WITH *LEISHMANIA TROPICA* ON THE DEVELOPMENT OF CUTANEOUS AND VISCERAL LEISHMANIASIS

Nourhane Msallem[1], Malek Trimeche[1], Ifhem Chelbi[1], Abhay Satoskar[2], **Elyes Zhioua**[1], Thouraya Boussofara[1]

[1]*Institut Pasteur de Tunis, Tunis, Tunisia,* [2]*Ohio State University, Columbus, OH, United States*

Canine leishmaniasis is a vector-borne zoonosis caused by protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). In dogs, the infection can be asymptomatic or symptomatic, and fatal if left untreated. Canine leishmaniasis is endemic in Tunisia. The isoenzymatic typing of the different strains isolated from dogs revealed the incrimination of *Leishmania infantum* zymodeme MON-1. Nevertheless, visceral canine infection with *Leishmania tropica*, the etiological agent of anthroponotic cutaneous leishmaniasis in neighboring countries such as Morocco, led us to explore the outcome of dog infection with *L. tropica*. The aim of this work is the development of a canine model for experimental infection with *L. tropica*. Dogs were infected intradermaly with 6 $x10^7$ metacyclic promatisgotes. A follow-up of the biochemical, hematological, parasitological, molecular and serological parameters, during 6 months post-infection, allowed us to conclude that *L. tropica* induce cutaneous lesions at the site of the injection as well as visceralization of the parasite. Dog infected experimentally with *L. tropica* could be used as a model to test new drugs or vaccines against cutaneous leishmaniasis and visceral leishmaniasis and the epidemiological role of dogs as a potential reservoir host of *L. tropica*.

1182

INEQUALITIES IN THE SOCIAL DETERMINANTS OF HEALTH AND CHAGAS DISEASE TRANSMISSION RISK IN INDIGENOUS AND CREOLE HOUSEHOLDS IN THE ARGENTINE CHACO

Maria P. Fernandez[1], Maria S. Gaspe[2], Ricardo E. Gürtler[2]

[1]*Columbia University, New York, NY, United States,* [2]*Universidad de Buenos Aires, Buenos Aires, Argentina*

The social determinants of health (SDHs) condition disease distribution and the ways they are handled. Socio-economic inequalities are closely linked to the occurrence of Neglected Tropical Diseases, but empirical support is limited in the case of Chagas disease, caused by the protozoan *Trypanosoma cruzi*. Herein we assessed the relationship between key structural SDHs and the risk of *T. cruzi* vector-borne transmission in rural communities of the Argentine Chaco occupied by creoles and an indigenous group (Qom). We used multiple correspondence analysis to quantify the household-level socio-economic position (social vulnerability and assets indices), access to health and sanitation services, and domestic host availability. We identified the most vulnerable population subgroups by comparing their demographic profiles, mobility patterns and distribution of these summary indices; then assessed their spatial correlation and household-level effects on vector domiciliary indices as transmission risk surrogates. Qom households had higher social vulnerability and fewer assets than creoles', as did local movers and migrant households compared with non-movers. We found significantly positive effects of social vulnerability and domestic host availability on infected-*Triatoma infestans* abundance, after adjusting for ethnicity. Access to health and sanitation services had no effect on transmission risk. Only social vulnerability displayed significant global spatial autocorrelation up to 1 km. A hotspot of infected vectors overlapped with an aggregation of most vulnerable households. This synthetic approach to assess socio-economic related inequalities in transmission risk provides key information to guide targeted vector control actions, case detection and treatment of Chagas disease, towards sustainability of interventions and greater reduction of health inequalities.

1183

HIGH VARIABILITY BETWEEN THE INCIDENCE OF CLINICAL LESION OF LEISHMANIASIS AMONG THREE HIGH ENDEMICITY NEIGHBORING VILLAGES OF DIEMA DISTRICT IN 2016: A WESTERN PART OF MALI

Oumar Thiero[1], Bourama Traoré[1], Ousmane Faye[1], Dieudonne Somboro[2], Adama Dicko[2], Cheick A. Coulibaly[1], Ibrahim M. Sissoko[1], Samake Sibiry[1], Sekou F. Traoré[1], Seydou Doumbia Doumbia[1]

[1]*International Center of Excellence in Research (ICER-MALI), University of Sciences, Techniques and Technology (USTTB) of Bamako, Bamako, Mali,* [2]*Centre National d'Appui à la Lutte contre la Maladie (CNAM), Bamako, Mali, Bamako, Mali*

Leishmaniosis is a neglected tropical-diseases(NTDs) which occurs commonly in the setting of extreme-poverty, rural and some disadvantaged urban populations. The disease is known to be highly-endemic in western Mali, with LST+prevalence=50.7%reported. This study was undertaken to estimate the incidence-rate and the risk-associated with Active-Cutaneous-Leishmanias (ACL) detected from participants with negative LST in three_villages of the Diema-Districts: Famoune-Nafadji, Guemou and Debo-Massassi. After initial screening by LST and clinical examination, the participants aged 0-18-year-olds were follow-up house-to-house visits for ACL through active case detection every three-months during-one-year-2016. The count of new-infected-subjects with clinical-lesion ACL were recorded for the incidence rate-per-1000-person-year (IR). The relative-risk (RR) was adjusted for the risk-factors, the time-to-exposure and time-to-event using Poisson-Regression-models and Generalized-Estimating-Equations for the repeated-measure, alpha was set-at-10%. From 1798-participants, the overall IR was 6.4 (11/1730). The age group-0-6-years-old (representing 52%), has the highest IR=10.1 (RR=6.6, p=0.08) vs. IR=1.6 (RR=ref) and IR=5.3 (RR=3.0,p=0.42) for 7-12-years-old and 13-18-years-old respectively. The female (53.7%) has significant IR=9.7 compared to male, IR=2.5, the (RR=3.9, p=0.08). The IR=13.1 among the sarakole(31.4%) ethnic-group, and IR=3.7 among the Bambara/Malinke(61.0%), (RR=1.5, p=0.6), no cases among the Peulh-ethnic-group. The IR varies among the three-villages: 11.2, 2.5 and 1.9 respectively for Famoune-Nafadji, Guemou and Debo-Massassi, (RR=4.1,p=0.23) and (RR=1.3,p=0.86), respectively, Debo-Massassi=reference. In conclusion, the discrepancy between high incidence of LST positive and the clinical incidence ACL may be due to immunity to leishmania parasite acquired through higher levels of antibodies to sand fly salivary proteins positively correlated with LST+. Further studies are needed to understand the difference in RR among ethnicity and villages.

1184

EVALUATING THE INCIDENCE OF LEISHMANIASIS SKIN TEST POSITIVITY (LST+) WITHOUT CLINICAL DISEASE IN DIEMA DISTRICT, IN 2016: A HIGH ENDEMICITY AREA IN WESTERN MALI

Oumar Thiero[1], Bourama Traoré[1], Ousmane Faye[1], Cheick A. Coulibaly[1], Sekou F. Traoré[1], Adama Dicko[2], Ibrahim M. Sissoko[1], Sibiry Samake[1], Seydou Doumbia[1]

[1]*International Center of Excellence in Research (ICER-MALI), University of Sciences, Techniques and Technology of Bamako (USTTB), Bamako, Mali,* [2]*Centre National d'Appui à la Lutte contre la Maladie (CNAM), Bamako, Mali*

Cutaneious leishmaniasis (CL) has been reported endemic disease in western region of Kayes, Mali. We carried out a study aimed to evaluate the risk factors associated with the conversion to Leishmaniasis Skin Test (LST) positiveness without active CL in volunteers in active follow up setting. Located at 215 km from Bamako in the western part of Mali, from Diema District, three villages, Famoune/Nafadji, Guemou and Debo/Massassi in Diema Districts, which shared the same topography and climate condition, were selected for this study. country. Participants with negative LST were retested after one year to estimate the annual incidence

rate (IR) and the relative risk (RR) of LST+. The data was stratified by village and the RR was adjusted using Poisson Regression and the Generalized estimating equation was used for parameters estimations. A total of 574, 381 and 463 of LST negatives aged 0-18 years-old from Famoune/Nafadji, Guemou and Debo/Massassi respectively, participated to the study. The IR for LST+ was respectively 230, 362 and 138, per 1000-person year. The 3 models indicated no significant gender association with LST+, p>0.05. The RR for age groups 13-18 vs 0-6 were 3.6, 3.8 and 3.5 (all p<0.01) for the 3 villages respectively. The RR for age groups 7-12 vs 0-6 were 2.4, 3.0 and 2.4 (all p<0.0001) for the 3 models, respectively in Famoune/Nafadji, Guemou and Debo/Massassi. The RR =0.28, p=000, for Ethnicity group Marka/sarakole vs Bambara/kakole was only significant in Famoune/Nafadji but no difference between ethnic groups elsewhere.The incidence rate between gender were similar within all the villages (all p>.1). In conclusion, although, this study has shown that age remain an important risk factor for LST+, the relative high IR for LST+ across groups without the clinical disease might be explained by the immunity acquired through the exposure to sand fly saliva as the antibody proteins was high correlated to LST+. Further investigation is needed to explore the co-infections effect on clinical leishmaniasis incidence.

1185

BRUCELLOSIS CONTROL IN TANZANIA - THE EFFECTS OF PASTORALISTS' PERCEPTIONS AND PRACTICES

Caroline Mwihaki Mburu[1], Salome Atieno Bukachi[1], Khamati Shilabukha[1], Mangi Ezekiel[2], Kathrin Heitz Tokpa[3]

[1]*University of Nairobi, Nairobi, Kenya,* [2]*Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [3]*Centre Suisse de Recherches Scientifiques en Cote d'Ivoire, Abidjan, Côte D'Ivoire*

Brucellosis, a highly contagious zoonotic disease is endemic in many countries in Africa. In Tanzania, brucellosis has been identified as one of the six high priority zoonotic diseases, especially in pastoral systems because of animal product handling and consumption practices, low awareness and difficulty in implementing control measures. Therefore, the control and prevention of brucellosis partly depends on a holistic approach to understand the interaction of factors such as lay perceptions and practices of the local communities especially those related to animal husbandry practices. Our study describes those beliefs and the associated customs that impact on the transmission of brucellosis among pastoralists in the Kilombero valley of Tanzania. The ethnographic study used information from participant observation, focus group discussions and in-depth interviews to identify the key issues related to perceived risk factors, awareness and interactions in the human-livestock-wildlife interface. The findings reveal insufficient knowledge of the risk factors and suggest that consumption habits were not considered as a potential pathway for infection with brucellosis in humans. This was related to the misdiagnosis of the disease by health providers, causing a lack of public concern for the zoonosis. This study also provides novel, in-depth information on the communities' awareness and risk perception related to human brucellosis. Understanding these dynamics is essential for the development of suitable health communication and mitigation strategies that are contextually specific and more likely to be adopted by the local communities.

1186

ONGOING RIFT VALLEY FEVER TRANSMISSION AT HUMAN-ANIMAL INTERFACES IN TWO DISTINCT CLIMATIC ZONES IN TANZANIA

Robert D. Sumaye[1], Goodluk Paul[2], Brian H. Bird[3], Christopher Kilonzo[3], David J. Wolking[3], Amina Abdalla[1], Peter I. Pazia[1], Walter Simon[2], Feisal Hassan[1], Honorati Masanja[1], Jonna K. Mazet[3], Rudovick R. Kazwala[2], Woutrina A. Smith[3]

[1]*Ifakara Health Institute, Ifakara, United Republic of Tanzania,* [2]*Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, United Republic of Tanzania,* [3]*One Health Institute, School of Veterinary Medicine, University of California, Davis, CA, United States*

Rift Valley fever (RVF) is a re-emerging vector-borne zoonotic disease that causes outbreaks in human and animals across the African continent. To better understand impacts at the human-animal interfaces, integrated surveillance and risk characterization in people, animals, and mosquitoes were conducted. The study aimed to use a One Health approach to strengthen local technical capacity for RVF surveillance and pathogen detection to better inform management and control strategies. We conducted multi-year sampling of people, livestock, and mosquitoes in two distinct Tanzanian climatic zones, the relatively drier Ruaha ecosystem with high-level human-livestock-wildlife interactions and the relatively wetter Kilombero River Valley area with annual flooding. Across these ecologic zones, a total of 4,824 acutely febrile human patients, 4,468 livestock, and 3,447 mosquito pools were sampled. Using molecular and serological laboratory testing (RT-qPCR and IgM/IgG ELISA), we then determined exposure in people and animals. Findings indicated ongoing RVF virus (RVFV) transmission as measured by seroconversion in sentinel livestock herds across study districts, evidence of exposure among humans throughout the study regions, and detection of RVFV among infected mosquitoes. Despite over 12 years since the last large reported RVF outbreak in 2006-2007, it is clear that RVF risk continues and remains an under-reported health challenge to people and animals in the region. The findings from this integrated One Health surveillance approach are a call to action to proactively reduce the burden of this disease through measures such as vaccination to reduce the impact of this significant health threat.

1187

THE EPIDEMIOLOGY OF RICKETTSIAL DISEASES, SCRUB TYPHUS AND Q FEVER IN BHUTAN: A FIRST REPORT

Tshokey Tshokey[1], John Stenos[2], David N. Durrheim[3], Keith Eastwood[3], Tenzin Tenzin[4], Kinzang Dukpa[4], Ratna Bdr. Gurung[4], Stephen R. Graves[2]

[1]*JDW National Referral Hospital, Thimphu, Bhutan,* [2]*Australian Rickettsial Reference Laboratory, Geelong, Australia,* [3]*Population Health, Hunter New England Local Health District, Newcastle, Australia,* [4]*National Centre for Animal Health, Thimphu, Bhutan*

Except for few scattered reports on scrub typhus (ST), no data were available on the Spotted Fever Group (SFG) & Typhus Group (TG) *Rickettsia* and Q fever (QF) in Bhutan prior to this study. Following an outbreak of ST in 2009, heightened awareness increased case notifications from 91(no deaths) in 2010 to 753 (3 deaths) in 2017. Another outbreak of ST in 2014 saw two deaths. In this study, blood (human & animal) and ticks from animals were collected from Bhutan and analyzed at the Australian Rickettsial Reference Laboratory using an immunofluorescence assay (IFA) and a qPCR. Findings suggest that about 15% (159/1044) of patients with acute undifferentiated fevers attending 14 Bhutanese hospitals were due to a rickettsiosis, ST being the commonest (6.7%), followed by SFG (4.4%), QF (2.8%) and TG (0.4%). About 49% of 864-healthy Bhutanese showed evidence of past exposure to ST (22.6%), SFG (15.7%), QF (6.9%) and TG (3.5%). ST and SFG exposure significantly increased with age and farming activities. Trongsa district residents had the highest exposure to ST but residents at altitudes >2000M were relatively protected. In domestic animals, overall seropositivity of 46% (106/294) with SFG (36%), ST (21%), TG (15%), and QF (4%) was seen. Seropositivity differences between animal species appeared to be significant. Two-hundred ticks from 155 domestic animals were identified; the commonest tick species being *Rhipicephalus microplus* followed by *R. haemaphysaloides*, Haemaphysalis sp. near ramachandrai, *H. tibetensis*, *H. bispinosa*, *Haemaphysalis sp*, Haemaphysalis sp. near davisi, *R. sanguineus*, *H. shimoga*, *H. hystricis*, *Ixodes ovatus* & *Amblyomma testudinarium*. Twenty-nine (15%) of the 188 ticks subjected to a qPCR to detect rickettsial DNA were positive and the *Rickettsia* belonged to three major groups: *R. sibirica*, *R. raoultii* and *R. japonica* in the phylogenetic tree. Our findings constitute baseline data for Bhutan & serve to prompt further

research. Health services should be equipped to manage these infections by developing diagnostic & clinical guidelines. Human-livestock sector collaborations should increase through a 'One Health' approach.

1188

TRENDS AND CLINICO-EPIDEMIOLOGICAL FEATURES OF HUMAN RABIES CASES IN BANGLADESH 2006-2018

Sumon Ghosh[1], Md. Sohel Rana[1], Md. Kamrul Islam[1], Sukanta Chowdhury[2], Najmul Haider[3], Mohammad Abdullah Kafi[2], Sayed Mohammed Ullah[1], Md. Rashed Ali Shah[1], Be-Nazir Ahmed[1], Umme Ruman Siddiqui[1], Sanya Tahmina[1]

[1]*Disease Control Unit, Communicable Disease Control, Directorate General of Health Services, Dhaka, Bangladesh,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Technical University of Denmark, Section for Epidemiology, National Veterinary Institutes, Copenhagen, Denmark*

Vaccinating dog against rabies is an effective means of reducing human rabies. We analyzed 1327 clinically diagnosed human rabies death and mass dog vaccination (MDV) data during 2006-2018 to quantify the impacts of MDV on human rabies incidence in Bangladesh and a subset of rabies death data (422) for clinico-epidemiological study. We found a positive and increasing trend of dog population immunization ($p=0.01$ and tau=0.71) and a negative and declining trend ($p<0.001$ and tau = -0.88) of human rabies cases. The rabies cases in humans and the number of immunized dogs were correlated (adjusted $R^2=0.55$) indicating that immunization in dogs helped in reducing the rabies cases in humans. Among 422 human rabies death cases, the majority (78%) of the victims sought treatment from traditional healers and 12% received post-exposure prophylaxis (PEP). The mean incubation period of rabies in cases with exposure sites on the head and neck (35 days) were shorter than upper limb (mean=64 days, $p=0.02$) and lower limb (mean=89 days, $p<0.01$). MDV is effective to reduce human rabies cases in Bangladesh. Increasing mass awareness, ensuring better accessibility and availability of rabies PEP, and continuing to scale up MDV can help to prevent human rabies deaths.

1189

MOLECULAR DETECTION OF *BARTONELLA* IN SOUTH AMERICAN FUR SEALS (*ARCTOCEPHALUS AUSTRALIS*) FROM CHILEAN PATAGONIA

Ananda Müller[1], Pedro Bittencourt[1], Mauricio Seguel[2], Sandra Pérez[3], Paulina Sepúlveda[3], Ricardo Gutiérrez[4], Yaarit Nachum-Biala[4], Shimon Harrus[4]

[1]*Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis,* [2]*University of Georgia, Athens, GA, United States,* [3]*Universidad Austral de Chile, Valdivia, Chile,* [4]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel*

The aim of this study was to perform a molecular survey of *Bartonella* spp. in free-ranging South American Fur Seals (*Arctocephalus australis*) from Guafo Island, Chilean Patagonia. A total of 69 blood samples taken from 11 lactating South American Fur Seals and 58 pups from on Guafo Island,southern Chile. DNA extracted from the samples was submitted to Real Time PCR (qPCR) for *Bartonella* spp. 16S–23S internal transcribed spacer (*ITS*). Positive samples on *ITS* qPCR screening were further submitted to four qPCR assays targeting *Bartonella* spp.: *16srRNA*, *rpoB*, *gltA* and *SsrA* gene fragments. All positive qPCR products were purified and subsequently sequenced for speciation. Two out of 11 (18%) Fur Seal adults were positive for *Bartonella* spp. *ITS* gene, and none of the 58 pups was positive. *ITS* fragments from sequenced *Bartonella* spp. showed that sample #H1 (142bp sequence, not deposited on GenBank) had 100-100% identity with *B. henselae* isolates and sample #H2 (124bp sequence, not deposited on GenBank) shared 100-99% identity to *B. clarridgeiae*. Of the two *ITS* qPCR-positive samples, none showed positive results in cPCR assays based on *gltA, SsrA* and *16s rRNA* genes. Sample #H2 was positive in the *rpoB* gene (169bp sequence, not deposited on GenBank) and shared 100% identity with *B. Rochalimae*. This is the first report of *Bartonella* positivity in *Arctocephalus australis*. Little is known about geographic distribution and potential reservoir of *Bartonella* species infecting marine mammals. *Bartonella henselae* was detected in harbor porpoises (*Phocoena phocoena*), captive and free ranging, hunter-harvested beluga whales (*Delphinapterus leucas*), harbor seals (*Phoca vitulina*) and captive cetaceans. Further studies are necessary to fully characterize which *Bartonella* species are present in South American Fur Seals and it's potential role in the ecology of those organisms.

1190

NEW HIGH RESOLUTION MELTING SYSTEM FOR GENOTYPIFICATION OF PATHOGENIC *LEPTOPIRA* SUBSPECIES IN URINE SAMPLES OF DOMESTICATED ANIMAL RESERVOIRS FROM BELEN, IQUITOS, PERU

Anika Guadalupe Eca Avila, Karen Lisbet Ocampo Cardenas, Jessica Ricaldi Camahuali, Katherine Torres Fajardo

Universidad Peruana Cayetano Heredia, Lima, Peru

Leptospirosis is widely prevalent in the Peruvian Amazon. Animal reservoirs contaminating water sources likely drive seasonal outbreaks of severe human leptospirosis. Knowing animal isolate genotypes would help us understand the transmission dynamics of leptospirosis in this environment. Culturing *Leptospira* from animals is challenging in this setting, so we aimed to develop a genotyping system based on High Resolution Melting (HRM) for the identification of pathogenic subspecies of *Leptospira*, directly from animal urine samples from a flood-prone area of Belen, Iquitos, Peru. HRM profiles of 19 *Leptospira* reference strains were determined using *lipL32, lpxC and secY* marker genes. Urine samples were collected from 63 dogs and 88 pigs from homes and a unlicensed slaughterhouse, and assayed using *lipL32*-qPCR to detect pathogenic *Leptospira*. Genotypes of DNA in positive samples were determined by HRM and verified by sequencing. The reproducibility, typing efficiency and discriminatory index (DI) values of our test were 100%, 85% and 0.94, respectively. In total, 14 dog urine samples (22.2%) and 13 pig urine samples (14.8%) were positive to *lipL32* qPCR. HRM showed 11 of the dog urine samples contained a genotype 2 leptospire (serovars: *L. interrogans* Australis, *L. interrogans* Autumnalis, *L. interrogans* Djasiman and *L. interrogans* Copenhageni), one sample showed serovar *L. kirschneri* Grippatyphosa. Only four pig urine samples contained genotype 2, however, four contained a *L. santarosai*, and one each *L. kirschneri* and *L. noguchii*. Two samples from dogs and three samples from pigs did not amplify. We show that HRM has high discriminatory power for genotyping pathogenic subspecies of *Leptospira* spp. Furthermore, three pathogenic *Leptospira* species (*L. santarosai, L. kirschneri and L. noguchii*) had not been previously identified in pigs in this area. In conclusion, HRM can be a useful tool to investigate the genetic epidemiology of leptospirosis when culture is not possible.

1191

AVIAN INFLUENZA SURVEILLANCE IN WILD BIRDS AT NORTHERN AND SOUTHERN PERU DURING MIGRATORY SEASON

J. Catherine Dupont-Turkowsky

Naval Medical Research Unit-[6]*, Callao, Peru*

Avian influenza is a public health relevant disease caused by influenza A virus which is responsible for widespread disease outbreaks in animals and human worldwide. Wild birds have been described as natural reservoirs, particularly the orders Anseriformes (waterfowl) and Charadriiformes (gull and shorebirds). Performing surveillance in wild birds contributes to the global avian influenza surveillance as part of a strategy to characterize influenza virus diversity. For this purpose, NAMRU- 6 began a sentinel surveillance in 2017 in wild birds in the south (Paracas) and northern coast of Peru (Piura). In January 2017 we began the collection of cloacal swab samples in Paracas and in December 2018, environmental fecal samples in Piura. Sampling was conducted during migratory season (December to March, 3 to 5 days per site per month) when hundreds of thousands of

individuals come together from North and South America and co-mingle, providing an opportunity for transmission of influenza. To date, we have collected 1060 samples belonging to 35 different bird species (i.e *Arenaria interpres, Calidris alba* and *Anas bahamensis*). Approximately, two thirds of the samples (623/1060) have been egg cultured so far and 4 (0.6%) were positive for hemagglutination inhibition. From those 4 positive samples, 3 resulted positive to Influenza A by real-time RT-PCR. Targeted amplification approach was used to obtain the 8 Influenza A virus segments and then they were sequenced on the Ilumina MiSeq platform. We identified Influenza A subtype H6N2 from a *Calidris alba* and subtype H2N6 from an *Arenaria interpres*. In the sample that resulted negative by RT-PCR, we conducted an unbiased amplification and sequencing approach identifying an Avulavirus APMV-15 from an *Arenaria interpres*. Both species, *Arenaria interpres* and *Calidris alba*, are migratory birds and were previously describe as reservoirs for the virus. These samples were collected from Paracas where surveillance has never been performed. With these results we can confirm the presence and circulation of avian influenza in migratory birds in Peru.

1192

POLITICAL ECONOMY OF BAT HUNTING: BAT-BORNE DISEASES PERSPECTIVE IN BANGLADESH

A.K.M. Dawlat Khan

University of Dhaka, Dhaka, Bangladesh

Bats harbor many infectious agents like Nipah virus (NiV) that spillover from bat to human through date palm sap, partially eaten fruits or bat's secretions. NiV also can transmit from person to person. Since 2001 in Bangladesh, NiV human outbreaks have occurred with a >70% case fatality. Butchering and consumption of bat meat caused of Ebola and a Nipah-like henipavirus infection to humans in Africa. Bat hunting is a common practice in several parts of Bangladesh. I conducted an ethnographic study among a 60-household bat hunter community in the central part of Bangladesh to understand political economy of bat hunting during May 2017-April 2018.Community people used to work as day laborer in jute mills and storehouses. During harvesting season, majority people had work to earn livelihood. Yet, during the non-harvesting season particularly in winter, the community people were in lack of work. Therefore, some of them hunted bats and sold meat. To the hunters, bat hunting was a laborious job as they had to climb up long trees to hang the net and with risk of falling to ground. They were compelled to do this work finding no other alternatives. During catching, slaughtering/ butchering; the hunters were exposed to bat urine and saliva. Moreover, this bat slaughtering was placed at their homestead that also could lead the risk of infections to family members. In Bangladesh no direct evidence of NiV cases found among bat hunters. However, one hunter had developed Nipah like encephalitis syndromes and died before tested. Improper disposal of carcasses could pose threat to other animals. NiV is a WHO listed 'Blueprint priority disease'. However in Bangladesh, poster and pamphlet were distributed to communities and its surroundings where a NiV case was found to discourage only the consumption of raw date palm sap. During my study I found 50% people did not have formal education unlike the national average of 30%.The per capita income was low (USD 530 vs. national USD 1751). Absence of effective drug or vaccine against NiV can worsen with its pandemic threat. We need to adopt a culture-sensitive intervention with economic activities for the hunters to reduce the threat.

1193

INFLUENZA SURVEILLANCE IN SMALL SCALE SWINE PRODUCTION SYSTEMS IN PERU

Maria Claudia Guezala[1], Yeny O. Tinoco[1], Jorge Mantilla[2], Maria E. Silva[1], Christopher D. Cruz[1], Gilda Troncos[1], Roger M. Castillo[1], Christian Quiroz[3], Eugenio J. Abente[1]

[1]*Naval Medical Research Unit-6, Callao, Peru,* [2]*Servicio Nacional de Sanidad Agraria - SENASA, Lima, Peru,* [3]*Servicio Nacional de Sanidad Agraria - SENASA, Callao, Peru*

Pandemic influenza A virus (IAV) strains wreak havoc on public health systems and most recently resulted in an estimated 284,400 (range of 151,700 to 575,400) deaths in the first year following the emergence of the 2009 H1N1 pandemic virus. All four reported IAV pandemics in humans have resulted from the introduction and adaptation of animal-origin IAV, including swine IAV in 2009, highlighting the need for sustained surveillance efforts to better understand the risk of emergence of future pandemic strains. In Peru, the lack of systematic surveillance in swine populations is an important gap to address. Of particular interest are small scale swine production systems which lack stringent sanitary control measures and account for 25% of the Peruvian pork market. Since August 2018, the Naval Medical Research Unit No. 6 has collaborated with the Peruvian Agriculture Health Service (SENASA) to monitor one of the largest small scale swine production areas in Lima, Peru that encompasses approximately 100,000 pigs. This pig population represents 17% of swine produced in Lima and 7% of the total swine production in the country. SENASA regularly performs syndromic surveillance and collects nasal swabs from respiratory symptomatic swine. From August 2018 through February 2019 a total of 151 nasal swabs were collected and tested for IAV by reverse-transcription PCR. One (1/151) sample that was collected in August 2018 was positive for IAV. Phylogenetic analysis identified the hemagglutinin gene as a pandemic 2009 H1 within the clade 1A.3.3.2. Continued surveillance in the swine population is critical to better characterize circulating IAV in swine in Peru to determine the potential risks of interspecies transmission to humans. Notably, it has been documented that human-origin IAV frequently infects and is sustained in swine where antigenic evolution and divergence in pigs could result in a virus with potential to re-emerge in humans with little population immunity, further warranting surveillance efforts.

1194

SELF-MEDICATION AS THE FIRST RECOURSE FOR THE CARE OF SICK ANIMALS IN COTE D'IVOIRE

Danielle Naugle[1], Natalie Tibbels[1], Abdul Dosso[2], William Benié[2], Walter Kra[3], Corinne Fordham[1], Mieko McKay[2], Valère Konan[4], Jeanne Brou[5], Jocelyne Nebre[5], Adaman Kouadio[4], Zandra Andre[6], Diarra Kamara[2], Stella Babalola[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Johns Hopkins University, Abidjan, Côte D'Ivoire,* [3]*Alassane Ouattara University, Bouaké, Côte D'Ivoire,* [4]*Department of Veterinarian Services Ministry of Animal Resources and Fisheries, Abidjan, Côte D'Ivoire,* [5]*National Institute of Public Hygiene, Abidjan, Côte D'Ivoire,* [6]*U.S. Agency for International Development, Abidjan, Côte D'Ivoire*

The 2014-2016 Ebola outbreak in West Africa shed new light on the epidemic potential of zoonotic diseases in the region. Though Côte d'Ivoire (CdI) was not directly affected by the Ebola epidemic, the threat of major infectious disease outbreaks persists, in part, due to socio-cultural practices that potentially expose populations to risk. It is within this framework that a qualitative study of socio-cultural practices and beliefs around care and management of sick animals was conducted to understand the determinants of risk and preventive behaviors. The study involved 32 focus groups, 32 individual interviews, 20 observations and 20 community mapping with 234 adult men and women from four urban sites. The study focused on risk and prevention behaviors around five priority zoonotic disease groups in CdI: rabies, mycobacteria, bacterial and parasitic diseases (i.e. brucellosis), viral hemorrhagic fevers, and respiratory

zoonoses like avian influenza. The interviews were recorded, transcribed, coded and analyzed according to key themes. The results indicate that the general population and those engaged in animal husbandry mainly use self-medication and rely on their own or their peers' past experiences in the care of sick animals. Veterinary technicians are only consulted when there is a deterioration in the animal's health, the animal is of value, and/or many animals are exhibiting the same symptoms. The accessibility of veterinary technicians, the perceptions that actors have of technicians, and the cost of technicians' services constitute barriers to the use of appropriate care. The widespread use of inappropriate medications likely contributes to the growing challenge that antimicrobial resistance poses in Sub-Saharan Africa. Social and behavior change programs should focus on interventions that promote the economic benefits of proper prevention and treatment for animals and should work with both veterinarians and chicken and livestock breeders to improve relationships between the two groups.

1195

EVALUATION OF THE ROLE OF MMPL3 GENE AS A CANDIDATE GENE FOR PYRAZINAMIDE RESISTANCE IN *MYCOBACTERIUM SMEGMATIS*

Luz A. Saavedra, Ricardo Antiparra, Mirko Zimic, Patricia Sheen

Universidad Peruana Cayetano Heredia, Lima, Peru

Efflux pumps are one of the principal systems used by bacteria to reduce antimicrobial activity. Since they prevent the accumulation of antibiotics and toxic metals, efflux pumps are highly conserved among bacterial species. For example, in *Mycobacterium tuberculosis* (MTB), this is a critical adaptation and involves a serious public health concern. It has been suggested that MTB uses this machinery to modulate the antimicrobial activity of the first-line drug pyrazinamide (PZA), by interfering with the normal efflux of its active form "pyrazinoic acid" (POA). However, the identity of these efflux pumps are still unknown. mmpL3 is a membrane transporter that is part of the RND family, widely associated with multidrug resistance. What is more, recent data from our group have found mutations in these gene associated to clinical resistant MTB isolates. Consequently, the main objective of this project was to study the function of its orthologous gene in the strain naturally resistant to PZA *Mycobacterium smegmatis* (Msm) by using the CRISPR interference (CRISPRi) system. First, we corroborated that expressing only the Cas9 protein had no silencing effects over mmpL3 expression ($p>0.05$). Next, we generated our recombinant Msm strain that harbored the complete CRISPRi system to knock down the gene mmpL3. Induction of the CRISPRi system was possible by a treatment with 100 ng/ml Anhydrotetracicline (ATc). After an incubation of 14 hours with ATc, this strain was used for further validation, enzyme activity essays and subsequent POA efflux rate quantification. In all the cases, comparisons were made between treated and non treated cultures. After inducing the CRISPRi system, our mmpL3 recombinant strain showed no phenotypic defect in growth on plate ($n=5$) and we could confirm a 10 fold RNA repression by RTqPCR ($p<0.0001$, $n=5$). Our results indicate that after knocking down the expression of mmpL3, there is no change in the POA efflux ve*loci*ty when compared to non treated cultures (0.66 mM POA/min Vs 0.61 mM POA/min; $p>0.05$, $n=3$). In conclusion, mmpL3 is not a POA efflux pump in Msm, which ultimately suggest an unlikely role in MTB.

1196

RISK FACTORS FOR UNFAVORABLE OUTCOMES IN DRUG-SUSCEPTIBLE *MYCOBACTERIUM TUBERCULOSIS* TREATMENT IN UGANDA

Michael S. Harper[1], Stella Zawedde-Muyanja[2], Yukari Manabe[1]

[1]*Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*Makerere University, Kampala, Uganda*

One of the targets of the WHO END TB Strategy is a treatment success rate of >90%. The success rate of treatment in Uganda, a TB high-burden country, is currently below this target. We sought to evaluate risk factors for unfavorable treatment outcomes for drug-susceptible *Mycobacterium tuberculosis* in 10 healthcare facilities in central and eastern Uganda. Patients diagnosed with bacteriologically confirmed TB using Xpert MTB/RIF testing between January 2018 and June 2018 at 10 facilities (3 Health Center IV, 4 District Hospitals, 3 Regional Referral Hospitals) with rifampicin-sensitive TB, and 15 years of age or older were included. Unfavorable treatment outcomes were defined as death, loss to follow up, or treatment failure during the study period. A total of 441 patients ages 15-90 were enrolled; 396 patients had treatment outcomes ascertained before the censor date and were included in the final analysis. The overall treatment success rate was 74% (293/396), with a mortality rate of 6.6% (26/396) similar to nationally reported treatment success rate, but below the 90% target set by WHO. Using multivariable logistic regression, there was no association with sex, delayed treatment, or distance from the healthcare facility with unfavorable treatment outcomes or mortality. Infection with HIV, however, was associated with unfavorable treatment outcomes (aOR 2.91, 95% CI 1.71 - 4.96) and mortality (aOR 10.3, 95% CI 3.49-30.4). The majority (70%) of HIV-infected patients were on ART at diagnosis, and 27 patients (22%) were started on ART during TB treatment. Initiation of ART after TB diagnosis was not associated with unfavorable treatment outcomes or mortality compared to patients already on ART. These data show that TB patients co-infected with HIV are particularly vulnerable to death and unfavorable outcomes during TB treatment. Improvements in TB/HIV care integration, which has previously been shown to reduce mortality in this population, may be needed to meet target treatment success rates.

1197

A STUDY OF THE DETERMINANTS OF TB DIAGNOSIS DELAY IN THE BAMBEY HEALTH DISTRICT IN 2017 (SENEGAL)

Jean S. Kaly

Bambey sanitary district, Bambey, Senegal

In Senegal, the diagnosis of tuberculosis faces two problems: under-screening and delayed diagnosis. The overall goal was to identify the determinants of delayed diagnosis of TB in the Bambey Health District in 2017. A comprehensive, cross-sectional, descriptive and analytical study of patients with pulmonary tuberculosis was conducted in the Bambey Health District from 3 to 7 September 2018. The data were collected at the Bambey Treatment Center with the help of a semi-structured questionnaire in an individual interview after informed consent. The capture and analysis was done using EPI INFO software version 3.5.3 with a 95% confidence interval, α risk of 0.05. A total of 229 patients were enrolled including 81.6% of TPM +. The average age was 35.1 years + or - 16; sex ratio 2.7. The knowledge of cough was 95.2%, contagiousness 83.3%, suspicion 45.4%, knowledge of the effectiveness of treatment 84.8% and the free 70.1%. The median consultation time was 30 days with a 75% delay. This delay was statistically related to: education (OR = 2.8, CI [1.09 - 8.62]), suspected tuberculosis (OR = 4.1, CI = [1.7-10, 2]), the use of traditional medicine (P = 0.0001, OR = 3.9) and the type of provider consulted (OR = 2.7, CI = [1.2 - 6.3]) and suspicion of tuberculosis (OR = 7.7, CI = [3.9-15.4]). In conclusion Early diagnosis of tuberculosis requires public awareness of the disease, training of health care providers and collaboration between traditional healers and health care providers.

1198

COMPARISON OF CLINICAL CHARACTERISTICS BETWEEN INFLUENZA A AND INFLUENZA B VIRUS INFECTIONS IN IQUITOS, PERU

Crystyan Siles[1], Joan Neyra[2], Anna Kawiecki[3], Stalin Vilcarromero[1], Amy C. Morrison[3], Carolina Guevara[2], Julia S. Ampuero[2]

[1]*U.S. Naval Medical Research Unit Six, Iquitos, Peru,* [2]*U.S. Naval Medical Research Unit Six, Lima, Peru,* [3]*University of California, Davis, CA, United States*

Infections with influenza A and B virus (IAV, IBV) are a worldwide public health concern that causes recurrent epidemics and unpredictable pandemics in humans. In 2009 an H1N1 virus, A(pH1N1)pdm09, caused a pandemic that resulted in severe disease and death mostly in children. It was recently reported that in the United States IBV is as clinically severe as IAV (H1N1, H3N2) infections although this has not been examined in Peru. We performed clinic-based surveillance at twelve health facilities in Iquitos, Peru to compare clinical symptoms among confirmed cases of influenza illness. From December 2010-November 2014, we enrolled 1,032 participants with acute influenza like-illness (ILI) from whom pharyngeal swabs, epidemiological and clinical information were collected. The samples were tested by virus isolation in cell culture and/or subtype/lineage specific real-time reverse transcription PCR (rt RT-PCR) for detection of influenza virus RNA. 41.2% of the samples were positive for influenza by one of the two methods. From the rt RT-PCR positive samples, we detected 115 A(H1N1)pdm09 (10.6%), 169 A(H3N2) (15.6%) and 141 IBV (12.2%). We divided our population into 4 groups: children (5-11 years), adolescents (12-17 years), young adults (18-29 years) and adults (30 years or more). For each age group, we assessed the association between symptoms and type of influenza virus detected utilizing prevalence odds ratio (POR). In children, nausea had a significant association with IBV (POR: 4.7, [2.2-10.15]). In adolescents, ear ache showed a significant association with IBV (POR: 4.9, [1.4-17.4]). In young adults, respiratory symptoms, such as cough and rhinorrhea were predominant, although only conjunctiva injection was associated with A(H1N1)pdm09 (POR: 3.0, [1.4-6.3]). In adults, myalgia was associated with A(H1N1)pdm09 (POR: 4.1, [1.4-12.4]). Although few differences were identified among clinical symptoms within age-groups, most clinical symptoms presented for ILI were not significantly different when comparing IAV and IBV.

1199

EPIDEMIOLOGY AND VIRAL ETIOLOGY OF ACUTE RESPIRATORY INFECTIONS IN AN ACTIVE SURVEILLANCE IN THE PERUVIAN AMAZON FROM 2009-2017

Isabel Bazán[1], Eugenio Abente[1], Amy C. Morrison[2], Carolina Guevara[1], Juan Pérez[1], Regina Fernández[1], Stalin Vilcarromero[1], Carlos Alvarez[3], Julia S. Ampuero[1]

[1]*U.S. Naval Medical Research Unit No. 6, Lima, Peru,* [2]*U.S. Naval Medical Research Unit No. 6 , University of California, Davis, USA, Iquitos, Peru,* [3]*Dirección Regional de Salud, Loreto, Perú, Iquitos, Peru*

Acute respiratory infection is one of the leading causes of morbidity and mortality worldwide. In developing countries it is an important public health concern because access to etiological diagnosis is limited, hindering effective treatment. The present study was carried out in Iquitos, Peru, which has a tropical climate and is surrounded by tributaries of the Amazon River. Active surveillance was conducted by going door-to-door three times per week in designated neighborhoods and volunteers who met the following inclusion criteria were enrolled: fever and one other respiratory symptom such as cough and/or sore throat. A total of 4,387 oropharyngeal swab samples were collected between September 2009 and December 2017. Oropharyngeal samples were assayed by real-time reverse transcription PCR (rt RT-PCR) and/or virus isolation and subsequent pathogen identification by immunofluoresence. A total of 1,961 pathogens were detected in forty-four percent of the total number of samples collected (1,914/4,387). The pathogen most frequently detected was influenza A virus (IAV; 1,574/1,961), although parainfluenza virus (154/1,961), adenovirus (98/1,961), enterovirus (81/1,961) , herpes simplex virus (26/1,961), human metapneumovirus (17/1,961) and respiratory syncytial virus (11/1,961) were also detected. IAV remains a significant burden and continued surveillance is warranted to better inform on viruses circulating to better match vaccine strains to viruses circulating and causing visits to clinics and hospitals. Additionally, our data supports use of IAV rapid diagnostics to guide use of anti-IAV therapeutics.

1200

LUNG ULTRASOUND FINDINGS IN PULMONARY TUBERCULOSIS

Matthew Fentress[1], Robert Gilman[2], David Moore[1], Cesar Ugarte-Gil[3]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Universidad Peruana Cayetano Heredia, Lima, Peru*

Current diagnostic algorithms for pulmonary tuberculosis (PTB) rely primarily on sputum microscopy, chest x-ray (CXR) and nucleic acid amplification tests (NAAT). Lung ultrasound is increasingly used for diagnosis of pneumonia with excellent performance characteristics compared to conventional radiography. Inexpensive portable ultrasound machines are increasingly available in resource-limited settings, but data on the use of lung ultrasound for diagnosis of PTB is limited. The aims of this pilot study are to describe abnormal sonographic lung findings in patients with confirmed PTB, describe the changes in these sonographic lesions during anti-tuberculosis treatment, and compare the sonographic findings with CXR. This multi-center prospective cohort study will consecutively enroll participants ≥18 years of age with microbiologically-confirmed PTB within 1 week of treatment initiation. Each participant will have a comprehensive lung ultrasound exam at enrollment by a clinician trained in the study protocol, and a repeat ultrasound at 8 weeks post-enrollment. Representative video clips will be saved and reviewed by an expert sonographer. Our results will describe the frequency and location of each of six sonographic lung lesions - consolidations, small subpleural consolidations, cavities, miliary patterns, pleural effusions and B-lines. We will also analyze the change in sonographic lesions between 0 and 8 week exams. Finally, we will compare ultrasound and chest x-ray for identifying consolidation, miliary pattern, cavitation and pleural effusion as binary variables. Preliminary data on the first 6 enrolled participants demonstrates excellent sensitivity (100%) of the ultrasound technique for PTB. Final results (predicted n=60) will be available October 2019. This pilot study will assess the utility of point-of-care lung ultrasound to detect pulmonary lesions in PTB. If findings are positive, further studies with symptomatic and asymptomatic controls will be indicated to determine what role portable lung ultrasound may play in point-of-care diagnostic algorithms for PTB.

1201

HUMAN BOCAVIRUS DETECTED IN UGANDAN CHILDREN WITH HYPOXEMIC PNEUMONIA: PATHOGEN OR BYSTANDER?

Jack Underschultz[1], Robert O. Opoka[2], Andrea L. Conroy[3], Sophie Namasopo[4], Michael Hawkes[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Makerere University, Kampala, Uganda,* [3]*Indiana University School of Medicine, Indianapolis, IN, United States,* [4]*Kabale Regional Referral Hospital, Kabale, Uganda*

Pneumonia is the leading infectious cause of childhood mortality globally, with high disease burden in sub-Saharan Africa. Human bocavirus (HBoV) has been detected in patients with pneumonia but its pathogenic role remains unclear. Here, we describe a detailed case series of Ugandan children under 5 years of age hospitalized in a resource-limited setting with hypoxemic pneumonia and in whom HBoV was detected. Respiratory samples from the children's nasopharynx were collected and analyzed using multiplex PCR. Clinical characteristics were abstracted from the chart

record and novel biomarkers were quantified from plasma by enzyme-linked immunosorbent assay (ELISA). HBoV was detected in 12/130 (9.2%) of children with hypoxemic pneumonia. In all cases, at least one other micro-organism was co-detected in the nasopharynx. Most patients experienced cough, difficulty breathing, tachycardia, tachypnea, nasal flaring, chest indrawing, and crackles on auscultation. Radiographic infiltrate was present in 6/11 (55%) cases. Elevated levels of C-reactive protein, Chitinase-3-like protein 1, Lipocalin 2, and surfactant protein D, previously associated with severe or fatal pneumonia, were present in 5 (42%), 3 (25%), 0, and 2 (17%) patients, respectively. All patients received antibiotics and supplemental oxygen (median duration2.0 days [IQR 1.6-3.0]). The median length of hospital stay was 3.0 days (IQR 2.7-4.5), and there were no fatalities. Clinical and radiographic signs, as well as host biomarkers suggest that HBoV may participate in the pathogenesis of severe pneumonia in African children. However, co-detection of other potential pathogens suggests that HBoV1 may be a co-pathogen or bystander.

1202

INFLUENZA INFECTION IN THE YUCATAN DURING THE YEAR 2018

Luis O. Bobadilla-Rosado[1], Rodrigo G Díaz-Novelo[1], Diego O. Quijano-Torres[1], **Nina Mendez-Dominguez**[1], Salvador Gómez-Carro[2], Barbara C. Hoil Vales[1]

[1]*Universidad Marista de Merida, Merida, Mexico,* [2]*Epidemiological Unit, General hospital Agustin O'horán, Merida, Mexico*

Introducción: Influenza viruses are notable for their adaptability which often results in a yearly appearance between October and February in the northern hemisphere. It is estimated that 15% of the world population becomes infected with the influenza virus each year, resulting in deaths worldwide. The clinical presentation of this disease is characterized by headache and severe, often generalized, myalgia, it debuts with inflammation of the nasal mucosa; the pharynx; and conjunctiva. The purpose of this study is to compare the signs and symptoms presented by patients with confirmed diagnosis who were treated hospitalized or as ambulatory. Methods.This descriptive, observational cross-sectional retrospective study included all hospitalized and ambulatory patients during the 2018 season. All data was obtained from the hospitals´ epidemiological surveillance service at the general hospital located in the city of Merida, Mexico. *Results.*A total of 264 influenza cases occurred in 119 (45%) were men and 145 (55%) were women, 11(7%) of the later were pregnant while infected. Patients mean age was 27, 172(67%) were adults, 43 (26%) were vaccinated. Ambulatory patients were out of which 8 (8%) had the influenza vaccine. No vaccinated patient died. The incidence during the year 2018 was 17.65 cases per 100 000 exposed people. Adults were more prone to be hospitalized when havingdyspnea (OR 4.70) and tachypnea (OR 8.22), while polypnea associated to pediatric hospitalizations (OR 16.93). Tachypnea was associated with the general mortality (p<0.000); when in both, adult (p<0.000) and pediatric patients (p<0.017). Rhinorrhea (OR 0.25).and vomit (OR 0.23) correlated inversely with hospitalization. *Discussion and conclusions.*Hospitalized patients were more prone to present with polypnea, tachypnea, dyspnea, while correlated inversely with headache rhinorrhea and vomit. Vaccination inversely associated with mortality and even when headache is considered a diagnostic clinical criteria, it did not predict hospitalization or death.

1203

IMPACT OF EBOLA OUTBREAK ON TB TREATMENT ADHERENCE AND OUTCOMES IN SIERRA LEONE

Kathryn M. Hogan[1], Jia-Fu Jiang[2], Henry S. Bangura[3], Stephen Sevalie[2], Ya-Jun Song[2], Yi Sun[2], Jing Li[2], Zhong-Peng Zhao[2], Jun Jiao[2], Foday Sahr[4]

[1]*George Mason University, Glendora, NJ, United States,* [2]*Beijing Institute of Microbiology and Epidemiology, Beijing, China,* [3]*Public Health Department,* [34] *Military Hospital, Wilberforce, Sierra Leone,* [4]*College of Medicine and Allied Health Sciences, Freetown, Sierra Leone*

The 2014-2015 Ebola outbreak was devastating to West Africa, with an estimated 28,637 cases and 11,315 deaths as of January 2016. Beyond this, the outbreak had detrimental effects on the already fragmented health care system in Sierra Leone. This data specifically examines the impact of the Ebola outbreak on treatment and health outcomes of patients infected with tuberculosis in Sierra Leone at the time. During the outbreak, healthcare systems experienced great stress, often halting services such as treatment delivery and testing due to curfews, border closures, and lack of healthcare workers. This retrospective study reviewed data from the 34 Military Hospital at the Wilberforce Barracks in the Western province of Sierra Leone, a facility that provides services for soldiers, their families, and civilians. Patient Hospital Register TB Records from 2012 to 2016 from 1,264 patients were analyze using STATA (v.14). Preliminary testing using chi-squared test of associations were executed to determine whether any demographic characteristics such as age, sex, and occupation were associated with entry point, HIV co-infections, loss to follow up of treatment, or death. Logistic regressions were used to assess the level of association between number of deaths, losses to follow up, or defaults and year, adjusting for age, sex, and entry point. Preliminary results showed an increase in the number of deaths and number of patients lost to follow up after the Ebola outbreak. Chi-squared tests also showed a significant association between entry point and loss to follow up (χ^2 =19.1, p< 0.001), and occupation and loss to follow up (χ^2 = 29.82, p < 0.001). Further statistical analysis is required to understand the magnitude and factors associated with the impact of the Ebola outbreak on TB patient follow up and survival. Information from this study will indicate the percent reduction in treatment rates and related number of deaths before and after the Ebola outbreak. This can be used to improve the understanding of the indirect impacts of the Ebola outbreak on diseases other than Ebola.

1204

A CLINICAL CHALLENGE TRIAL DELIVERING AEROSOL BCG AS A CONTROLLED HUMAN INFECTION IN HEALTHY BCG-NAÏVE, UK ADULTS: ESTABLISHING OPTIMAL DOSE AND EVALUATING SAFETY

Julia L. Marshall[1], Iman Satti[1], Stephanie Harris[1], Rachel Wittenberg[1], Raquel Lopez Ramon[1], Michael Riste[1], Pedro Folegatti[1], Rebeccca Powell-Doherty[1], Alison M. Lawrie[1], Samantha Vermaak[1], Morven Wilkie[1], Paul Moss[2], Henry Bettinson[3], Helen McShane[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*NIHR / Wellcome Trust Birmingham Clinical Research Facility, Birmingham, United Kingdom,* [3]*Oxford Centre for Respiratory Medicine, Oxford, United Kingdom*

Human challenge models are a quick cost-effective way to assess candidate vaccine efficacy. Bacille Calmette-Guérin (BCG), a live-attenuated strain of *Mycobacterium bovis*, could be used as a surrogate *M. tuberculosis* challenge. BCG is licensed for human use and is protective against *M. tuberculosis* in UK populations. Intradermal BCG challenge can distinguish BCG naïve and prior-BCG vaccinated in UK adults, however an aerosol model would better mimic the natural infection route. We therefore undertook a randomized control trial to establish safety and optimal dose of aerosol BCG challenge in healthy mycobacteria-naive UK adults. Following dose escalation and satisfactory safety reviews, blinded volunteers were randomized to receive aerosol BCG with intradermal

saline placebo, or intradermal BCG with aerosol saline. Volunteers underwent a bronchoscopy 14 days post-challenge, completed a symptom diary, and were followed up for 6 months. 46 volunteers were enrolled and 12 volunteers inhaled the target dose of $1x10^7$ cfu BCG Bulgaria. There was no significant difference in the frequency or severity of either systemic or respiratory adverse events (AEs) by infection route. In both groups, most volunteers experienced at least one AE in the two weeks following BCG infection, the majority of which were mild and all of which were transient. There were no SUSARs and all bronchoscopies following aerosol BCG were normal. Local mucosal immune responses following BCG aerosol inhalation were higher than the corresponding systemic response. We have developed a controlled human mycobacterial challenge model and demonstrate here that delivery of inhaled BCG at doses of up to $1x10^7$ cfu is safe and feasible.

1205

NATIONAL MAPPING OF SOIL-TRANSMITTED HELMINTHIASIS AND SCHISTOSOMIASIS IN ETHIOPIA

Gemechu Tadesse Leta[1], Kalkidan Mekete[1], Yonas Wuletaw[1], Abeba Gebretsadik[1], Heven Sime[1], Sindew Mekasha[1], Adugna woyessa[1], Oummer Shafi[2], Michael French[3], Jozef Vercruysse[4], Bruno Levecke[4], Jack Grimes[5], Lasely Drake[5], Iain Gardiner[5], Wendy Harisson[6], Alan Fenwick[6]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*Federal Ministry of Health, Addis Ababa, Ethiopia,* [3]*RTI International, USA, Washington, DC, United States,* [4]*Ghent University, Merelbeke, Belgium,* [5]*Partnership for Child Development, London, United Kingdom,* [6]*Schistosomiasis Control Initiative, London, United Kingdom*

Geographical distribution of both soil-transmitted helminths (STHs; *Ascarislumbricoides*, *Trichuristrichiura*, and the hookworms (*Necatoramericanus* and *Anycylostomaduodenale*) and schistosomes (SCH; *Schistosomamansoni* and *S. haematobium*) is pivotal to design and implement mass drug administration programs. Currently, up to date data on the distribution of both STH and SCH is not available for Ethiopia. Between 2013 and 2015, we assessed the distribution of STHs and SCH in a nationwide survey covering 153,282 school aged children (age 5 to 15) from 833woredas (districts) representing all 9 Regional States and 2 City Administrations of Ethiopia. From these disease maps, recommendations were made on the implementation and frequency of mass drug administration programs. The prevalence of any STHs across the study population was 21.7%, with *A. lumbricoides* (12.8%) being the most prevalent, followed by hookworms (7.5%) and *T. trichiura* (5.9%). The prevalence for any SCH was 3.8%, with *S. mansoni* (3.5%) being the dominant and *S. haematobium* (0.3%). STHs were more prevalent in south west Ethiopia, whereas SCH most found in the west and north-east of the country. The prevalence of moderate-to-heavy intensity infections equalled 2% for STHs and 1.6% for SCH. Based on the disease maps for STH, 215 out of 833woredas(25.8%)require mass drug administration once a year and 279(33.5%)twice a year. For SCH, mass drug administration once a year is recommended for 69 woredas (8.2%) and once every two years for 153woredas (18.4%). The results confirm that Ethiopia is endemic to both STHs and SCH, and both diseases pose problem to public health. Following the WHO recommendations on mass drug administration, 18 and 14 million school age children are in need for mass drug administration for STHs and SCH respectively.

1206

EVIDENCE OF HYBRIDIZATION BETWEEN *SCHISTOSOMA HAEMATOBIUM* AND *S. BOVIS* IN CÔTE D'IVOIRE

Kpongbo Etienne Angora[1], Jean-François Allienne[2], Olivier Rey[2], Hervé Menan[3], William Yavo[3], André Offianan Touré[4], Jean Coulibaly[5], Giovanna Raso[1], Jürg Utzinger[1], Oliver Balmer[1], Jérôme Boissier[2]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*IHPE, University Montpellier, CNRS, Ifremer, University Perpignan Via Domitia, Perpignan, France,* [3]*Université Félix Houphouët-Boigny, BPV* [34]*, Abidjan, Côte D'Ivoire,* [4]*Institut Pasteur de Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [5]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire*

Schistosomiasis is the most prevalent neglected tropical disease in sub-Saharan Africa. In Côte d'Ivoire, *Schistosoma haematobium* causes urogenital schistosomiasis in humans, and *S. bovis* causes intestinal schistosomiasis in livestock. Both species are phylogenetically closed and hosted by the same freshwater snails of the genus *Bulinus*. There is no data available about hybridization between these species. So, this study aims to identify hybrids between *S. haematobium* and *S. bovis* in urines from schoolchildren in Côte d'Ivoire. A cross-sectional study was carried out in four locations in Côte d'Ivoire from January to April 2018. Urine samples from schoolchildren were examined to detect *S. haematobium* eggs using filtration method. Miracidia or eggs from positive samples were collected on Whatman® FTA cards and transferred to «Interactions Hôtes- Pathogènes- Environnement» (IHPE) laboratory in Perpignan (France) for genetic analysis. A RD-PCR test using mitochondrial Cox1 gene was performed and some miracidia DNA were sequenced. Of 1187 schoolchildren included in the survey, 116 (13.98%) were infected with *S. haematobium* microscopically. A total of 2688 miracidia or eggs were collected from 90 positive schoolchildren. The molecular analysis of schistosome miracidia or eggs confirmed infection with *S. haematobium* but also *S. haematobium-S. bovis* hybrids. Forty-eight percent of the miracidia or eggs were hybrids and 52 % were pure *S. haematobium*. Hybridization between schistosomes is present in Côte d'Ivoire. Therefore, further large-scale studies will be needed to identify all potential hybrids zones. Understanding such inter-species interactions is essential for optimizing control strategies.

1207

EFFECT OF SCHISTOSOMIASIS ON THE HEMATOLOGICAL PROFILE OF SCHOOL CHILDREN LIVING IN LAMBARÉNÉ, A SEMI-URBAN AREA IN GABON

Jean Claude Dejon Agobé[1], Yabo Josiane Honkpehedji[1], Jeannot Fréjus Zinsou[1], Jean-Ronald Edoa[1], Bayodé Roméo Adégbitè[1], Bertrand Lell[1], Peter Gottfried Kremsner[2], Ayola Akim Adegnika[1], Martin Peter Grobusch[3]

[1]*Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon,* [2]*Institut für Tropenmedizin, Eberhad Karls Universität Tübingen and German Center for Infection Research (DZIF), Tübingen, Germany,* [3]*Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands*

Schistosomiasis is a highly-prevalent parasitic infection in sub-Saharan Africa where school-aged children bear the brunt of the burden of disease. Schistosomiasis is a chronic disease, and the presence of blood in urine and/or in stool - occurring by blood-spilling during egg excretion - is the main symptom of the disease. In endemic areas where the full blood count (FBC) is very often used by clinicians as orientating examination in case of suspicion of an infectious disease, we assessed the impact of urogenital schistosomiasis on the hematology profile. A cross-sectional study was conducted among school children living in Lambaréné. Urine filtration technique was performed for the detection of *Schistosoma* eggs. Rapid Deep Stick (Combur[10] test) was performed for the detection of blood in urine while hematology parameters were evaluated using a Pentra ABX 60® analyzer. A total of 614 school children were included in the analysis. Mean age was 10.9 (SD = 2.7) years, with a 1.05 girl-to-boy sex ratio. The prevalence of schistosomiasis was 26%. Full blood counts (FBC) showed a decrease of hemoglobin levels (mean: 11.3 g/dL *vs* 11.7 g/dL, *p-value* = 0.0006), an increase of WBC (mean: 7.9 10^3/mm^3 *vs* 7.1 10^3/mm^3, *p-value* = 0.0008) and of platelet (mean: 258 10^3/mm^3 *vs* 231 10^3/mm^3, *p-value* = 0.001) levels among the schistosoma-infected children compared to their non-infected counterparts. Hematuria was found to be associated with schistosomiasis (RR=5.54, *p*-value < 0.001) with a sensitivity and specificity of 60% and 92%, respectively. Predictive positive and negative values were 73% and 87%, respectively. The prevalence of

schistosomiasis is moderate in Lambaréné, where children with infections exhibit a distinct FBC profile, indicating the necessity to take into account schistosomiasis as a co-morbidity when managing other infectious diseases in schistosomiasis-endemic areas.

1208

ASSOCIATION OF RIVERINE PRAWNS AND INTERMEDIATE HOST SNAIL AND CORRELATION WITH HUMAN SCHISTOSOMIASIS IN TWO RIVER SYSTEMS IN SOUTHEASTERN COTE D'IVOIRE

Nana Rose Diakite epse Ngoran

Universite Felix Houphouet Boigny, Abidjan, Côte D'Ivoire

The current emphasis of schistosomiasis control is placed on preventive chemotherapy using praziquantel. However, reinfection may occur rapidly in the absence of complementary interventions. Recent studies from Senegal suggest that predatory prawns might feed on intermediate host snails and thus impact on schistosomiasis transmission. We designed a study with four repeated cross-sectional surveys pertaining to prawns and snails, coupled with a single cross-sectional parasitological survey among humans. We assessed for potential associations between the presence/density of prawns and snails and correlation with Schistosoma infection in a composite sample of school-aged children and adults. The study was carried out between October 2015 and December 2016 in 24 villages located near the Agnéby and Mé coastal river systems in south-eastern Côte d'Ivoire. At each site, snails and prawns were collected, and in each village, 150 individuals were subjected to stool and urine examination for the diagnosis of Schistosoma mansoni and S. haematobium. We found peaks of relative abundance of intermediate host snails in the villages of the Agnéby River system, while predatory prawns were predominantly recorded in the Mé River system. A negative association was observed between intermediate host snail densities and riverine prawns; however, no pattern was found between this trend in the predator-prey relationship and the prevalence of human schistosomiasis.

1209

PREGNANCY INCREASES RISK OF *SCHISTOSOMA HAEMATOBIUM* INFECTION AND DISEASE SEVERITY AMONG REPRODUCTIVE AGE WOMEN IN MUNYENGE, SOUTH WEST REGION, CAMEROON. A CASE-CONTROL STUDY

Judith K. Anchang-kimbi[1], Godlove B. Wepnje[1], Vicky D. Ndassi[1], Irene U. Sumbele[1], Helen K. Kimbi[2]

[1]*University of Buea, Buea, Cameroon,* [2]*The University of Bamenda, Bambili, Cameroon*

Pregnancy is found to be associated with increased parasitic infections. In this study, we hypothese that pregnancy predisposes reproductive age women to higher *S. haematobium* infection rate and disease severity. The study was a case-control study carried out from November 2017 to January 2018. Pregnant women who reported for antenatal care clinic were enrolled as cases. Non-pregnant reproductive aged (15-45 years) females were selected from the same community and sampling period as the cases. Two control were enrolled for each case. Semi-structured questionnaires was used to collect information on socio-demographic data and water contact behaviour. Urine samples were obtained and analysed for the presence of microhaematuria and/or *Schistosoma haematobium* ova using test strip and filtration methods respectively. Haemoglobin levels were measured using haemoglobinometer. Data were analysed using univariate and multivariate regression analyses. A total of 300 (100 pregnant: 200 non-pregnant) females were enrolled into the study among whom 11.7% (35) were diagnosed for urogenital schistosomiasis (UGS). In univariate analysis, pregnancy status ($\chi^2 = 15.54$; $p < 0.001$), maternal age ($\chi^2 = 8.04$; $p = 0.018$), stream usage ($\chi^2 = 4.08$; $p = 0.043$) and degree of contact with stream (domestic activity and/or bathing) ($\chi^2 = 17.03$; $p < 0.001$) were factors associated with occurrence of the infection. Logistic regression analysis revealed pregnancy status as the only predictor of UGS (aOR = 7.83; 95% CI: 3.14 - 19.52) where pregnant females were seven-fold more likely to be infected than their non-pregnant counterparts. Fewer (< thrice/day) or no visit to stream (aOR = 0.49; 95% CI = 0.30 - 0.83) reduced exposure to infection. Haematuria ($\chi^2 = 9.99$; $p = 0.002$) and anaemia ($\chi^2 = 26.23$; $p < 0.001$) were more common among pregnant (15.0% vs 67%) than non-pregnant (4.5% vs 35.6%) females respectively. Urogenital schistosomiasis affects non-pregnant and pregnant women alike, but the later may be susceptible to greater disease severity as a result of their physiological state. Efforts to curtail maternal UGS in this setting cannot be overemphasized.

1210

EVALUATION OF THE EFFECT OF ARTEMISININ-BASED COMBINATION THERAPIES ON URINARY SCHISTOSOMA HAEMATOBIUM WHEN ADMINISTERED FOR THE TREATMENT OF MALARIAL CO-INFECTION

Dearrie Glory Okwu, Rella Zoleko Manego, Michael Ramharter, Ghyslain Mombo-Ngoma

Centre de Recherche Médicales de Lambaréné (CERMEL), Lambaréné, Gabon, Lambarene, Gabon

Schistosomiasis(STS) is the most prevalent water borne disease affecting 230 million people worldwide, of which 90% live in Africa. In 2007, a study presented the efficacy of Artemisinin based Combination Therapies (ACTs) in the management of STS when administered in the treatment of uncomplicated malaria. Praziquantel (PZQ) is presently the drug of choice for treating all forms of STS, however, this drug is found to be ineffective in immature stages. Additionally, parasite resistance and consequent rapid reinfection require the development of new therapies. ACTs could be combined with PZQ thus targeting different stages of parasite development to improve treatment outcomes. The evaluation of the efficacy of Artemisinin derivatives in the treatment of schistosomes is of great importance. The study is an open-label non- randomized trial to assess the efficacy and safety of artemisinin derivatives versus non- artemisinin drugs on *S. haematobium* infection while administered for the treatment of malaria. Study population included all patients in the study area diagnosed with malaria-STS co-infection. Those excluded from the study were treated with PZQ in the preceding 6 weeks, have drug intolerance or are pregnant. Basic demographic data and history of hematuria were recorded. During the follow up visits, urine samples were collected on day 28 and day 42 (6 weeks post treatment). Post treatment assessment was done on urine samples collected on at least two consecutive days. All subjects were treated with single dose PZQ (40mg/kg) as recommended by the WHO at the end of follow up. A total of 331 subjects were screened, 40 positive subjects were enrolled and treated with artesunate-pyronaridine. Median age is 12 years (Interquartile Range (IQR) 9-17), sex ratio of Male: Female is 2:1, Median baseline eggs is 30(IQR 6-256) while the median post treatment eggs is 22(IQR 4-304). One of the subjects cleared eggs in the urine by day 43.The study demonstrates some effect of ACTs on STS. Preliminary results are going to be presented at the ASTMH

1211

PERFORMANCE OF THE POINT OF CARE URINE CIRCULATING CATHODIC ANTIGEN TEST IN A SCHISTOSOMIASIS CONTROL PROGRAM SETTING IN WESTERN KENYA, 2017-2018

Anne Straily[1], Emmy K. Awino[2], Madeline Usey[1], Susan P. Montgomery[1], Ryan E. Wiegand[1], Alie Eleveld[2], Alex Mwaki[2], William E. Secor[1], Maurice R. Odiere[3]

[1]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [2]*Safe Water and AIDS Project (SWAP), Kisumu, Kenya,* [3]*Kenya Medical Research Institute (KEMRI), Kisumu, Kenya*

Schistosoma mansoni control programs have traditionally used the Kato-Katz fecal exam method (KK) for monitoring and evaluation of infection control programs. The commercially available point of care urine circulating cathodic antigen (POC-CCA) test might be an improvement over KK but before transitioning to the POC-CCA, it is necessary to determine how

it performs in a program setting. Single urine and stool specimens were collected from students in 30 schools in western Kenya prior to mass treatment with praziquantel and again one year later. Prevalence and intensity of infection were determined by both KK and POC-CCA. We compared changes in prevalence and intensity of the two tests among all the schools as well as when groups of schools were separated according to baseline KK prevalence (0-10%, >10-20%, >20%). Prevalence by POC-CCA was higher than by KK for all schools at both time points. Mean POC-CCA score and mean intensity decreased after one year when all schools were analyzed collectively ($p<0.0001$). The proportion of egg-positive (by KK) and POC-CCA positive individuals was significantly different between baseline and one year ($p<0.001$); notably, with POC-CCA there were significantly fewer "trace" results and more +2 and +3 results. The POC-CCA results did not vary in proportion to the KK results thus, the translation of historical data and programmatic guidelines based on KK results to the POC-CCA may not be straightforward. The reduction in the proportion of "trace" results suggests that persons with light infections were less likely to be re-infected during the course of the following year. POC-CCA may be useful as tool to evaluate the effectiveness of schistosomiasis control programs, but where programs wish to switch to the POC-CCA from historical KK testing use, it may be necessary to establish new prevalence maps and programs using only the POC-CCA.

1212

UTILIZATION OF THE COVERAGE SUPERVISION TOOL DURING SCHISTOSOMIASIS MASS DRUG ADMINISTRATION

Ibrahim Kargbo Labour[1], Habib I. Kamara[2], Mohamed Turay[2], Abdul Conteh[1], Abdulai Kandeh[2], Victoria Redwood-Sawyer[2], Mohamed Kallon[2], **Mustapha Sonnie**[2], Mary Hodges[2]

[1]*Neglected Tropical Disease Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, Freetown, Sierra Leone*

Since 2009 mass drug administration (MDA) with praziquantel has been used for schistosomiasis (SCH) control and independent monitoring used to help monitor the MDA process and validate reported coverage. World Health Organization guidelines recommend a Supervisor's Coverage Tool (SCT) for use in supervising the MDA to ensure effective coverage. This study in 2018 was to assess coverage and factors affecting compliance in 7 districts using CST for the first time in Sierra Leone. HKI recruited and trained supervisors at district level in Makeni. The chiefdoms with SCH prevalence >20% at the last national survey (2016) were selected and assigned into 16 Supervision Areas (SAs): Bombali: 1, Kenema: 4, Kailahun: 4, Kono: 2, Koinadugu: 4 and Tonkolili: 1. 20 villages per SA were randomly selected. One household per village was randomly selected. One school-aged child (5-14 years) was randomly selected and interviewed. If the child was <10 years, their caregiver was interviewed. In total 320 children were selected (boys: 51.6%, girls: 48.4%). Overall coverage was 79.4% (boys: 82.4%, girls: 76.1%). Six SAs reached acceptable coverage (≥18 children treated). However, 7 SAs failed to conclusively reach acceptable coverage (13-17 children treated) and 3 SAs had inadequate coverage (≤12 children treated). Reasons for noncompliance (66) were: not offered drug (31), community not reached (18), out of area (7), sick (4), no knowledge of MDA (3), afraid of side effects (2) and mother not allowing child to take the drug (1). Findings were relayed to the health units responsible for the catchment communities, the district NTD focal person and the national NTDP in order that the mop-up MDA could be performed. All SAs with inadequate coverage were from Karene (Bombali) and Falaba (Koinadugu) which are notorious for being underserved by health facilities per square kilometer and health workers per population and are in the process of being designated as new districts in recognition of the need. Implementation of the SCT was smooth and quickly determined villages where MDA had been ineffective.

1213

POST-TREATMENT SURVEILLANCE CRITERIA FOR *SCHISTOSOMA MANSONI*: WILL ELIMINATION OR RESURGENCE OCCUR AFTER STOPPING MASS DRUG ADMINISTRATION?

Jaspreet Toor[1], James E. Truscott[1], Marleen Werkman[1], Hugo C. Turner[2], Anna E. Phillips[1], Charles H. King[3], Graham F. Medley[4], Roy M. Anderson[1]

[1]*Imperial College London, London, United Kingdom,* [2]*Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,* [3]*Case Western Reserve University, Cleveland, OH, United States,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Schistosomiasis remains an endemic parasitic disease affecting millions of people around the world. The World Health Organization has set elimination (breaking transmission) as the end goal for schistosomiasis. However, there is currently a lack of guidance on what treatment programs should do once very low prevalence levels have been reached to determine whether elimination or resurgence of the infection will occur after stopping mass drug administration (MDA). Using a mathematical model and positive predictive values, we define the post-treatment surveillance criteria which can be used to detect *Schistosoma mansoni* elimination. We determine the prevalence threshold, i.e. prevalence of infection, below which the parasite cannot maintain transmission leading to elimination. We find that a prevalence threshold of 0.5% by Kato-Katz is sufficient for surveillance six months after the last round of MDA. However, as such a low prevalence can be difficult to measure in the field, we recommend using a prevalence threshold of 1% by Kato-Katz at two years (or later) after the last round of MDA with a sample size of 200 individuals across the entire community. Higher prevalence thresholds can be used but require a longer post-treatment surveillance period. For treatment programs where elimination is highly likely (due to maintenance of high MDA coverage), higher thresholds could be used sooner. Overall, our results provide guidance on the post-treatment surveillance which needs to be carried out when approaching elimination of *S. mansoni* transmission in a defined area.

1214

OPTIMIZING SURVEY STRATEGIES FOR PRECISION MAPPING OF SCHISTOSOMIASIS TO GUIDE MASS DRUG ADMINISTRATION: A VALUE-OF-INFORMATION ANALYSIS

Nathan C. Lo[1], Yi Liu[2], Giovanna Raso[3], Jean T. Coulibaly[4], Hugh J.W. Sturrock[5], Jürg Utzinger[3], Isaac I. Bogoch[6], Jason R. Andrews[7]

[1]*Stanford University School of Medicine; University of California San Francisco, Stanford; San Francisco, CA, United States,* [2]*University of Chicago, Chicago, IL, United States,* [3]*Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [4]*Swiss Tropical and Public Health Institute, University of Basel, Université Félix Houphouët-Boigny, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [5]*University of California San Francisco, San Francisco, CA, United States,* [6]*University of Toronto, Toronto, ON, Canada,* [7]*Stanford University School of Medicine, Stanford, CA, United States*

WHO surveillance guidelines for schistosomiasis are designed to estimate prevalence to inform a decision on mass drug administration (MDA). However, the current sampling strategy for these prevalence surveys do not account for prior sources of data (e.g. historical surveys, geospatial risk maps), the 10% prevalence threshold for an MDA decision, or the costs and health impact of a "right" or "wrong" decision. We developed a value-of-information model that integrated prior information on prevalence, sampling theory, and cost-effectiveness analysis to improve efficiency of sampling. The model used prior prevalence data to estimate the optimal sample size for district prevalence surveys to guide MDA decisions based on value-of-information. We defined "value" of collecting stool sample data in US$ based on whether this information changed an MDA decision. We used person-level data on S. mansoni from Côte d'Ivoire (< 2011) to construct a prior prevalence estimate by district.

Simulations computed the value of collecting stool samples (N=0-5,000) from schools testing all possible prevalences and using Bayesian updating. The model used a dynamic transmission and cost-effectiveness model to estimate the total programmatic cost (US$) and health impact (disability adjusted life-years) of the MDA decision over 5 years. We evaluated the model optimal sample size and classification accuracy compared to WHO surveillance guidelines using person-level data on S. mansoni from 25 districts in Côte d'Ivoire (2011-2012). The model recommended a mean sample size of 45 total children (range: 0-61 children) chosen amongst 2-4 schools per district, compared to WHO recommendation of 250 children chosen amongst 5 schools. The model correctly classified the district MDA decision in 90% of cases with the full dataset as reference, while reducing total sampling by 82% (95%UI: 80-87%). Prevalence surveys using a value-of-information approach to estimate sample size can reduce sampling effort with high classification accuracy for MDA decisions, which can be applied to precision mapping of endemicity at a community level and future WHO surveillance guidelines.

1215

A BETTER UNDERSTANDING OF THE BASIC PARASITE LIFE CYCLE AND TRANSMISSION DYNAMICS IS CRUCIAL TO MOVE TOWARD THE ELIMINATION OF SCHISTOSOMIASIS

Louis-Albert Tchuem Tchuenté

University of Yaoundé I, Yaoundé, Cameroon

Within the past decade, significant progress has been made on the control and elimination of neglected tropical diseases (NTDs). Today, the 'preventive chemotherapy' is the primary strategy for the control of 4 helminthiasis from the 20 diseases that are listed by the World Health Organization as the group of NTDs, i.e. schistosomiasis, soil-transmitted helminthiasis, onchocerciasis and lymphatic filariasis. The major intervention for schistosomiasis control is mass administration of praziquantel to those shown to be, or presumed to be, at-risk of infection, on the basis of disease mapping. However, using praziquantel alone has not been proven to be able to lead to elimination in high transmission settings. The interruption of schistosomiasis transmission involves several challenges due to multiple epidemiological and ecological factors such as climatic and physical conditions, type of water body, snail species and population dynamics, impact of reservoir hosts, and intensity of water contacts. Schistosomes have a complex life cycle and their transmission is highly focal. Reaching the endgame requires the implementation of alternative integrated multi-interventions approach, guided by a better understanding of the transmission of schistosomiasis in the targeted settings. The presentation will highlight and discuss some of the key factors and considerations that should guide optimizing our interventions to overcome the impact of schistosomiasis and accelerate toward the elimination of schistosomiasis in sub-Saharan Africa.

1216

INDIVIDUAL AND VILLAGE-LEVEL CONNECTIVITY AND RISK OF *SHISTOSOMA JAPONICUM* INFECTION IN SICHUAN, CHINA

Andrea Geri Buchwald[1], Elise Grover[1], Julia Van Dyke[1], Ding Lu[2], Yang Liu[2], Bo Zhong[2], Elizabeth J. Carlton[1]

[1]*University of Colorado School of Public Health, Aurora, CO, United States,* [2]*Sichuan Centers for Disease Control, Chengdu, China*

Human population movement (HPM) is vital to pathogen spread and transmission. *Schistosoma* parasites have a complex life cycle: transmission is dependent on a variety of ecological pathways including transport of larval stages via water and movement of snails and mammalian hosts. Human migration has been shown to contribute to schistosome emergence, and accounting for short-scale parasite movement improves performance of schistosomiasis transmission models, suggesting that HPM also plays a role in schistosomiasis maintenance and transmission. However, the mechanisms by which HPM contributes to individual-level *Schistosoma* infection risk are poorly characterized. In endemic areas, travel outside the region may be protective against infection, while certain types of travel may increase exposure and infection risk. Utilizing data collected from detailed monthly travel surveys from residents in 28 communities in Sichuan, China where *S. japonicum* is present, we aimed to describe short-term HPM and to determine the strongest travel-related predictors of *S. japonicum* infection. Candidate predictors included timing, frequency, distance, duration, and purpose of recent travel, as well as known demographic risk factors. Village connectivity was measured as travel distance from nearest city and frequency of travel outside the village by residents. As this was a large set of correlated variables, Random Forest was used to investigate the contribution of HPM variables to individual-level infection risk, and multi-level general estimating equations (GEE) were used to disentangle the effects of individual vs community-level HPM on population prevalence. Age, the number of years a person has lived in the village, travel in August and September, and mean number of days traveled were the factors most predictive of *S. japonicum* infection. In GEE models, village-level predictors of distance from the city and increasing frequency of travel were more strongly associated with increased individual *S. japonicaum* risk, suggesting that community connectivity plays a more important role than individual travel in *S. japonicum* transmission.

1217

BIOGEOGRAPHICAL CHARACTERISTICS OF *SCHISTOSOMA MANSONI* ENDEMIC AREAS IN ETHIOPIA

Keerati Ponpetch[1], Berhanu Erko[2], Lindsay Richards[3], Yang Yang[4], Song Liang[1]

[1]*Department of Environmental and Global Health, University of Florida, Gainesville, FL, United States,* [2]*Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia,* [3]*Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, United States,* [4]*Department of Biostatistics, University of Florida, Gainesville, FL, United States*

Schistosomiasis is a neglected tropical disease affecting 258 million people in 78 countries worldwide with over 90% of cases occurring in sub-Saharan Africa. In Ethiopia, schistosomiasis is caused by *Schistosoma mansoni* and *S. hamaetobium* with the former being widely distributed and prevalent - more than 4 million people are infected by *S. mansoni* annually with 35 million at risk of infection. Although many school- and community-based epidemiological surveys were conducted in the past decades, the national distribution of schistosomiasis endemic areas and associated socio-environmental determinants remain little understood. The present study aims to delineate biogeographical characteristics of *S.mansoni* endemic areas in Ethiopia. Through a systematic review, we compiled a comprehensive dataset on *S.mansoni* surveys. A total of 95 survey sites were identified and geo-referenced. Sites are distributed in six regional states - Southern Nations, Nationalities, and Peoples' Region (SNNPR), Tigray, Addis, and Beneshangul Gumuz, with the majority of surveys in Amhara and Oromia. The surveys exhibited a wide range of prevalence of infections from 0.43% to 95.06%, and 20% of the sites had >50% prevalence of infection, followed by 47% and 33% of the sites in the ranges of 10%-50% and <10%, respectively. A meta-analysis using a random effect model generated pooled estimates of 45.67%, 24.56%, and 19.84% for the prevalence in SNNPR, Tigray, and Oromia regions, respectively. Based on landscape features, these areas are broadly classified into the following ecozones: Afroalpine, East Montane, Eastern, Northeastern, Rift Valley, Western, and West Mountain. The Western Ecozone had the highest prevalence at 38.4%, followed by 38.14% and 34.8% in Rift Valley and East Montane Ecozones, respectively. In addition, endemic sites with >50% prevalence of infection were found at altitudes from 1,010 to 2,016 meters above sea level, annual mean temperatures between 18.6 - 25.64 degree Celsius, annual cumulative precipitation between 652 - 1754 millimeters, and normalized difference vegetation index between 0.28 - 0.74.

1218

MODELLING THE IMPACT OF VACCINATION STRATEGIES ON THE TRANSMISSION OF SCHISTOSOMIASIS

Klodeta Kura, James Truscott, Jaspreet Toor, Roy Anderson

Imperial College London, London, United Kingdom

Schistosomiasis is one of the most important neglected tropical diseases (NTDs) affecting millions of people in 79 different countries. The World Health Organization (WHO) has specified two control goals to be achieved by 2020 and 2025. These are morbidity control and elimination as a public health problem goals. Mass drug administration (MDA) is the major method for schistosomiasis control but it has proved difficult in the past to both secure adequate supplies of the most efficacious drug praziquantel to treat the millions infected either annually or biannually, and to achieve high treatment coverage in targeted communities in regions of endemic infection. The development of alternative control methods remains a priority at present. Using an individual based stochastic model, we analyze whether the addition of a novel vaccine alone or in combination with drug treatment, is a more effective control strategy, in terms of achieving the WHO goals, as well as the time and costs to achieve these goals when compared to MDA alone. The key objective of our analyses is to help facilitate decision making for moving a promising candidate vaccine through the phase I, II and III trials in humans to a final product for use in resource poor settings. The simulation studies indicate that a vaccine is needed to both reduce the probability of reinfection and eliminate transmission within a reasonable time frame. The value of the vaccine depends on vaccine efficacy, the duration of vaccine protection, the goal (reduction in prevalence of infection or prevalence of heavy-intensity infection) and the cost of the treatment. In high transmission settings, vaccination alone may achieve the morbidity control goal, but integrated MDA plus vaccination is required to achieve the elimination as a public health problem goal.

1219

SCHISTOSOMIASIS IN BURKINA FASO: TIME TO CHANGE CONTROL STRATEGY IN FOUR PERSISTENT HOTSPOTS

Hamado Ouédraogo[1], Clarisse Bougouma[1], Dieudonné Nare[2], Jean-Paul Djiatsa[2], Amy Veinoglou[3], Achille Kaboré[4], Fanny Yago-Wienne[2], Yaobi Zhang[5]

[1]*NTD Control Program, Ministry of Health, Ouagadougou, Burkina Faso,* [2]*Helen Keller International, Ouagadougou, Burkina Faso,* [3]*Helen Keller International, New York, NY, United States,* [4]*Family Health International 360, Washington, DC, United States,* [5]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

The baseline mapping of schistosomiasis in Burkina Faso in 2004-2005 showed high level of endemicity for *Schistosoma mansoni* and *Schistosoma hematobium*, with prevalence ranging from 1.67% to 89.4% across the country. The country has three ecological zones with the following baseline prevalence: a) Sahelian zone with the highest prevalence of 89.4% in the North and Sahel regions, b) Sahelo-Sudanese zone with a prevalence of 51.1% in the Center East, Haut Bassins and Boucle du Mouhoun regions, and c) Sudanese zone with a prevalence of 34.3% in the South West. Mass treatment campaigns with praziquantel have been implemented since 2004. *S. hematobium* is predominant in the Sahel region whereas *S. mansoni* is more prevalent in the south and Haut Bassins. After more than 10 years of mass drug administration (MDA), the prevalence from sentinel sites decreased significantly from between 1.7% and 89.4% (mean prevalence of 53.9%) to between 0% and 23.9% (mean prevalence of 5.6%) in 2016. By 2017, the prevalence in previously high endemic region dropped to less than 5%. As of June 2016, the villages of Panamaso (Haut-Bassins), Nagbingou, Tougouri (Center-East) and Dori (Sahel) had prevalence of 26.85%, 29.9%, 20% and 14.81% respectively. In the district of Dafra, two sentinel site villages were surveyed (Sogossagasso and Panamasso). All positive cases were found in the village of Panamasso which was previously documented as a persistent hotspot with increase in prevalence (8.3% in 2005, 25% in 2013 and 26.9% in 2017) despite annual MDA with good coverage (>75%). Although MDA has been implemented with satisfactory coverage (>75%) for over 14 years, schistosomiasis remains a public health issue in these few isolated areas in the country. Some behavior issues might be contributing to frequent reinfection of people in these hotspots. The situation calls for a change in treatment strategies, for socio-science investigation and for the roll-out of multi-sectoral interventions involving WASH, intensive health education or snail control, to permanently bring the prevalence down in the hotspots.

1220

EFFICACY OF BIENNIAL TREATMENT OF SCHISTOSOMIASIS IN MODERATE AND HIGH ENDEMIC AREAS IN BURKINA FASO

Hamado Ouédraogo[1], Clarisse Bougouma[1], Dieudonné Nare[2], Jean-Paul Djiatsa[2], Amy Veinoglou[3], Achille Kaboré[4], Fanny Yago-Wienne[2], Yaobi Zhang[5]

[1]*NTD Control Program, Ministry of Health, Ouagadougou, Burkina Faso,* [2]*Helen Keller International, Ouagadougou, Burkina Faso,* [3]*Helen Keller International, New York, NY, United States,* [4]*Family Health International 360, Washington, DC, United States,* [5]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

The World Health Organization (WHO) guidelines recommend mass drug administration (MDA) with praziquantel to control schistosomiasis (SCH) according to district level prevalence. In districts with prevalence between 10 and 50%, WHO recommends biennial MDA (once every 2 years). Baseline mapping in 2004 in Burkina Faso showed an overall prevalence of between 1.7% and 89.4% in high endemic zones (Sahel region). Burkina Faso adopted a biennial treatment strategy across the regions targeting school age children (5 to 15 years old) and adults. At baseline, 23 sentinel sites (schools) were established, among which 5 had prevalence above 50%, 15 had prevalence between 10% and 50% and 3 had prevalence below 10%. For sentinel site survey, a systematic sampling method was applied to select children in each school, 160 children aged 7 to 11 (32 children in each classroom with equal number of girls and boys). For urinary schistosomiasis, one urine specimen was collected from each child to determine *S. haematobium* infection using the filtration method and microscopy. For intestinal schistosomiasis, a single stool sample was collected from each child. Duplicate Kato-Katz slides were prepared from each sample and examined for *S. mansoni* infections. Out of 22 sentinel sites surveyed between 2016 and 2018, 18 have SCH prevalence less than 5%. The other 4 have prevalence of 26.9%, 23.9, 6.3% and 5.6% respectively. After more than 10 years, the biennial MDA with satisfactory coverage (>75%) has been successful in controlling SCH not only in moderately endemic districts (10-50% baseline prevalence) but also in 4 out of 5 highly endemic districts (>50% baseline prevalence). The Strategy has brought the prevalence down to less than 1% and 5% respectively in 13 and 4 sentinel sites (out of a total of 22). Only 4 sentinel sites have prevalence above 5% in 2018. It is likely that the country could achieve elimination of SCH as public health problem by 2025 if more efforts are deployed to maintain the gains and to wipe out the last areas where SCH is still prevalent.

1221

DECISION MAKING FOR MASS DRUG ADMINISTRATION FOR SCHISTOSOMIASIS AFTER IMPACT SURVEYS IN SENEGAL, 2016-2018

Boubacar Diop[1], Fatou Ndiaye Badiane[1], Amadou Doucoure[1], Mawo Fall[2], Daniel Albert Cohn[3], Achille Kabore[3]

[1]*Direction de Lutte contre la Maladie, Ministère de la Santé et de l'Action Sociale, Dakar, Senegal,* [2]*RTI International, Dakar, Senegal,* [3]*FHI 360, Washington, DC, United States*

Since 2016, Senegal's National Bilharzia and Soil-Transmitted Helminths Control Program (PNLBG) has closely followed World Health Organization guidelines for decisions about the frequency or stopping of treatment

for schistosomiasis (SCH) in districts already under treatment with praziquantel. From 2016-18 the PNLBG conducted SCH evaluations in districts that had completed 5-6 rounds of MDA, per WHO guidelines. The evaluations followed the standard protocol, adapted to the local context (at each site, 50 children aged 7-14 years were selected), and used standard diagnostic techniques (Kato-Katz for *S. mansoni*; reagent strips and urine filtration for *S. haematobium*). Sites were selected from those previously surveyed, choosing those with the highest prevalence of SCH (of either species). The highest-prevalence finding in each homogeneous ecological zone (2016 evaluations) or district (2017-18), by site, by species, and by diagnostic, was retained for the entire zone or district. The 2016 evaluation, in three ecological zones of the Senegal River Basin, found that the Haut Bassin (10 districts), the Vallée (6 districts), and the Delta (8 districts) zones all had SCH prevalence ≥50%, meaning MDA should be conducted twice per year (per WHO guidance). The 2017 evaluation, in 8 districts, found 1 district with prevalence ≥50%, (twice per year MDA); 2 districts with prevalence ≥10% and <50% (continue MDA with same frequency); 3 districts with prevalence ≥1% and <10% (biennial MDA); and 2 districts with prevalence <1% (no further MDA). The 2018 evaluation, in 3 districts, found 1 district with prevalence ≥50% (twice per year MDA); and 2 districts with prevalence ≥10% and <50% (continue MDA with same frequency). The PNLBG has maintained or adjusted the frequency of district-level MDA in line with WHO guidance based on the prevalences found, except where twice-yearly MDA is recommended; in these areas the PNLBG has maintained once-yearly treatment for reason of available internal and external resources, including the donation of praziquantel. This approach has enabled Senegal's NTD Control Program to focus its resources where they are most needed.

1222

RELATIONSHIP BETWEEN GUT MICROBIOTA AND *FASCIOLA HEPATICA* INFECTION IN CHILDREN FROM A COMMUNITY IN CAJAMARCA, PERU

Hugo Carrillo-Ng[1], Yordi Tarazona[1], Miguel A. Aguilar-Luis[2], Wilmer Silva-Caso[2], Carmen Tinco-Valdez[1], Carlos Palomares-Reyes[2], Ronald Aquino-Ortega[1], Johanna Martins-Luna[1], Isaac Peña-Tuesta[1], Juana M. del Valle-Mendoza[2]

[1]*Instituto de Investigacion Nutricional, Lima, Peru,* [2]*Investigation Center and Innovation of the Health Sciences Faculty, Universidad Peruana de Ciencias Aplicadas (UPC), Lima, Peru*

Fasciola hepatica infection is one of most important zoonosis affecting the liver in Peru. The highest prevalence of human fascioliasis has been reported in the Andean valleys with a prevalence that can reach up to 27%. The intestinal microbiota is an essential part of the gut homeostasis, it participates in the development of intestinal mucosa and immune system of the host. Recent studies have shown that parasitic infections can cause a negative effect on gut microbiota. However, *Fasciola hepatica* infection and its relationship with the gut microbiota have not been studied yet. The aim of this study was to describe the prevalence of 13 representative bacteria from the gut microbiota in stools samples from children with *Fasciola hepatica* infection and children without the infection. A prospective cross-sectional study was conducted in 103 children between 5 and 12 years old from a community in San Pablo (Cajamarca,Peru). A stool sample was collected from each patient. The presence of *Fasciola hepatica* was determined by ELISA using the commercial kit Bio-X Fasciola hepatica antigenic ELISA kit (Bio-X diagnostics, Belgium) . DNA extraction was performed using the commercial kit innuPREP Bacteria DNA kit (Analytik Jena, Germany) and 13 bacterial pathogens were amplified using PCR. Of all 103 patients, 16 (15.53%) cases were positive for *Fasciola hepatica.* The most common isolated bacteria from patients with *Fasciola hepatica* infection were Proteobacteria (68.5%), followed by Bacteroides (37.5%), Lactobacillus (37.5%) and Clostridium (12.5%), these pathogens were significantly lower compared with patients negative for *Fasciola hepatica* with prevalence of Proteobacteria (86.2%), Bacteroides (70.1%), Lactobacillus (72.4%) and Clostridium (69%). The prevalence of *Fasciola hepatica* infection was associated with contact with animals, this being significant with 15/16 cases (94.62%). *Fasciola hepatica* infection has a high prevalence in school-aged children from Cajamarca, Peru. It has a negative impact on the presence of some gut bacteria such as Bacteroides, Lactobacillus, Proteobacteria and Clostridium.

1223

ACCESSIBLE-OMICS: ADVANCES IN SCRNA TRANSCRIPTIONAL SIGNALING AND THE CREATION OF A TRANSCRIPTOMIC ATLAS OF ZAMBIAN ADULTS WITH LIKELY ENVIRONMENTAL ENTEROPATHY

Thomas Wallach[1], Conner Kummerlowe[2], Travis Hughes[2], Paul Kelly[3], Alex Shalek[2], Zev Gartner[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Massachusetts Institute of Technology, Cambridge, MA, United States,* [3]*University Teaching Hospital, Lusaka, Zambia*

The intestinal epithelium comprises a diverse population of cells that must collaborate to maintain gut function. Understanding the normal cellular composition of this population, and how it might differ between the developing and developed worlds, is key for addressing poorly understood non-focal conditions such as Environmental Enteropathy (EE). EE is characterized by poor growth and increased intestinal permeability, and typically found in areas with poor access to clean water. Its effects persist into adulthood, with persistent intestinal epithelial changes and increased risk of infection and metabolic syndrome. Its restriction to low-income regions of the world has limited application of cutting-edge molecular profiling techniques until the recent advent of Seq-Well. Seq-Well is a massively-parallel single-cell RNA-Seq platform that leverages advances in materials technology to allow for on-site, simple processing without complicated peripherals, facilitating creation of cDNA libraries anywhere. This allows for shipment of cDNA to facilities with the equipment for sequencing and associated computational approaches. We have successfully demonstrated single cell analysis of both duodenal and jejunal epithelium obtained from biopsies from 17 Zambian adults residing in high EE risk regions. We have identified enteroendocrine L cells via PYY production, but noted an absence of GLP1 and GLP2 transcripts. We see a high level of Enteroendocrine D cells with high somatostatin production. Decreased GLP2 is correlated with increased issues with insulin insensitivity (Amato et al, 2016), and somatostatin has been shown to support barrier function while inhibiting proliferation, these findings support a possible compensatory mechanism to recurrent enteric infection consistent with secondary findings of EE such as increased risk of metabolic syndrome and diminished crypt:villus ratio. Characterization of these pathways can be used for identification of not only new possibilities for non-invasive disease assessment and monitoring, but also possible therapeutic or prophylactic interventions.

1224

A WATERBORNE DISEASE INDEX (WBDI) FOR RURAL COMMUNITIES IN THE CARIBBEAN

Akilah T. Stewart[1], Vrijesh Tripathi[1], Azad Mohammed[1], Catherine Seepersad[1], Adrian Cashman[2], Dave D. Chadee[1], Adesh Ramsubhag[1]

[1]*The University of the West Indies, St. Augustine, Trinidad and Tobago,* [2]*The University of the West Indies, Cavehill, Barbados*

Within rural communities, socio-economic resource issues can impact access to potable water. However, these issues are often not well captured at a national level but need to be considered in developing national water resource management policies. A Waterborne Disease Index (WBDI) is a new tool that can be used by policy makers to make decisions on allocation of critical water resources. It integrates water stress and scarcity measures from a Water Poverty Index (WPI) survey instrument with microbiological water quality data. In this study a WBDI was developed for rural communities in the southern Caribbean based on a study in communities of Carriacou, Grenada, Nariva, Trinidad and Speightstown, Barbados. A structured questionnaire was administered to 606 households and included five key sections: Demographics, Water

Use & Water Quality, Environmental Health, Agricultural Activities and Climate Change Perceptions. Microbial data from 303 domestic water samples were analysed for pathogens of human health interest. Calculated WPI values scores out of 100 were similar in the mid-range for the three communities. The categories of Use (the different ways in which water is used) and Environment (health status of the environment) values account for the major vulnerabilities within these communities. The microbial water quality results also showed widespread occurrence of DNA from pathogenic organisms from all the rural communities suggesting potential exposure concerns which is reflected in the WBDI values. This tool can be more widely applied in the Caribbean region to characterize water resource status as it includes data beyond water scarcity and can highlight areas that require improvement. These results can complement health data to develop a comprehensive picture of water resources issues in the study areas of interest.

1225

RECOMMENDATIONS FOR BUCKET CHLORINATION IMPLEMENTATIONS IN EMERGENCY CONTEXTS AND CHOLERA OUTBREAKS

Gabrielle String, Mustafa Sikder, Yarmina Kamal, Annie Huang, Karin Gallandat, Daniele Lantagne

Tufts University, Medford, MA, United States

Bucket chlorination (BC) is a commonly implemented intervention for water treatment, particularly in cholera outbreaks. In bucket chlorination, agents stationed near a water source dose beneficiaries' water containers with chlorine. International guidelines suggest two recommendations for chlorine dosage: a fixed dosage (2 mg/L for <10 NTU, 4 mg/L for >10 NTU) or a variable dosage that provides a free chlorine residual (FCR) of 0.5 mg/L 30 minutes after dosing. To understand the effectiveness of these guidelines and the factors that impact their implementation, we investigated BC in laboratory studies and field evaluations. In the laboratory, using spread plates and viability qPCR, we assessed the inactivation of *V. cholerae* in prepared challenge waters after treatment with three different chlorine types at the two recommended dosages. Preliminary results indicate that all chlorine types and dosages achieved >3-log reduction in *V. cholerae* 30 minutes post-treatment. At 24 hours, culturable *V. cholerae* was present in ≥5 NTU water when it was not detected at 30 min due to incomplete inactivation. Variable dosages provided higher FCR at 30 min than fixed dosages for high organic demand water. Finalized results will include whether recommended dosages inactivate *V. cholerae* and maintain 0.2 mg/L FCR 24 hours post-treatment. In field evaluations, we conducted key informant interviews, structured observations of chlorination points, chlorine solution testing, focus groups, and household surveys. Drinking water samples were collected at the household and source. Four evaluations were completed in DRC (2), Cox's Bazar (1), and Haiti (1), including 40 chlorination points and 702 households. Preliminary results indicate variability in average chlorine solution concentration (0.18-3%). *E. coli* levels were generally reduced from source to storage in a majority of households 30 minutes post-treatment; however, presence of total coliforms and non-detect FCR (80% and 11% of households respectively) indicates recontamination risk. Recommendations on chlorine efficacy, dosage, manufacture, and monitoring will be presented.

1226

IDENTIFYING PSYCHOSOCIAL DETERMINANTS OF WASH BEHAVIORS FOR THE DEVELOPMENT OF THE EVIDENCE-BASED BABY WASH INTERVENTIONS (REDUCE PROGRAM)

Ronald Saxton[1], Jennifer Kuhl[1], Jamie Perin[1], Nicole Coglianese[2], Elizabeth Thomas[1], Sarah Bauler[2], Anthony Koomson[2], Phil Moses[2], Geoffrey A. Nyakuni[3], Amagana Togo[3], Ruthly Francois[1], Patrick Mirindi[3], Lucien Bisimwa[3], Christine Marie George[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Food for the Hungry, Phoenix, AZ, United States,* [3]*Food for the Hungry, Bukavu, Democratic Republic of the Congo*

To develop theory-based behavior change techniques for a Baby WASH intervention, we examined psychosocial factors associated with caregiver WASH behaviors in an ongoing USAID Office for Food for Peace funded cohort study in South Kivu, Democratic Republic of Congo. Caregivers of children <5 years (N=385) answered a 54-item psychosocial factor questionnaire derived from the IBM-WASH, and RANAS models. The following WASH behaviors were assessed by 5-hour structured observation: 1) handwashing with soap; 2) stopping children from mouthing fomites and food with visible dirt; and 3) water treatment (caregiver report). Caregivers that strongly agreed that their child contracting diarrhea would severely impact their life (perceived severity) were significantly more likely to stop their child from putting visibly dirty food in their mouth. Caregivers that strongly agreed that their child was at high risk of diarrhea (perceived susceptibility) were significantly more likely to wash their hands with soap. Caregivers that strongly agreed that visibly clean hands had no germs on them (dirt reactivity) were significantly less likely to wash their hands with soap. Caregivers that strongly agreed that it was hard to find wood or charcoal were significantly less likely to report boiling their water. Caregivers that strongly agreed that visitors would respect them more if they provided boiled water for drinking (injunctive norms) were significantly more likely to report boiling their water. We developed pictorial modules to target these significant psychosocial determinants of WASH behavior including: 1) a child mouthing pictorial module emphasizing the high risk for diarrhea and impaired growth from children mouthing dirt and dirty items and encouraging caregivers to supervise their child during play; 2) handwashing with soap pictorial module showing that hands that appear clean can have microbes and that not washing hands with soap puts children at risk for diarrhea; and 3) a pictorial module showing chlorine as a water treatment method not requiring fuel. This study presents an evidence-based approach for development of a Baby WASH intervention.

1227

EVIDENCE-BASED DEVELOPMENT OF BABY WASH INTERVENTIONS TO REDUCE EXPOSURE TO FECAL PATHOGENS (REDUCE PROGRAM)

Jennifer Kuhl[1], Ronald Saxton[1], Jamie Perin[1], Nicole Coglianese[2], Elizabeth Thomas[1], Sarah Bauler[2], Anthony Koomson[2], Phil Moses[2], Geoffrey A. Nyakuni[3], Amagana Togo[3], Ruthly Francois[1], Patrick Mirindi[3], Lucien Bisimwa[3], Christine Marie George[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Food for the Hungry, Phoenix, AZ, United States,* [3]*Food for the Hungry, Bukavu, Democratic Republic of the Congo*

Findings from large scale WASH intervention trials focused on "F diagram" fecal exposure pathways have not shown expected improvements on child health, highlighting the need for further research identifying the unique exposure pathways to fecal pathogens for pediatric populations, and WASH interventions tailored to young children. The REDUCE study focuses on identifying exposure pathways to fecal pathogens that are significant contributors to morbidity for young children in the Democratic Republic of the Congo, and on developing and evaluating scalable interventions to reduce fecal contamination from these pathways. Our recent cohort study found unsafe feces disposal, child mouthing of contaminated fomites, and contact with animals to be associated with diarrhea and

stunting among young children. Formative research, including 31 semi-structured interviews and 6 focus group discussions, was conducted to inform development of interventions to target these identified high risk behaviors. Contextual, psychosocial, and technological factors identified were analyzed using the IBM-WASH framework to inform intervention development. Lack of adequate child supervision due to parents working outside the home, and lack of clean child play spaces were factors limiting caregivers' ability to stop children from mouthing contaminated fomites. Using rice bags and plastic flooring as a playmat for young children emerged as a locally sourced solution to prevent children touching and ingesting dirt. Fear of theft, lack of resources to separate animals and children, and a long generational history of living with animals were factors driving the high contact observed between children and small animals. Locally sourced hutches for small animals were recommended to separate children from animals. Pictorial modules were developed to target the contextual, psychosocial, and technological factors identified. Pilot-testing of these Baby WASH interventions in 50 households is ongoing. This study presents a theory and evidence-based approach for the development of a Baby WASH intervention. This study was funded by the USAID Office of Food for Peace.

1228

COMPARISON OF SANIPATH EXPOSURE ASSESSMENTS IN LOW-INCOME URBAN AREAS IN EIGHT COUNTRIES

Wolfgang Mairinger, Yuke Wang, Suraja Raj, Habib Yakubu, Casey Siesel, Jamie Green, Sarah Durry, **Christine Moe**

Emory University, Atlanta, GA, United States

The SaniPath exposure assessment tool compares risks of exposure to fecal contamination in urban environments across multiple exposure pathways. The tool has been deployed in 39 neighborhoods in 8 cities: Accra, Ghana, Vellore, India; Maputo, Mozambique; Siem Reap, Cambodia; Dhaka, Bangladesh; Atlanta, United States; Lusaka, Zambia; and Kampala, Uganda. Ten exposure pathways were investigated (open drains, ocean water, surface water, floodwater, public latrines, soil, bathing water, raw produce, drinking water, and street food) through behavior surveys and environmental sample analyses. Exposure was expressed as monthly dose (average amount of fecal contamination ingested as measured by *E. coli* colony-forming units [CFU]) and the percent of population exposed to fecal contamination for each pathway. Magnitude of fecal contamination, frequency of exposure behavior, and estimated fecal exposures were compared across pathways, neighborhoods and cities. The most common dominant exposure pathways for adults were raw produce, open drains, and street food and for children were open drains, produce, and floodwater. For produce, the dose was usually very high (>10^6 CFU/month), and a large percent of the population was exposed (>80%). For street food, average *E. coli* concentration ranged from $10^{1.3}$ CFU/serving in one neighborhood in Lusaka, Zambia to $10^{5.5}$ CFU/serving in one neighborhood in Dhaka, Bangladesh. Exposure to open drains resulted in high doses (>10^4 CFU/month), but the population exposed varied (5%-92%) even within the same city. Exposure to fecal contamination via floodwater, usually affected a high percent of population (>80%) but had variable doses ($10^{2.5}$-10^{10} CFU/month). Both dose and percent of population exposed varied for public latrines and municipal piped water. This information can help city governments choose effective interventions to reduce the risk of exposure to fecal contamination. Widespread risks from contaminated produce and street food within and across cities underscore the link between excreta management and food safety and need for global action.

1229

RANDOMIZED CONTROLLED TRIAL OF THE CHOBI7 MOBILE HEALTH PROGRAM TO REDUCE PEDIATRIC DIARRHEA

Christine Marie George[1], Fatema Zohura[2], Shirajum Monira[2], Elizabeth Thomas[1], Tasdik Hasan[2], Tahmina Parvin[2], Maynul Hasan[2], Khaled Hasan[1], Mahamud-ur Rashid[2], Md. Sazzadul Islam Bhuyian[2], Camille Morgan[1], Peter J. Winch[1], Ronald Saxton[1], Alain Labrique[1], Kelsey Zeller[1], Farzana Begum[1], David A. Sack[1], R. Bradley Sack[1], Jamie Perin[1], Munirul Alam[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh*

The Cholera-Hospital-Based-Intervention-for-7-Days (CHoBI7) is a handwashing with soap and water treatment intervention program delivered by a health promoter bedside in a health facility and through home visits to diarrhea patients and their household members during the 7 days after admission to a health facility. In a RCT of cholera patient households in Bangladesh, the CHoBI7 program significantly reduced cholera and led to sustained improvements in drinking water quality and handwashing with soap 12-months post intervention. In an effort to develop a low cost scalable approach to deliver this program in Bangladesh without the need for in-person visits, we have developed a mobile health (mHealth) module for the CHoBI7 program to reinforce the WASH behavioral recommendations given in the health facility. To evaluate the efficacy of this program in reducing pediatric diarrhea, we are conducting a RCT of 825 children under 5 years in Dhaka, Bangladesh. Diarrhea patients and their household members presenting at icddr,b Dhaka Hospital or Mugda Hospital were recruited for this study. The "Standard Message" arm received the standard message in Bangladesh given to diarrhea patients on the use of oral rehydration solution. The "Hospital Visit + mHealth" arm received: (1) the standard message; (2) one visit by a promoter to deliver the CHoBI7 pictorial module at the health facility during the time of illness; and (3) CHoBI7 mHealth voice and text messages at least once every two weeks for 12 months. The "Hospital/Home Visits + mHealth" arm received these same activities plus two home visits by a promoter during the first week of intervention delivery. Monthly diarrhea surveillance was conducted. In this abstract we present the findings from the first 11 months of this ongoing 12 month trial. The CHoBI7 mHealth program resulted in a significant reduction in pediatric diarrhea in both the Hospital Visit + mHealth arm (Prevalence Ratio (PR): 0.80, 95% CI: 0.67, 0.95) and the Hospital/Home Visits + mHealth arm (PR: 0.71, 95% CI: 0.60, 0.85). This result suggests WASH mHealth programs present a promising approach to reduce diarrhea among young children.

1230

FORMATIVE RESEARCH FOR THE DESIGN OF THE CHOBI7 BABY WASH MOBILE HEALTH PROGRAM

Shwapon Biswas[1], Jahed Masud[1], Elizabeth D. Thomas[2], Fatema Zohura[1], Tasdik Hasan[1], Tahmina Parvin[1], Md. Sazzadul Islam Bhuyian[1], Fatema Tuz Johura[1], Marzia Sultana[1], Jamie Perin[2], Shirajum Monira[1], Munirul Alam[1], Christine Marie George[2]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States*

Mobile Health (mHealth) programs present a promising low-cost, scalable approach to improve WASH practices and reduce diarrhea. The recent RCT of the CHoBI7 WASH mHealth program significantly improved WASH behaviors and reduced pediatric diarrhea. Building on this work, this USAID funded WASHPaLS study aims to develop three Baby WASH mHealth modules targeting safe child feces disposal, improved food hygiene, and safe child mouthing practices. Formative research was conducted in Dhaka, Bangladesh for the development of the mHealth program, including 5 focus group discussions, 21 semi-structured interviews, 6 mHealth workshops, and a pilot study of 50 households.

Drivers of child feces disposal, food hygiene, and child mouthing behaviors were identified using the Integrated Behavioral Model for Water, Sanitation and Hygiene framework. Self efficacy, existing habits, perceived disease risk, and availability of child potties were drivers of child feces disposal behaviors. Gender roles, hot and cold weather, access to soap, fly covers, and gas supply, perceptions that storing food in a food rack was sufficient to keep food safe, and availability of a refrigerator were drivers of food hygiene behaviors. Caregivers said children always play outside and that it is difficult to control what they put in their mouth during play. Descriptive norms around child mouthing behaviors, lack of adequate support for supervision, lack of clean play spaces, and elders perceiving that eating soil was good for child health were drivers of child mouthing behavior. Mobile messages were developed targeting "System 1" drivers (relatively automatic, cue-driven drivers) of behavior change to leverage context changes, highlight descriptive norms around key behaviors, piggy back on to existing behaviors, and manage availability of enabling technology. Dr. Chobi, the sender of program messages, was well received and considered a credible source of health information. This study presents a theory and evidence-based approach for intervention development that can be implemented for the development of future Baby WASH mHealth programs in low-resource settings.

1231

A LONGITUDINAL STUDY OF CHRONIC LEAD EXPOSURE IN BENINESE CHILDREN

Shukrullah Ahmadi[1], Roméo Zoumenou[2], Barbara Le Bot[3], Séverine Durand[3], Nadine Fievet[2], Pierre Ayotte[4], Achille Massougbodji[5], Michel Cot[6], Philippe Glorennec[3], **Florence Bodeau-Livinec**[3]

[1]*INSERM U*[1153]*, Paris, France,* [2]*IRD MERIT, Cotonou, Benin,* [3]*EHESP, Paris, France,* [4]*INSPQ, Québec, QC, Canada,* [5]*IRCB, Cotonou, Benin,* [6]*IRD MERIT, Paris, France*

Lead is a well-known neurotoxic metal. Children are particularly vulnerable to its adverse neurocognitive effects. Within a birth cohort, elevated Blood Lead Levels (BLL) (>50 μg /L) were observed in one-year-old infants in Benin in 2011-13. Sources of exposure included the presence of paint chips in the house and consumption of animals killed by lead bullets. We aimed to investigate lead exposure in the same children at six years of age in 2016-18. 424 children with BLL at one year of age and reassessed at six years of age were included in the analysis. Blood samples were drawn and analyzed by inductively coupled plasma mass spectrometry. The study took place in the district of Allada, Benin. We estimated geometric mean BLL. Using the Wilcoxon rank-sum test BLL between boys and girls are compared. The proportion of children with BLL above 50 μg/L and above 100 μg/L are described and compared at both periods. Among 424 children (208 boys and 215 girls), the geometric mean BLL in children was 56.5 μg/L (95% CI: 53.5-59.7) at one year of age, and 56.3 μg/L (95% CI: 53.9 - 58.6) at six years of age. The distribution of BLL between boys and girls was not statistically different at any given age. The proportions of children with BLL >50 μg/L at one and at six years of age were not statistically different (55.0% versus 59.7%, respectively; McNemar's p=0.1183). However, the proportion of children with BLL >100 μg/L was higher at one year of age than at six years of age (14.2% versus 8.3%, respectively; McNemar's p=0.002). Children continue to suffer from elevated BLL and thus constitute an important public health problem in this population of children, and deserves preventive strategies. Sources of exposure may evolve and merits further investigation.

1232

IDENTIFICATION OF COCCIDIAN ISOLATES PATHOGENIC TO HUMANS IN SOURCES OF POTABLE WATER IN CAPE COAST METROPOLIS, GHANA

Priscilla Ankamaa Opare[1], Samuel Addo Akwetey[2], Joana C. Silva[3], Godwin Kwakye-Nuako[1]

[1]*University of Cape Coast, Department of Biomedical Sciences, School of Allied Health Sciences, College of Health and Allied Sciences, Cape Coast, Ghana,* [2]*University of Cape Coast, Department of Microbiology and Immunology, School of Medical Sciences, College of Health and Allied Sciences, Cape Coast, Ghana,* [3]*University of Maryland School of Medicine, Institute for Genome Sciences and Department of Microbiology and Immunology, Baltimore, MD, United States*

Water is a necessity of life. However, millions of humans die annually as a result of waterborne diseases, with 88% associated with unsafe water supply, poor sanitation, and lack of good hygienic practices. Possible contaminants making drinking water unsafe include microorganisms, chemicals and sewage. Most of the microorganisms that are present in water sources are a result of fecal matter contamination from sewage discharges, runoffs from animal lots as well as seepages from septic tanks. The aim of this study is to detect human-pathogenic coccidians, namely *Microsporidia*, *Cryptosporidia* and *Cyclospora*, in various potable water in Cape Coast Metropolitan District, in Ghana. A total of 100 samples from different sources of drinking water were sampled within the Cape Coast Metropolis for the detection of human-pathogenic coccidian taxa, including well water, pipe-borne water, boreholes, streams, underground water and harvested rain water. Each sample was initially stained with modified Zeihl-Neelsen stain and observed microscopically. Total genomic DNA of the various water samples have been extracted using the Ezup Column Blood Genomic DNA Extraction Kit in preparation of PCR-based taxonomic validation. Microscopy analysis revealed that about 60% of the samples collected were contaminated with microorganisms. The breakdown among coccidians was as follows; 23% *Microsporidium*; 13% *Cryptosporidium* and 11% *Cyclospora* oocysts. Other parasites of medical importance included *Sarcocystis*, present in 3% of the samples. These preliminary data obtained thus far suggest that water consumed by the majority of the Cape Coast population is contaminated with parasites and may be the predominant cause of diarrheal cases in the metropolis as well as of other asymptomatic infections. The study is to be expanded to PCR amplification and sequencing of the 18S rDNA and other *loci* and phylogenetic analysis.

1233

IDENTIFYING BARRIERS TO ADOPTION OF HOUSEHOLD DISINFECTION KITS FOR ENVIRONMENTAL INFECTION CONTROL OF CHOLERA TRANSMISSION

Camille Heylen, Daniele Lantagne

Tufts University, Medford, MA, United States

Cholera is a disease resulting from infection by *Vibrio cholerae* that can cause death from dehydration if untreated. Environmental infection control interventions are key to interrupting within household cholera transmission in outbreaks and two approaches for household disinfection (HD) currently exist: 1) household spraying (HS), where a dedicated team sprays patients' households with chlorine; and, 2) distribution of household disinfection kits (HDK), which contain cleaning materials so that household members can perform disinfection themselves. Despite the fact that international agencies no longer recommend HS, this intervention remains widely implemented. Therefore the main objectives of this research are: 1) To identify the barriers for adopting HDKs by interviewing 15 key-informant responders on the decision-making and implementation of HD Interventions in their organization; 2) to assess the training of beneficiaries to use HDKs, an identified barrier to adopting HDKs; and, 3) to inform the ongoing discussion on HD interventions. To date, 10 of 15 planned key-informant interviews (KII) have taken place and whereas participants indicated many barriers that make the implementation of HDK difficult (e.g. context adaptability, uncertainty of the method, etc.), one commonly cited barrier was beneficiary training to educate households on how to utilize the kit appropriately. Trainings should be effective to prevent cholera but logistically feasible and therefore a trade-off between a fast, inexpensive and easy-to-implement training and an effective hands-on training should be considered. To assess the effectiveness of these two training modalities, we are planning a trial in Haiti in Summer 2019. Study personnel will visit households and conduct (before and after clean with HDK) household surveys and environmental sampling for *E. coli*on

surfaces. This presentation will include results from KIIs with responders about household disinfection interventions and the field evaluation of HDK training, and will present a comparison of the benefits and challenges of HS and HDK interventions to inform outbreak responders.

1234

PREVALENCE OF FECAL PATHOGENS IN SOIL, FOOD, HAND AND SURFACE SAMPLES FROM HOUSEHOLDS IN SLUMS OF DHAKA, BANGLADESH (CHOBI7 TRIAL): EVIDENCE-BASED DEVELOPMENT OF BABY WASH INTERVENTIONS

Fatema Tuz Johura[1], Christine Marie George[2], Indrajeet Barman[1], Fatema Tuz Jubyda[1], Mohd. Riajul Islam[1], Jarin Tasnim[1], Sahitya Ranjan Biswas[1], Kazi Sumaita Nahar[1], Md. Wali Ullah[1], Shirajum Monira[1], Fatema Zohura[1], Tasdik Hasan[1], Tahmina Parvin[1], Md. Sazzadul Islam Bhuyian[1], Shwapon Biswas[1], Jamie Perin[2], Elizabeth Thomas[2], Marzia Sultana[1], Munirul Alam[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States*

Diarrheal disease is the second leading cause of death in children under five years of age globally. Findings from large scale WASH intervention trials focused on "F diagram" fecal exposure pathways have not shown expected improvements on child health. This has highlighted the need for further research identifying the unique exposure pathways to fecal pathogens for pediatric populations, and WASH interventions tailored to young children. The objective of this USAID WASHPaLS funded study was to identify pathways of fecal contamination for a susceptible pediatric population in Bangladesh, and to develop interventions to target these pathways. This study is nested within the CHoBI7 Baby WASH mobile health program. A total of 259 soil, food, hand, and surface samples were collected from households with a child under 5 years of age. Soil samples were collected from spaces where children were observed playing during structured observation, hand rinse samples were collected from children under 5 years of age and their caregiver, samples of the food given to the child under 5 years of age in the home was collected, and surface samples were collected of objects children put in their mouth during structured observation. All samples were analyzed using the IDEXX Quanti-Tray System (Colilert-18 media; IDEXX Laboratories, Inc., Westbrook, ME) to enumerate *Escherichia coli* using the most probable number (MPN) method. The median concentration of *E. coli* in soil was 134.6 (N=46) (Range: 1-2500). Thirty three percent (N=63) of child hand rinse samples had *E. coli* (Median: 0, Range: 0-2500), and 46% (N=28) of caregiver hand rinse samples collected had *E. coli* (Median: 0, Range: 0-613.1). Thirty five percent (N=66) of surface samples had *E. coli* (Median: 0, Range: 0-2500) among them balls were the most commonly contaminated item. Twenty nine percent (N=56) of food samples had *E. coli* (Median: 0, Range: 0-2500) among them rice was the most commonly contaminated food item. These results indicate that WASH interventions are needed to reduce child contact with contaminated fomites and to promote food hygiene and handwashing with soap practices.

1235

A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE ASSOCIATION BETWEEN WATER, SANITATION, HYGIENE AND FOOD EXPOSURES AND TYPHOID FEVER IN CASE-CONTROL STUDIES

Sarah Brockett[1], **Marlene Wolfe**[1], Asa Hamot[1], Grace Appiah[2], Eric Mintz[2], Daniele Lantagne[1]

[1]*Tufts University, Medford, MA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Typhoid transmission occurs through ingestion of fecally-contaminated food or water; case-control studies are often used to identify outbreak sources and transmission vehicles. The majority of typhoid cases occur where access to safe water, sanitation, and safe food practices is limited. There is currently no summary of the associations between water, sanitation, hygiene (WASH) and food exposures from case-control studies of typhoid outbreaks. We conducted a systematic review and meta-analysis of case-control studies to evaluate the associations between typhoid fever with 13 predicted WASH and food exposure risk factors and 7 predicted protective factors. Overall, 19 manuscripts describing 22 case-control studies were included. In quality assessment, two studies were categorized as having low risk of bias, one as medium risk, and 19 as high risk. In total, 9 of 13 predicted risk factors were associated with increased odds of typhoid while 5 of 7 predicted protective factors were associated with lower odds. For WASH exposures, good household hygiene and water treatment demonstrated the greatest reductions in odds of typhoid (OR=0.52 and 0.59, respectively), while poor hygiene and untreated water demonstrated the greatest increases (OR=2.2 and 2.4, respectively). Protective food practices such as cooking food thoroughly and using hygienic practices in food preparation were significantly associated with lower odds of typhoid (OR=0.74), while risky food practices and eating and drinking outside the home were associated with significantly higher odds (OR=1.6-1.7). Results highlight: the importance of household hygiene transmission pathways; the need for further research around appropriate food interventions and the risk of consuming specific foods and beverages outside the home; and, the absence of any observed association between sanitation exposures and typhoid fever. The high bias of identified studies was a limitation of this meta-analysis. Household hygiene and water treatment interventions can reduce typhoid risk; more detailed information about WASH and food exposures could inform more targeted interventions.

1236

HRP2 AND HRP3 ANTIGEN CROSS-REACTIVITY ON HRP2-BASED MALARIA RAPID DIAGNOSTIC TESTS AND ITS IMPLICATIONS FOR *HRP2* GENE DELETION SURVEILLANCE

Michael Aidoo[1], Amy Kong[1], Scott Wilson[2], Ah Yong[2], Jeffrey Glenn[1], Eric Rogier[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*The CDC Foundation, Atlanta, GA, United States*

Plasmodium falciparum histidine rich protein 2 (HRP2) is targeted by majority of malaria rapid diagnostic tests (RDTs) because of its abundant production by parasites, stability under high temperature conditions and detection sensitivity. However, reports from many countries indicate some parasites have the *hrp2* gene deleted, making them undetectable by HRP2 RDTs. HRP2 is paralogous to *P. falciparum* HRP3, and antibodies to HRP2 can detect similar epitopes on HRP3. HRP2/HRP3 cross-reactivity on HRP2 RDTs has not been systematically determined. Parasites have been described with deletions of one or both of *hrp2* and *hrp3* genes and how the various combinations of *hrp2* and *hrp3* deletions affect RDT sensitivity is unclear. Serial dilutions ranging from 100,000 to 0.01 parasites/μL of four *P. falciparum* culture parasites, 3D7 (*hrp2*+/*hrp3*+), Dd2 (*hrp2*-/*hrp3*+), HB3 (*hrp2*+/*hrp3*-) and 3BD5 (*hrp2*-/*hrp3*-) were prepared. HRP2 and *Plasmodium* lactate dehydrogenase (pLDH) concentrations were determined for the diluted samples using a multiplex bead assay. The samples were tested on three RDT products from the same manufacturer that detect *P. falciparum* by HRP2 alone or in combination with pLDH. At clinically relevant parasite densities (≥1000 parasites/μL), the *hrp2*-/*hrp3*+ parasite Dd2 was always detected on HRP2-based RDTs. With Dd2 even at a density of 100 parasites/μL, which is often associated with asymptomatic infection in high transmission areas, HRP2 bands on the three RDTs were reactive, although at low band intensities. An additive effect of intact *hrp2* and *hrp3* was observed, with 3D7 having much higher measured HRP2 concentration at 1000 parasites/μL (47.8 ng/mL) when compared with HB3 (3.0 ng/mL), Dd2 (0.198 ng/mL) and 3BD5 (0.0 ng/mL). Only the *hrp2*/*hrp3* double-deleted parasite 3BD5 caused HRP2 RDTs to give false negative results at all parasite densities. These results suggest substantial cross-reactivity between HRP2 and HRP3 antibodies and that studies of *hrp2* deletions and their possible effects on HRP2 RDTs must consider the presence or absence of *hrp3*. It remains to be tested how product-specific antibody types influence reactivity

1237

SURVEILLANCE FOR *PFHRP2/3* DELETIONS AND NON-FALCIPARUM MALARIA IN THREE PROVINCES IN MOZAMBIQUE, 2018

Mateusz Plucinski[1], Baltazar Candrinho[2], Mercia Dimene[2], James Colborn[3], Austin Lu[4], Doug Nace[5], Rose Zulliger[6], Eric Rogier[5]

[1]*CDC Malaria Branch and United States President's Malaria Initiative, Atlanta, GA, United States,* [2]*National Malaria Control Program, Maputo, Mozambique,* [3]*Clinton Health Access Initiative, Maputo, Mozambique,* [4]*Georgia State University, Atlanta, GA, United States,* [5]*CDC Malaria Branch, Atlanta, GA, United States,* [6]*CDC Malaria Branch and United States President's Malaria Initiative, Maputo, Mozambique*

Rapid diagnostic tests (RDTs) that detect the *Plasmodium falciparum*-specific histidine rich protein 2 (PfHRP2) antigen are the primary method for malaria diagnosis in Mozambique. However, these tests do not detect infections with non-falciparum malaria or *Pfhrp2/3*-deleted *P. falciparum* parasites. To assess the appropriateness of PfHRP2-only RDTs for malaria diagnosis in Mozambique, samples collected during a health facility survey conducted in Mozambique were screened with serological and molecular techniques. Samples from 1861 outpatients of all ages and symptoms attending 117 randomly-selected public health facilities in April and May 2018 in Maputo, Zambézia, and Cabo Delgado Provinces were analyzed with an ultra-sensitive bead-based immunoassay for the presence and concentration of PfHRP2, pan-*Plasmodium* Aldolase (pAldo), and pan-*Plasmodium* lactate dehydrogenase (pLDH). The concentration of PfHRP2 in patient blood was compared to the results of PfHRP2-based RDTs performed during the routine health facility consult and during the survey exit re-examination. Samples with discordant antigen profiles were further characterized by PCR. Using the bead-based laboratory assay as the gold standard, the sensitivity of the RDTs administered during the health facility consult and the exit interview were 90% and 83%, respectively, and specificity was 91% and 97%. Of 710 samples positive for at least one antigen, 704 (99.2%) were positive for PfHRP2. Of the 6 (0.8% of total) discordant samples lacking PfHRP2 but positive for pAldo and/or pLDH, 3 (0.4% of total) were *P. ovale* mono-infections or co-infections where *P. ovale* was the dominant species. The remaining 3 discordant samples were negative by PCR. The sensitivity and specificity of the RDTs performed in the health facility consults and survey exit interviews were acceptable, and there was no evidence of *Pfhrp2/3*-deleted parasites. Mono-infections with non-falciparum malaria species comprised <1% of total malaria infections. Nearly all malaria antigen-positive patients had detectable PfHRP2, confirming this as an appropriate malaria diagnostic target for Mozambique.

1238

AUTOMATED DIGITAL MICROSCOPY USING ARTIFICIAL INTELLIGENCE FOR THE POINT-OF-CARE MALARIA DIAGNOSIS

Hans-Peter Beck[1], Armin Passecker[1], Youngmin Shin[2], Chae Y. Bae[2], Younghoon Song[2], Jiyeon Lee[2], Mijin Kim[2], Raeeun Chung[2], Douglas Lungu[3], Donyoung Lee[2]

[1]*Swiss Tropical and Public Health Institution, Basel, Switzerland,* [2]*Noul Inc. Ltd., Yongin-si Gyeonggi-do, Republic of Korea,* [3]*Wezi Medical Centre, Mzuzu, Malawi*

Besides the very frequent use of rapid diagnostic tests (RDTs) for malaria diagnosis in the field, expert microscopy is still used for reliable diagnosis. In contrast to microscopy, RDTs are unable to quantify parasitemia, identify gametocytes, and cannot distinguish all malaria species. Furthermore, the rapid spread of *Plasmodium* parasites having the *hrp2/hrp3* gene deleted compromises the reliable use of RDTs. Here we present an innovative platform that is designed to be employed in endemic areas that automatically generates microscopic blood smears termed miLab. These smears are liquid-free stained using a hydrogel-based stamping technology coupled with automatic digital microscopy. All images captured are being analysed by a machine-learned algorithm to quantify and identify malaria parasites. We have validated the instrument on full human blood samples spiked with various numbers of synchronized and non-synchronized cultured *P. falciparum* parasites. In addition, we have used cultures and induced gametocytes for training the algorithm to also detect gametocytes. After training and validation the platform was employed in a clinical field study in Lilongwe, Malawi in which collected clinical samples were analysed. For further performance tests of the miLab platform, dried blood spots were collected on filter paper for subsequent quantitative PCR and slides were prepared for expert microscopy testing at Swiss TPH. We will present and describe the platform, its performance characteristics and initial results from this field study conducted in Malawi.

1239

A NOVEL LATERAL FLOW ASSAY FORMAT WITH INTEGRATED CATCH-AND-RELEASE BIOMARKER CONCENTRATION FOR DETECTION OF LOW PARASITEMIAS AT THE POINT-OF-CARE

Nathaniel Z. Piety, Carson P. Moore, Kristina A. Pieterson, David W. Wright

Vanderbilt University, Nashville, TN, United States

Malaria control and elimination strategies require the detection of *Plasmodium* parasites in both symptomatic and asymptotic individuals in order to guide treatment and disrupt transmission. Benchtop techniques such as polymerase chain reaction or microscopic diagnosis are both sensitive and specific, but are prohibitively expensive and complex for the resource-limited regions where the burden of malaria is highest. Lateral flow assays (LFAs) are easy-to-use, low-cost, rapid and do not require equipment, and have therefore become a principal method of malaria screening in resource-limited settings. However, most conventional LFAs have limits of detection (LOD) of ~200 parasites/μL, which is not sufficiently low to detect malaria in asymptomatic individuals who may have parasitemias in the single digits. As such, asymptomatic infections may go undetected and untreated by malaria control and elimination campaigns guided by conventional LFAs and continue to serve as transmission reservoirs. To address the need for LFAs with lower LOD, we have developed an LFA format which utilizes a sliding, zinc-iminodiacetic acid (Zn-IDA) functionalized cellulose membrane for catch-and-release concentration of malaria biomarkers *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) and *Plasmodium* lactate dehydrogenase (*P*LDH). The affinity between divalent zinc ions and histidine residues enabled membrane capture of *Pf*HRP2 with 99% efficiency, and histidine$_6$-tagged anti-pLDH antibodies enabled simultaneous capture of *P*LDH with 80% efficiency. Both biomarkers could then be released with >80% efficiency using an EDTA buffer which chelates zinc. By flowing a sample volume ~50× times larger than conventional LFAs could accommodate through the functionalized membrane into absorbent pads, then sliding the membrane – a single additional user step compared to conventional LFAs – and eluting captured biomarkers onto the LFA in a smaller volume, the amount of biomarker delivered to the LFA was significantly increased and the LOD was improved to 3.2 parasites/μL for *Pf*HRP2. This improved LOD is sufficient to detect most asymptomatic infections.

1240

AUTOMATIC BLOOD SMEAR ANALYSIS WITH ARTIFICIAL INTELLIGENCE AND SMARTPHONES

Hang Yu[1], Stefan Jaeger[1], Feng Yang[1], Kamolrat Silamut[2], Richard Maude[2]

[1]*National Institutes of Health, North Bethesda, MD, United States,* [2]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand*

MalariaScreener is the first software using artificial intelligence, in particular deep learning, to process both thin and thick blood smear images on smartphones. When running on a phone attached to the eyepiece of a microscope, MalariaScreener can detect parasites in thick smear images captured by the smartphone's camera. It can also discriminate between infected and uninfected red blood cells in thin blood smear images. The idea is to provide a cost-effective alternative for malaria

diagnosis in resource-limited regions that does not depend on expert knowledge. This could also help in standardizing parasite counts, which often depend on the experience and skill of the microscopist. We trained our system on hundreds of thousands of manually annotated parasites and blood cells, in images from hospitals and clinics in Bangladesh and Thailand, so that it captures the typical shape and appearance of parasites and cells. The trained system can detect parasites in thick smears and discriminate between infected and uninfected red blood cells in thin smears. For thick smears, we measure an area under the ROC curve (AUC) of 85% on patient level. For thin smears, using ensemble techniques, we achieve an AUC of 99.92% for cell classification (infected/uninfected). The underlying algorithms utilize customized convolutional neural network models (CNNs). An embedded database system can store patient data, screening results, disease severity, and patient histories. Moreover, researchers can save blood smear images captured during the screening process together with their manual counts of parasites and blood cells, which can then be used later for training to improve future automated screening. The internal algorithms have been optimized for fast processing speed and leave a small memory footprint. The user interface allows easy access to the image data and review of all stored information. MalariaScreener is currently being field-tested in several countries. Due to its modular and flexible design, Malaria Screener could be extended to support screening of other blood-based diseases and beyond, when given the properly trained machine learning models.

1241

DETECTION OF SUB-MICROSCOPIC BLOOD LEVELS OF *PLASMODIUM FALCIPARUM* USING TANDEM OLIGONUCLEOTIDE REPEAT CASCADE AMPLIFICATION (TORCA) ASSAY WITH AN ATTOMOLAR DETECTION LIMIT

Andrey L. Ghindilis[1], Olga Chesnokov[2], Billy Ngasala[3], Maria W. Smith[1], Kenneth Smith[1], Andreas Mårtensson[4], **Andrew V. Oleinikov**[2]

[1]*TORCATECH, LLC, Mukilteo, WA, United States,* [2]*Florida Atlantic University, Boca Raton, FL, United States,* [3]*Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [4]*International Maternal and Child Health (IMCH), Uppsala University, Uppsala, Sweden*

Tandem Oligonucleotide Repeat Cascade Amplification (TORCA) approach is a novel, isothermal, highly sensitive, and low-cost method of target nucleic acid detection. TORCA based on signal rather than target amplification under isothermal conditions. The initial signal is generated by hybridization of single stranded DNA targets to immobilized recognition probes followed by hybrid cleavage with specific restriction endonuclease (REase), and release of trigger oligonucleotides (Tr1), attached to recognition sequence. The released Tr1 is transferred into signal amplification chamber. The signal amplification chamber contains two bead types (each carrying single-stranded "amplification probe") and two "amplification REases". The probes consist of multiple tandem repeats of either Tr1 or another trigger Tr2, with the tandem-Tr1 anchored to the beads through the antisense Tr2 linker and vice versa. Addition of the recognition reaction solution and Tr1 hybridization to the anti-Tr1 linkers initiates cleavage and release of Tr2. Hybridization of released Tr2 to the anti-Tr2 linkers initiates cleavage and release of Tr1. Because each Trigger sequence is also attached to horseradish peroxidase (HRP), release and transfer of this enzyme into detection chamber generates signal measured colorimetrically. Thus, the cleavage cascade results in exponential signal amplification proportional to the initial amount of target sequence in the recognition chamber. This TORCA assay was developed for detection of *Plasmodium falciparum* parasites in blood using Pf-specific recognition probes. It demonstrated the detection limit in the attomolar concentration range, successfully detecting sub-microscopic *P. falciparum* infections in laboratory and field samples down to less than 0.075 infected erythrocytes per microliter. Further TORCA optimization will likely produce the quantitative isothermal alternative to PCR at a fraction of its cost.

1242

PROSPECTIVE PERFORMANCE EVALUATION OF A COMBINED MALARIA/CRP RAPID DIAGNOSTIC TEST IN INDIA

Sandra Incardona[1], Bina Srivastava[2], Supriya Sharma[2], Stefano Ongarello[1], Shubhada Shenai[3], Prabakaran Loganathan[3], Sanjay Sarin[3], Anupkumar R. Anvikar[2], Sabine Dittrich[1]

[1]*FIND, Geneva, Switzerland,* [2]*National Institute of Malaria Research, New Delhi, India,* [3]*FIND India, New Delhi, India*

The integrated management of febrile patients is difficult in low resource settings where good quality diagnostics are not always available. This emerged as a particular challenge in low malaria endemic settings where the number of malaria-negative tests is increasing, especially in Asia and South East Asia. Recent studies in this region have shown that C-Reactive Protein (CRP) can be a suitable marker to guide antibiotic treatment for non-malarial patients with fever alone or with respiratory symptoms. Currently, there is no integrated test that combines both malaria and CRP; yet such a tool is envisioned to improve individual care, triage decisions and to support malaria elimination efforts through continued malaria testing and surveillance. To address this gap, FIND partnered with SD Biosensor to develop a simple RDT combining malaria diagnosis (pfHRP2 and panLDH antigens) with semi-quantitative detection of CRP at a 20mg/L cut-off to provide an indication of bacterial infections. A prospective clinical trial is being conducted in India (North-East, South, and Central) to assess the performance (sensitivity, specificity, positive and negative predictive values) of a Malaria/CRP combination test when used at the point-of-care, with a target sample size of 1800 febrile patients (expected accuracy: 95% CI of ± 5%, 80% power). The malaria test lines will be assessed against expert malaria microscopy and nested PCR as the reference standards, while the CRP test line will be assessed against a commercial CRP quantification test on a chemistry analyzer. The ease of use and acceptability of this new test will also be evaluated through interviews with standardized questionnaires. The results of this trial will be instrumental to confirm that this test meets all technical needs for implementation and use, especially in low malaria endemic countries. The data from this study, combined with work that evaluates the utility and safety of CRP alone will inform priority implementation efforts and can be used towards a dossier to support the use of this and similar tools in the frame of an integrated fever management approach more widely.

1243

TRANSPLACENTAL ANTIBODY TRANSFER AMONG WOMEN LIVING WITH HIV

Lisa M. Bebell[1], Mark J. Siedner[1], Joseph Ngonzi[2], Audrey L. Butler[3], Julian Adong[2], Sepideh Dolatshahi[3], Ingrid V. Bassett[1], Drucilla J. Roberts[1], Galit Alter[3]

[1]*Massachusetts General Hospital, Boston, MA, United States,* [2]*Mbarara University of Science and Technology, Mbarara, Uganda,* [3]*The Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard, Cambridge, MA, United States*

HIV-exposed, uninfected (HEU) children have an elevated risk of severe early-life infections and increased immune activation compared to HIV-unexposed children. Immunologic differences in HEU children may be linked to changes in transplacental transfer of IgG subclasses and the Fcγ receptors (FcγR) they bind. Altered transplacental antibody transfer in women living with HIV (WLWH) may contribute to HEU infection risk and has implications for maternal and infant vaccination strategies. We used a systems serology approach to define features of transplacental antibody transfer in maternal-umbilical cord pairs from 176 WLWH in Uganda and 176 HIV-uninfected comparators. We used multiplexed Luminex assays to quantify antigen-specific antibody subclasses (IgG1-IgG4) and binding to FcγR 2A and 3A for a wide range of vaccine-preventable and common pathogens. We used t-tests to compare antibody transfer ratios and partial least squares discriminant analysis (PLS-DA) to identify correlates of antibody profile differences by maternal HIV serostatus. Compared to HIV-uninfected women, WLWH transferred significantly less IgG1 for

polio, hepatitis A, HSV 1 and 2, and CMV. Transfer of IgG1 for pertussis, RSV, Hib, EBV, measles, mumps, rubella, adenovirus, and tetanus toxoid was not affected by maternal HIV serostatus. Compared to HIV-uninfected women, WLWH transferred significantly more FCγR3A-binding antibodies for measles, but significantly less for CMV. PLS-DA revealed that IgG1 titer, FcγR3A, and FcγR2A binding separated HEU antibody profiles in an antigen-specific manner. Transplacental antibody transfer differs by HIV serostatus and may contribute to HEU immune activation and infection susceptibility. Differences observed by maternal HIV serostatus in the quality of maternal humoral immune profiles suggest these alterations may be related to placental changes among WLWH, even in the presence of effective antiretroviral therapy. Further studies may reveal specific mechanisms leading to altered immunity and novel opportunities to design specific therapeutics and vaccines to selectively enhance immunity in HEU children.

1244

HUMAN MILK OLIGOSACCHARIDES AND GROWTH IN HIV EXPOSED UNINFECTED INFANTS IN KENYA

Christine J. McGrath[1], Judd L. Walson[1], Lars Bode[2], Chloe Yonemitsu[2], Rose Bosire[3], James A. Berkley[4], Dorothy Mbori-Ngacha[5], Grace C. John-Stewart[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*University of California San Diego, San Diego, CA, United States,* [3]*Kenya Medical Research Institute, Nairobi, Kenya,* [4]*KEMRI/Wellcome Trust Collaborative Research Programme, Kilifi, Kenya,* [5]*United Nations Children's Fund (UNICEF), Nairobi, Kenya*

Human milk oligosaccharides (HMOs) are the third largest solid component in breast milk and serve as prebiotics, supporting growth of commensal gut bacteria and influencing the immune system. Recent studies implicate perturbations in the infant gut microbiome as a critical mechanism for increased risk of growth faltering and infectious morbidity in HIV-exposed uninfected (HEU) infants. We investigated the association between HMO composition and growth in HEU infants during the first year of life. Total and specific HMO concentrations were quantified from breast milk samples collected at one month postpartum from a random subset of 124 HIV-infected women enrolled in a pre-antiretroviral therapy cohort in Nairobi, Kenya. Linear mixed effects models were used to determine the influence of specific HMOs on the rate of change in monthly weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ) z-scores. At 32 weeks' gestation, median maternal age was 24 years (Interquartile range [IQR]=22, 27) and median CD4 count was 471 cells/μL (IQR=316, 664). Of the 124 HEU infants, median birth weight was 3.1 kg (IQR=2.9, 3.4) and median breastfeeding duration was 11.8 months (IQR=7.5, 12.0). Weight remained stable from birth to age 12 months (-0.1 WAZ/mo, 95% confidence interval [CI]= -0.3, 0.01, p=0.32; -0.1 WLZ/mo, CI= -0.03, 0.01, p=0.24), while length gradually declined over time (-0.04 LAZ/mo, CI= -0.06, -0.02, p<0.001). Higher concentrations of sialylated HMOs, 6-sialyllactose and sialyllacto-*N*-tetraose c, were associated with a faster rate of increase in WLZ (0.06 WLZ/mo, CI=0.01, 0.10, p=0.02; and 0.07 WLZ/mo, CI=0.22, 0.11, p=0.004, respectively). There was a trend toward an association between higher lacto-*N*-fucopentaose III concentration and faster declines in weight and length growth (-0.04 LAZ/mo, CI= -0.9, 0.004, p=0.08; -.05 WAZ/mo, CI= -0.10, 0.01, p=0.11). These data suggest a relationship between specific oligosaccharides and postnatal growth in breastfed HEU infants. Understanding the mechanisms by which HMOs and gut microbiota interact is critical to inform interventions to optimize growth in HEU children.

1245

SOCIAL FACTORS ASSOCIATED WITH VIROLOGIC SUPPRESSION IN CHILDREN AND ADOLESCENTS LIVING WITH HIV INITIATED ON ANTIRETROVIRAL THERAPY IN LILONGWE, MALAWI

Bryan J. Vonasek[1], Tsogolo Itaye[2], Joseph Mhango[2], Andrea Dean[1], Peter Kazembe[2]

[1]*Baylor College of Medicine, Houston, TX, United States,* [2]*Baylor College of Medicine Children's Foundation Malawi, Lilongwe, Malawi*

Across a wide range of settings and with many diseases, there are strong links between health outcomes and the circumstances in which people live, known as the social determinants of health (SDH). Especially for children and adolescents living with HIV (CALHIV), there is very little evidence about the impact of SDH on successful virologic suppression upon initiation of antiretroviral therapy (ART). Understanding this dynamic in low resource countries like Malawi is key to achieving the third of the UNAIDS' 90-90-90 goals. This was a retrospective cohort study of subjects less than 20 years old initiating ART at the Baylor College of Medicine Abbott Fund Children's Center of Excellence in Lilongwe, Malawi between January 2016 and January 2018. Clinical and social variables were abstracted from the site's electronic medical record and these were compared to virologic suppression. Virologic suppression was defined as documented viral load less than 1000 copies/mL within 8 months of ART initiation. Over this period, 285 CALHIV were initiated on ART at this site with a median age of 7.5 years and 48% female. Within eight months of ART initiation, 93 subjects (33%) had documented virologic suppression. Social factors that were associated with virologic suppression were living in a home with electricity, living in a home with a refrigerator, and using a personal vehicle to travel to clinic visits. However, with multiple logistic regression, the only social variable associated with virologic suppression was using a personal vehicle to travel to clinic visits (adjusted odds ratio 3.40, 95% confidence interval 1.35-8.55). Initial WHO stage of III or IV was also independently associated with virologic suppression (aOR 0.321, 95% CI 0.128-0.807). This initial analysis shows one social factor, mode of transport to clinic, associated with virologic suppression for this cohort of CALHIV. Ongoing research to further identify key social factors associated with the third of the UNAIDS' 90-90-90 goals will be important for developing interventions to improve rates of virologic suppression for CALHIV.

1246

VIROLOGICAL SUPPRESSION AMONG HIV INFECTED ADOLESCENTS & YOUTHS RECEIVING ART IN THE NATIONAL TEACHING AND REFERRAL HOSPITAL IN KENYA

James M. Kangethe

Kenyatta National National/University of Nairobi, Nairobi, Kenya

Virological suppression among HIV infected adolescents and youths receiving ART in the National teaching and referral hospital in Kenya. HIV virological suppression is poor among the adolescents and youths which may be related to several factors including adherence to antiretroviral therapy. This study aimed to determine the HIV virological response and the associated risk factors among adolescents and youths on ART. This was a cross-sectional study among adolescents and youths aged 10 to 24 years in Kenyatta National Hospital who were on ART for at least six months. Patient characteristics were captured in a questionnaire and viral load was abstracted from electronic medical records. Viral suppression was presented as a proportion based on viral load less than 1000 copies per milliliter of plasma. Viral suppression rate was associated with categorical independent factors using chi square test and means were compared using independent T –test. The mean age was 17 years (SD 4.3 years) and 55.6% were females. The median CD4 count was 573 cells per micro liter of blood (IQR: 344- 1780). A total of 227 (74.2%) HIV infected adolescents and youths were virologically suppressed (viral load less than 1000 copies/ml blood). As compared to children 10-14 years old who had 83.2% suppression rate, adolescents 15-19 years had poorer suppression

rate at 69.6% [OR 0.5 (95% CI 0.2-0.9), P= 0.022]. Similarly youths 20-24 years had a lower suppression rate at 70.8% compared to the children [OR 0.5 (95% CI 0.2-0.9), P= 0.022]. Only 56.2% of the study participants had undetectable HIV viral RNA (as per UNAIDS 90-90-90 strategy). RNA Viral suppression rate was lower among ART defaulters (47.2%), those defaulting clinic appointments (51.7%) and those not honoring ART refill (50%). Majority of the participants (86.3%) were in WHO stage I whereas 2% were in WHO stage IV. Among those with unsuppressed viral loads, 20.7% had been diagnosed with Tuberculosis. None of the study participants had Hepatitis B virus infection. In conclusion, HIV viral suppression among adolescents and youths was low and even much lower among 15 to 24 year-olds.

1247

SERUM VITAMIN D IS DIFFERENTIALLY ASSOCIATED WITH SOCIOEMOTIONAL ADJUSTMENT IN EARLY SCHOOL-AGED UGANDAN CHILDREN ACCORDING TO PERINATAL HIV STATUS AND *IN UTERO* ORPERIPARTUM ANTIRETORIVAL EXPOSURE HISTORY.

Amara E. Ezeamama[1], William Yakah[1], Jenifer Fenton[1], Robert Tuke[1], Sarah K. Zalwango[2], Alla Sikorskii[1], Bruno Giordani[3], Michael J. Boivin[1], Philippa M. Musoke[4]

[1]*Michigan State University, East Lansing, MI, United States,* [2]*Kampala Capital City Authority, Kampala, Uganda,* [3]*University of Michigan, Ann Arbor, MI, United States,* [4]*Makerere University School of Medicine, Kampala, Uganda*

It is unknown whether vitamin-D insufficiency (VDI) is associated with socio-emotional adjustment (SEA) in school-aged children. This study examines the hypothesis that deficits in SEA are related to VDI using longitudinal data from 254 children that are perinatally HIV-infected (PHIV), HIV exposed uninfected (HEU) or HIV-unexposed uninfected (HUU). Four caregiver reported age-and sex-standardized measures of SEA were defined at months 0, 6 and 12 for dependent children aged 6-10 years –externalizing problems (EPSI), internalizing problems (IPSI), behavioral symptoms index (BSI) and adaptive skills index (ASI) - per the Behavior Assessment System for Children. Higher scores reflected worse SEA for all but ASI. Serum vitamin-D was measured and analyzed in quartiles in relation to SEA using linear mixed effects models implemented in statistical analysis software SAS 9.4 . VDI was highly prevalent (74%, n=188), and its association with change in SEA measures (i.e., BSI, IPSI and EPSI) over 12 months varied by HIV-status (VDI*HIV, all p-values <0.03). Among PHIV children, there was no association between VDI and change in any SEA measure. Among HUU, BSI (β=-0.32, 95%CI: -0.50, -0.13), IPSI (β=-0.28, 95%CI: -0.47, -0.09) and EPSI (β=-0.20, 95%CI: -0.37, -0.02) all declined modestly per quartile increment in VD. Among HEU, VD relationship to change in BSI, IPSI and EPSI varied according to whether or not these children were exposed to ART *inutero*/peripartum (*Early* ART*VDI all p<0.02). Specifically, in absence of *Early* ART higher VDI predicted a modest to moderate declines in problematizing SEA domains including EPSI (β=-0.26, 95%CI: -0.51, -0.02). However among HEU with early ART exposure higher VDI predicted elevated problems - BSI (β=0.39, 95%CI: 0.00, 0.78) and IPSI (β=0.48, 95%CI: 0.05, 0.92). VDI may be associated with worse SEA among HUU and HEU without *early* ART exposure. The interaction between VD and *early* ART for SEA outcomes warrants further investigation. Improvement of vitamin D levels may be a possible intervention target to remediate SEA impairments among HUU and HEU Ugandan children.

1248

HIV CARE CASCADE REVIEW: CASE REPORTS FROM KENYA CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE NETWORK (CHAMPS) PROGRAM

Victor Akelo[1], Emily Zielinski-Gutierrez[2], Aggrey Igunza[3], Dickens Onyango[4], Dianna M. Blau[5], Pratima L. Raghunathan[5], Robert F. Breiman[6], Beth A. Tippett Barr[1]

[1]*Centers for Disease Control and Prevention, Kisumu, Kenya,* [2]*Centers for Disease Control and Prevention, Nairobi, Kenya,* [3]*Kenya Medical Research Institute, Kisumu, Kenya,* [4]*Kisumu County Public Health Department, Kisumu, Kenya,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*Emory Global Health Institute, Emory University, Atlanta, GA, United States*

HIV-associated child mortality remains a major public health concern, the magnitude of which is affected by mother-to-child transmission (MTCT) rates. In Kenya, an estimated 4,800 HIV-infected children (<14 years) died in 2016. CHAMPS is a multi-country surveillance program that systematically tracks causes of under-five mortality from a defined catchment area. Between May 2017 and November 2018, causes of death (COD) were determined by a panel of experts for 103 deceased children 0-59 months in western Kenya using data from post-mortem tissue specimen testing, clinical records and verbal autopsy. HIV status and viral load (VL) were determined from whole blood collected within 24 hours of death. Of 103 child deaths, 21 (20%) were HIV-associated (mother or child was HIV-positive); HIV DNA (by polymerase chain reaction) was detected in 6/103 (5.8%). We reviewed the HIV care continuum for the six HIV-infected child deaths to identify areas for improvements. Three (50%) HIV-infected children had unknown HIV diagnosis before death, 2 had prior diagnosis and were on ART while one HIV diagnosis was made during last hospital admission. In 4 (67%) cases, maternal HIV status was either unknown or undocumented; one case had only one maternal HIV negative test in 2nd trimester (no repeat test); and the other maternal HIV status was positive but ART status was undocumented. Four (67%) children died of pneumonia; etiologies for each case were cytomegalovirus [CMV], respiratory syncytial virus [RSV], Streptococcus spp, and in one case, coinfection with RSV, CMV and Streptococcus was considered to have caused the death. Two children died of cerebral malaria (one with salmonella sepsis co-infection). CHAMPS highlighted gaps including missed child and maternal HIV diagnosis, treatment, and HIV-related conditions. The investigation revealed HIV-associated pediatric deaths that would have otherwise been unreported. Improved, focused antenatal care, a high index of clinical suspicion for HIV infection, and routine clinical audits of the HIV care cascade should be encouraged to eliminate pediatric HIV deaths and improve maternal HIV care

1249

THE SAFETY AND ACTIVITY OF POMALIDOMIDE IN THE TREATMENT OF KAPOSI SARCOMA IN INDIVIDUALS WITH OR WITHOUT HIV: LONG-TERM OUTCOMES

Ramya Ramaswami[1], Mark N. Polizzotto[1], Thomas S. Uldrick[1], Kathryn A. Lurain[1], Anaida Widell[1], Kathleen M. Wyvill[1], Priscila H. Goncalves[1], Vikram Khetani[2], Ken Arakawa[2], Jerome B. Zeldis[2], Robert Yarchoan[1]

[1]*National Institutes of Health, Bethesda, MD, United States,* [2]*Celgene Corporation, Summit, NJ, United States*

Kaposi sarcoma (KS) is caused by Kaposi sarcoma herpesvirus ((KSHV), also known as human herpesvirus 8 (HHV-8)). Its incidence is markedly increased in HIV-infected persons, but also arises in individuals without HIV. KS is one of the most common tumors in sub-Saharan Africa and is the second most common HIV-associated tumor in the United States. Patients with HIV-associated KS are treated with antiretroviral therapy (ART). Chemotherapy, which is required in severe cases of KS, may be administered intermittently for years, as KS is often a relapsing and remitting condition. However, current agents are limited by toxicities, such as cumulative cardiotoxicity and peripheral neuropathy. In initial

results of a Phase I/II study, we showed that pomalidomide, an oral immunomodulatory derivative of thalidomide, was safe and active in patients with KS. Here, we present complete outcomes including progression free survival data. KS patients with or without HIV were treated with pomalidomide 5mg once daily for 21 days per 28-day cycle with aspirin 81mg once daily was administered for thromboprophylaxis. Patients were treated until complete response, plateau or progression from the best response, defined by a modified version of the AIDS Clinical Trial Group KS criteria. We evaluated adverse events on the study, time to progression from best response and long-term outcomes. Twenty-eight male patients were enrolled. Median age was 53 years, 18 (64%) were HIV-positive, 21 (75%) had advanced (T1) disease and 23 patients had prior treatment for KS excluding antiretroviral therapy. The most common grade 3/4 adverse event attributable to therapy was neutropenia (37 cycles, 14 patients). Twenty patients had a complete or partial response (71%; 95% confidence interval (CI) 51-87%); including 12 of 18 HIV-positive patients (66%; 95% CI: 41-87%) and 8 of 10 HIV-negative patients (80%; 95% CI: 44-97%). The median progression free survival was 10.1 months (95% CI: 7.6-15.8 months). Pomalidomide is a safe and active chemotherapy-sparing agent for the treatment of KS among individuals with or without HIV.

1250

TENSION IN THE RED BLOOD CELL MEMBRANE REGULATES *PLASMODIUM FALCIPARUM* INVASION: FROM SINGLE CELL HOST/PATHOGEN LIVE IMAGING TO RESISTANCE IN HUMAN POPULATIONS

Viola Introini[1], Yen-Chun Lin[1], Silvia N. Kariuki[2], Alejandro Marin-Menendez[3], Jurij Kotar[1], Thomas N. Williams[2], Julian C. Rayner[3], Pietro Cicuta[1]

[1]*University of Cambridge, Cambridge, United Kingdom,* [2]*KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya,* [3]*Wellcome Sanger Institute, Cambridge, United Kingdom*

Live imaging of the invasion of red blood cells by malaria parasites has become possible in the last few years, revealing new detail about the short and complex invasion steps. This process takes place within just a few minutes and is one of the most crucial, yet least understood, stages of malaria infection. Blood stage parasites cause the symptoms of malaria, and are also exposed to the host immune system; they thus represent a target for vaccines and drug development. In our research, we developed biophysical methods to investigate invasion characteristics, and properties of the red blood cells. We considered a novel blood group variant, Dantu, a rare polymorphism found mainly in East Kenya that provides up to 70% protection against severe malaria in homozygous individuals. This is a similar level of protection to sickle cell trait but without negative side-effects. With real-time live microscopy we measured the morphology and kinetics of invasion, and with optical tweezers we measured the parasite-red blood cell attachment force during invasion. By analysing red blood cell membrane fluctuations, we established that the membrane tension of the red blood cell strongly correlates with invasion efficiency, identifying a tension threshold above which invasion does not occur: cells with higher tension are protected from invasion, while cells with lower tension are preferentially parasitized. The Dantu cells have on average a higher tension than non-Dantu cells. Finally, we explored the relation between red blood cell biophysical properties such as bending modulus, tension, radius, and viscosity, and *Plasmodium* invasion in other malaria-protective genetic polymorphisms, such as thalassemia. This observation potentially answers a long-standing mystery in the malaria field, why *Plasmodium* parasites prefer young red blood cells, which are known to have low membrane tension values. This paves the way to a potentially novel therapeutic approach, where manipulation of red cell tension properties could provide malaria protection.

1251

SHED EBA-175 MEDIATES RED BLOOD CELL CLUSTERING THAT ENHANCES MALARIA PARASITE GROWTH AND ENABLES IMMUNE EVASION

Nichole D. Salinas[1], May M. Paing[2], Yvonne Adams[3], Anna Oksman[2], Anja T. Jensen[3], Daniel E. Goldberg[2], Niraj H. Tolia[1]

[1]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [2]*Washington University School of Medicine, St. Louis, MO, United States,* [3]*Centre for Medical Parasitology at Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, Copenhagen, Denmark*

Erythrocyte Binding Antigen of 175 kDa (EBA-175) has a well-defined role in binding to glycophorin A (GpA) during *Plasmodium falciparum* invasion of erythrocytes, and cell signaling that leads to rigidity changes within the red blood cell (RBC). However, EBA-175 is shed post invasion and a role for this shed protein has not been defined. We show that EBA-175 shed from parasites promotes clustering of RBCs, and EBA-175-dependent clusters occur in parasite culture. The RBC clustering is dependent on EBA-175 binding to GpA and the Region II of EBA-175 is sufficient for clustering. These clusters are capable of forming under physiological flow conditions, across a range of concentrations. EBA-175-dependent RBC clustering provides daughter merozoites ready access to uninfected RBCs enhancing parasite growth. Clustering also provides a general method to protect the invasion machinery from immune recognition and disruption as exemplified by protection from neutralizing antibodies that target the unrelated invasion proteins AMA-1 and RH5. Cluster formation may sterically block antibody access to antigens or could limit the time that the merozoite is exposed to the immune system. Indeed, severe malaria often results in patient death due to high parasite burdens that are protected from immune recognition. These findings provide a mechanistic framework for the role of shed proteins in RBC clustering, immune evasion, and severe malaria. Defining the role of shed proteins in malaria pathogenesis will open new avenues for intervention and control.

1252

PVDBP AMPLIFICATION PROTECTS *PLASMODIUM VIVAX* AGAINST ANTI-PVDBP HUMORAL IMMUNITY

Jean Popovici[1], Camille Roesch[1], Lenore Carias[2], Nimol Khim[1], Amelie Vantaux[1], Ivo Mueller[3], Chetan Chitnis[3], Christopher L. King[2], Benoit Witkowski[1]

[1]*Institut Pasteur of Cambodia, Phnom Penh, Cambodia,* [2]*Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States,* [3]*Institut Pasteur, Paris, France*

Multiple copies of the gene coding for PvDBP have been observed in clinical isolates from diverse geographic areas. This gene amplification might have evolved to facilitate *Plasmodium vivax* (Pv) invasion of Duffy negative reticulocytes. However, isolates with high PvDBP gene copy number occur in areas without Duffy negative individuals (such as in Cambodia) suggesting that this amplification must have other function for the parasite. Here we examine the hypothesis that PvDBP amplification evolved to circumvent host humoral immune response. First we show that the higher the gene copy number of the parasite, the higher the PvDBP gene expression in mature schizonts. Second, through short-term *in vitro* cultures, we show that human monoclonal antibodies (mabs) that target the PvDBP binding interface with the Duffy receptor on reticulocytes blocked Pv invasion of reticulocyte. However the concentration of mAb required to block invasion by 50% (IC_{50}) was on average > 20-fold higher using Pv isolates with three *pvdbp* copies versus single *pvdbp* copies ($P<0.0001$). Invasion inhibition for isolates with two *pvdbp* copies was intermediate between single copy and three copies parasites. Furthermore, the level of PvDBP RNA expressed in the schizonts used for the assays was significantly related to the level of inhibition by mabs. Third, we screened plasma from 267 individuals with acute vivax malaria (for which we had determined the PvDBP amplification status of the infecting parasites) to

identify eight individuals with high levels of naturally acquired, strain-transcending antibodies that inhibit PvDBP binding to erythrocytes. Seven of eight individuals (88%) were infected with *pvdbp* multi-copy parasite while only one was single copy (P=0.010). One out of 151 (0.66%) patients infected with single-copy parasites had high levels of binding-blocking antibodies to PvDBP while for patients infected by multi-copies parasites, 6% (7/116) had high levels of binding inhibitory antibodies (P=0.023). This work indicates that PvDBP gene amplification evolved, at least in part, to escape host humoral immunity.

1253

MULTIPLE *PLASMODIUM FALCIPARUM* K13 MUTATIONS CONFER ARTEMISININ RESISTANCE AND MODULATE PARASITE FITNESS IN ASIAN AND AFRICAN STRAINS

Barbara H. Stokes[1], Kelly Rubiano[1], Nina F. Gnädig[1], Judith Straimer[1], Tim J. Anderson[2], Frédéric Ariey[3], Didier Ménard[4], Sachel Mok[1], David A. Fidock[5]

[1]*Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY, United States,* [2]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [3]*Cochin Institute, University Paris Descartes, Paris, France,* [4]*Malaria Genetics and Resistance Group, Pasteur Institute, Paris, France,* [5]*Department of Microbiology and Immunology and Division of Infectious Diseases, Department of Medicine, Columbia University Medical Center, New York, NY, United States*

Plasmodium falciparum resistance to the first-line drug artemisinin (ART) has spread at an alarming rate across Southeast Asia since its initial detection in Cambodia a decade ago. Recent reports have suggested *de novo* emergence of ART resistance (ART-R) in South America, India and, of particular concern, Africa, where the disease burden is highest. ART-R results primarily from specific point mutations in the C-terminal propeller of the Kelch protein K13. The most important of these is C580Y, which has reached fixation in much of the Greater Mekong Subregion. To date, only a handful of *K13* alleles have been genetically validated as drivers of ART-R. Several other mutations in K13 have been associated with delayed parasite clearance in ART-treated patients and/or with increased parasite survival in the *in vitro* ring-stage survival assay ($RSA_{0\text{-}3h}$), however these have not been assessed by gene editing. Here we report a novel CRISPR/Cas9-mediated system for *K13* editing, which has allowed us to rapidly and efficiently introduce mutations into distinct parasite genetic backgrounds. Of particular interest is M579I, recently identified in Equatorial Guinea, which we report yields moderate ART-R in lab strains and several African isolates, at levels comparable to C580Y. These results provide evidence that K13 mutations can indeed mediate ART-R in African parasites. *In vitro* co-culture assays show that M579I and C580Y confer a significant fitness cost in all parasite backgrounds tested. This reduced fitness may staunch the spread of ART-R, particularly in high-transmission settings. We also observe very low $RSA_{0\text{-}3h}$ survival rates for parasites bearing the Southeast Asian F446I, P553L, or P574L mutations. Moderate $RSA_{0\text{-}3h}$ survival rates were obtained with the R561H mutation, which was previously prevalent in Thailand. Intriguingly, we find that two clinical isolates bearing the E252Q mutation, which lies outside the K13 propeller region, have elevated survival in the $RSA_{0\text{-}3h}$. However, introducing E252Q into various strains was not sufficient to confer ART-R, strongly suggesting that resistance can be multifactorial and background-dependent.

1254

VESICULAR MECHANISMS PROVIDE PHENOTYPIC ASSAYS OF ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA

Niraja Suresh[1], **Maisha Khair Nima**[1], Isabelle Coppens[2], Souvik Bhattacharjee[3], Mehdi Ghorbal[1], Kasturi Haldar[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Johns Hopkins University, Baltimore, MD, United States,* [3]*Jawaharlal Nehru University, New Delhi, India*

Artemisinin resistance threatens world-wide malaria control and elimination. Our studies show that *P. falciparum* Kelch13 (K13), a primary marker of artemisinin resistance and mutations in which cause resistance, localizes to vesicles in the parasite's endoplasmic reticulum (ER), as detected by cryo-immunoelectron microscopy. Emerging data from multiple labs suggest additional mechanisms that regulate ER vesiculation and export, independent of K13 mutation, also induce artemisinin resistance. Together these data suggest that ER vesicular dynamics may provide phenotypic markers of resistance in both K13-dependent and independent resistance. But phenotypic assays that may be used to detect K13-dependent and independent pathways of resistance have remained elusive. We integrated findings of proteomic studies with transcriptomic and metabolomics analyses to reveal just two parasite pathways that are selectively refractory to artemisinins in drug-resistant but not sensitive ring-stage parasites, the stage of clinical artemisinin resistance. Validation of the first, an ER proteostatic pathway suggests multiple substrates, whose vesicular dynamics in presence of both mutation as well as drug provide novel phenotypic readouts of resistance that may be deployed without establishing (long or short term) cultures from clinical samples.

1255

STUDY OF BIOLOGICAL MECHANISM OF REDUCED ARTEMISININ SUSCEPTIBILITY IN WEST AFRICAN *PLASMODIUM FALCIPARUM* ISOLATES

Aabha Sharma[1], Allison R. Demas[2], Selina Bopp[1], Sarah V. Volkman[1], Daniel L. Hartl[3], Dyann F. Wirth[1]

[1]*Harvard T. H. Chan School of Public Health, Boston, MA, United States,* [2]*Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard, Cambridge, MA, United States,* [3]*Harvard University, Cambridge, MA, United States*

Current emergence of artemisinin resistance threatens the success achieved by artemisinin combination therapies (ACTs) in battling malaria. If this resistance spreads from Southeast Asia to Africa, the continent with the highest global malaria burden, efforts to control malaria will be in serious jeopardy. We sought to investigate artemisinin resistance in the African context through *in vitro* selection of West African parasites using dihydroartemisinin (DHA) pulses. After thirteen rounds of DHA selection, we obtained two independent artemisinin resistant parasite lines Pikine_R and Thiès_R and identified ten mutations in seven genes by whole genome sequencing. Of the seven genes, mutations found in two *loci* (*pfcoronin Pf3D7_1251200* and a conserved *Plasmodium* gene of unknown function *Pf3D7_1433800*) were shared by both parasites while the rest of the changes were unique for each of the two selected lines. Using CRISPR/Cas9 gene editing, we established that WD-40 propeller domain mutations in *pfcoronin* are sufficient to confer artemisinin resistance measured by *in vitro* Ring-stage Survival Assay (RSA) in West African parasites and the 3D7 laboratory strain. Consistent to the observations in *pfkelch13* mutants, parasite genetic background was crucial to the level of resistance observed in *pfcoronin* mutants. To test the potential resistance contribution of *Pf3D7_1433800,* the other gene of unknown function, we have introduced the mutations (I575M and S1054F) separately into the 3D7 wildtype laboratory strain and are reverting these mutations in both Thiès_R (I575M) and Pikine_R (S1054F). We are currently investigating the *in vitro* artemisinin resistance level of *Pf3D7_1433800* revertant parasites and 3D7 mutants by RSA. Additionally, we are testing the potential synergy between *pfkelch13* and *pfcoronin* by introducing

the *pfkelch13* mutation (C580Y) into the *pfcoronin* mutant (R100K & E107V) background, with phenotypic evaluation by RSA. Investigation of *pfkelch13*-independent biological mechanisms of artemisinin resistance will make significant contributions to our currently limited understanding of artemisinin resistance.

1256

HIDDEN SPECIES BOUNDARIES AMONG MOSQUITOES OF THE MALARIA-TRANSMITTING *ANOPHELES GAMBIAE* COMPLEX FROM BURKINA FASO

Jacob A. Tennessen[1], Victoria A. Ingham[2], Hyacinthe K. Toé[3], N'Falé Sagnon[3], Hilary Ranson[2], Daniel E. Neafsey[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso*

The *Anopheles gambiae* complex consists of several morphologically indistinguishable mosquito species representing the most important vectors of the malaria parasite *Plasmodium falciparum* in sub-Saharan Africa. Continued exploration of this diverse clade has included the discovery of the cryptic taxon GOUNDRY in eastern Burkina Faso in 2011, along with other newly described species. The ecological, immunological, and reproductive differences among these species are essential to understand, as they will critically impact population responses to intervention strategies and environmental changes. Recently, we examined whole-genome sequencing data from a longitudinal study of putative *A. coluzzii* in western Burkina Faso, with the goal of detecting evolutionary changes associated with insecticide resistance. Surprisingly, we discovered that many of our samples are genetically divergent from *A. coluzzii* and all other Anopheles taxa. Population genetic analysis suggests that GOUNDRY sensu stricto represents an admixed population descended from both *A. coluzzii* and this new taxon. Thus, a cryptic anopheline species inhabits Burkina Faso and is both more geographically widespread, and more evolutionarily distant from other species, than suggested by the original GOUNDRY discovery. Misidentified cryptic taxa could seriously confound the various extensive ongoing studies of Anopheles ecology and evolution in western Africa. Reproductive barriers between cryptic species may also complicate efforts at vector control, for example via gene drives, and thwart predictions about disease epidemiology and the spread of insecticide resistance.

1257

ENGINEERED RESISTANCE TO DENGUE AND ZIKA VIRUSES IN TRANSGENIC *AEDES* OVER-EXPRESSING RNAI PATHWAY

Yuemei Dong, Shengzhang Dong, Nahid Borhani Dizaji, Natalie Rutkowski, Mary Gebhardt, George Dimopoulos

Johns Hopkins University, Baltimore, MD, United States

Mosquito transmitted arboviruses have become the most important causes of reemerging epidemic disease worldwide. The spread of insecticide resistance, lack of drugs and limited availability of licensed vaccines, has accentuated the need for the development of novel control strategies that can prevent arbovirus transmission. Similar to other insects, mosquitoes use their innate immune systems, especially the RNA interference (RNAi) antiviral pathway to control arboviral infection and transmission. We have developed genetically modified *Ae. aegypti* mosquitoes that activate the antiviral RNAi pathway in the midgut, by over-expressing RNAi pathway factors Dicer2 or R2D2 by the blood meal inducible carboxypeptidase A (*AeCpA*) promoter. Transgenic over-expression of *Dicer2* and *R2D2* inhibits both Dengue virus serotype 2 (DENV2) and Zika virus (ZIKV) significantly in the midguts, as well as systemically and in the salivary glands. Transient activation of *Dicer2* and *R2D2* in the gut tissue has only a minimal impact on key fitness parameters under laboratory conditions. RNA-seq based full genome transcriptomic analysis showed broad transcriptome regulation in the midgut tissue upon *Dicer2*- and *R2D2* over-expression, and a significant elevated expression of antimicrobial peptides (AMP) genes, suggesting a cross-talk between the RNAi pathway and other immune pathways including Toll and IMD pathways. Over-expression of *Dicer2* and *R2D2* in the midgut tissue in these transgenic mosquitoes also limited the proliferation of the midgut microbial flora, and increased mosquitoes' resistance to systemic bacterial challenges. The midgut-specific activation of the RNAi pathway resulted in a significant resistance to both DENV2 and ZIKV infection, thereby indicating a broad-spectrum antiviral role of RNAi pathway.

1258

20-HYDROXYECDYSONE (20E) PRIMES *ANOPHELES GAMBIAE* INNATE IMMUNE RESPONSE TO BACTERIA AND MALARIA PARASITES

Rebekah Reynolds, Hyeogsun Kwon, Ryan Smith

Iowa State University, Ames, IA, United States

Mosquito blood feeding is driven by the requirement of a nutrient-rich blood meal for egg development, which as a result, can potentially expose mosquitoes to blood-borne pathogens. Shortly after a blood meal, levels of the hormone 20-hydroxyecdysone (20E) increase stimulating yolk protein production in a process known as vitellogenesis. 20E reaches peak levels ~24 hours after blood feeding, coinciding with the timing of malaria parasite invasion. While well-studied with respect to insect development, mating, and reproduction, little is known about the influence of 20E on mosquito immune responses. Here we report the effects of 20E on the innate immune system of *Anopheles gambiae*, further defining the role of 20E in priming mosquito immunity for pathogen challenge. In addition to its influence on phagocytosis, RNA-seq analysis identified that 20E application upregulated expression of antimicrobial peptides, suggesting that 20E influences anti-bacterial immunity. We demonstrate that blood feeding can reduce *E. coli* survival, an effect reconstituted by 20E priming. Similarly, 20E priming also significantly reduces *Plasmodium berghei* survival. We demonstrate that the regulation of the anti-microbial peptide (AMP) cecropin 3 is induced following 20E priming and contributes to both anti-bacterial and anti-*Plasmodium* immunity, offering a potential mechanism for how 20E stimulates mosquito innate immunity. Together, these results suggest that the increased levels of 20E following a blood meal stimulate antimicrobial peptide production and prime mosquito immunity to prevent development of pathogens present in the blood meal. Current experiments are underway to investigate the effect of disrupting 20E synthesis on *Plasmodium* survival. With potential translational applications to reduce malaria transmission, these studies offer important insights into the contributions of mosquito hormones in shaping vector competence.

1259

IR8A MUTANT MOSQUITOES LOSE STRONG ATTRACTION TO HUMANS

Joshua Raji

Florida International University, Miami, FL, United States

Mosquitoes use olfaction as a primary means of detecting their hosts. Previously, the functional ablation of a family of *Aedes aegypti* olfactory receptors, the Odorant Receptors (ORs), was not sufficient to reduce host-seeking in the presence of carbon dioxide (CO_2). This suggests the olfactory receptors that remain, such as the Ionotropic Receptors (IRs), could play a significant role in host detection. To test this, we disrupted the Ir8a co-receptor in *Ae. aegypti* using CRISPR/Cas9. *Ir8a* mutant female mosquitoes are not attracted to lactic acid, a behaviorally active component of human odor, and lack odor-evoked responses to acidic volatiles. The loss of Ir8a reduces mosquito attraction to humans and their odor. We show that the CO_2-detection pathway is necessary but not sufficient for IR8a to detect human odor. Our study reveals that the IR8a pathway is crucial for an anthropophilic vector mosquito to effectively seek hosts.

1260

SEX-SPECIFIC YEAST INTERFERING RNA LARVICIDES FOR EFFECTIVE SORTING OF MALE DISEASE VECTOR MOSQUITOES

Molly Duman-Scheel, Longhua Sun, Ping Li, Joseph Roethele, Limb K. Hapairai, Keshava Mysore

Indiana University School of Medicine, South Bend, IN, United States

Efficient means of sex-sorting prior to mass release of male mosquitoes is critical for a number of mosquito control technologies. However, effective and affordable sex-sorting methods that can be readily employed for mass rearing of mosquitoes at remote or resource-limited locations have not yet been established. We performed a small interfering RNA (siRNA) larval soaking screen that uncovered >30 previously uncharacterized putative female-specific larval lethal genes in *Aedes aegypti. Saccharomyces cerevisiae* shRNA expression strains corresponding to these siRNAs were developed to functionally assess the hypothesis that yeast-mediated RNAi silencing can be employed for simple, effective, affordable, and scalable sex-sorting of male larvae. Use of the yeast system also facilitated down-selection of screen hits to be characterized in further detail. These studies revealed several high-priority target genes that encode long non-coding RNA (lncRNA). Use of yeast interfering RNA larvicide technology revealed that silencing these *lncRNA* genes, including one that will be presented in more detail here, result in female larval mortality without impacting the fertility or fecundity of adult male survivors. The yeast larvicide studies also revealed a high priority protein-encoding target gene that is conserved in multiple species of disease vector mosquitoes. Silencing this gene in *A. aegypti* also resulted in mortality of female larvae without impacting the fertility or fecundity of male survivors. RNAi silencing of the orthologs of this gene in other mosquito species indicated that this strategy can facilitate male sex-sorting in other vector insects. In addition to functionally validating *lncRNAs* as a new class of genes that regulate sex-specific development in mosquitoes, the results of this investigation indicate that yeast-based RNAi larvicides may represent an affordable, effective, and scalable technology that could facilitate mass-rearing of multiple species of male mosquitoes in remote and resource-limited regions throughout the world.

1261

CIRCADIAN GENE KNOCKOUT REDUCES FITNESS AND ALTERS BEHAVIOR IN *AEDES AEGYPTI*

Jacob I. Meyers, Michel A. Slotman

Texas A&M University, College Station, TX, United States

The circadian activity of mosquito disease vectors is well-documented and much of the behavior, physiology and a considerable amount of gene expression of *Aedes aegypti* follows 24-hour cycles. *Aedes aegypti* mosquitoes are active diurnally, with peaks of activity at the beginning and end of the light phase. We hypothesized that these circadian peaks in activity rely on the circadian molecular clock and that disrupting this clock will reduce behavioral activity and overall mosquito fitness. The circadian gene *cycle*, along with its partner *clock*, encode proteins that dimerize to function as a transcription factor which drives circadian gene expression. In *Drosophila*, knocking out *cycle* disrupts circadian clock gene expression causing arrhythmic activity, behavior and reduced fitness. To determine the impact of the circadian clock on *Ae. aegypti* behavior and fitness, we used CRISPR/Cas9 mutagenesis to create a 10bp deletion within the coding sequence of *cycle*, resulting in a premature STOP codon and defective protein product. We used qRT-PCR to measure gene expression of seven circadian genes across the day/night cycle. Surprisingly, *cyc-/-* mutants still had rhythmic expression of clock genes, albeit with a different pattern and intensity. None-the-less, *cyc-/-* mutants have altered flight activity and lack the characteristic activity peaks at the start and end of the light phase, indicating a dysfunctional circadian clock. The effect of a dysfunctional clock on host-seeking activity was measured across the light/dark cycle using an olfactometer. Initial results show that *cyc-/-* mutants display significantly reduced host-seeking activity during end of the light phase, when this mosquito typically host-seeks. We also examined *cycle-/-* mutant fitness by measuring developmental success and rates, as well as adult survivorship. *Cyc-/-* mutants have reduced developmental rates and survival from egg to adult. Additionally, *cyc-/-* mutants have reduced adult survivorship. Our results show that knocking out *cycle* disrupts the circadian clock in *Ae. aegypti* and strongly affects mosquito behavior and fitness.

1262

USING EVOLUTIONARY APPROACHES TO DISSECT THE GENETIC BASIS OF *WOLBACHIA*-MEDIATED BLOCKING OF DENGUE VIRUS IN *AEDES AEGYPTI*

Suzanne Ford[1], Scott Allen[2], Aswathy Sebastian[1], Istvan Albert[1], Stephen Chenoweth[2], **Elizabeth McGraw**[1]

[1]*Pennsylvania State University, University Park, PA, United States,* [2]*The University of Queensland, Brisbane, Australia*

Wolbachia is an intracellular bacterium that blocks virus replication in insects and that has been introduced into the mosquito, *Aedes aegypti* for biocontrol of the viruses: dengue, Zika and chikungunya. In field-releases, *Wolbachia* has reduced the local incidence of dengue fever in humans, however it is unknown if blocking will remain stable, or if resistance will evolve. The mechanism of *Wolbachia*-mediated viral blocking is yet to be fully elucidated. Here we carry out artificial selection upon natural variation in *Wolbachia*-mediated dengue blocking in *A. aegypti* and demonstrate a 45% reduction. We find evidence that stronger blocking genotypes are maintained at high frequencies and have an inherently faster population growth rate and so may be maintained by natural selection. Using genome wide association, we dissect the genetic basis of the changes in blocking phenotype and reveal novel candidate pathways for the basis of blocking, that include aspects of immunity.

1263

GENOME-WIDE ASSOCIATION STUDY OF CRYPTOSPORIDIOSIS IN BANGLADESHI INFANTS REVEALS ROLE FOR *PKRCA*

Genevieve Wojcik[1], Poonum Korpe[2], Chelsea Marie[3], Beth D. Kirkpatrick[4], Rashidul Haque[5], William A. Petri[3], Priya Duggal[2]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*University of Virginia School of Medicine, Charlottesville, VA, United States,* [4]*University of Vermont Larner College of Medicine, Burlington, VT, United States,* [5]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh*

Cryptosporidiosis is a leading cause of diarrhea and is responsible for greater than 200,000 deaths in young children in South Asia and Sub-Saharan Africa each year. The majority of human infectious are caused by *Cryptosporidium hominis* and *C. parvum*, with disease sequelae including morbidity, linear growth faltering, and cognitive defects. To investigate the role of human genetics in cryptosporidiosis, we conducted a genome-wide association study of symptomatic cryptosporidiosis within the first year of life among 873 Bangladeshi infants in three independent birth cohorts. Cases were defined as children with at least one diarrheal episode positive for cryptosporidium within the first year of life (N=183), while controls were children without any positive stool samples (N=690). A total of 6.5 million single nucleotide polymorphisms (SNPs) were evaluated for association with cryptosporidiosis, controlling for, height-for-age Z-score (HAZ_{12}) at 12 months of age, sex, and population substructure by including the first two principal components. We identified a significant association on chromosome 17 within the gene *PRKCA* (protein kinase C alpha) at rs58296998 ($P=3.78x10^{-8}$), with each additional T allele conferring 2.4 times the odds of cryptosporidiosis. We observe consistent effects across all three studies ($P_{het}=0.11$). The Gene-Tissue Expression (GTEx) project, a publicly available resource, previously identified this SNP as being associated with PRKCA expression. When coupled with our results, the direction of effects indicated decreased expression of PRKCA

to be correlated with increased risk of cryptosporidiosis. Genetic variants in *PRKCA* have previously been found to be associated with other infections, including *Staphylococcus aureus*, progression of sepsis, toxoplasmosis, *Burkholderia cenocepacia* in cystic fibrosis patients, and hepatitis E virus replication. Our results further suggest a role for *PRKCA* in the immune system's defense against pathogens, specifically cryptosporidiosis. Future work is needed to better understand the biological mechanism of this gene in intestinal infection.

1264

GIARDIA DUODENALIS INFECTIONS IN THE CONTEXT OF A WASH AND DEWORMING TRIAL IN TIMOR-LESTE

Naomi E. Clarke[1], Jessica Aw[2], James S. McCarthy[3], Rebecca J. Traub[4], Archie C. Clements[5], **Susana Vaz Nery**[1]

[1]*University of New South Wales, Kensington NSW, Australia,* [2]*Australian National University, Canberra ACT, Australia,* [3]*QIMR Berghofer Medical Research Institute, Brisbane QLD, Australia,* [4]*University of Melbourne, Parkville VIC, Australia,* [5]*Curtin University, Perth WA, Australia*

Giardiasis - caused by the protozoon *Giardia duodenalis* - represents one of the most prevalent intestinal protozoon infections worldwide. It is transmitted through the faecal-oral route and is therefore prevalent in low-income countries with poor access to safe water, sanitation and hygiene (WASH), where other neglected tropical diseases (NTDs) are frequently co-endemic. However, there is limited experimental evidence of the impact of community-based WASH interventions on *G. duodenalis* infections. Furthermore, there is limited evidence regarding the impact of regular periodic deworming with albendazole on *G. duodenalis* infections, despite such treatment being regularly delivered in low-income countries for control of other NTDs, such as soil-transmitted helminths and lymphatic filariasis. In the WASH for WORMS cluster-randomized controlled trial, rural communities in Timor-Leste were randomly allocated to receive either a community-based WASH and deworming intervention, or community-based deworming alone. All community members in both study arms received a single dose of albendazole, every six months for two years. We collected stool samples from all willing community members every six months for two years, and diagnosed *G. duodenalis* infections using quantitative PCR. Among 1971 participants at study baseline, the prevalence of *G. duodenalis* infections was 13.1% (95% CI 10.9-16.0), with highest prevalence among those aged 1-5 years (26.4%, 95% CI 20.4-33.5). After four rounds of deworming at six-monthly intervals, *G. duodenalis* prevalence was 15.6% (95% CI 12.8-18.9), not significantly different from baseline prevalence (p=0.165). Compared to deworming alone, the combined WASH and deworming intervention had no impact on *G. duodenalis* infections over a two-year-period (RR 1.18, 95% CI 0.79-1.75, p=0.413). Our findings suggest that periodic deworming has no impact on *G. duodenalis* prevalence and highlights the ongoing high burden of this protozoon in Timor-Leste, particularly among young children. WASH interventions may require higher coverage and adherence to reduce the burden of *G. duodenalis* infection.

1265

TRANSMISSION OF *CRYPTOSPORIDIUM* SPP. IN CONTACT NETWORKS IN SUB-SAHARAN AFRICA

Daniel Eibach[1], Ralf Krumkamp[1], Simone Caccio[2], Akim Adegnika[3], John Amuasi[4], John Lusingu[5], Raphael Rakotozandrindrainy[6], Jürgen May[1]

[1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany,* [2]*Istituto Superiore di Sanità, Rome, Italy,* [3]*Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon,* [4]*Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana,* [5]*National Institute for Medical Research, Korogwe, United Republic of Tanzania,* [6]*Université d'Antananarivo, Antananarivo, Madagascar*

High prevalence and mortality from Cryptosporidiosis among children in sub-Saharan Africa has been shown in recent years, however transmission dynamics and reservoirs are yet to be investigated. This multicentre study traces back *Cryptosporidium* positive children to their close human and animal contacts in order to identify transmission networks and reservoirs. Stool samples from children below 5 years with diarrhoea were collected at hospitals in Gabon, Ghana, Madagascar and Tanzania. *Cryptosporidium* positive and negative initial children were followed to the community, where stool samples from all household members, neighbouring children (<5 years) and animal contacts were obtained. Samples were screened for *Cryptosporidium spp*. by PCR-RFLP analysis and sequence analysis of the 60 kDa glycoprotein gene for *C. hominis* and *C. parvum*. Contact networks were identified and rate ratios (RR) calculated. Among 1,363 initial children 44 (20%), 47 (11%), 25 (11%) and 68 (14%) were diagnosed with *Cryptosporidium* spp. in Gabon, Ghana, Madagascar and Tanzania, respectively. Most frequently *C. hominis* (n=144; 79%), *C. parvum* (n=26; 14%) and *C. meleagridis* (n=10; 5%) were detected. Among 108 contact networks *gp*60 subtyping established 37 clusters, which contained 49% and 54% of *Cryptosporidium* positive household members and neighbours, respectively, but only 18% of *Cryptosporidium* positive animals. In comparison to *Cryptosporidium* negative initial children, positive initial children had an increased risk of having positive household members (RR = 2.5; 95%-Confidence Interval (CI): 1.5–5.2) or positive neighbouring children (RR = 2.7; 95%-CI: 1.6–4.8), but no risk of having positive animals (RR = 1.3; 95%-CI: 0.8–2.1) in their contact network. In conclusion, Cryptosporidiosis in rural sub-Saharan Africa is characterized by clusters among human contacts, to which zoonotic transmission seems to contribute only marginally. Public health programmes need to focus on improving hygiene and sanitation practices, particularly in the context of infant and childcare.

1266

THE EPIDEMIOLOGY AND IMPACT OF *ENTEROCYTOZOON BIENEUSI* AND *ENCEPHALITOZOON INTESTINALIS* INFECTIONS AMONG CHILDREN FROM LOW RESOURCES SETTINGS IN THE MALED COHORT

Amidou Samie[1], Elizabeth Rowgoskii[2], Mal-ed Network Investigators[1]

[1]*University of Venda for Science and Technology, Thohoyandou, South Africa,* [2]*University of Virginia, Charlottesville, VA, United States*

Microsporidia are fungal pathogens though originally known as protozoan parasites. They are responsible for chronic diarrhea among HIV as well as non HIV patients. However, there is a dearth of information on their occurrence and impact on child grow in community settings. In the present study we used qPCR assays to detect *E. bieneusi* and *E. intestinalis* from 41,327 monthly asymptomatic stools as well as stool collected during diarrheal episodes from 1715 children in the MalEd cohort. Anthropometric data were collected on a monthly basis for up to two years. Descriptive analysis of the data provided the overall distribution of microsporidia. We estimated the effects of microsporidia carriage on linear growth at 2 years of age. We also looked at the impact of other socioeconomic factors on the occurrence and distribution of microsporidia in the study population. The overall prevalence of *E. bieneusi* was 5.9% varying from 0.7% in Brazil to 13% in Tanzania while that of *E. intestinalis* was 0.4% with the highest in India (1%) and lowest in Brazil (0%). *E. bieneusi* was significantly high in Tanzania, Pakistan and Bangladesh (p<0.001) while it was less prevalent in Brazil, India and South Africa. Overall there was a significant difference between the prevalence of *E. bieneusi* in male (5.5%) and females (6.3%) (p<0.001). Children who have taken rotavirus vaccine tended to be protected against *E. bieneusi* (OR=0.694; 95% CI: 0.564 - 0.854). *E. bieneusi* was not associated with diarrhea, dehydration, fever, loss of appetite and vomiting. However, *E. bieneusi* infections were significantly associated with stunting (7.3% vs 4.9%) (p<0.0001; OR=1.524, 95%CI: 1.405 - 1.652), underweight (7.5% vs 5.1%) (p<0.0001; OR=1.429; 95%CI: 1.303 - 1.566) and wasting (6.9% vs 5.8%) (p=0.012; OR=1.209; 95%CI: 1.043 - 1.401). Risk factors for *E. bieneusi* included lower income, poor sanitation, low maternal education, poor floor quality, keeping cattle and chicken in the

household and poor quality of drinking water source. *E. bieneusi* infection was associated with faltering growth and low socio-economic factors encouraged the spread of infections among the children.

1267

GIARDIA DUODENALIS MODULATES IMMUNE RESPONSE TO *TOXOPLASMA GONDII* DURING MURINE CO-INFECTION

Camila H. Coelho[1], Aline Sardinha-Silva[1], Marc Fink[2], Diego L. Costa[1], Pedro Gazzinelli-Guimaraes[1], Michael E. Grigg[1], Steven M. Singer[2]

[1]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States,* [2]*Georgetown University, Department of Biology, Washington, DC, United States*

Enteric infections in the developing world are typically poly-microbial rather than due to a single pathogen. Data from the Global Enteric Multicenter Study (GEMS) suggested that co-infection with Giardia duodenalis reduces the incidence of severe diarrhea due to other pathogens. We have used oral infection with Toxoplasma gondii in mice with prior G. duodenalis infection as a model to understand how these parasites interact with each other and their host. We infected $C^{57}BL/^{6}$ mice with ^{1} million G. duodenalis trophozoites (strain GS, assemblage B) three or seven days prior to oral infection with ^{10} cysts of T. gondii (type II strain ^{76}K GFP-Luciferase). After five days of T. gondii infection, we assessed parasite burden daily by in vivo imaging and qPCR for ^{18}S gene. Nine days post-infection we collected tissue from the duodenum and ileum for analyses. In the duodenum, qPCR and Luminex revealed reduced levels of IL-^{6}, TNF-α, IFN-y and IL-$^{1}\beta$ in the co-infected groups compared to the controls. Flow cytometry of lamina propria cells revealed reduced levels of Tbet expression and the reduction of Treg population usually detected in Toxoplasma-infected mice was prevented by Giardia. Histology analyses demonstrated reduced inflammatory infiltrate in the co-infected group compared to tissues from animals infected only with T. gondii. We also observed an increased burden of T. gondii in mice previously infected with Giardia. Current analyses are being performed to compare weight loss, survival and microbiome changes after co-infection. In conclusion, our preliminary results suggest that prior Giardia infection modulates rodent immune response during acute toxoplasmosis.

1268

HIGH-THROUGHPUT SEQUENCING-BASED ANALYSES OF 3,528 INFANT DIARRHEAL SAMPLES FROM THE GLOBAL ENTERIC MULTICENTER STUDY (GEMS) TO IDENTIFY NOVEL PATHOGENIC VIRUSES AND PARASITES

Matthew V. Cannon, Claudia Perez, Jennifer Jones, GEMS consortium, Sharon M. Tennant, David Serre

University of Maryland, Baltimore, Baltimore, MD, United States

More than 800,000 infants die due to diarrheal diseases every year. Diarrhea results from an intestinal infection that can be caused by a wide variety of pathogens, including bacteria, viruses and eukaryotic parasites. A better and more comprehensive understanding of the pathogens causing these diseases would facilitate targeted treatments and could guide the development of vaccines. Unfortunately, traditional detection approaches have important limitations and, even in very large and well-controlled studies, the cause of most diarrhea cases cannot be explained. Indeed, despite screening for more than 40 putative pathogens in >22,000 individuals, 56% of the moderate-to-severe diarrheal cases in the Global Enteric Multicenter Study (GEMS) could not be attributed to any pathogen. Here, we use a novel high-throughput sequencing approach to analyze 3,528 stool samples from infants under the age of five recruited by the GEMS in Mali, Kenya, Bangladesh and Pakistan. By combining multiplex PCRs with next-generation sequencing, our assay enables the sensitive detection and identification of eukaryotic parasites from a wide range of taxonomic groups - including Amoebas, Apicomplexans, Diplomonads, Kinetoplasts, Parabasalia, Platyhelminthes and Nematodes - as well as adenoviruses. Importantly, since our approach relies on PCR amplification of conserved regions, it enables detection and identification of known and previously uncharacterized organisms from these taxa. The results of this study will provide a global perspective on the adenoviruses and eukaryotic parasites present in the intestinal tract of children from these four countries, while the unique case/control design of the GEMS study will enable a first assessment of the respective contribution of these organisms to gastroenteritis.

1269

DIRECT VALIDATION OF SCREENING HITS IN A CRYPTOSPORIDIOSIS *IN VIVO* EFFICACY MODEL

Dale Robinson[1], Natalie Hawryluk[1], Stacie Canan[1], Joseph Camardo[2], Robert K.M. Choy[3], Eugenio L. de Hostos[3], Wesley C. Van Voorhis[4], Matthew A. Hulverson[4], Ryan Choi[4], Molly C. McCloskey[4], Grant R. Whitman[4], Lynn K. Barrett[4], Samuel L.M. Arnold[4]

[1]*Celgene Global Health, San Diego, CA, United States,* [2]*Celgene Global Health, Summit, NJ, United States,* [3]*PATH, San Francisco, CA, United States,* [4]*Center for Emerging and Re-emerging Infectious Disease, Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA, United States*

Cryptosporidiosis is a global diarrheal illness caused by the microscopic parasite, *Cryptosporidium*, and is recognized as one of the most common waterborne diseases in humans and animals. Individuals with weakened immune systems (malnourished children, patients receiving cancer chemotherapy, patients with HIV/AIDS, etc.) can develop serious, life-threatening illness from this infection. *Cryptosporidium* is the second most common pathogen in malnourished 6 to 18 month-old children with severe diarrhea in resource limited environments, is a major cause of diarrhea mortality, and is associated with stunting and poor development. Yet, effective anti-*Cryptosporidium* drugs do not exist for malnourished and immunocompromised individuals. A diverse set of compounds from the Celgene chemical library, selected based on principal component analysis (PCA), were screened directly against the parasite of interest, *Cryptosporidium parvum*. After confirmation of hits and expansion of the structure-activity relationship (SAR) with a selection of near neighbor analogues, three compounds from three distinct series were identified with favorable potency, physiochemical properties, and pharmacokinetic (PK) profiles to move directly into *in vivo* proof-of-concept (POC) studies. All three compounds displayed *in vivo* efficacy in the *C. parvum*-infected interferon-γ knockout mouse model, with two of the compounds outperforming the *in vivo* positive control, BKI-1369. SAR around one of the series is currently being explored with the immediate goal of progressing compounds into a calf model of cryptosporidiosis for further validation in a model that fully recapitulates human clinical symptoms.

1270

ATP-BINDING ABILITY OF RIOK-2 PROTEIN KINASE IS ESSENTIAL FOR *STRONGYLOIDES STERCORALIS* EGG HATCHING

Huan Zhou, Weiqiang Lei, Jinyang Hu, Ying Zhang, Min Hu

Huazhong Agricultural University, Wuhan, China

Parasitic nematodes impose a significant impact on economic development and human welfare and the intensive emergence of drug resistance prompted us to develop new drugs and vaccines to control parasitic diseases, which requires a deep understanding of the key molecules' functions in the biological processes of these parasites. Protein kinases have been reported as potential drug targets in various fields. As an atypical protein kinase, RIOK-2 is a non-ribosomal factor essential for processing the 18S pre-rRNA in both yeast and human cells, and depletion of yeast RIOK-2 resulted in the accelerated mitotic exit. Although RIOK-2 have been studied in yeast and mammalian cells, little is known about

its function in parasites. Our previous work investigated the sequence characteristics and expression profiles of RIOK-2 encoding gene (*Ss-riok-2*) of *Strongyloides stercoralis*, a human and canine parasitic nematode. In the present study, extending from the previous work, the function of this gene has been explored using transgenesis combined with the mutation technique. The tissue expression pattern of *Ss*-RIOK-2 was detected in the intestine, hypodermis and pharynx of larvae, and mainly expressed in the cytoplasm. At the same time, the location was shifted from the intestine to the hypodermis with the development of larvae from the first-stage larvae to the third-stage larvae. Bacterially expressed recombinant *Ss*-RIOK-2 with a mutation at its catalytic site (D288A) or a mutation at its ATP-binding site (K123A) lost the kinase auto-phosphorylation activity *in vitro*. The D288A mutation caused the delayed larval growth and development. Interestingly, the K123A mutation induced the localization of *Ss*-RIOK-2 transferred from the cytoplasm to the nucleus, which caused an egg hatching disorder, and this defect can be effectively rescued by the wild-type *Ss*-RIOK-2. Our findings demonstrated that the ATP-binding ability but not the catalytic activity of *Ss*-RIOK-2 is essential for the egg hatching of *S. stercoralis*.

1271

IDENTIFICATION OF LONG NONCODING RNAS IN *STRONGYLOIDES STERCORALIS*

Ying Zhang, Huan Zhou, Weiqiang Lei, Jinyang Hu, **Min Hu**

Huazhong Agricultural University, Wuhan, China

Long noncoding RNAs (lncRNAs) are transcripts generally longer than 200 nucleotides with no or poor protein coding capability, and most of their functions are poorly characterized. Recently, an increasing number of studies have shown that lncRNAs are involved in various critical biological processes such as organism development and cancer progression. However, little is known about their effects in helminth parasites, such as *Strongyloides stercoralis,* a human and canine parasitic nematode which poses a threat to global health and is a major challenge in terms of diagnosis and clinical management. The present study employed a computational pipeline to identify and characterize lncRNAs from RNA-seq data of *S. stercoralis* infective third-stage larvae (iL3) and parasitic females (pF). In total, 1206 new putative lncRNAs were identified through the utilization of different criteria such as genome localization, exon number, gene length, and expression level, including 635 putative lncRNAs differentially expressed between iL3 and pF worms. The new putative lncRNAs were categorized into four types: 586 antisense, 497 intergenic, 106 sense and 17 intronic lncRNAs. Additionally, the credibility of RNA-seq results were successfully confirmed by real-time PCR analysis of 8 randomly selected lncRNAs. The Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to predict lncRNAs' co-localized and co-expressed target genes, revealing that they were enriched in several pathways involved in the interaction between host and *S. stercoralis*, eg signal transduction, translation, and metabolism. Our findings demonstrated that these lncRNAs could play important regulatory roles during the infection of *S. stercoralis*.

1272

IL-13RA1 SIGNALING DRIVEN BY ALLERGEN SENSITIZATION TRIGGERS EOSINOPHIL-DEPENDENT LUNG-SPECIFIC ARREST OF HELMINTH DEVELOPMENT

Pedro Gazzinelli-Guimaraes[1], Rafael de Queiroz Prado[1], Alessandra Ricciardi[1], Sandra Bonne-Annee[1], Joshua Sciurba[1], Erik Karmele[1], Ricardo Fujiwara[2], Thomas Nutman[1]

[1]*NAID, National Institutes of Health, Bethesda, MD, United States,* [2]*UFMG, Belo Horizonte, Brazil*

We have previously shown, using a murine model of house dust mite (HDM)-induced allergic asthma inflammation followed by *Ascaris* infection, that pre-existing allergen sensitization markedly limits Ascaris larval development and reduces up to 70% the parasite burden in the lung. This reduction in parasite burden is driven by an eosinophil-dependent pulmonary type-2-immune response (Th2 cells, IL-33, IL-4, IL-13 and mucus). To explore further the role played by IL-13/IL-13R signaling in mediating this eosinophil-dependent phenomenon, we show that in HDM-sensitized-IL-13Ra1 deficient mice, there was a significant reduction in tissue eosinophil number (a reduction similar to that found in HDM-sensitized eosinophil-deficient mice) that, as a consequence, failed to limit parasite development or numbers. RNA-seq analyses of *Ascaris* larvae isolated from the lungs of allergen pre-sensitized mice compared to larvae from non-allergic mice demonstrated marked alterations between in the 2 sets of larvae with 1330 genes being significantly differentially expressed. Using L3 and L4 larval stage-specific signature mRNA profiles, it could be shown that the *Ascaris* larvae recovered in the lungs/airways of non-allergic mice showed a transcriptomic-signature of a larvae transitioning between the L3-lung stage and the L4 intestinal stage whereas the larval stages recovered in the airways of HDM-pre-sensitized mice had a molecular signature of L3-liver stage. Taken together, our data suggest that HDM-induced allergic sensitization drives a response that mimics a primary *Ascaris* infection, such that lung-specific IL-13Ra1 signaling driven by allergen sensitization mediates eosinophil-dependent helminth larval killing in the tissue occurs. This study provides a window through which the mechanisms underlying tissue-specific responses that can drive a protective response against the early stages of helminth parasites can be seen. It also helps identify at a molecular level potential gene products crucial for larval development that could be used as potential targets for the prevention of chronic Ascaris infections in the host.

1273

VACCINATION WITH AN ATTENUATED HOOKWORM VACCINE: PRELIMINARY RESULTS FROM A PHASE 1B CLINICAL TRIAL

Paul R. Chapman[1], Paul Giacomin[2], Peter O'Rourke[1], Stacey Llewellyn[1], Christian Engwerda[1], Alex Loukas[2], James S. McCarthy[1]

[1]*Queensland Institute of Medical Research - Berghofer, Herston, Australia,* [2]*Australian Institute of Tropical Health and Medicine, Cairns, Australia*

Control of human hookworm infection through mass drug administration is impeded by high rates of reinfection, leading to the proposal for a human hookworm vaccine. We hypothesized that percutaneous exposure to *N. americanus* larvae (L_3) attenuated with ultraviolet C (UVC) light would result in protective immunity. We conducted a phase 1b clinical trial using an attenuated L_3 vaccine delivered via dermal application to healthy human volunteers. Immune response and protective efficacy were secondary endpoints. Fifteen healthy, hookworm-naive volunteers were randomized (1:2) to receive placebo (tabasco sauce) or vaccine (50 attenuated L_3) via dermal application. L_3 were attenuated by exposure to 700uJ of UVC. Two cycles of placebo or vaccine were administered 6 weeks apart, followed 6 weeks later by challenge (30 unattenuated L_3). Fecal samples were collected from each subject 10-11 weeks post challenge for larval culture by modified Harada-Mori and for fecal hookworm DNA intensity using qPCR. No serious AEs occurred. Following challenge, dermal erythema at day 3 measured a median of 20mm vs 32.5mm (p=0.007) and persisted for a median of 2.0 days vs 5.5 days (p=0.007) in controls and vaccinated subjects respectively. A significant difference in the median peripheral eosinophil count was observed at day 38, 0.49 vs 1.44 (p=0.008) which persisted until day 71 post challenge, 1.13 vs 2.32 (10^9), (p=0.014), in controls and vaccinated subjects respectively. Dermal application of attenuated L_3 was well tolerated. Vaccinated subjects had more robust dermal reactions and peripheral eosinophilia than control subjects, consistent with immune sensitization following vaccination. Significantly fewer larvae were recovered per gram of cultured feces from vaccinated than control subjects (0.78 vs 10.16; p=0.014). Vaccinated subjects had lower fecal hookworm DNA intensity than control groups (cycle time 18.67 vs 16.63; p=0.87). Serological responses to vaccine and challenge will be presented (analysis pending). Lower fecal hookworm DNA intensity and recovery of significantly fewer larvae are suggestive of a protective effect in this pilot study.

1274

REPEATED CONTROLLED HUMAN HOOKWORM INFECTION IMPROVES VARIABILITY IN EGG EXCRETION: THE ROAD TO TESTING VACCINES

Marie-Astrid Hoogerwerf[1], Jan Pieter Koopman[1], Jacqueline Janse[1], Eric Brienen[1], Marijke Langenberg[1], Yvonne Kruize[1], Luc Coffeng[2], Sake de Vlas[2], Leo Visser[1], Lisette van Lieshout[1], Maria Yazdanbakhsh[1], **Meta Roestenberg**[1]

[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Erasmus Medical Center, Rotterdam, Netherlands*

Hookworm control programs fall short due to high rates of re-infection and insufficient coverage of mass drug administration. A vaccine would aid in bringing the ultimate goal of elimination in sight. However, field testing of potential vaccine candidates is costly and hampered by limited infrastructure and large sample sizes. Experimental infection of humans with hookworm, can provide a more controlled setting for preliminary testing of vaccine efficacy and downselection of the most promising candidates, but egg counts should reflect the natural infection setting. This trial aimed to achieve a stable and high egg secretion in a controlled human hookworm model for future vaccine testing. Twenty-four healthy, male and female volunteers were randomized double blind to receive either one, two or three doses of 50 L3 *Necator americanus* larvae at 2-week intervals divided over four infection sites. Volunteers visited the trial center weekly for twenty weeks after which they were treated with albendazole . Adverse events were collected at each visit, eosinophil counts were measured weekly and faecal samples were collected for Kato-Katz slides and PCR from week 6 onwards. Three volunteers received early treatment because of severe abdominal adverse events. Skin adverse events were extremely mild. There was a trend towards a longer duration of adverse events in the highest dose group which received 150 L3. All volunteers showed patent infection by Kato-Katz 6-9 weeks after first exposure to larvae. At 12 to 16 weeks after first exposure egg counts reached a plateau phase. Median egg count for the group exposed to 50L3 for this plateau was significantly lower (480 epg) than the median count for the 100L3 and 150L3 group (both 1200 epg). Variability in egg excretion was highest in the 50L3 group and lowest in the 150L3 group. Repeated infection reduces the variability in egg excretion without a concomitant increase in adverse events. We conclude that doses of 100 or 150 L3 larvae are well tolerated and result in mild-to-moderate level infections according to WHO criteria, resembling field worm burdens.

1275

A COMPARISON OF QUANTITATIVE PCR, KATO-KATZ TECHNIQUE, AND SODIUM NITRATE FLOTATION FOR THE DIAGNOSIS OF HOOKWORM INFECTIONS IN VIETNAM

Naomi Clarke[1], Dinh Ng-Nguyen[2], Rebecca Traub[3], Archie Clements[4], Roy Anderson[5], Susana Vaz Nery[1]

[1]*University of New South Wales, Kensington NSW, Australia,* [2]*Tay Nguyen University, Dak Lak, Vietnam,* [3]*University of Melbourne, Parkville VIC, Australia,* [4]*Curtin University, Perth WA, Australia,* [5]*Imperial College London, London, United Kingdom*

There are concerted global efforts underway to scale up soil-transmitted helminth control efforts. Accurate diagnostic capabilities are crucial to these efforts. However, the current standard diagnostic test, the Kato-Katz technique, has a number of shortcomings. Quantitative PCR (qPCR) and sodium nitrate flotation represent alternative promising diagnostic techniques. This study aimed to compare the diagnostic sensitivity and infection intensity measurements of three different diagnostic techniques: the Kato-Katz technique, qPCR, and sodium nitrate flotation. We obtained stool samples from 430 schoolchildren, recruited from 13 primary schools in Dak Lak province, Vietnam. Samples were analyzed using the Kato-Katz technique and sodium nitrate flotation, with infection intensity measurements given in eggs per gram (epg) of feces. Samples were also analyzed using qPCR, with infection intensity measurements given in Ct values that were converted to epg. Diagnostic sensitivity of each technique was calculated by considering an individual to be a "true positive" if their stool sample was hookworm-positive using any of the three techniques. The prevalence of hookworm infection among the study population was 13.3% (95%CI 10.4-16.9) by Kato-Katz, 16.3% (12.9-20.3) by sodium nitrate flotation, and 21.3% (17.7-25.5) by qPCR. All qPCR-positive individuals had *Necator americanus*, and 6 of these individuals additionally had *Ancylostoma ceylanicum*. Diagnostic sensitivity of the Kato-Katz technique was 54.8%, sodium nitrate flotation 63.3%, and qPCR 87.5%. Egg counts were typically lowest for sodium nitrate flotation, followed by Kato-Katz, and highest for qPCR. This study confirmed the high diagnostic sensitivity of qPCR compared to microscopy-based techniques, and showed superior diagnostic sensitivity for sodium nitrate flotation compared with the Kato-Katz technique. Fecal egg counts, on the other hand, were higher for the Kato-Katz technique compared with sodium nitrate flotation. Correlations between infection intensity measurements for the three techniques will also be presented.

1276

VALIDATION OF A MULTIPLEX REAL-TIME PCR GASTROINTESTINAL HELMINTH PANEL

Jason Kwan[1], Kimberley Marks-Beaubrun[1], Rachel Lau[2], Filip Ralevski[2], Amanda Wang[2], Ruben Cudiamat[2], Ellen Min Chen[2], Krista Orejana[2], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario, Toronto, ON, Canada*

Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens, however, it requires high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We validated a commercial multiplex parasitic real time PCR panel detecting 7 helminths: *Ascaris* spp. *(As)*, *Enterobius vermicularis (Ev)*, *Hymenolepis* spp. *(Hy)*, *Necator americanus (Na)*, *Strongyloides* spp. *(St)*, *Taenia* spp. *(Ta)* and *Trichuris trichiura (Tt)* at Public Health Ontario, Canada. We analyzed 86 banked frozen fecal specimens including: 86 specimens without any pre-treatment and 86 specimens pre-treated with ASL buffer, containing *As* (n=23), *Ev* (n=13), *Hy* (n=1), *Ta* (n=4), St (n=33), *Tt* (n=10), and 3 mixed infections. A panel of protozoa and helminth specimens not covered in these assays was used for cross reactivity evaluation. DNA extraction and PCR were conducted with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms. Where fully evaluable due to sufficient numbers, sensitivity, specificity, positive predictive-, and negative predictive values without pre-treatment were: 48%, 100%, 100% and 84% for *As*; 77%, 100%, 100% and 96% for *Ev*; 57%, 98%, 95% and 77% for *St*; and 100%, at all metrics for *Hy* and *Ta*; and with ASL pre-treatment were: 65%, 100%, 100% and 89% for *As*; 100% at all metrics for *Hy* and *Ta*; and 53%, 100%, 100% and 75% for *St*. No cross-reactivity was observed with other protozoa or helminths. The platform had high sensitivity for detection of a small number of *Ta* and *Hy,* but suboptimal sensitivity for *Ev* and *St*. Further validation with greater numbers of specimens is required for performance determination with other helminths and those without sufficient numbers to report in this analysis.

1276

VALIDATION OF A MULTIPLEX REAL-TIME PCR GASTROINTESTINAL HELMINTH PANEL

Jason Kwan[1], Kimberley Marks-Beaubrun[1], Rachel Lau[2], Filip Ralevski[2], Amanda Wang[2], Ruben Cudiamat[2], Ellen Min Chen[2], Krista Orejana[2], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario, Toronto, ON, Canada*

Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens, however, it requires high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We validated a

commercial multiplex parasitic real time PCR panel detecting 7 helminths: *Ascaris* spp. *(As)*, *Enterobius vermicularis (Ev)*, *Hymenolepis* spp. *(Hy)*, *Necator americanus (Na)*, *Strongyloides* spp. *(St)*, *Taenia* spp. *(Ta)* and *Trichuris trichiura (Tt)* at Public Health Ontario, Canada. We analyzed 86 banked frozen fecal specimens including: 86 specimens without any pre-treatment and 86 specimens pre-treated with ASL buffer, containing *As* (n=23), *Ev* (n=13), *Hy* (n=1), *Ta* (n=4), St (n=33), *Tt* (n=10), and 3 mixed infections. A panel of protozoa and helminth specimens not covered in these assays was used for cross reactivity evaluation. DNA extraction and PCR were conducted with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms. Where fully evaluable due to sufficient numbers, sensitivity, specificity, positive predictive-, and negative predictive values without pre-treatment were: 48%, 100%, 100% and 84% for *As*; 77%, 100%, 100% and 96% for *Ev*; 57%, 98%, 95% and 77% for *St*; and 100%, at all metrics for *Hy* and *Ta*; and with ASL pre-treatment were: 65%, 100%, 100% and 89% for *As*; 100% at all metrics for *Hy* and *Ta*; and 53%, 100%, 100% and 75% for *St*. No cross-reactivity was observed with other protozoa or helminths. The platform had high sensitivity for detection of a small number of *Ta* and *Hy,* but suboptimal sensitivity for *Ev* and *St*. Further validation with greater numbers of specimens is required for performance determination with other helminths and those without sufficient numbers to report in this analysis.

1277

COMPREHENSIVE ANTIBODY PROFILING IN NODDING SYNDROME: CONTINUED ASSOCIATION BETWEEN ONCHOCERCA-INDUCED ANTIBODIES AND CROSS-REACTIVE AUTOANTIBODIES TO HUMAN BRAIN EXPRESSED LEIOMODIN-1 AND DJ-1

Joseph Kubofcik[1], Rodney Ogwang[2], Thomas B. Nutman[1], Richard Idro[2]

[1]*National Institutes of Health, Bethesda, MD, United States,* [2]*Makerere University College of Health Sciences, Kampala, Uganda*

Although the underlying etiology of the Nodding syndrome (NS) is unknown, the most compelling data suggest an association between antibody responses to the filarial parasite *Onchocerca volvulus*(Ov)that cross-react with the human brain-expressed leiomodin-1 (LMOD-1). To explore more comprehensively the role of cross-reactive autoantibodies, we examined IgG responses in the serum of 240 NS patients and 154 unaffected village controls (UVC) to Ov-16 and Ov-3261, 2 antigens that assess exposure to O. volvulus parasites ,human (Hs) LMOD-1 and DJ-1, the 2 brain expressed antigens previously shown to be higher in NS patients, and their OV homologues, Ov-tropomodulin/helminth tropomyosin and Ov-DJ-1. As shown previously, the antibody responses to Ov-16 and Ov-3261 was significantly elevated in NS patients compared to the UVC. Using ROC-based cutoffs to determine positive responses, more NS patients had positive response to human LMOD 1 than did UVC. The response to HsDJ-1 was equivalent between those with NS and the UVC. By correlation analyses, there was a highly significant correlation between the responses to HsLMOD-1 and the Ov homologue (helminth tropomyosin; p= 1e-9) and between the responses to HsDJ-1 and Ov-DJl (p=0.0007). These data provide corroborating evidence that cross-reactive antibodies driven by Ov exposure/infection underlies some of the pathogenesis of NS. Profiling of the autoantibodies in cerebral spinal fluid is underway, as CSF antibody profiling may provide a more CNS specific understanding of the mechanisms underlying the NS.

1278

A TRIAL OF REPEATED DOSES OF IVERMECTIN VERSUS ALBENDAZOELE PLUS IVERMECTIN FOR TREATMENT OF ONCHOCERCIASIS

Nicholas Opoku[1], Seidu A. Mahmood[2], Simon K. Attah[2], James W. Kazura[3], Katiuscia O'Brian[4], Kerstin Fischer[4], Peter U. Fischer[4], Gary J. Weil[4], Christopher L. King[3]

[1]*University of Health and Allied Sciences, Hohoe, Ghana,* [2]*College of Health Sciences, University of Ghana Medical School, Korle-Bu Accra, Ghana,* [3]*Case Western Reserve University, Cleveland, OH, United States,* [4]*Washington University School of Medicine, St. Louis, MO, United States*

An improved treatment for onchocerciasis is needed to accelerate elimination in Africa. This study examined whether annual or semiannual treatment with ivermectin (IVM) plus albendazole (ALB) is superior to IVM alone in killing and/or sterilizing female adult worms. This trial was performed in the Ghana Volta Region and enrolled 375 microfiladermic (Mf)-positive participants not previously treated with IVM and randomized to 5 treatment arms: annual treatment with IVM (200ug/kg) alone or IVM (200ug/kg) in combination with ALB 800mg at 0, 12, and 24 months, or semiannual treatment with IVM alone, IVM+ALB 400mg or IVM+ALB 800mg at 0, 6, 12, 18 and 24 months. Microfiladermia was tested determined pre-treatment and at 6, 12, 18, 24 and 36 months. The primary outcome was the proportion of fertile and viable female adult worms excised from onchocercomata at 36 months. The proportion of living worms with normal embryogenesis based on nodule histology was similar among treatment arms; 32 of 245 (13.1%) in IVM annual, 43 of 309 (13.9%) in IVM semiannual, 46 of 268 (17.1%) in IVM+ALB 800mg annual, 33 of 260 (12.7%) in IVM+ALB 400mg semiannual and 51 of 264 (19.3%) in IVM+ALB 800mg semiannual groups (p=0.231, chi-square analysis). Proportions of dead worms also did not differ between the 5 groups (p=0.37). There was no difference in the proportion of participants without skin Mf at 36 months among treatment groups; 34 of 69 (49%) after annual IVM, 36 of 68 (53%) after semiannual IVM, 26 of 64 (40%) after IVM+ALB 800mg annually, and 31 of 65 (48%) after semiannual IVM+ALB 400mg, and 26 of 62 (42%) after semiannual IVM+ALB 800mg (P=0.411). Thus the combination treatment with IVM plus ALB was no better than IVM alone for the sterilizing and killing of adult worms or achieving sustained Mf clearance. However, semiannual treatment with either regimen was superior to annual treatment for achieving overall reduction in skin Mf during the course of the study.

1279

DRUG DEVELOPMENT FOR THE TREATMENT AND CONTROL OF ONCHOCERCIASIS: A POPULATION PHARMACOKINETIC ANALYSIS OF EMODEPSIDE (BAY 44-4400) IN HEALTHY VOLUNTEERS

Frauke Assmus[1], Richard M. Hoglund[1], Ivan Scandale[2], Joel Tarning[1]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*Drugs for Neglected Diseases initiative, Geneva, Switzerland*

Programs for the elimination of onchocerciasis (river blindness) rely on mass drug administration of ivermectin, targeting the larvae (microfilariae) and temporarily sterilizing, but not killing the adult parasite. Hence, repeated (bi)-annual treatment during the reproductive lifespan of the adult worm (more than a decade) is required to stop transmission and halt disease progression. In order to reduce the treatment duration, a short-course macrofilaricidal drug that kills the adult worm is needed urgently. Emodepside has shown macrofilaricidal efficiency against a variety of nematodes. Following a repurposing strategy, the veterinary anthelmintic is currently under clinical development for treatment of onchocerciasis and three Phase I studies have been completed. The aim of this study was to characterize the population pharmacokinetic properties of emodepside in healthy subjects and propose a dosing regimen for a planned phase II clinical trial. Plasma concentration-time data was obtained from 142 subjects enrolled in the three Phase I studies, including a single-dose, and

a multiple-dose, dose-escalation study as well as a relative bioavailability study. All cohorts were pooled (doses: 1mg, 2.5mg, 5mg, 10mg, 20mg, 40mg, formulations: oral solution/two new immediate-release tablet formulations, food: fasted/fed) and analyzed using nonlinear mixed-effects modelling (NONMEM v7.3). Different absorption, disposition, variability and covariate models were evaluated and the final population pharmacokinetic model was used to simulate clinical dosing scenarios. Emodepside pharmacokinetics was well described by a transit-compartment absorption model, followed by a 3-compartment disposition model. Body weight was included as an allometric function and both food state and formulation had a significant impact on absorption rate and relative bioavailability (decreased by food intake and by administration of tablet formulations in comparison to the oral solution). Pharmacokinetic modelling and simulation was used to derive an optimized dosing regimen for a planned Phase II clinical trial in sub-Saharan Africa.

1280

ANTI-*WOLBACHIA* CANDIDATE ABBV-4083: PHASE 1 SAFETY AND PHARMACOKINETICS CLINICAL TRIAL IN HEALTHY ADULTS

Negar Niki Alami, David C. Carter, Nisha V. Kwatra, Weihan Zhao, Linda Snodgrass, Ariel R. Porcalla, Cheri E. Klein, Daniel E. Cohen, Loretta A. Gallenberg, Robert A. Carr, Kennan C. Marsh, Dale J. Kempf

AbbVie, North Chicago, IL, United States

Onchocerciasis is caused by the filarial nematode *Onchocerca volvulus.* Doxycycline has demonstrated efficacy against *O. volvulus* by depleting *Wolbachia*, an endosymbiotic bacterium essential for worm development and fertility. ABBV-4083, a macrolide antibacterial with activity against *Wolbachia*, is intended to sterilize and eliminate *O. volvulus* by a similar mechanism but with a shorter treatment course and improved safety profile vs. doxycycline (pregnancy Category D; contraindicated for age <8; photosensitivity). This First in Human phase 1 trial evaluated the pharmacokinetics, food effect, and safety of escalating single and multiple doses of ABBV-4083 in healthy subjects. Seventy-eight healthy subjects were exposed to ABBV-4083 (36 in single ascending dose at 40, 100, 200, 400 or 1000mg; 12 in Food Effect at 1000mg; and 30 in multiple ascending daily dose at 100mg for 7 days, 200mg for 7 or 14 days, or 400mg for 7 or 14 days). Maximum concentrations (C_{max}) of ABBV-4083 were achieved around 1-2 hours, with a half-life less than 4 hours at doses ≤ 400 mg. C_{max} and AUC increased predictably in a slightly more than dose-proportional manner, with similar exposures after multiple dose administration. The mean fractions of ABBV4083 doses recovered in urine were < 1% for a 24-hour collection interval. The most common adverse events (AEs) reported were nausea (8/78, 10%) and headache (6/78, 8%). Two subjects given a single dose of ABBV-4083 1000mg in the food effect portion experienced asymptomatic and reversible ALT and AST elevations (Grade 2 in one and Grade 4 in the other), without bilirubin elevations, deemed related to study drug. The effect of food on exposure parameters was minimal. No treatment-related serious AEs were reported. ABBV-4083 400mg was well tolerated in this Phase 1 study in healthy adults. Based on preclinical pharmacokinetic/pharmacodynamic modeling, ABBV-4083 400mg daily for 7 or 14 days is expected to be an efficacious dose. A Phase 2 proof of concept study with ABBV-4083 will be conducted in Africa by Drugs for Neglected Diseases *initiative*.

1281

PRECLINICAL EFFICACY OF THE NOVEL MACROFILARICIDAL DRUG CANDIDATE ABBV-4083

Marc P. Hübner[1], Thomas W. von Gedern[2], Kennan Marsh[2], Sabine Specht[1], Marianne Koschel[1], Alexandra Ehrens[1], Stefan J. Frohberger[1], Emma Gunderson[3], Christina Bulman[3], KC Lim[3], Mark J. Taylor[4], Joseph D. Turner[4], Stephen A. Ward[4], Judy Sakanari[3], Dale Kempf[2], Achim Hoerauf[1]

[1]*University Hospital Bonn, Bonn, Germany,* [2]*AbbVie, North Chicago, IL, United States,* [3]*University of California San Francisco, San Francisco, CA, United States,* [4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

The tylosin analogue ABBV-4083 represents a novel drug candidate that is currently considered for phase 2 clinical trials for the treatment of onchocerciasis and lymphatic filariasis. ABBV-4083 targets the *Wolbachia* endosymbionts of filariae. Depletion of *Wolbachia* by the present gold standard doxycycline leads after 4-5 weeks of daily treatment to permanent sterilization and long-term death of adult filariae causing onchocerciasis and lymphatic filariasis. Oral treatment of *L. sigmodontis*-infected mice with ABBV-4083 reduced the *Wolbachia* in female adult worms as soon as three days after treatment start with continued declining *Wolbachia* levels throughout a 3-week washout phase. Two weeks of ABBV-4083 treatment in patently *L. sigmodontis*-infected jirds led to a >99.9% *Wolbachia* depletion in female adult worms, the inhibition of filarial embryogenesis and clearance of peripheral microfilaremia. Furthermore, *Wolbachia* reduction in microfilariae coincided with the *Wolbachia* reduction in adult worms, indicating that *Wolbachia* depletion in microfilariae represents a potential surrogate marker for efficacy in upcoming phase 2 clinical studies. Importantly, continuous daily treatment with ABBV-4083 was not required, as subsequent completion of missed treatments did not impair the *Wolbachia* depletion efficacy. Combination of ABBV-4083 with albendazole allowed lower doses of ABBV-4083 and shorter treatment regimens of 7 days, leading to an improved *Wolbachia* depletion and maintained clearance of peripheral microfilaremia compared to treatments with ABBV-4083 or albendazole alone. Ongoing experiments are now evaluating the number of albendazole treatments required to boost ABBV-4083 efficacy. Confirmation of the *Wolbachia* depletion in adult worms, the inhibitory effect on filarial embryogenesis and the synergistic effect with albendazole by ABBV-4083 treatment was further obtained in *Brugia pahangi*-infected jirds. Thus, ABBV-4083 represents a promising anti-*Wolbachia* candidate that is efficacious in animal models of onchocerciasis and was recently tested in a phase 1 clinical trial.

1282

MANSONELLA PERSTANS IN LYMPHATIC FILARIASIS HOTSPOTS IN SIERRA LEONE

Yakuba M. Bah[1], Mustapha Sonnie[2], Abdulai Conteh[1], Victoria Sawyerr[1], Alhassan Konneh[2], Amy Veinoglou[3], **Mary Hodges**[2], Yaobi Zhang[4]

[1]*Neglected Tropical Disease Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, Freetown, Sierra Leone,* [3]*Helen Keller International, New York City, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Sierra Leone is endemic for lymphatic filariasis (LF) in all 14 districts, with prevalence of antigenemia by immunochromatographic test cards from 3.1% to 52% in 2005. The highest was in Bombali (52%), Koinadugu (46%), Kono (30%), Port Loko (20.5%) and Kailahun (19.1%). It is known that *Mansonella perstans* is also present in Sierra Leone. After a minimum of five rounds of mass drug administrations (MDA) with ivermectin and albendazole, nine districts successfully reached the criteria of stopping MDA in 2017/18. However, four districts failed to pass the pre-transmission assessment survey (pre-TAS) with microfilaremia (mf) prevalence >1% in 2013 in at least one site: Bombali, Koinadugu, Kailahun and Kenema districts. After a further three rounds of MDA, a re-pre-TAS was conducted in these four districts with one sentinel and one

spot check site per district (2 spot checks in Bombali) in 300-350 persons aged ≥5 years per site using Filariasis Test Strips (FTS). All positive results were confirmed by a second FTS test. Antigenemia prevalence by site was 9.1%, 16.7% and 25.9% in Bombali, 7.5% and 19.4% in Koinadugu, 6.1% and 2.9% in Kailahun, and 1.3% and 2.3% in Kenema. In order to investigate potential interference of *M. perstans* infections on FTS tests, a follow-up study was conducted before the next MDA by tracing the 296 FTS positive cases. Among these, 236 provided a midnight blood sample and 232 provided a midday blood sample (80% response rate). Microscopy was performed in the field and all slides double checked by a senior technician from Ghana. Nine persons were positive for *W. bancrofti* mf (2 each in Bombali and Kailahun and 5 in Koinadugu) and 13 persons were positive for *M. perstans* (Bombali 4, Koinadugu 7 and Kailahun 2). This follow-up study suggested that the presence of *M. perstans* may have interfered with the FTS tests, however further study is needed to investigate the use of FTS in the presence of *M. perstans.*

1283

MORBIDITY MANAGEMENT AND SURVEILLANCE OF LYMPHATIC FILARIASIS PATHOLOGY AND ACUTE DERMATOLYMPHANGIOADENITIS (ADLA) ATTACKS USING A MOBILE PHONE-BASED TOOL BY COMMUNITY HEALTH VOLUNTEERS IN GHANA

Linda Batsa Debrah[1], Jubin Osei-Mensah[2], Yusif Mubarik[2], Aliyu Mohammed[3], Olivia Agbenyega[4], Nana Kwame Ayisi-Boateng[1], Janina M. Kuehlwein[5], Ute Klarmann-Schulz[5], Achim Hoerauf[5], Alexander Yaw Debrah[6]

[1]*School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana,* [2]*Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana,* [3]*School of Public Health, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana,* [4]*Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana,* [5]*Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital Bonn, Bonn, Germany,* [6]*Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana*

Health surveillance using mobile phone technology is a burgeoning area in both infectious and non-infectious diseases. Current programmes to control lymphatic filariasis and onchocerciasis rely on sustained delivery of standard anti-filarial drugs to affected communities via mass drug administration (MDA). MDA uses a community-directed treatment strategy where community members with varying backgrounds are trained to distribute drugs. The success of this strategy rests on the resourcefulness of community health volunteers (CHVs). Previous pilot studies in Ghana and Tanzania which tested a short messaging service (SMS) tool that enabled CHVs to report basic information on identified lymphedema (LE) and hydrocele cases, such as village, age, sex, condition and severity, proved to be more efficient than the traditional way of reporting. However, because of high illiteracy rates in Ghana and other African countries, illiterate CHVs could not use the SMS tool. We therefore decided to explore the use of mobile phone voice messaging to overcome this challenge. This mobile phone surveillance uses a simple algorithm. It is designed using an Interactive Voice Response (IVR) system, an automated mobile telephony with the capacity to provide two-way voice communication. The system gives instructions in either English or Twi (a local dialect). The CHVs provide responses by pressing the mobile phone keypad numbers. The pre-coded responses provided by the CHVs are automatically stored on a secured server and extracted in csv format for further analysis. In our ongoing pilot study in Ghana, which is one of the workpackages of the TAKeOFF consortium, CHVs have been engaged to report LE and hydrocele cases as well as ADLA using the IVR. This study has reported 384 pathology cases and 174 ADLA within 6 weeks by both illiterate and literate CHVs. The system, when fully developed, will overcome underreporting of pathology cases and ADLA in endemic communities and people with ADLA could get immediate help from the drone services being introduced in Ghana now. The system has the potential to be further expanded to other communicable or non-communicable diseases.

1284

MULTI-COUNTRY ANALYSIS OF REPORTED AND SURVEYED COVERAGE FROM 222 MASS DRUG ADMINISTRATIONS IN 15 COUNTRIES TO FACILITATE DECISION-MAKING IN NEGLECTED TROPICAL DISEASE PROGRAMS

Kathryn L. Zoerhoff[1], Pamela S. Mbabazi[2], Katherine Gass[3], John Kraemer[4], Brian Fuller[5], Lynsey Blair[6], Roland Bougma[7], Aboulaye Meité[8], Nebiyu Negussu[9], Bizuayehu Gashaw[10], Scott Nash[11], Nana-Kwadwo Biritwum[12], Jean Frantz Lemoine[13], Helena U. Pangaribuan[14], Eksi Wijayanti[14], Karsor Kollie[15], Clara F. Rasoamanamihaja[16], Lazarus Juziwelo[17], John Chiphwanya[17], Pradip Rimal[18], Issa Gnandou[19], Bocar Diop[20], Ameyo M. Dorkenoo[21], Rachel Bronzan[22], Edridah M. Tukahebwa[23], Fatima Kabole[24], Violetta Yevstigneyeva[25], Lauren Courtney[1], Joseph Koroma[26], Egide Ndayishimye[27], Richard Reithinger[1], Margaret Baker[1], Fiona Fleming[6]

[1]*RTI International, Washington, DC, United States,* [2]*World Health Organization, Geneva, Switzerland,* [3]*Task Force for Global Health, Decatur, GA, United States,* [4]*Georgetown University, Washington, DC, United States,* [5]*Helen Keller International, Washington, DC, United States,* [6]*Schistosomiasis Control Initiative, London, United Kingdom,* [7]*Burkina Faso Ministry of Health, Ouagadougou, Burkina Faso,* [8]*Côte d'Ivoire Ministry of Health, Abidjan, Côte D'Ivoire,* [9]*Ethiopia Federal Ministry of Health, Addis Ababa, Ethiopia,* [10]*Amhara Regional Health Bureau, Federal Ministry of Health, Amhara, Ethiopia,* [11]*The Carter Center, Atlanta, GA, United States,* [12]*The Bill & Melinda Gates Foundation, Seattle, WA, United States,* [13]*Haiti Ministry of Public Health and Population, Port-au-Prince, Haiti,* [14]*Indonesia Ministry of Health, Jakarta, Indonesia,* [15]*Liberia Ministry of Health and Social Welfare, Monrovia, Liberia,* [16]*Madagascar Ministry of Public Health, Antananarivo, Madagascar,* [17]*Malawi Ministry of Health, Lilongwe, Malawi,* [18]*Nepal Ministry of Health and Population, Kathmandu, Nepal,* [19]*Niger Ministry of Public Health, Niamey, Niger,* [20]*Senegal Ministry of Health and Social Action, Dakar, Senegal,* [21]*Togo Ministry of Health, Lome, Togo,* [22]*FHI 360, Washington, DC, United States,* [23]*Uganda Ministry of Health, Kampala, Uganda,* [24]*Zanzibar Ministry of Health, Zanzibar City, United Republic of Tanzania,* [25]*United States Agency for International Development, Washington, DC, United States,* [26]*Consultant, Freetown, Sierra Leone,* [27]*FHI 360, Accra, Ghana*

Mass drug administrations (MDAs) are the main intervention in the control and elimination of preventable chemotherapy neglected tropical diseases (NTDs), specifically lymphatic filariasis (LF), trachoma, onchocerciasis, schistosomiasis and soil transmitted helminths. MDA treatment coverage is used to identify where modifications to the MDA approach are necessary, and is a main criterion for determining when to conduct impact surveys to assess whether MDAs can be stopped. Coverage can be measured through routinely reported programmatic data or population-based coverage evaluation surveys. Reported coverage is often the easiest and least expensive way to estimate coverage, by utilizing NTD program systems and staff at national, district and subdistrict levels; however, it is prone to inaccuracies due to errors in data compilation by persons often with low levels of education, imprecise denominators, and in some cases actually measures treatments offered as opposed to treatments swallowed. We compared data from coverage surveys implemented in 15 countries between 2008–2017 and compared these results to routinely reported coverage for 222 LF, trachoma, onchocerciasis, schistosomiasis and soil transmitted helminths MDAs. Each of these diseases has a minimum coverage threshold. Coverage estimates using routine reports and surveys gave the same result in terms of whether the minimum threshold was reached 70% of the time in the Africa region and 52% in Asia region. The median absolute difference between coverage point estimates using the two data sources was 14 percentage points (IQR 6–25 percentage points). We conclude that in many cases, based on the concordance with respect to reaching the minimum threshold coverage, routinely reported data

should continue to be used for decision-making, while working to improve the quality of these data. Coverage evaluation surveys are a useful tool and should be systematically used to periodically assess the accuracy of routinely reported data.

1285

ASSESSING THE RESILIENCE OF COMMUNITY DRUG DISTRIBUTORS (CDDS) CONDUCTING MASS DRUG ADMINISTRATION (MDA) FOR LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN CÔTE D'IVOIRE

Daniel Dilliott[1], David Addiss[2], Margaret Gyapong[3], Deborah McFarland[4], Mary Amuyunzu-Nyamongo[5], Esther Comoe[6], Adam Mama Djima[6], Amos Wung Buh[1], Alison Krentel[1]

[1]*Bruyère Research Institute, Ottawa, ON, Canada,* [2]*The Task Force for Global Health, Decatur, GA, United States,* [3]*University of Health and Allied Sciences, Ho, Ghana,* [4]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [5]*African Institute for Health and Development, Nairobi, Kenya,* [6]*Ministère de la Santé et de l'Hygiène Publique, Abidjan, Côte D'Ivoire*

Volunteer community drug distributors (CDDs) are pivotal to the success of national neglected tropical disease (NTD) elimination programs. Through mass drug administration (MDA), they distribute millions of preventive chemotherapy treatments to endemic communities. However, CDDs can encounter considerable challenges during MDA, such as massive workload and uncooperative community members. CDDs must be resilient to overcome these challenges and meet NTD elimination and control goals, yet little is known about CDD resiliency. The purpose of this study was to assess resilience among CDDs conducting MDA in Côte d'Ivoire using an international standardized tool and compare their resilience to that of frontline health workers (FLHWs). Samples of CDDs (N=133; 63.9% male, 36.1% female) and FLHWs (N=21) involved in the 2018 MDA in the Abidjan and N'zi Iffou regions were purposively selected. A survey was administered following the MDA to assess participants' demographics and experiences during MDA; resilience was assessed using the Connor-Davidson Resilience Scale 25 (CD-RISC-25). Mean CD-RISC-25 scores were calculated based on 25 self-assessed indicators. Variables individually associated with mean CD-RISC-25 score ($p<0.25$) were included in a multiple regression model. CDDs were found to have lower mean resilience scores than their FLHW counterparts in both Abidjan (73.5 to 86.5, $p<0.05$) and N'zi Iffou (71.2 to 81.1, $p<0.05$). The multivariable regression model revealed that CDDs who reported having an easy relationship with their community were significantly more resilient than those who did not ($p<0.05$). Mean resilience scores were unaffected by variations in district, age, gender, and length of involvement with the NTD program. This study establishes the CD-RISC-25 as a useful tool for assessing the resilience of NTD program volunteers and will assist Ivorian NTD program managers in augmenting CDD training modules and developing interventions to improve CDD-community relationships. Further research is needed to understand, promote, and support the resilience of this valuable health workforce, upon which NTD programs depend.

1286

EVALUATING THE IMPACT OF INTERVENTIONS ON SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS IN NORTH-CENTRAL NIGERIA

Emily Griswold[1], Abel Eigege[2], John Umaru[2], Solomon Adelamo[2], Bulus Mancha[2], Andrew Nute[1], Obiageli Nebe[3], Chukwuma Anyaike[3], Evelyn Ngige[3], Jonathan Kadimbo[4], Jacob Danboyi[5], Emmanuel Miri[2], Frank Richards[1]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*The Carter Center, Jos, Nigeria,* [3]*Federal Ministry of Health, Abuja, Nigeria,* [4]*Plateau State Ministry of Health, Jos, Nigeria,* [5]*Nasarawa State Ministry of Health, Lafia, Nigeria*

Statewide mapping surveys of schistosomiasis (SCH) and soil-transmitted helminths (STH) were conducted in 2013 in Nasarawa and Plateau states of north-central Nigeria, sampling 11,332 children. Varying combinations of mass drug administration for school-aged children followed for the next five years. We repeated these assessments in 2018 in the same schools, sampling 9,660 children aged 6 to 14 from 202 schools. We analyzed overall parasite prevalence and intensity, as well as any associations with gender, age, behaviors, water and sanitation, and treatment history. Schistosomiasis (*S. haematobium* or *S. mansoni*) prevalence was 15.0% (95% CI: 12.0% - 18.6%) in 2013 in Nasarawa state, and 11.3% (9.5% - 13.4%) in Plateau state. Prevalence in 2018 was 6.5% (4.6% - 9.0%) in Nasarawa and 6.3% (4.6% - 8.6%) in Plateau. Six districts saw significant changes in prevalence of *S. mansoni,* while 11 saw significant changes in *S. haematobium.* Stool samples were also analyzed for presence of *A. lumbricoides, T. trichiura,* and hookworm. The 2013 prevalence of any infection was 14.5% (11.8% - 17.8%) in Nasarawa and 8.0% (6.6% - 9.7%) in Plateau. In 2018, prevalence in Nasarawa was 11.2% (9.1% - 13.6%), whereas it was 8.2% (6.4% - 10.4%) in Plateau. In contrast to SCH, only one district saw a statistically significant changes. Intensity (measured as mean eggs per gram of stool) was measured at both timepoints in stool samples only; egg counts in urine were only done in 2018. Of children infected with *S. mansoni*, heavy infections were found in 2013/2018 in 17.0% (9.9% - 27.7%)/8.1% (3.8% - 16.3%). Of children with *S. haematobium* (2.5% [1.8% - 3.5%] in 2018), 21.3% (13.7% - 31.7%) had heavy infections. Heavy intensity infections were found in <1% of children with hookworm, and none were found in children with *A. lumbricoides* or *T. trichiura* in either study. Water and sanitation data were only available in 2018; latrine coverage was poor: less than half (43.6%) of schools had a latrine and only 14.4% had handwashing facilities. Although progress is evident, heavy schistosomiasis infections remain in Nasarawa and Plateau state.

1287

TREND ANALYSIS OF SOIL TRANSMITTED HELMINTHS AND SCHISTOSOME INFECTIONS PREVALENCE OVERLAID WITH PROGRAMMATIC TREATMENT COVERAGE STRATIFIED BY COUNTIES IN KENYA: LONGITUDINAL STUDY DESIGN

Collins Okoyo[1], Suzy J. Campbell[2], Sammy Njenga[1], Simon J. Brooker[3], Charles Mwandawiro[1]

[1]*Kenya Medical Research Institute, Nairobi, Kenya,* [2]*Evidence Action, Washington, DC, United States,* [3]*London School of Hygiene and Tropical Medical Medicine, London, United Kingdom*

Kenya's national school-based deworming programme began in 2012 and aimed at reducing infection and morbidity associated with soil-transmitted helminth (STH) and schistosome infections. It has rich dataset from its monitoring and evaluation arm conducted during baseline, midterm, endline and sixth year evaluation surveys in 20 counties over the last six years. We conducted trend analysis using six years secondary data collected between 2012 and 2018 from this programme to understand the associations between prevalence of infection and the treatment coverage, by county, over time. The surveys utilized a series of pre- and post-MDA intervention, repeat cross-sectional surveys in a representative, stratified, two-stage sample of 200 schools in 20 counties. In overall, prevalence of 32.3% and 12.9% were observed for the undifferentiated STH infections during baseline and year six evaluation surveys respectively, translating into a significant decline in the prevalence (RR = 60.2%, $p<0.001$). Differentiated STH infections reductions were at differing rates; hookworms (RR = 93.6%, $p<0.001$), *Ascaris lumbricoides* (RR = 46.1%, $p<0.001$) and *Trichuris trichiura* (RR = 45.5, $p<0.001$). For schistosomes, prevalence reduction was observed for *Schistosoma haematobium* (RR = 97.8, $p<0.001$) but not for *Schistosoma mansoni*. Overall significant association between undifferentiated STH prevalence and coverage (Fisher's exact test, $p=0.032$) was observed, however, no significant associations were observed for each of the differentiated STH species as well as for schistosome species. High treatment coverage was seen to be nonsignificantly correlated with low *S. haematobium* infection ($r = -0.1498$, $p=0.7486$). Heterogeneity in county-level associations between prevalence and coverage was observed. The findings of this secondary

analysis will allow the national control programme to make informed decisions regarding maintaining high level treatment coverage as the country rallies towards elimination of these infections.

1288

SUCCESSFUL INTEGRATION OF STH SURVEY WITH LF TRANSMISSION ASSESSMENT SURVEYS IN TEN EVALUATION UNITS IN MALI

Massitan Dembélé[1], Mahamadou Traoré[1], Benoit Dembélé[2], Boubacar Guindo[2], Mama Niélé Doumbia[2], Seydou Goita[2], Modibo Keita[2], Yaya I Coulibaly[3], Salif S Doumbia[3], Moussa Sacko[4], Renion Saye[4], Abdoulaye Guindo[1], Abdoul Karim Sidibé[1], Steven Reid[5], Fama Kondo[2], Mohamed Lamine Yattara[2], Yaobi Zhang[6]

[1]*Directorate General of Health, Ministry of Health and Public Hygiene, Bamako, Mali,* [2]*Helen Keller International, Bamako, Mali,* [3]*Filariasis Unit, International Center of Excellence in Research, Faculty of Medicine and Odontostomatology, Point G, Bamako, Mali,* [4]*Institut National de Recherche en Santé publique, Bamako, Mali,* [5]*Helen Keller International, New York, NY, United States,* [6]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Mass drug administration (MDA) for lymphatic filariasis (LF) with ivermectin and albendazole has also targeted soil-transmitted helminths (STH) in Mali. As recommended by WHO, after a minimum of five years of effective MDA, a series of transmission assessment surveys (TAS) is conducted and the national NTD program should also collect data on STH when a TAS is conducted to inform STH program decision-making. In 2018, Mali combined the TAS2 and STH assessments in 10 evaluation units (EUs), consisting of 27 HDs, according to the WHO guidelines. The Survey Sample Builder (SSB) was used to determine the minimum sample size (for TAS 759-1692 and for STH 332) and to generate the A and B random lists for sample selection in the field. The maximum positive case threshold (critical cut-off) was also determined by the SSB for LF. The target population was based on the type of survey: for STH assessments it was children aged 8-10 years in school and 6-7 years in households, while for LF, the target was children aged 6-7 years. A joint team of surveyors and technicians from two national programs conducted the field assessments. Filaria Test Strips (FTS) for LF and Kato-Katz thick smears for STH were used as the diagnostic tests. Three EUs were surveyed in schools and seven EUs were in communities. In total, 17,263 children were tested for TAS and 3,468 for STH in the 10 EUs. All the EUs evaluated for LF passed TAS2 with the number of positive cases ranging from 1 to 3 in each EU, below the critical cut-off of either 18 or 20 positive results. One or two positive children for STH were diagnosed in 3 EUs (all in community-based surveys), giving an STH prevalence of between 2% and 10% (according to WHO guidance on TAS-STH results). No positive cases for STH were found in the other 7 EUs, indicating that these EUs have an STH prevalence of <2%. These results confirmed the low prevalence of STH in Mali found by sentinel site assessments integrated with schistosomiasis surveys previously. These TAS-STH results indicate the continued success of the Malian LF program and the reduction in STH prevalence and the results show that MDA for STH may no longer be required in these HDs.

1289

COMMUNITY EFFECTIVENESS AND INDIVIDUAL EFFICACY OF IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS, SCABIES AND SOIL TRANSMITTED HELMINTHS IN FIJI

Myra Hardy[1], Josaia Samuela[2], Mike Kama[2], Meciusela Tuicakau[2], Lucia Romani[3], Margot Whitfeld[3], John Kaldor[3], Leanne J. Robinson[4], Andrew Steer[1]

[1]*Murdoch Children's Research Institute, Melbourne, Australia,* [2]*Ministry of Health and Medical Services, Suva, Fiji,* [3]*Kirby Institute, Sydney, Australia,* [4]*Burnet Institute, Melbourne, Australia*

Despite repeated rounds of mass drug administration (MDA) using diethylcarbamazine and albendazole (DA), lymphatic filariasis (LF) remains a public health problem in many parts of Fiji, where scabies and soil-transmitted helminths are also co-endemic. Based upon evidence from other settings, the addition of ivermectin to DA (IDA) was hypothesised to be more effective at eliminating *W. bancrofti* and scabies. A trial conducted in 2017 randomized 35 villages from two Fijian islands, Rotuma and Gau, to receive DA (n=1216) or IDA (n=2396) in a 1:2 ratio, confirming that IDA has an acceptable safety profile in Fiji. Permethrin as scabies treatment was given to 513 in the DA group, and as an ivermectin MDA alternative to 236 in the IDA group. The impact on community prevalence of LF microfilaremia and antigenemia, scabies and soil transmitted helminths (STH) was assessed at 12 months, as well as clearance of microfilaremia in infected individuals from baseline. At 12 months, 2942 participants were re-enrolled, 870 were lost to follow up, and 961 were new residents, resulting in a total of 3903 participants. From baseline to 12 months the prevalence of LF microfilaremia declined from 3.8% to 1.9% (absolute reduction (AR) of 1.9%) in DA and 3.9% to 1.9% (AR 2%) in IDA group. Scabies prevalence declined from 13.6% to 1.1% (AR 12.5%) in DA and 13.4% to 1.9% (AR 11.4%) in IDA group. STH prevalence declined from 13.1% to 4.6% (AR 8.4%) in DA and 21% to 16% (AR 5%) in IDA group. In those individuals who were infected with LF at baseline, clearance of microfilaremia at 12 months was 67.5% in DA versus 62.5% in IDA arm. However this varied between island groups: for DA, clearance in Rotuma was 60% and in Gau 80%; and for IDA, clearance in Rotuma was 57.8% and in Gau 100%. There was a reduction in community prevalence of all three neglected tropical diseases 12 months following MDA of either DA or IDA. However, there was no additional reduction by adding ivermectin to the standard DA MDA for community burden of disease or for clearance of LF microfilaremia in infected individuals. Further analysis is ongoing to understand these findings.

1290

PHARMACOKINETIC STUDY OF YAWS AND LYMPHATIC FILARIASIS DRUGS INTERACTIONS

Lucy Ninmongo John[1], Catherine Bjerum[2], Christopher King[2], Darryl Murry[3], Oriol Mitja[4], Michael Marks[5]

[1]*National Department of Health, Port Moresby, Papua New Guinea,* [2]*Case Western Reserve University, Cleveland, OH, United States,* [3]*Nebraska University Medical Centre, Omaha, NE, United States,* [4]*University of Barcelona, Barcelona, Spain,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Pharmacokinetic data are a pre-requisite to the integrated implementation of large-scale mass drug administration (MDA) of medicines for neglected tropical diseases (NTDs). We investigated the safety and drug interactions of integration of azithromycin (AZI), targeting yaws, with the newly approved Ivermectin, Albendazole, Diethylcarbamazine (IDA) regime for LF. A small, randomized, 3-arm (12 subjects per arm) pharmacokinetic interaction study in adult volunteers with equal gender participation was carried out in Lihir Island, Papua New Guinea in September 2018. Healthy adult participants were recruited and randomized to (1) IDA alone (2) AZI alone, and (3) IDA combined with AZI. The primary outcomes were safety (i.e. differences in adverse events (AE)) and drug interactions (i.e. differences in area under the curve (AUC)). Thirty-seven (37) participants were randomized and completed the study. There was no significant difference in the frequency of AEs across study arms (AZI and IDA alone arms 9/12 (75%), co-administration arm 12/13 (92%); p = 0.44). All adverse events were grade 1 and self-limiting. There were no significant drug-drug interactions between the study arms. The mean AUC0-∞ adjusted to dose (hr*ng/mL) in the separate compared to co-administration arms were: Albendazole 273.7 vs 257.4 (difference 16.3, 95%CI -199.7 to 232.3); DEC 25332.4 vs 22477.3 (diff 2855.1, -3872.2 to 9582.3); Ivermectin 2348.7 vs 2009.5 (diff 339.2; -359.1 to 1037.5) and AZI 14820.6 vs 16024.0 (diff -1203.4, -5432.1 to 3025.3). There were no serious adverse events in any of the study arms. Co-administration of AZI with IDA did not show evidence of significant drug-interactions. Our data support the need for a larger field-study on the safety of integrated MDA for the control of NTDs.

1291

A RANDOMIZED CLINICAL TRIAL OF THE SAFETY AND IMMUNOGENICITY OF A 2-DOSE HETEROLOGOUS EBOLA VACCINE REGIMEN WITH AD26.ZEBOV AND MVA-BN®-FILO IN HEALTHY AND HIV+ AFRICAN ADULTS

Houreratou Barry[1], Gaudensia Mutua[2], Hannah Kibuuka[3], Zacchaeus Anywaine[4], Jennifer Serwanga[4], Joseph Blehou[5], Christine Bétard[6], Laura Richert[6], Georgi Shukarev[7], Cynthia Robinson[7], Auguste Gaddah[8], Dirk Heerwegh[8], Viki Bockstal[7], Kerstin Luhn[7], Maarten Leyssen[7], Sirima Sodiomon[9], Omu Anzala[2], Salim Wakabi[3], Nicolas Meda[1], Serge Eholie[9], Macaya Douoguih[8], Rodolphe Thiebaut[6]

[1]*Centre MURAZ, Bobo-Dioulasso, Burkina Faso,* [2]*KAVI - Institute of Clinical Research University of Nairobi, Nairobi, Kenya,* [3]*Makerere University - Walter Reed Project, Kampala, Uganda,* [4]*Medical Research Council/ Uganda Virus Research Institute and London School of Hygiene & Tropical Medicine Uganda Research Unit, Entebbe, Uganda,* [5]*Programme PACCI/ EBOVAC*[2]*, CHU Treichville, Centre Medical SAPH Toupah, Toupah, Côte D'Ivoire,* [6]*INSERM, U*[1219] *Bordeaux Population Health research centre, and Euclid/F-CRIN Clinical Trials Platform, University Bordeaux, Bordeaux, France,* [7]*Janssen Vaccines and Prevention, Leiden, Netherlands,* [8]*Janssen Research & Development, Beerse, Belgium,* [9]*Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Unité de Recherche Clinique de Banfora, Ouagadougou, Burkina Faso*

The West African 2014-16 Ebola epidemic and current outbreaks in DRC highlight the need for safe and effective Ebola vaccines. A 2-dose prophylactic Ebola vaccine regimen based on Ad26.ZEBOV (Ad26) and MVA-BN®-Filo (MVA) is in late stage clinical development. We report a Phase 2, randomized clinical trial of safety and immunogenicity (EBL2002) conducted in Burkina Faso, Côte d'Ivoire, Kenya and Uganda. Healthy and HIV-infected adults were vaccinated with Ad26 (dose 1) on Day 1 followed by MVA (dose 2) on Day 29 (Group 1), Day 57 (Group 2) or Day 85 (Group 3, healthy adults only). Serious adverse events (AEs) were assessed until study end, AEs until 28 days post-dose and solicited AEs until 7 days post-dose. Binding antibody responses were evaluated by EBOV GP FANG ELISA at baseline, pre-dose 2, 21 days post-dose 2 and 1 year post-dose 1. A total of 668 healthy adults (cohort 1) and 142 HIV-infected adults (cohort 2) were enrolled to receive Ad26, MVA (N=559; N=118, respectively) or placebo (N=109; N=24). All Ad26, MVA regimens were well-tolerated with no significant safety signals. In both cohorts, most solicited AEs were mild to moderate. Serious AEs were considered unrelated to study vaccination. A robust humoral immune response was observed in all healthy adults, with 77-81% responders (defined as >2.5x over positive baseline or >2.5x over LLOQ) post-dose 1 and 98-100% responders post-dose 2. Geometric mean binding antibody concentrations (GMC) at 21 days post-dose 2 ranged from 3085 EU/mL (group 1) to 7518 EU/mL (group 2) and 7300 EU/mL (group 3). In HIV-infected adults, 81-88% responders were detected post-dose 1 and 100% post-dose 2. The GMCs at 21 days post-dose 2 were 4207 EU/mL in group 1 and 5283 in group 2. Binding antibody responses persisted in at least 78% of the healthy and 86% of the HIV-infected adults up to at least 1 year post-dose 1. Ad26, MVA regimens were safe, well tolerated and induced robust immune responses in healthy and HIV+ adults. Humoral responses persisted at least up to 1 year post-vaccination in the majority of participants. Ad26, MVA vaccine regimens may be suitable for vaccination strategies in healthy and HIV-infected individuals.

1292

RVSVΔG-ZEBOV-GP EBOLA VACCINE (MERCK & CO., INC., KENILWORTH, NJ, USA): UPDATED SAFETY, IMMUNOGENICITY, AND EFFICACY

Jakub Simon, Matthew Onorato, Kenneth Liu, Rebecca Grant-Klein, Sheri Dubey, Melissa Hughes, Sharon Rudo, Jayanthi Wolf, Beeth-Ann Coller

Merck & Co., Inc., Kenilworth, NJ, United States

Ebola continues to cause significant morbidity and mortality in regions of the world where the provision of healthcare and vaccine are difficult. The vaccine from Merck & Co., Inc., Kenilworth, NJ, USA is a live recombinant vesicular stomatitis virus (VSV) containing the Zaire Ebola virus glycoprotein (GP) in place of the VSV GP (rVSVΔG-ZEBOV-GP). Vaccine safety, efficacy, or immunogenicity have been evaluated in 13 Phase 1-3 clinical trials, 4 additional Phase 2 clinical trials are planned or ongoing; and expanded access protocols allow for the delivery of vaccine in outbreak situations before licensure. More than 90,000 subjects have received rVSVΔG-ZEBOV-GP vaccine in these clinical trials as of 26 Feb 2019 (time of abstract preparation). Common adverse events included injection-site reactions, fever, fatigue, myalgia, arthralgia, and headache, which were mostly mild to moderate in intensity and of short duration. Arthritis, which was also generally mild to moderate in intensity, has been reported at frequencies <5% in most trials. Immune responses, based on GP antibodies detected by GP-ELISA and neutralizing antibodies detected by plaque reduction neutralization test (PRNT), were detectable at 14 days in most vaccinated subjects, with a high proportion of subjects demonstrating a seroresponse by 28 days. Durability of the anti-GP antibody responses was demonstrated through 24 months. Updated safety, immunogenicity, and efficacy data from across the program will be discussed as well as the use of the vaccine in the context of outbreak response.

1293

SAFETY AND IMMUNOGENICITY OF A 2-DOSE HETEROLOGOUS VACCINE AGAINST EBOLA IN AFRICAN CHILDREN AND ADOLESCENTS

Muhammed Afolabi[1], Gaudensia Mutua[2], Zacchaeus Anywaine[3], Hannah Kibuuka[4], David Ishola[1], Bailah Leigh[5], Frank Baiden[1], Kwabena Owusu-Kyei[1], Omu Anzala[2], Mohamed Samai[1], Joseph Blehou[6], Brian Greenwood[7], Daniela Manno[1], Viki Bockstal[8], Auguste Gaddah[9], Dirk Heerwegh[9], Georgi Shukarev[8], Babajide Keshinro[8], Kerstin Luhn[8], Maarten Leyssen[8], Cynthia Robinson[8], Debby-Watson Jones[1], Rodolphe Thiebaut[10], Macaya Douoguih[9], Houreratou Barry[11]

[1]*Clinical Research Department, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*KAVI-Institute of Clinical Research University of Nairobi, Nairobi, Kenya,* [3]*Medical Research Council/Uganda Virus Research Institute and London School of Hygiene & Tropical Medicine Uganda Research Unit, Entebbe, Uganda,* [4]*Makerere University Walter Reed Project, Kampala, Uganda,* [5]*College of Medicine and Allied Health Sciences (COMAHS), COMAHS Secretariat, New England, Freetown, Sierra Leone,* [6]*Centre Medical SAPH Toupah, Toupah, Côte D'Ivoire,* [7]*Department of Disease Control, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [8]*Janssen Vaccines and Prevention B.V., Leiden, Netherlands,* [9]*Janssen Research & Development, Beerse, Belgium,* [1011]*INSERM, U*[1219] *Bordeaux Population Health research centre, and Euclid/F-CRIN Clinical Trials Platform, University Bordeaux, Bordeaux, France,* [11]*Centre MURAZ, BoboDioulasso, Burkina Faso*

The West African 2014 Ebola epidemic and the recent outbreaks in DRC highlight the need for safe and effective Ebola vaccines. A heterologous 2-dose prophylactic Ebola vaccination regimen consisting of Ad26.ZEBOV (Ad26) followed by MVA-BN®-Filo (MVA) is now in Phase 3 development. In EBL2002 (Burkina Faso, Cote D'Ivoire, Uganda, Kenya) and EBL3001 (Sierra Leone) studies, three age cohorts (adolescents [12-17 years], children [4-11 years] and toddlers [1-3 years]) were vaccinated with Ad26 or placebo (dose 1) followed by MVA or placebo (dose 2), 28 or 56 days

later (EBL2002) or 56 days later only (EBL3001). Placebo was inactive apart from dose 1 EBL3001 where MenACWY was used. Serious adverse events (SAEs) were assessed until end of study, unsolicited and solicited AEs were assessed until 28 and 7 days post-dose, respectively. Overall, 838 participants (adolescents: 322, children: 324, toddlers: 192, Burkina Faso: 121, Cote D'Ivoire: 30, Uganda: 70, Kenya: 42, Sierra Leone: 575) were enrolled. The Ad26, MVA regimen was well tolerated and no safety signals were identified. Most solicited AEs were mild to moderate. One vaccine-related (active or MenACWY) SAE was reported. In toddlers, the frequency of any febrile response was similar between the Ad26, MVA regimen and the control regimen (range 16.7-22.9%, respectively). In children, the frequency of any fever was 14.7% and 5.6% for active or control, respectively. Overall, frequency of grade 3 fever was <2%. Robust humoral immune responses were observed in all age cohorts. The geometric mean binding antibody concentration (GMC) at day 21 post dose 2 was 22452 EU/mL in toddlers, 10212, 7735 and 17388 EU/mL in children (EBL3001, EBL2002 0,28 and 0,56, respectively), and 9929, 7022 and 13532 EU/mL in adolescents (EBL3001, EBL2002 0,28 and 0,56, respectively). Antibody levels were shown to persist up to 6 months post-MVA in children and adolescents. These data indicate that the Ad26, MVA vaccination regimen is well tolerated and produced robust vaccine-induced immune responses and may be suitable for pediatric populations.

1294

AD26.ZEBOV EBOLA BOOSTER VACCINATION INDUCES A STRONG ANAMNESTIC RESPONSE IN PREVIOUSLY VACCINATED PEOPLE AND PROVIDES RAPID PROTECTION AGAINST LETHAL EBOLA VIRUS CHALLENGE IN NHP

Viki Bockstal[1], Ramon Roozendaal[1], Daniela Manno[2], Zacchaeus Anywaine[3], Muhammed Afolabi[2], Gaudensia Mutua[4], Frank Baiden[2], Houreratou Barry[5], Kwabena Owusu-Kyei[2], David Ishola[2], Brian Greenwood[6], Bailah Leigh[7], Mohamed Samai[7], Omu Anzala[4], Brett Lowe[8], Sodiomon Sirima[9], Cynthia Robinson[1], Auguste Gaddah[10], Dirk Heewegh[10], Laura Solforosi[1], Jenny Hendriks[1], Roland Zahn[1], Kerstin Luhn[1], Rodolphe Thiebaut[11], Deborah Watson-Jones[2], Maarten Leyssen[1], Macaya Douoguih[1]

[1]*Janssen Vaccines & Prevention, Leiden, Netherlands,* [2]*Clinical Research Department, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Medical Research Council/Uganda Virus Research Institute and London School of Hygiene & Tropical Medicine Uganda Research Unit, Entebbe, Uganda,* [4]*KAVI-Institute of Clinical Research University of Nairobi, Nairobi, Kenya,* [5]*Centre MURAZ, Bobo-Dioulasso Dioulasso, Burkina Faso,* [6]*Department of Disease Control, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [7]*College of Medicine and Allied Health Sciences (COMAHS), COMAHS Secretariat, New England, Freetown, Sierra Leone,* [8]*University of Oxford, Oxford, United Kingdom,* [9]*Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Unité de Recherche Clinique de Banfora, Ouagadougou, Burkina Faso,* [10]*Janssen Research & Development, Beerse, Belgium,* [11]*INSERM, U*[1219] *Bordeaux Population Health research centre, and Euclid/F-CRIN Clinical Trials Platform, University Bordeaux, Bordeaux, France*

The heterologous 2-dose vaccine regimen of Ad26.ZEBOV (Ad26), MVA-BN®-Filo (MVA) is well tolerated, induces robust immune responses, persisting antibodies, and protection in NHP against lethal EBOV challenge. As the duration of protection is unknown, the potential benefit of a 'pre-exposure' Ad26 booster vaccination was evaluated in adults who received the 2-dose regimen. We describe human clinical data with immunogenicity and efficacy data obtained in NHP challenge studies. Study EBL2002 took place in Burkina Faso, Côte d'Ivoire, Kenya & Uganda and EBL3001 in Sierra Leone. Healthy adults (N=102) were vaccinated with Ad26 (dose 1) on Day 1 followed by MVA (dose 2) on Day 57 (EBL2002 and 3001), or Day 29 in EBL2002. An Ad26 booster vaccination was given 1 year (EBL2002) or 2 years (EBL3001) post-dose 1. Binding antibody responses were assessed via EBOV GP FANG ELISA at baseline and post vaccination. 21 days post-MVA, binding antibody responses were observed in 98-99% of subjects with geometric mean binding antibody concentrations of 3810 EU/ml (EBL3001) to 7518 EU/ml (EBL2002). Binding antibody responses persisted to 1 year (EBL2002) and 2 years (EBL3001) post-dose 1 with pre-booster GMCs ranging from 279 (EBL3001) to 366 EU/ml (EBL2002). Booster vaccination with Ad26.ZEBOV induced ~40-fold increase in binding antibody levels within 7 days. 21 days post-booster dose, binding antibody levels were ~10-fold greater than peak post-MVA levels (GMC: 29238 EU/ml [EBL3001] and 41643 EU/ml [EBL2002]). To test Ad26 booster vaccination protection against Ebola challenge, NHP were boosted at 17 months post-dose 2, 3 or 7 days before EBOV Kikwit infection (IM, 100 pfu). All NHP boosted with Ad26 3 or 7 days prior to EBOV challenge survived. Both in people and NHP, the 2-dose heterologous Ad26, MVA vaccine regimen induces a strong and lasting memory response that is rapidly re-activated by a booster of Ad26. Exposure to EBOV GP in the context of EBOV infection may lead to a similarly strong anamnestic response. The data also suggest that a booster with Ad26 could be considered for individuals at imminent risk of exposure who previously received the 2-dose regimen.

1295

THE EFFECT OF INTENSIVE CARE TREATMENT BUNDLE ON SERUM CYTOKINES AND VIRAL LOAD DURING EBOLA VIRUS (ZAIRE) INFECTION

Paul W. Blair[1], Karen A. Martins[2], Mark G. Kortepeter[3], Michael W. Keebaugh[2], Isaac L. Downs[2], Anthony P. Cardile[2]

[1]*Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, MD, United States,* [3]*University of Nebraska College of Public Health, Omaha, NE, United States*

Intensive care treatment bundles (ICTBs) are thought to improve survival from Ebola virus disease (EVD). However, the effects of ICTBs on EVD immunopathogenesis are unknown. Therefore, we sought to evaluate the effects of an ICTB on select cytokine levels and serum viral load in a non-human primate (NHP) model. This post-hoc analysis included an ICTB group (N=5), a levofloxacin plus intravenous fluids group (N=2), and a control group (N=4) of rhesus macaques. NHPs were inoculated intramuscularly with a target dose of 1000 PFU *Zaire ebolavirus* (Kikwit). The ICTB group received intravenous fluids, levofloxacin, acetaminophen, norepinephrine, and hydrocortisone following the onset of fever based on a clinical algorithm. We measured serum IL-6, IL-10, GM-CSF, MCP1, MIP1β, IFNγ, and TNFα levels with a Luminex MAGPIX cytokine panel at baseline and daily starting day 3 post-exposure until euthanasia. Linear mixed effects regression models were selected with random slopes and intercepts, using Akaike information criterion, to evaluate the effects of ICTB on serum $\log_{10}$ viral load and serum $\log_{10}$ cytokine levels. These models included an interaction term between days post-fever and treatment group, and models were adjusted for days post-fever and baseline dependent variable values. When evaluating the effect of a supportive care treatment bundle, there were decreases in IL-6 -0.44 $\log_{10}$ pg/mL per day (95% CI: -0.77, -0.10; p-value = 0.01), IFNγ -0.20 $\log_{10}$ pg/mL per day (p-value = 0.005), MCP1 -0.19 $\log_{10}$ pg/mL per day (p-value = 0.006) and GM-CSF -0.11 $\log_{10}$ pg/mL per day (95% CI: -0.19, -0.02; p-value = 0.01) compared to controls. Surprisingly, viral load was observed to decrease at a rate of -1.16 $\log_{10}$ GE/mL per day (95% CI: -1.80, -0.51; p-value < 0.001) in the supportive care group compared to controls. Further research is needed to evaluate the effect of immunomodulating therapies on viral dissemination during EVD.

1296

EBOLA EDUCATIONAL OUTREACH LED BY LOCAL MEDICAL STUDENTS IN EASTERN DEMOCRATIC REPUBLIC OF CONGO

Michael T. Hawkes[1], Kasereka Masumbuko Claude[2]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Université Catholique du Graben, Butembo, Democratic Republic of the Congo*

The second largest outbreak of Ebolavirus is currently ongoing in Eastern DRC. The epidemic is characterized by social resistance to foreign-led response teams. Trusted local health practitioners, including medical

students, may be valuable social mobilizers in this challenging context. We report on a student-led educational campaign to increase community awareness & engagement in EVD control efforts. We evaluated student and community participant satisfaction using standardized questionnaires. The outreach was conducted in November 2018, involving 600 students and reaching 5-10,000 community members with parades, speeches, branded banners and T-shirts, and interpersonal interactions in public spaces. Key messages, linked to previously identified resistant attitudes, included: "Ebola exists in Butembo," "Bring infected family members to the Ebola Treatment Unit," and "Leave burials to the official team." Medical students (n=355) and community participants (n=319) evaluated the outreach campaign. Satisfaction was high: 320 (90%) students agreed that medical students could contribute to the EVD response effort, and 233 (73%) community members agreed that the students had helped them understand Ebola in the area. Lower satisfaction scores were associated with self-reported "resistant" attitudes (e.g., intention to hide infected family member from authorities, ρ=-0.25, p<0.0001), denial of the existence of Ebola in the area (ρ=-0.17, p=0.0018), and mistrust of the foreign response team (e.g., belief in mercenary motive, ρ=-0.11, p=0.042). Higher satisfaction scores were associated with the view that local engagement was critical to ending the epidemic (ρ=+0.13, p=0.017). Both students (77%) and community members (71%) agreed that they were more motivated to combat Ebola as a result of the outreach, suggesting that the activities fostered empowerment. Medical students can lead satisfactory community engagement and educational activities during an EVD epidemic. As trusted local health agents, medical students may be valuable allies in building public trust and cooperation in this epidemic complicated by social resistance.

1297

FINDINGS FROM POST EBOLA SURVEILLANCE: ACUTE FEBRILE ILLNESS IN TWO HIGH VOLUME HEALTHCARE FACILITIES IN MONROVIA, LIBERIA, 2019

Lekilay Tehmeh[1], Elijah Paa Edu-Quansah[2], Terrence Lo[3], Daniel Martin[3], Jolie Dennis[4], Gulu Gwesa[4], John Dogba[5], April Baller[6], Eric Houpt[7], Jie Liu[7], Darwin Operario[7], Maame Pokuah Amo-Addae[2], Davis Ashaba[2], Barry Fields[3], Mosoka Fallah[5], Desmond Williams[4]

[1]*Ministry of Health, Monrovia, Liberia,* [2]*African Field Epidemiology Network, Monrovia, Liberia,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Centers for Disease Control and Prevention, Monrovia, Liberia,* [5]*National Public Health Institute of Liberia, Monrovia, Liberia,* [6]*World Health Organization, Monrovia, Liberia,* [7]*University of Virginia, Charlottesville, VA, United States*

Clinical diagnoses for fever-causing diseases in Liberia have been limited to malaria and typhoid fever. With the recent emergence of Ebola, there is a need to expand the search for other known and unknown fever-causing pathogens, particularly for those with epidemic and high consequence potential, that may be circulating in Liberia. We report here our findings from acute febrile illness (AFI) surveillance from a hospital and health center in urban Monrovia that both saw Ebola patients in the 2014 epidemic. From December 2018 through March 2019, outpatients and inpatients ≥2 years with documented temperatures ≥37.5 °C or a history of fever within the previous 7 days were enrolled from two high volume health facilities. Blood samples were collected and tested at the National Reference Laboratory using PCR-based TaqMan Array Cards (TAC) with AFI assays to detect 27 pathogens. Demographics and exposure data were collected from participants. Among the 257 enrolled participants, the median age was 16 years (range: 2-83 years), 176 (68%) were female, of which 77 (44%) were pregnant. Preliminary results from 257 samples yielded 41% positivity on at least one target. Malaria was most commonly detected: 97 (38%) were *Plasmodium spp.* with 84 (33%) *Plasmodium falciparum*. Malaria cases were primarily female [n=74 (76%)] with 48 (65%) of them pregnant. Other pathogens detected included 5 (2%) rickettsia, 6 (2%) dengue, 2 (1%) *Streptococcus pneumoniae*, and 2 (1%) Lassa fever. Co-infections with malaria were found among 5 (2%) cases. One Lassa fever case presented without hemorrhagic symptoms and would not have been otherwise suspected. The most common AFI pathogens detected were malaria, but dengue and rickettsia also were detected: pathogens that are newly identified in Liberia. This suggests a need for increased focus on these pathogens. Patient management also should consider possible co-infections with malaria. As Liberia continues to recover post-Ebola, early detection of febrile illness causing pathogens is critical and addressing public health threats must remain a priority.

1298

MODELING AND MAPPING PATHOGEN-SPECIFIC ENTERIC INFECTIOUS DISEASE RISK USING EARTH OBSERVATION-DERIVED AND HOUSEHOLD-LEVEL COVARIATES

Josh M. Colston[1], Benjamin Zaithchik[2], Margaret Kosek[3], Hamada Badr[2], Gagandeep Kang[4], Tahmeed Ahmed[5], Pablo Peñataro Yori[3], Aldo Lima[6], Esto Mduma[7], Prakash S. Shrestha[8], Pascal Bessong[9], Karen Kotloff[10], Anna Roose[10], Imran Nisar[11], Uma Onwuchekwa[10], AS Faruque[5], Jahangir Hossain[12], Inácio Mandomando[13]

[1]*Johns Hopkins School of Public Health, Baltimore, MD, United States,* [2]*Johns Hopkins Krieger School of Arts and Sciences, Baltimore, MD, United States,* [3]*University of Virginia, Charlottesville, VA, United States,* [4]*Christian Medical College, Vellor, India,* [5]*International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh,* [6]*Federal University of Ceará, Fortaleza, Brazil,* [7]*Haydom Global Health Institute, Haydom, United Republic of Tanzania,* [8]*Institute of Medicine of Tribhuvan University, Kathmandu, Nepal,* [9]*University of Venda, Thohoyandou, South Africa,* [10]*University of Maryland, Baltimore, MD, United States,* [11]*Aga Khan University, Karachi, Pakistan,* [12]*MRC Unit The Gambia, Basse, Gambia,* [13]*Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique*

Despite considerable progress in recent years, diarrheal disease remains a major cause of mortality and morbidity in childhood and its sequelae lead to poor health and economic outcomes in adulthood. New diagnostic methods, as well a number of ambitious multi-site, population-based studies have started to shed light on the pathogen-specific etiologies of diarreal disease, revealing a complex picture. Numerous microbial agents each with distinct biologies that interact with host factors and environmental and meteorological risk factors cause enteric infections which may be symptomatic or sub-clinical. Under a NASA-funded collaboration, data from multiple multi-site studies have been compiled into a dataset consisting of results from around 90,000 stool samples from 33,000 infants in 28 different locations around the world. Presence of specific enteric pathogens in stool samples was ascertained by multiple diagnostic methods, and samples were matched by date and location to earth observation-derived estimates of hydrometeorological parameters extracted from global models. These along with individual- and household-level covariates were used with modified Poisson regression to model their associations with symptomatic and asymptomatic infections with specific enteric pathogens. This approach is able to explain up to 22% of the variability in a single outcome (rotavirus). The results are being used to create high resolution maps of pathogen-specific enteric disease transmission risk, which will be made available through an online platform for use in programmatic and policy decision-making.

1299

THE GEOGRAPHIC DISTRIBUTION OF CHOLERA IN BANGLADESH

Sonia T. Hegde[1], Ashraf Khan[2], Fahima Chowdhury[2], Md. Taufiqul Islam[2], Joshua Kaminsky[1], Emily S. Gurley[1], Justin Lessler[1], Firdausi Qadri[2], Andrew Azman[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh*

Meeting the ambitious WHO-backed target of reducing cholera as a public health threat by 2030 will require significant reductions in cholera incidence in hyper endemic countries like Bangladesh. Despite years of research on cholera, we know little about the incidence and variability of cholera across the country. Most data informing cholera burden estimates

are based on passive surveillance of acute watery diarrhea cases, which are rarely laboratory confirmed. Using detailed data from systematic laboratory confirmation of acute watery diarrhea cases at 22 sentinel surveillance hospitals across Bangladesh from 2014-2018 with data on acute watery diarrhea visits from all 64 district Ministry of Health facilities from 2014-2018, we sought to estimate cholera incidence to better characterize the geographic distribution of disease and provide critical information for the development of the national cholera control strategy in Bangladesh. We estimated country-wide annual incidence and incidence at a 7 x 7 km grid cell level using generalized additive models in a Bayesian framework with spatially correlated errors, and identified priority areas for future surveillance efforts where both incidence estimates and their degree of statistical uncertainty are high. Considering an incidence of ≥ 1.5 per 1,000 as high risk, preliminary estimates 23.1 million (14.3%) of the Bangladesh population live in high risk cholera areas, while 68.9 million (42.8%) live in moderate risk cholera areas (incidence of ≥ 0.5 to 1.5 per 1,000). We identified high-risk areas in Central (south of Dhaka), Western (near Rajshahi district), and Southeastern (near Chittagong district) Bangladesh. This fine-scale incidence mapping provides a new framework for combining detailed sentinel surveillance data with national datasets on diarrhea and can lead to efficient targeting of oral cholera vaccine, water, sanitation, and hygiene interventions. Only with continued surveillance can we improve our understanding of high-burden cholera hotspots, track changes in incidence and further improve the efficiency of cholera interventions.

1300

GEOGRAPHIC VARIATION IN ORAL REHYDRATION THERAPY COVERAGE IN LOW- AND MIDDLE-INCOME COUNTRIES, 2000—2017

Kirsten E. Wiens, Paulina Lindstedt, Mathew Baumann, Brigette Blacker, Aniruddha Deshpande, Simon I. Hay, Robert C. Reiner, Jr

University of Washington, Seattle, WA, United States

Oral rehydration solution (ORS) is a treatment for diarrhea with the potential to dramatically reduce child mortality, yet less than half of children with diarrhea in low- and middle-income countries (LMICs) received ORS in 2017. Recommended home fluids (RHF) are an alternative to ORS; however, it is unclear whether RHF prevent child mortality. Previous studies have shown considerable variation between countries in ORS and RHF use, but subnational distribution patterns of ORS and RHF coverage are unknown. The aim of this study was to produce the first high-resolution geospatial estimates of oral rehydration therapy (ORT) coverage (use of ORS and/or RHF) in LMICs. We used Bayesian geostatistical modeling techniques and an extensive geolocated dataset to estimate the proportion of children under 5 with diarrhea who received ORS or RHF in LMICs from 2000 to 2017. Wherever possible, we tailored these methods to take into account factors that may contribute to variation in ORT coverage, using spatially-resolved covariates to estimate for areas with sparse data. These techniques produced estimates on continuous continent-wide surfaces, which we aggregated to policy-relevant administrative units. We found that, while ORS use among children with diarrhea increased in most locations, coverage remained below 50% in many administrative units. We identified various countries with even subnational distributions of ORS and RHF; however, we also identified countries with broad inequalities across geographic subdivisions, including countries such as Nigeria, Sudan, and Afghanistan where diarrhea mortality rates remain high. Moreover, increases in ORS coverage over time were correlated with declines in RHF coverage and declines in diarrheal mortality in the majority of administrative units. These results, combined with detailed subnational estimates of diarrheal mortality, can assist in determining where new or increased efforts to improve ORS coverage are needed. Over 50 years after the discovery that led to this simplest of therapies, large gains in reducing mortality could be made by reducing geographic inequalities in ORS coverage.

1301

THE PERSISTENT IMPACT OF THE EBOLA EPIDEMIC ON HEALTH SEEKING BEHAVIOR IN KENEMA, SIERRA LEONE

Mikaela R. Koch[1], Lansana Kanneh[2], Foday Alhasan[2], Robert F. Garry[3], Jeffrey G. Shaffer[3], John S. Schieffelin[3], Donald S. Grant[4]

[1]*Stanford University, Stanford, CA, United States,* [2]*Viral Hemorrhagic Fever Program, Kenema Government Hospital, Kenema, Sierra Leone,* [3]*Tulane University, New Orleans, LA, United States,* [4]*Ministry of Health and Sanitation, Freetown, Sierra Leone*

The West African Ebola epidemic of 2014-2015 killed over 11,000 people and devastated healthcare infrastructure in Liberia, Guinea, and Sierra Leone. Whether, and how, the epidemic impacted health-seeking behavior (HSB) among citizens in these Lassa fever (LF) endemic areas is unknown. Kenema Government Hospital (KGH) is located in the Eastern Province of Sierra Leone, a region considered to have the highest per capita incidence of LF in the world. An advanced clinical and laboratory research infrastructure has been established at KGH, which serves as the primary referral hospital for LF cases in the region. KGH was also an early epicenter of the Ebola outbreak. The numbers of LF patients presenting to KGH was similar from 2008-2012. A retrospective review of KGH records from 2012-2017, analyzed the number of suspected LF patients, number admitted, and noted age, sex, outcome, and district of residence. Trend and proportion analysis showed a statistically significant decrease in suspected Lassa cases presenting to KGH from pre- to post- 2014/2015 Ebola epidemic (2012-2013 vs 2016-2017, with Pearson Correlation -.934 (CI -.9460 to -.9406). The decline pre- vs post- Ebola was statistically significant across all age groups. Furthermore, to assess attitudes of HSB in the region, a validated HSB questionnaire was adapted and delivered to 194 residents from 8 villages in Kenema District living within 32 miles of KGH. 78% of respondents stated they felt hospitals were safer post Ebola with no meaningful difference between religious background, age, gender or education. An accurate assessment of HSB can influence how local health care systems provide care, and, in turn, improve health care delivery and hospital experiences. Post Ebola epidemic, HSB as measured by admissions to the LF ward declined and overall confidence was low. To improve the care in regions confronted with highly pathogenic hemorrhagic fevers, re-establishing trust in health care services will require efforts beyond rebuilding infrastructure/personnel and require concerted efforts to rebuild the trust of local residents who may be wary of seeking care post epidemic.

1302

BEHAVIORAL, ECOLOGICAL AND SOCIO-DEMOGRAPHIC CORRELATES FOR VISCERAL LEISHMANIASIS TRANSMISSION IN BARINGO, KENYA

Hellen Nyakundi[1], Mwatela Kitondo[1], Moses Atuko[2], Elijah Elijah[2], Joseph K. Wang'ombe[1], Damaris Matoke[3], Daniel Masiga[4], Richard Wamai[5]

[1]*School of Public Health, University of Nairobi, Nairobi, Kenya,* [2]*Kaperur Community-Based Organization, Chemolingot, Kenya,* [3]*Kenya Medical Research Institute, Nairobi, Kenya,* [4]*International Centre of Insect Physiology and Ecology, Nairobi, Kenya,* [5]*Northeastern University, Boston, MA, United States*

Visceral leishmaniasis (VL) or Kala-azar is among the neglected tropical diseases (NTDs) endemic in Kenya. The 2015-2020 national NTD program identifies a critical need to determine behavioral correlates of VL and its clinical, epidemiological, entomological and health systems statuses to drive control interventions in the country. Our study sought to determine the behavioral, ecological and socio-economic characteristics of household members in VL endemic remote rural villages in East Pokot Baringo. We used a cross-sectional design to select four high-burden village clusters from a list of hospital records of all villages with diagnosed and treated cases of VL in year 2017-2018. The study sites were assumed to have the greatest transmission risk and increased potential to benefit from control strategies. A knowledge, attitudes and practices (KAP) survey was

implemented using a standard household questionnaire, key informant interviews and focus group discussions. Data analysis was with Epi-Info and excel spreadsheets. A total of 139 households participated in the KAP survey with a slightly larger female representation (72 /52%). Most respondents (84%) were living below the national poverty line and were illiterate (116/83.4%). Most (129/93%) were aware of Kala-azar and of these 110 (79%) knew of someone who had the disease and 8 (5.8%) knew of someone who had died as a result. A large majority (101/73%) knew there is a cure and also knew where to seek treatment. What worried respondents most about Kala-azar is fear of death (28/20%) and challenges of accessing treatment due to distance and cost of transport (44/32%). A socio-ecological observation reveals most houses (82%) had stick walls and grass-thatched roofs. Termite mounds were present in all households. Acacia trees are the main vegetation. These factors make this semi-arid area conducive for sandfly vector breeding and human contact. In conclusion: KAP studies are important determinants of community participation in the control of Kala-azar. An integrated control and prevention strategy using household dynamics will have the greatest impact.

1303

DECLINING MASS DRUG ADMINISTRATION COVERAGE FOR LYMPHATIC FILARIASIS IN PORT-AU-PRINCE, HAITI: A PROGRAMMATIC CASE STUDY AND RECOMMENDATIONS

Breanna K. Wodnik[1], Didié H. Louis[2], Michel Joseph[3], Lee T. Wilkers[1], Susan D. Landskroener[1], Jean F. Lemoine[2], James V. Lavery[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Ministry of Public Health and Population, Port-au-Prince, Haiti,* [3]*Radio Caraibes, Port-au-Prince, Haiti*

The World Health Organization (WHO) defines an effective round of mass drug administration (MDA) for lymphatic filariasis (LF) as one that reaches at least 65% of the target population. In its first round of MDA in 2011-2012, the National Program to Eliminate LF in Haiti achieved a 79% epidemiological coverage in urban Port-au-Prince. In 2013, coverage dropped below the WHO threshold and has declined year-over-year to a low of 41% in 2017. We conducted a retrospective qualitative case study to identify key factors behind the year-over-year decline in coverage in Port-au-Prince and ways to address them. Our findings suggest that the main contributors to the decline in MDA coverage, year-over-year, appear to be the absence of effective documentation of practices, reporting, analysis, and program quality improvement—i.e., learning mechanisms—within the program's design and implementation strategy. In particular, the lack of capacity to identify and resolve challenges for MDA drug distribution teams, and to address aspects of the program that had a significant negative influence on prospective participants' experience of the program, makes the progressive decline in coverage seem almost inevitable. In addition to their contribution to the program's failure to meet its coverage targets, these deficits have resulted in a high cost for the program in both lost momentum and depleted morale. Through a proposed operating logic model, we explore how the pathway from program inputs to outcomes is influenced by a wide array of mediating factors, which shape potential participants' experience of the program and, in turn, influence their reasoning and decisions to take, or not take, the pills. Our model suggests that the decisions and behavior of individuals are a reflection of their overall experience of the program itself, mediated through a host of contextual factors, and not simply the expression of a fixed choice or preference. This holistic approach offers a novel and potentially valuable framing for the planning and evaluation of MDA strategies for LF and other diseases, and may be applicable in a variety of global health programs.

1304

GENDER EQUITY IN MASS DRUG ADMINISTRATION CAMPAIGN FOR NEGLECTED TROPICAL DISEASES (NTDS) IN MALI

Mahamadou Traoré[1], Massitan Dembélé[1], Benoit Dembele[2], Boubacar Guindo[2], Mama Niélé Doumbia[2], Seydou Goita[2], Modibo Keita[2], Abdoulaye Guindo[1], Adboul Karim Sidibé[1], Steven D. Reid[3], Fama Kondo[2], Mohamed Lamine Yattara[2], Yaobi Zhang[4]

[1]*National Direction of Health, Ministry of Health and Public Hygiene, Bamako, Mali,* [2]*Helen Keller International, Bamako, Mali,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Mali has made significant progress in the control and elimination of NTDs through mass drug administration (MDA). Mali adopted community-based and school-based platforms for delivering treatments to eligible populations in endemic health districts (HDs). MDAs target the minimum required treatment coverage for each NTD in the general population without a specific emphasis on gender. To understand gender equity during MDA in Mali, we analyzed the reported MDA data in 2016, which had the most complete integrated MDA data for lymphatic filariasis (LF), onchocerciasis and soil transmitted helminths and MDA for schistosomiasis (SCH) since 2012. District-level coverage data were analyzed in 61 districts (HDs) for LF MDA and 47 HDs for SCH MDA and overall, in urban vs rural areas and secure vs unsecure areas. The Kruskal Wallis test was used to compare MDA coverage. For LF MDA in 61 HDs, the median coverage for females was 77% (range: 5-100%), which was higher than the 74% coverage (range: 6-98%) reported for males ($p<0.01$). For SCH MDA the median coverage was 91% (range: 36-229%) for girls and 90% (range: 34-253%) for boys ($p>0.05$). In urban areas, the LF median coverage was 79% (range: 37-100%) for females and 79% (range: 23-98%) for males and in rural areas, 75% (range: 5-91%) for females and 71% (range: 6-90%) for males (both $p>0.05$). For SCH MDA, the median coverage was 98% (range: 71-229%) for females and 92% (range: 65-253%) for males in urban areas, and 90% (range: 36-123%) for females and 84% (range: 34-125%) for males in rural areas (both $p>0.05$). There was also no statistical difference in coverage between females and males in secure and insecure HDs: LF females 79% (range: 37-100%) and males 76% (range: 23-98%) in secure areas and females 66% (range: 5-100%) and males 64% (range: 6-86%) in insecure areas, and SCH females 94% (range: 36-130%) and males 92% (range: 34-132%) in secure areas and females 84% (range: 40-229%) and males 81.5% (range: 60-253%) in insecure areas. The results indicate that current MDA strategies in Mali successfully reached both males and females in the eligible populations and there is no major gender imbalance during MDA intervention.

1305

ESTIMATION OF INCIDENCE RATE OF MORTALITY FOR ANTILEISHMANIAL THERAPIES: A SYSTEMATIC REVIEW OF PUBLISHED LITERATURE FROM 1980 TO 2018

Sauman Singh[1], Prabin Dahal[1], Roland Ngu[1], Brittany Maguire[1], Piero Olliaro[1], Kasia Stepniewska[1], Christine Halleux[2], Fabiana Alves[3]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*World Health Organization, Geneva, Switzerland,* [3]*DNDi, Geneva, Switzerland*

The safety of antileishmanial chemotherapies is not well characterised and reported, despite historical records of poor tolerability and serious adverse events. This systematic review aimed to estimate the incidence rate of mortality in patients treated with antileishmanial therapies within 30 days of commencing therapy. We identified therapeutic clinical trials for visceral leishmaniasis published between 1980 and 2018 by searching four databases: clinicaltrials.gov, WHO ICTRP, Cochrane Library, and PubMed. We estimated the incidence rate of death within the first 30 days of treatment initiation for each of the study regimen with confidence interval using exact Poisson method. The pooled incidence rate, expressed per 1,000 person-days, was derived from random effects

meta-analysis after applying continuity corrections to handle structural zeros. We identified 152 published studies (1980 to 2018) enrolling 28,593 patients in 339 treatment arms. Amphotericin B formulations were administered in 147 (43.4%) arms (10,544 patients), pentavalent antimonial in 73 (21.5%, 7,054 patients), and combination therapies in 35 (10.3%, 2,609 patients). In all, 382 deaths were reported during treatment and follow-up, of which 212 (55.5%) occurred during the first 30 days of starting treatment. The overall expected incidence rate of death regardless of the treatment regimen was estimated at 0.656 [95% CI: 0.550 - 0.783] per 1,000 person-days from 291 arms which excluded (or did not describe) HIV patients. This was 2-fold higher in study arms with HIV patients: 1.494 [95% CI: 0.793-2.812] from 13 arms. The incidence rates were comparable between treatment arms: 0.593 [95% CI: 0.489 - 0.719] for Amphotericin B formulations, 0.813 [95% CI: 0.540 - 1.224] for pentavalent antimonial, and 0.630 [95% CI: 0.415 - 0.956] for the combination therapies. This comprehensive review allowed estimating the incidence rate of mortality in VL patients treated with a range of therapies while highlighting the heterogeneity in reporting death post treatment under trial settings and the need for more systematic pharmacovigilance reporting.

1306

LOCAL AND REGIONAL TRANSMISSION DYNAMICS OF VISCERAL LEISHMANIASIS AND INDICATORS OF ONGOING TRANSMISSION

Luc E. Coffeng, Johanna Munoz, Epke A. Le Rutte, Sake J. De Vlas

Erasmus MC, University Medical Center, Rotterdam, Netherlands

Visceral leishmaniasis (VL) is currently targeted to be eliminated as a public health problem on the Indian subcontinent (ISC), which is defined as less than 1 reported case per 10,000 capita per year at sub-district level. However, it is not well understood whether and how VL transmission on the ISC will continue once this target is reached. Mathematical models have highlighted how VL transmission on the ISC may continue in the presence of potential reservoirs of infection such as 1) cases of post-kala-azar dermal leishmaniasis (PKDL), who have been shown to be infective towards sandflies, and 2) asymptomatically infected individuals (if infectious towards sandflies). However, the main limitation of current mathematical models is that they do not yet correctly capture VL transmission dynamics in finite populations and/or when the number of cases is low. Therefore, to investigate the stability of VL transmission in finite populations, we developed a stochastic model for transmission and control of VL. Using the model, we simulated VL transmission dynamics under various assumptions about transmission conditions, human mobility between villages in a region, the risk of developing PKDL, and infectiousness of asymptomatic cases. We then assessed to what extent reported numbers of cases and prevalence of DAT and PCR positivity are indicative of ongoing transmission. Model predictions suggest that with intervention strategies as currently recommended by the World Health Organization, VL transmission may be sustained at levels below the current target (i.e. with few or no reported clinical cases), with small local outbreaks that move around a larger region over the course of years. Model results further suggest that the combination of time since last VL case combined with current prevalence of DAT or PCR positivity is predictive of ongoing transmission. If PKDL is the only reservoir of infection outside of VL cases, intervention strategies targeting the identification and treatment of PKDL cases would significantly increase the chance of interrupting VL transmission.

1307

DEEP SEQUENCING TO EXPLORE CONGENITAL TRANSMISSION OF CHAGAS DISEASE

Natalie M. Bowman[1], Freddy Tinajeros[2], Oksana Kharabora[1], Edith Malaga Machaca[3], Manuela Verastegui[3], Nery Tirabante[3], Maria del Carmen Meduina[4], Billy Scola[2], Cristian Roca[3], Edward Valencia Ayala[3], Steven R. Meshnick[1], Jonathan J. Juliano[1], Robert H. Gilman[5]

[1]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Asociacion Benefica PRISMA, Lima, Peru,* [3]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [4]*Hospital Maternidad Percy Boland, Santa Cruz, Plurinational State of Bolivia,* [5]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Nearly 6 million people remain infected with *Trypanosoma cruzi*, the agent of Chagas disease, and thousands of infected infants may be born each year. The pathogenesis of congenital Chagas disease is not well understood, and clinicians remain unable to predict which ~5% of infected women will vertically transmit the parasite to their newborns. Since May 2016, our group has screened all consenting women delivering at Hospital Maternidad in Santa Cruz, Bolivia for Chagas disease with a rapid serological test (InBios), with positive tests confirmed by IHA and/or ELISA. Infants of *T. cruzi*-infected women provided samples of umbilical cord blood and peripheral blood at 1, 3, 9, and 12 months. Infant samples were tested by micromethod, quantitative polymerase chain reaction (qPCR), and serology. *T. cruzi* DNA was extracted from blood clot and quantified by qPCR using Cruzi 1 and 2 primers and Cruzi 3 probe. From selected parasitemic maternal and infant samples, we amplified the single-copy nuclear gene *tcsc5d*; amplicons of 684 bp were barcoded and purified then sequenced (2x300) on Illumina MiSeq. Sequences were clustered into haplotypes using a heuristic clustering algorithm in the software SeekDeep. Alpha and beta diversity metrics were calculated with EstimateS. More than 5000 women have been enrolled, and 21.5% were seropositive for *T. cruzi*. 58 infants were confirmed infected; these were offered treatment with benznidazole, and qPCR was used to assess for cure. Infected women were 3 times more likely to transmit to twins than to singletons. We sequenced 74 samples from 32 live infants (19 male, 13 female) including 5 sets of twins, 23 mother-infant pairs, and 14 infants with longitudinal samples. Mean age of mothers was 28 years (range 15-40) and only one had been treated for Chagas disease. In one infant, we found evidence of reinfection by a different strain of *T. cruzi* several months after successful treatment, indicating ongoing vector-borne transmission. We have sequenced the largest sample of mother-infant dyads of which we are aware, and our study is unique in its application of sequencing to evaluate *T. cruzi* transmission in multiple sets of twins.

1308

SEQUENCE HETEROGENEITY IN *LEISHMANIA* RNA VIRUS-1 (LRV-1) DETECTED IN STRAINS OF LEISHMANIA VIANNIA SPP.

Ruwandi Kariyawasam[1], Rachel Lau[2], Eric Shao[3], Braulio M. Valencia[4], Alejandro Llanos-Cuentas[5], Andrea Boggild[3]

[1]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario Laboratories, Toronto, ON, Canada,* [3]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [4]*Kirby Institute, University of New South Wales, Sydney, Australia,* [5]*Instituto de Medicina Tropical "Alexander von Humboldt", Lima, Peru*

Leishmania RNA Virus (LRV) is classified as a Group III dsRNA virus belonging to the family *Totiviridae,* containing a 5284 nucleotide sequence. LRV-1 in the New World has 14 subtypes (LRV-1-1 - LRV-1-14) predominantly isolated from the Amazon basin. Since the detection of LRV-1 in a patient with cutaneous satellite lesions and lymphatic involvement after visiting Suriname, the notion that LRV-1 in the parasite might be causing more severe disease has been the focus of evaluation over the past few decades. We wanted to understand whether sequence heterogeneity within the LRV-1 virus could contribute to the severe

phenotype observed in some patients infected by the *Leishmania Viannia* spp. Nucleic acid was extracted from clinical cultured cells for species identification and LRV-1 detection using quantitative real-time PCR (qPCR). Cultures positive by qPCR were confirmed by end-point PCR and Sanger Sequencing using primers targeting LRV-1-1 and LRV-1-4 subtypes primarily known to circulate in Latin America. Of 109 available clinical cultures, 32 were positive by qPCR. To date, 3/32 (9.4%) LRV-1 positive clinical cultures have been confirmed by end-point PCR with sufficient sequence product. The following species were identified: 2/3 (67%) *L. V. braziliensis* and 1/3 (33%) *L. V. panamensis*. A phylogenetic molecular analysis was performed using the Maximum Likelihood method (BioEdit version 7.2.5) post ClustalW Multiple alignment using the three previously mentioned clinical cultures, ATCC© 50126 *L. V. guyanensis* and NCBI reference genomes: NC002063.1 and NC00306.1. An unrooted tree was produced 2 distinct clusters whereby the 2 LRV-1 positive *L. V. braziliensis* and 1 ATCC© 50126 *L. V. guyanensis* strains were branched from the same node (length = 0.08639, $p < 0.01$) while the LRV-1 positive *L. V. panamensis,* NC00306.4 and NC002063.1 strains branched from an unrelated node (length = 1.23789, $p < 0.01$). Further analysis of the remaining LRV-1 positive cultures will provide more insight into the divergence of LRV-1 between species as well as implications into the severity of disease in ATL.

1309

TRANSMISSION DYNAMICS OF VISCERAL LEISHMANIASIS IN INDIA: ROLE OF ASYMPTOMATICALLY INFECTED INDIVIDUALS

Shyam Sundar

Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Visceral leishmaniasis (VL) is the most fatal form of leishmaniasis caused by protozoan parasite *Leishmania donovani* complex and transmitted by the bites of blood sucking vector sand fly *Phlebotomous argentipes*. Contribution of the various subsets of infected individuals in propagating disease transmission, especially those without clinical illness, is unknown. In the present study we investigated the role of *L. donovani* exposed individuals from across the infection spectrum in driving the transmission in an endemic area for VL. Direct xenodiagnosis using 10 male and 30 female *P. argentipes* sand flies was performed on each of the active VL patients (n=74), before and after successful treatment, Asymptomatic (with high antibody titres) individuals (n=183) and Post Kala-azar Dermal Leishmaniasis (PKDL) patients (n=26). After 60 hours of blood meal, flies were dissected and infections in sand flies was evaluated by microscopy. We found that sand fly infection positively correlated with severity of disease in VL patients, ranging from 0.4 % (95% CI 0.14-1.44) to 24.02 % (95% CI 3.76- 71.92) in splenic scores 1+ to 5+, respectively. After 30 days of successful treatment with a single dose (10 mg/kg) of liposomal amphotericin B, none of these patients were able to transmit infection to the sand flies. PKDL patients were infective to sand flies, however, nodular PKDL forms are more infective to sand flies (56.3% p=0.031, two sided) than macular forms (p=0.5, two sided). None (of 183) of the asymptomatic subjects transmitted infection to sand flies, and assuming that if we had fed more flies on the subjects they still would not have transmitted, then the estimated probability that an asymptomatic subject can transmit is 0 with 95% CI (0-0.023). Results of this study confirms that while active VL and PKDL patients transmit infection in the vector, asymptomatic individuals play little role in disease transmission.

1310

NOVEL DETECTION OF *LEISHMANIA* RNA VIRUS-1 (LRV-1) IN *LEISHMANIA VIANNIA PANAMENSIS* CLINICAL ISOLATES

Ruwandi Kariyawasam[1], Rachel Lau[2], Braulio M. Valencia[3], Alejandro Llanos-Cuentas[4], Andrea Boggild[5]

[1]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario Laboratories, Toronto, ON, Canada,* [3]*Kirby Institute, University of New South Wales, Sydney, Australia,* [4]*Instituto de Medicina Tropical "Alexander von Humboldt", Lima, Peru,* [5]*Tropical Disease Unit, Toronto General Hospital, Toronto, ON, Canada*

American tegumentary leishmaniasis (ATL) comprises a discrete set of clinical presentations of leishmaniasis endemic to Central and South America. *Leishmania*RNA virus-1 (LRV-1) is a double stranded RNA virus identified in 20-25% of the *Leishmania Viannia*braziliensis and *L. V. guyanensis,* and is believed to be a predictive biomarker of severe ATL. We describe the novel detection of LRV-1 in *L. V. panamensis*and its associations with clinical phenotypes of ATL. Clinical isolates were identified from Public Health Ontario Laboratory (PHOL) and the *Leishmania* Clinic of the Instituto de Medicina Tropical "Alexander von Humboldt" between 2012 and 2018. Banked clinical isolates were species identified by PCR, RFLP analysis, and Sanger sequencing. Clinical isolates identified as *L. V. panamensis*were screened for LRV-1 by real-time PCR. Patient isolates were stratified according to clinical phenotype: localized cutaneous leishmaniasis (LCL) was defined as "non-severe" ATL, whereas "severe ATL" was defined as mucosal or mucocutaneous leishmaniasis (ML/MCL); erythematous, purulent, or painful ulcers and/or lymphatic involvement (inflammatory ulcers); or multifocal/disseminated ulcers (≥ 4 in ≥2 anatomic sites). Of 22 patients with confirmed *L. V. panamensis*, 9 (41%), 7 (32%), 5 (23%), and 1 (0.5%) had travel history to or resided in: Peru, Costa Rica, Ecuador and Panama, respectively. Nine (41%) patients had the severe phenotype while 13 (59%) had a non-severe phenotype. Three (33%) of 9 severe cases and 4 (31%) of 13 non-severe cases were positive for LRV-1, respectively (p=0.90). Median age of patients did not differ by clinical phenotype (median age 45.8 years in severe ATL vs. 31.9 years in non-severe ATL, p=0.09), or LRV-1 status (median age 34.1 years in LRV-1 positive patients vs. 38.3 years in LRV-1 negative patients, p=0.64). No differences in sex were observed for clinical phenotype (p=0.60) and LRV-1 status (p=0.52). We describe the novel detection of LRV-1 in*L. V. panamensis,* a species that has been documented predominantly in Central America.

1311

PREVALENCE OF CHRONIC CO-MORBIDITIES AMONG PEOPLE WITH CHAGAS DISEASE IN LOS ANGELES, CALIFORNIA

Salvador Hernandez, Colin J. Forsyth, Gisele Munoz, José Amadeo Flores, Michelle Toruno Alonso, Lesner Suncin Rivas, Sheba K. Meymandi

Center of Excellence for Chagas Disease, Sylmar, CA, United States

Estimates suggest over 300,000 people with Chagas disease (CD) live in the United States, with 30-45,000 suffering from Chagas cardiomyopathy. In Los Angeles, the prevalence of CD in the Latin America-born population was found to be 1.24% in a large-scale screening study of 4,755 individuals, and was 19% in a separate study of individuals with nonischemic cardiomyopathy. However, little is known about the prevalence of co-morbidities in this population. In this study, via chart review, we assessed the prevalence of seven chronic co-morbidities in a convenience sample of 216 patients with CD at the Center of Excellence for Chagas Disease (CECD) at Olive View-UCLA Medical Center in Los Angeles. Over half the sample (57.4%) was female; the most frequent countries of origin were El Salvador (49.5%) and Mexico (28.2%). Mean age at diagnosis was 50.5. Three in ten patients had developed chronic Chagas cardiomyopathy. The most common co-morbidities were hypertension (40.2%), obesity (32.3%), hyperlipidemia (31.0%), and diabetes mellitus (19.1%). Over 70% of the sample had been diagnosed with at least one co-morbidity, while 35.2% had three or more. Patients with cardiomyopathy had a mean of 3.6 co-morbidities, compared to 1.5 among patients without cardiomyopathy ($p<0.001$). Males also exhibited more co-morbidities than females (2.6 vs 1.8, p=0.007). The number of co-morbidities was significantly correlated with age ($p<0.001$), and individuals over 70 had an average of 3.1 comorbidities, compared to 0.6 in individuals under 40. In a multivariable linear regression, age and presence of Chagas cardiomyopathy were significantly associated with the number of comorbidities. Rates of alcohol (4.3%), tobacco (6.2%),

and illicit drug use (2.4%) were low. Patients with CD in Los Angeles face a significant burden of chronic comorbidities, which are more frequent in older individuals and those who have CD cardiomyopathy. This points to an urgent need for comprehensive care of these patients and for earlier, more proactive screening and treatment of this largely hidden disease.

1312

EXTENSIVE PHENOTYPIC DIVERSITY OF NORTH AMERICAN POWASSAN VIRUS

Bekah McMinn[1], Erica Normandin[2], Sam R. Telford[3], Anne Piantadosi[2], Gregory D. Ebel[1]

[1]*Colorado State University, Fort Collins, CO, United States,* [2]*Broad Institute, Cambridge, MA, United States,* [3]*Tufts University, N. Grafton, MA, United States*

Powassan virus (POWV) is a neuroinvasive tick-borne flavivirus that is maintained in nature by Ixodid ticks. Over the past decade, the number of human cases has quadrupled, and wildlife studies suggest that the intensity of enzootic transmission is also increasing. POWV is marked by diverse transmission cycles and disease outcomes. The virus consists of two distinct phylogenetic clades, and evidence of infection has been observed in several tick and vertebrate species. POWV infection in humans results in variable clinical symptoms, ranging from asymptomatic to severe encephalitis and death, with no apparent correlation to age or co-morbidity. Experimental studies of the relationships between POWV genetic diversity, vector competence, and disease outcome are currently lacking. Therefore, we sequenced several low-passage POWV isolates using NGS and evaluated their ability to replicate in well-characterized mammalian cell lines (Vero and BHK), as well as a human neuronal cell line (SH-SY5Y). Genetically similar POWV isolates vary significantly in *in vitro* replication, though these differences do not correlate with geographical or temporal characteristics. In human neuronal cells, peak titers ranged from 10^5 to 10^8 PFU/mL, the day of peak titer ranged from 4 to 10 dpi, and the degree of cytopathic effects in the monolayers was similarly variable. These results support the observation that the North American POWV population may be highly genetically and phenotypically diverse. The degree to which *in vitro* phenotype reflects transmission and pathogenesis remains to be determined.

1313

WITHIN-SITE HETEROGENEITY OF LARVAL BLOODMEAL SOURCES FOR NYMPHAL DEER TICKS

Heidi Goethert, Sam Telford

Tufts Cummings School of Veterinary Medicine, N. Grafton, MA, United States

Mouse targeted interventions to reduce the risk of Lyme disease have yielded mixed results, likely because of site specific differences for reservoir hosts . We determined the larval bloodmeal hosts for questing nymphal deer ticks within two sampling transects (brush edge and within thickets) 350m apart on the Nantucket Field Station site. Other than edge vs. within thicket, the habitat was physiographically identical. DNA from ticks was extracted by alkaline lysis and the larval blood meal remnants identified by real time PCR targeting host-specific retrotransposons. Retrotransposons are transposable elements that are represented by many thousands of copies in mammalian genomes making them uniquely suited for targets of the very sparse blood meal remnant. Ticks were also tested by PCR for evidence of infection by *Borrelia burgdorferi, Babesia microti* and *Anaplasma phagocytophilum*. Although white footed mouse was the most common host for both sets of ticks, their relative contribution differed between these two transects (P<0.001). In the edge transect, 89% (95% CI=79-95) had fed on mice. The only other major host was shrews (8%). In contrast, within the thicket transect, only 52% (95% CI=39-64) of ticks had fed on mice, with the other major hosts being deer (19%), birds (6%) and unknown (16%). The prevalence of *B. burgdorferi* and *B. microti* combined were generally greater in the edge-collected ticks (26%) that had focused their bites on mice or shrews than thicket ticks (13%) where hosts were more diverse (P=0.07). The prevalence of *Anaplasma* was slightly greater in ticks from the brush transect (22% vs 18%). Bloodmeal sources for larval deer ticks differed at a small habitat scale within what would otherwise be considered a single sampling site. Environmental interventions to reduce the risk of Lyme disease may have to account for microscale habitat heterogeneity of potential reservoir hosts.

1314

VECTOR COMPETENCE OF THE HUMAN FLEA *PULEX IRRITANS* TO TRANSMIT *YESINIA PESTIS*

Adelaide Miarinjara, David M. Bland, Joseph B. Hinnebusch

Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States

Pulex irritans is closely associated with human environments, prevalent in plague endemic regions, and *P. irritans* infected with *Yesinia pestis* have been detected during epidemics. Despite these facts, *P. irritans* is generally considered to be a poor vector and receives very little attention from public health policy makers. However, systematic studies evaluating its ability to transmit *Y. pestis* are needed to establish its potential role in plague epidemiology. We evaluated the vector competence of *P. irritans* collected from foxes and owls in Montana and Idaho. Wild-caught fleas were maintained in laboratory and infected with either human or rat blood containing ~1 x10^8 to 7x10^8 *Y. pestis* CFU/ml, using an artificial feeding system. Infected fleas were allowed to feed on sterile blood every 1-3 days and monitored for mortality rate, feeding rate, infection rate, bacterial load per flea, biofilm formation in the foregut (proventricular blockage), and transmission efficiency. Overall, *P. irritans* was challenging to maintain in laboratory conditions, but uninfected control groups survived longer than the infected fleas. *P. irritans* was susceptible to infection, with > 50% infection rates and high bacterial loads for up to 15 days post infection (dpi). Infected *P. irritans* transmitted *Y. pestis* as early as 3 dpi and proventricular blockage was observed in some fleas by 6 dpi. However, transmission efficiency was influenced by host blood source and feeding frequency, as observed in other flea species. *P. irritans* infected and then fed daily (its natural feeding behavior) using human blood transmitted bacteria less efficiently than those fleas which were infected and fed using rat blood. Higher blockage rates and greater numbers of CFUs transmitted per flea were recorded in groups fed intermittently with rat blood. Our results indicate *P. irritans* is a competent vector, but its role in plague epidemics or epizootics likely depends on host species selection and availability.

1315

DIFFERENTIAL EXPRESSION OF *IXODES SCAPULARIS* SALIVARY FACTORS DURING POWASSAN VIRUS-INFECTED TICK FEEDING

Meghan Hermance[1], Jose M. Ribeiro[2], Steven G. Widen[3], Saravanan Thangamani[1]

[1]*SUNY Upstate Medical University, Syracuse, NY, United States,* [2]*National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [3]*The University of Texas Medical Branch, Galveston, TX, United States*

Powassan virus (POWV) is a North American flavivirus transmitted by the Lyme Disease vector, *Ixodes scapularis*. It is the causative agent of a severe neuroinvasive disease with neurological sequelae displayed in over 50% of survivors. In recent years, an expanded geographic range of *Ixodes* ticks has been documented in the United States, coupled with an increase in reported POWV human cases. Transmission of POWV to a host can occur within 3 hours of *I. scapularis* feeding. Through a process known as saliva-assisted transmission, pharmacologically-active tick saliva factors modulate the host environment making it more favorable for the transmission and establishment of a pathogen. In a previous study, *I. scapularis* saliva was shown to facilitate POWV infection and influence disease outcome in mice inoculated with a low dose of POWV. The objective of this study was to characterize the *I. scapularis* microRNAs (miRNAs) and messenger RNAs

expressed during the first 6 hours of POWV-infected tick feeding. POWV-infected and uninfected *I. scapularis* females were fed on naïve mice for 1, 3, and 6 hours, and Illumina next generation sequencing was used to examine miRNA and messenger RNA profiles of POWV-infected versus uninfected ticks. 78 salivary gland miRNAs were significantly up-regulated and 46 miRNAs were significantly down-regulated in response to POWV infection. The majority of significantly modulated miRNAs were unique to each time point and not shared between time points. To investigate the potential role of salivary gland miRNAs in POWV replication, miRNA inhibitors were transfected into VeroE6 cells to profile POWV replication in mammalian cells over time. *In vitro* data from testing 9 miRNA inhibitors demonstrated their role in regulating POWV infection. *In-silico* analysis revealed potential mechanisms by which the tick salivary miRNAs modulate POWV infection during blood feeding. Concurrently, we are analyzing the differential expression of messenger RNAs and the outcome of this study will lead to identification of salivary factor/s with the potential to enhance/regulate POWV transmission to a mammalian host.

1316

SCRUB TYPHUS PROTOTYPE STRAINS OF *ORIENTIA TSUTSUGAMUSHI*: CURRENT STATUS AND THEIR RELATIONSHIP TO RECENT ISOLATES

Allen L. Richards[1], Daryl J. Kelly[2], Paul A. Fuerst[2]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*Ohio State University, Columbus, OH, United States*

Scrub typhus, a mite-borne febrile illness, due to infection with the obligate intracellular bacterium *Orientia tsutsugamushi,* occurs primarily in countries of the Asia-Pacific and islands of the Western Pacific and Indian Ocean. Research on scrub typhus has relied on the availability of several prototype strains of*O. tsutsugamushi*, which were isolated from human cases in the 1940´s and 1950´s. We present the history, current status and relationship to recent *Orientia*isolates of the three original, and most important, prototype strains, Gilliam, Karp and Kato, including information on their isolation, their culture history, their clinical characteristics, their importance within the research literature on scrub typhus, and recent advances in elucidating their molecular genomics. The importance of these strains to the researchand development of clinical tools related to scrub typhus will also be presented. We finally examine whether the strains have been genetically stable since their isolation, and examine by DNA comparisons whether the alternative duplicate samples of prototype strains used by different laboratories are actually equivalent. By using genetic information archived in the international DNA databases, we show that the prototype strains used by different laboratories are essentially identical, and that the strains have retained their genetic integrity at least back to the 1950's.

1317

TICK GUT MICROBIOTA-GATEWAYS OR GATE KEEPERS?

Sukanya Narasimhan, Rajeevan Nallakkandi, Ming-Jie Wu, Kathleen DePonte, Morven Graham, Erol Fikrig

Yale University, New Haven, CT, United States

Ixodes scapularis, an obligate hematophagous arthropod transmits bacterial, viral and protozoan parasites, including *Borrelia burgdorferi*, the agent of Lyme disease to the mammalian host. There is no vaccine against these tick-borne pathogens. My presentation will focus on the vector-host-pathogen interface that is generated in the tick gut when the tick takes a bloodmeal. This represents a pivotal interface in the vector-pathogen life cycle and present opportunities to interrupt the life cycles of the vector and consequently of the pathogen. A less understood player at this interface is the resident bacterial microflora and their impact on the process of *Borrelia* colonization, growth and migration in the tick gut. Our work suggests that resident tick gut microbiota modulate the integrity of the glycan-rich peritrophic matrix (PM), a mucus-like layer that separates the gut lumen from the epithelium, and that likely presents a critical bottleneck in pathogen colonization. Our work also suggests that tick gut microbiota is actively managed by the tick innate immune responses. Whether tick gut microbiota also modulate transmission is not known, and our ongoing efforts to elucidate this is briefly summarized.

1318

PREDICTING THE EXPANSION OF LYME DISEASE TO IDENTIFY GAPS IN CASE REPORTING IN NORTHEASTERN U.S.

Donal Bisanzio[1], **Maria P. Fernandez**[2], Elisa Martello[3], Richard Reithinger[1], Maria Diuk-Wasser[2]

[1]*RTI International, Washington, DC, United States,* [2]*Columbia University, New York, NY, United States,* [3]*Independent researcher, Beeston, United Kingdom*

In this study, we modeled the spatio-temporal expansion of LD case reporting at a county level from 2000-2017 in northeastern and Upper midwestern U.S., by combining county-level environmental factors associated with tick presence and the spatial relationship between counties. The main goal was to predict the probability of reporting a LD case in counties not reporting LD in 2000, and derive the direction and spread ve*loci*ty of LD by 2017. We performed a two-step modeling approach, where we used a logistic regression model to identify the relevant explanatory variables and then used them in a stochastic spatially-explicit diffusion model. Reporting a first case of LD was associated with a county's and neighbors' forest cover, elevation, percentage of population living in wildland-urban interface, tick presence, county's population, and proportion of neighbors reporting cases and neighbors' years since first reporting. The LD spread simulated by the model showed high similarity to the observed data, but 30.1% of not reporting counties by 2017 had a high probability to report according to the model. The model also showed a more rapid temporal diffusion, with the first case of LD predicted to occur 5.5 years earlier on average compared to the observed data. The mean spread ve*loci*ty of LD was 27.4 km year^{-1}, spreading faster in South-eastern counties than in the upper Midwest counties. Variations in case reporting might explain the mismatch between the model predictions and observed data in some counties. If updated regularly and expanded geographically, this predictive model can serve as a public health tool to guide LD surveillance efforts by identifying potential gaps in reporting in those counties that are predicted to report LD in the near future.

1319

PRIOR DENGUE VIRUS INFECTION IS ASSOCIATED WITH INCREASED VIRAL LOAD IN PATIENTS INFECTED WITH DENGUE BUT NOT ZIKA VIRUS

Tyler M. Sharp[1], Gilberto Santiago[1], Eli Rosenberg[2], Iris I. Sosa Cardona[1], Luisa Alvarado[3], Gabriela Paz-Bailey[1], Jorge L. Munoz-Jordan[1]

[1]*Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*University at Albany School of Public Health, Department of Epidemiology and Biostatistics, Rensselear, NY, United States,* [3]*San Lucas Episcopal Hospital, Ponce, PR, United States*

Antibody dependent enhancement (ADE) is a proposed mechanism whereby dengue virus (DENV) infection in an individual previously infected with a heterologous DENV results in increased viral load, immune response, and disease severity. Multiple *in vitro* studies as well as findings from immunocompromised mouse models have suggested that circulating antibodies against DENV can enhance Zika virus (ZIKV) infection. To evaluate potential ADE of ZIKV among patients with prior DENV infection, we compared viral loads and disease severity among patients who tested positive by RT-PCR for infection with ZIKV (n=1,070) in 2016, or DENV-2 (n=312) or DENV-3 (n=260) in 2005-6, and had defined status of primary vs. secondary flavivirus infection (i.e., serostatus). Whereas most patients infected with ZIKV (87%) or DENV-2 (88%) were experiencing secondary infection, frequency of secondary infection was less common among those infected with DENV-3 (55%). Patients with secondary DENV-2 infection were hospitalized more frequently than patients with primary DENV-2 infection (53.3% vs. 31.6%, respectively; p = 0.01), whereas

frequency of hospitalization among patients infected with DENV-3 did not differ significantly by serostatus (39.6% vs. 49.1%, respectively; p = 0.13). Frequency of hospitalization was not different among patients with primary vs. secondary ZIKV infection (1.4% vs. 2.0%, respectively; p = 0.62). Compared to patients without prior DENV infection, patients with prior DENV infection had significantly higher mean viral loads for DENV-2 (10.6 vs. 11.6 $\log_{10}$ GCE/mL, respectively; $p < 0.0001$) and DENV-3 (10.3 vs. 10.9 $\log_{10}$ GCE/mL; $p < 0.0001$), but not ZIKV (4.7 vs. 4.7 $\log_{10}$ GCE/mL; p = 0.959). In a multivariable model of viral load, day post-illness onset of specimen collection, infecting virus, and serostatus were significantly associated with viral load, indicating virus- and serostatus-specific effects on viral load over time. In summary, although viral load was higher among dengue patients with prior DENV infection, similar findings were not observed for ZIKV, arguing against *in vivo* enhancement of ZIKV by anti-DENV antibodies.

1320

FLAVIVIRUS NS1 PROTEINS FACILITATE VIRUS DISSEMINATION THROUGH ENDOTHELIAL CELLS AND ENHANCE VIRUS INFECTION

Scott B. Biering[1], Henry Puerta-Guardo[1], Michael J. DiBiasio-White[2], Chunling Wang[1], Thu Cao[2], Diego A. Espinosa[1], Dustin R. Glasner[1], Richard J. Kuhn[2], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Department of Biological Sciences, Purdue University, West Lafayette, IN, United States*

A pathogenic hallmark of severe dengue virus (DENV) infection is vascular leak. Recently, we found that DENV non-structural protein 1 (NS1) directly triggers vascular leak *in vivo* and endothelial hyperpermeability *in vitro*. We further determined that NS1 from multiple flaviviruses trigger vascular leak in a tissue-specific manner reflecting disease tropism. We next explored if and how NS1 might facilitate virus dissemination into target organs. To do this, we first utilized an *in vitro* model consisting of a Transwell system with endothelial cell monolayers derived from different organs seeded above target cells. Here, we demonstrate that NS1 from different flaviviruses (DENV, Zika virus [ZIKV], and West Nile virus) mediates dissemination of DENV, ZIKV, and Kunjin virus across distinct human endothelial cell monolayers and infection of target monocytes or astrocytes on the basolateral side. Intriguingly, we found increased viral infection of cells when evaluating homologous pairs of NS1 and virus, suggesting an interaction between flaviviruses and their homologous NS1 proteins that enhances infectivity. Further, virion attachment to endothelial cells as well as direct interaction of NS1 with virus particles was observed and was enhanced to the greatest degree in the presence of homologous NS1. A single amino acid mutation of NS1 that disrupts interaction of NS1 and the virion but retains its ability to trigger endothelial hyperpermeability ablated enhanced viral infection. Further, a distinct single amino acid mutation that eliminates NS1-triggered endothelial hyperpermeability also eliminated infection of target cells in the basolateral chamber. Thus, NS1-mediated viral dissemination appears to be a combination of non-specific diffusion through disrupted barriers as well as specific interaction between NS1 and the virion. These studies present a proviral explanation for the evolutionary conservation of NS1-mediated endothelial permeability of multiple flaviviruses and highlight the interaction between the flavivirus virion and NS1 as a new target for antiviral therapeutics.

1321

THE WING DOMAIN OF DENGUE VIRUS NON-STRUCTURAL PROTEIN 1 IS REQUIRED FOR BINDING TO ENDOTHELIAL CELLS AND INDUCING HYPERPERMEABILITY

Nicholas T.N. Lo, Chunling Wang, Scott B. Biering, Kendall E. Lee, Mark Patana, Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Vascular leak is a hallmark of severe dengue caused by the positive-sense RNA flavivirus, dengue virus (DENV). DENV non-structural protein 1 (NS1) is a secreted glycoprotein that causes endothelial hyperpermeability directly by disrupting the endothelial glycocalyx-like layer (EGL) *in vitro*, as well as inducing vascular leak *in vivo*. We have shown that NS1 proteins from different flaviviruses bind to human endothelial cells (EC) and induce vascular leak in a tissue-specific manner, consistent with their respective disease tropism. For example, DENV NS1 but not West Nile virus (WNV) NS1 binds to human pulmonary microvascular endothelial cells (HPMEC), as DENV but not WNV induces leakage in the lungs. However, the molecular determinants of NS1 binding to ECs remain unknown. We recently identified immunodominant regions in the "wing" domain of NS1 in human and mouse DENV infections; this domain contains both conserved and variable regions. Using chimeric NS1 proteins, we exchanged a 35-amino acid (aa) section (101-135) within the wing domain of DENV NS1 with that of WNV NS1. This $DENV^{WNV101-135}$ NS1 chimera lost its ability to bind and induce endothelial permeability in HPMEC. We examined this region further, generating another NS1 chimera ($DENV^{WNV110-122}$) that exchanged only the portion containing highly conserved poly-lysines and a conserved 3-aa motif (110-122). The $DENV^{WNV110-122}$ NS1 mutant retained the ability to bind HPMECs. Further, mutation of the 3-aa motif to Ala reduced binding, EGL disruption, and endothelial hyperpermeability. Interestingly, the reverse exchange of the 101-135 region from DENV into WNV NS1 ($WNV^{DENV101-135}$) did not restore NS1 binding to HPMEC, suggesting that specific residues in this 35-aa section are necessary but not sufficient for DENV NS1 binding to HPMEC. These results support the idea that specific residues in the NS1 "wing" region mediate its broad, as well as tissue-specific, EC binding capacity and identify the residues in DENV NS1 that may confer binding to human ECs, which are required for induction of EC hyperpermeability. Further work is underway to elucidate the implications in viral infection.

1322

CAUGHT ON CAMERA: VISUAL AND BIOCHEMICAL EVIDENCE OF ENDOTHELIAL GLYCOCALYX DISRUPTION IN DENGUE AND ASSOCIATION WITH PLASMA LEAK SEVERITY

Lam K. Phung[1], Angela Mcbride[1], Quyen T. Nguyen[1], Duyen T. Huynh[1], Hans Vink[2], Bridget Wills[3], Sophie Yacoub[1]

[1]*Oxford University Clinical Research Unit - HCMC, Ho Chi Minh City, Vietnam,* [2]*Maastricht University, Maastricht, Netherlands,* [3]*University of Oxford, Oxford, United Kingdom*

The endothelial glycocalyx layer lines all microvessels and provides vital barrier functions to capillary networks. In-vitro studies suggest dengue NS1 mediated degradation of this layer may underlie the capillary leak syndrome associated with severe disease, but there are a lack of human studies to support this. We performed an observational study in two hospitals in Vietnam, to evaluate the sublingual microcirculation using sidestream dark field imaging in patients a) presenting early (<72 hours fever) with clinically suspected dengue b) hospitalized with dengue and c) with other febrile illnesses (OFI). The glycocalyx depth was analyzed using the software application: GlycoCheck™. The Perfused Boundary region (PBR High Flow, PBR_HF), which is inversely proportional to the depth of the glycocalyx and corrected for interpatient microvascular flow differences, and the MicroVascular Health Score (MVHS) were measured at three time-points during the illness. Plasma glycocalyx degradation products including syndecan-1 (SDC1) and endocan were measured at the same time-points. Of the 70 patients enrolled, 45 had GlycoCheck

analysis performed. In dengue patients, we found the PBR_HF was increased in patients with severe plasma leakage versus those with no leakage during the critical phase, 1.36μm (1.22, 1.57) vs. 1.96μm (1.90, 1.98) ($p<0.001$), and MVHS was generally lower in patients with more severe plasma leakage ($p<0.001$). Plasma SDC1 levels were raised in patients with the most severe leakage compared to patients with no plasma leakage, particularly during the critical phase (median: 125.9 versus 2613 ng/ml, $P<0.001$). SDC1 levels had a positive correlation with the PBR_HF but this did not reach statistical significance at the 0.01 level. SDC1 levels were higher in the acute phase of dengue compared to OFI, but we found no significant difference in the PBR_HF or MVHS between patients with dengue versus OFI. In summary, we have shown the first human in-vivo evidence of glycocalyx disruption in dengue patients, with a thinner glycocalyx and increased plasma glycocalyx degradation products associated with worse plasma leakage severity.

1323

PRIMARY DENGUE SEROTYPE 2 INFECTION IN HUMANS IMPRINTS A BLOOD TRANSCRIPTOMIC PROFILE AFTER VIRUS CLEARANCE

Sean A. Diehl[1], John Hanley[1], Korin Eckstrom[1], Dorothy M. Dickson[1], Nicholas Selig[1], Stephen S. Whitehead[2], Anna P. Durbin[3], Kristen K. Pierce[1], Beth D. Kirkpatrick[1], Julie Dragon[1], Donna Rizzo[1], Sam V. Scarpino[4]

[1]*University of Vermont, Burlington, VT, United States,* [2]*National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Johns Hopkins University School of Public Health, Baltimore, MD, United States,* [4]*Northeastern University, Boston, MA, United States*

Dengue disease is caused by any of the four dengue virus (DENV) serotypes and severe disease is most often associated with secondary heterotypic infection. Given the impact of primary DENV exposure as a pre-disposing risk factor for secondary disease, it is important to define systems-level impacts of the immune response to infection and through convalescence. To study this, we used genome-wide RNA-Seq to determine the blood transcriptomic profile of 11 healthy flavivirus-naïve adults that were challenged with an underattenuated DENV2 virus. We captured transcriptomics at baseline, during viremia (day 8 after infection) and at convalescence (Day 28) and used a systems approach to assess transcriptional changes associated with infection and resolution thereof in the context of fine-mapped, longitudinal virological, serological, and clinical findings. We leveraged RNA-Seq replicates for each subject to analyze gene regulation on an individual basis. This novel approachyielded a small set of 99 differentially regulated genes (95 up and 4 down) that changed in the same direction in all 11 subjects between baseline and Day 8 after infection, a timepoint which captured all peak viremias within ± 2 days. At day 28 after infection, neutralizing antibodies were generated, all viremia and clinical signs were resolved, and 93 genes were downregulated in all subjects, including 56 that were upregulated during acute infection. Thus, we found a group of 43 genes that, once regulated by acute infection (39 up and 4 down), did not return to baseline in convalescence in any of the subjects, suggesting evidence of systemic imprinting after primary dengue infection. This residually-activated set of genes included myeloid and immune regulation factors. Since leukocyte responses were re-establishing towards baseline shortly after viremia resolved, this suggested an imprinted profile in remaining cells which may reflect a refractory period of immune activation early in convalescence after primary infection.

1324

DENGUE VIRUS 2 GENOTYPIC VARIATION MEDIATES NEUTRALIZATION SENSITIVITY TO HUMAN ANTIBODY RESPONSES

David R. Martinez, Boyd Yount, Ralph S. Baric

University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Dengue virus (DENV) infection incidence rates in humans have increased recently with up to 390,000 new infections each year worldwide. Yet, the only licensed DENV vaccine, Dengvaxia, does not perform equally well against all four Dengue virus serotypes, with particularly poor vaccine efficacy against Dengue virus serotype 2 (DENV2). It is not clear why vaccine-elicited responses against DENV2 provide lower efficacy compared to DENV serotypes 1, 3, and 4. Thus, more studies are needed to better understand dynamics of DENV2 and host immune responses. To begin to answer this question, we used reverse genetics to generate a ten-virus isogenic panel of precursor membrane (prM) and envelope (E) of the distinct DENV2 genotypes, Asian I, Asian II, Asian-American, Cosmopolitan, Sylvatic African, Sylvatic Asian, and grew them in C6/36 insect cells. Interestingly, these DENV2 genotypic variants showed considerable amino acid residue variability within E domain I, II, and III, which are key targets for neutralizing antibodies. We then tested the neutralization sensitivity of these distinct DENV2 genotypic variants to natural infection human sera, NIH monovalent DENV vaccine human sera, tetravalent DENV vaccine human sera, and human monoclonal antibodies in a Vero cell-based microneutralization assay. Interestingly, distinct DENV2 genotypic variants were differentially neutralized by natural infection sera, vaccine-elicited sera, and monoclonal antibodies, suggesting that DENV2 genotypic variation modulates neutralization sensitivity. Our data suggests that amino acid variation within the prM and E of the different DENV2 genotypes mediates differential neutralization by host-elicited antibodies in both the settings of natural infection and vaccination. Our findings also suggest that the poor vaccine efficacy observed against DENV2 may in part be due to the natural genotypic variation within the DENV2 serocomplex. These findings have important implications for ongoing clinical trials testing the efficacy of DENV vaccine candidates and argue for the use of DENV vaccines with improved coverage against the different genotypes.

1325

ANTI-FLAVIVIRUS ANTIBODY DYNAMICS BEFORE, DURING, AND AFTER THE ARRIVAL OF ZIKA IN THE AMERICAS

Leah Katzelnick[1], Damaris Collado[2], Douglas Elizondo[2], Juan Carlos Mercado[3], Lionel Gresh[2], José Victor Zambrana[2], Amy Schiller[4], Sonia Arguello[2], Raquel Burger-Calderon[2], Tatiana Miranda[2], Sergio Ojeda[2], Nery Sanchez[2], Brenda Lopez[2], M. Elizabeth Halloran[5], Josefina Coloma[1], Aubree Gordon[4], Guillermina Kuan[6], Angel Balmaseda[3], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua,* [4]*Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, United States,* [5]*Department of Biostatistics, University of Washington, Seattle, WA, United States,* [6]*Centro de Salud Sócrates Flores Vivas, Ministry of Health, Managua, Nicaragua*

Dengue viruses 1-4 (DENV1-4) were reintroduced to the Americas in the last 50 years and now circulate endemically. A closely related flavivirus, Zika virus (ZIKV), was introduced in 2014 and caused epidemics across the continent. We evaluated how the arrival of ZIKV impacted the immune landscape to flaviviruses in a longitudinal pediatric dengue cohort study (2004-2018) in Nicaragua. Using an Inhibition ELISA, we first measured dynamics of anti-DENV antibodies (DENV-Abs) in participants with different DENV infection histories in the pre-ZIKV era. Mixed-effects generalized additive models revealed that DENV-Abs reached a stable set-point by 1 year after primary (1°) DENV infection but waned rapidly for 2 years

after secondary (2°) DENV infections before reaching a steady state. This finding differs from the long-held hypothesis that waning DENV-Abs explain changes in risk of severe dengue after 1° DENV infection. Instead, individual DENV-Ab set-point titer is important: those with low titers are at elevated risk of severe dengue, whereas those with high titers are protected. When ZIKV arrived, there was concern that DENV-Abs could also enhance Zika disease. However, we find DENV-Abs are protective against uncomplicated Zika. In contrast, the effect of ZIKV infection on dengue risk is unknown. We built mixed-effects linear models of DENV-Ab titers in the first 2 years following ZIKV infection in those with different DENV infection histories. DENV-Abs were higher after 1° DENV than 1° ZIKV infection but both groups showed evidence for a set-point. Large fractions of both groups had titers within the severe dengue risk range. In contrast, those with 2° ZIKV infections (i.e., DENV-ZIKV) had comparably high DENV-Abs titers as those with 2° DENV. Both groups experienced comparable DENV-Ab decay within 2 years of infection. Thus, while DENV-Abs can partially protect against Zika, ZIKV infection may provide protection against future dengue in those with prior DENV infections while placing many previously naïve individuals at elevated risk of severe dengue. Such asymmetric immune interactions may affect efficacy and safety of DENV and ZIKV vaccines.

1326

INTERACTION OF LIVE ATTENUATED *LEISHMANIA* PARASITES INFECTED NEUTROPHILS WITH DENDRITIC CELLS AUGMENTS CD4$^+$TH1 CELL PRIMING IN C57BL/6 MOUSE

Parna Bhattacharya, Nevien Ismail, Subir Karmakar, kazuyo takeda, Ranadhir Dey, Hira L. Nakhasi

Food and Drug Administration, Silver Spring, MD, United States

Visceral leishmaniasis (VL), is a vector-borne disease with no available vaccine. We have previously reported the protective role of live attenuated centrin gene-deleted *L. donovani* (*LdCen-/-*) parasite vaccine in animal models. *LdCen-/-* induces strong innate immunity which leads towards protective Th1 response. Neutrophils are involved in initial steps of most responses to pathogens and can also enhance various T cell responses either directly or indirectly through activation of dendritic cells (DCs). Recently we have demonstrated neutrophil's direct antigen presenting potential in generating early host protective immune response to *LdCen$^{-/-}$*. However, neutrophil mediated immunomodulation of DC function in response to live attenuated *Leishmania* vaccine has not been studied yet. Hence, we evaluated the interaction between neutrophils and DCs during *LdCen-/-* infection and compared with *LdWT* both *in vitro* and *in vivo*. Robust chemo attractive activity for DCs was detected in the supernatants of neutrophils exposed to *LdCen-/-*compared to *LdWT in vitro. Additionally,* uptake of *LdCen-/-mCherry*-infected neutrophils by DCs augmented their expression of costimulatory molecules and heightened CD4$^+$T cell priming in comparison with DCs with *LdWTRFP* infected neutrophils *in vitro*. Intradermal immunization in the ear with *LdCen-/-* induces synchronized higher neutrophil and DC recruitment compared to *LdWT* parasites. Furthermore, to investigate neutrophil-DCs interactions *in vivo*, we evaluated dermal DCs recovered from mice 24h after infection with *LdWTRFP / LdCen-/-mCherry* parasites. We observed, DCs which have *LdCen-/-* parasitized neutrophils have higher CD4$^+$T cell priming capacity thereby promoting heightened Th1 response compared to *LdWT*. Also, depletion of neutrophil significantly abrogates antigen presenting potential of DC in *LdCen$^{-/-}$* infected mice. This study suggests that interaction of infected neutrophils with DCs plays pivotal role in shaping the vaccine induced host protective adaptive immune response.

1327

CHAGAS DISEASE IN THE NEW YORK CITY METROPOLITAN AREA

Crystal Zheng[1], Orlando Quintero[2], Elizabeth K. Revere[3], Michael B. Oey[3], Fabiola Espinoza[4], Yoram A. Puius[2], Diana Ramirez-Baron[5], Carlos R. Salama[6], Luis F. Hidalgo[7], Fabiana S. Machado[8], Omar Saeed[2], Jooyoung Shin[2], Snehal R. Patel[2], Christina M. Coyle[9], Herbert B. Tanowitz[9]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Montefiore Medical Center, Bronx, NY, United States,* [3]*Donald and Barbara Zucker School of Medicine at Hofstra, Manhasset, NY, United States,* [4]*Metro Infectious Diseases Consultants, Burr Ridge, IL, United States,* [5]*Grameen VidaSana Clinic, Queens, NY, United States,* [6]*Icahn School of Medicine at the Mount Sinai Hospital, Queens, NY, United States,* [7]*University of Kentucky College of Medicine, Lexington, KY, United States,* [8]*Federal University of Minas Gerais, Belo Horizonte, Brazil,* [9]*Albert Einstein College of Medicine, Bronx, NY, United States*

Chagas disease, caused by the parasite *Trypanosoma cruzi*, once considered a disease confined to Latin America, is now an emerging global public health problem. An estimated 300,000 immigrants in the United States are chronically infected with *T. cruzi*. However, awareness of Chagas disease among the medical community in the United States is poor. We review our experience managing sixty patients with Chagas disease in hospitals throughout the New York City metropolitan area and describe screening, clinical manifestations, electrocardiogram (ECG) findings, imaging, and treatment. The patients were evenly split by sex (30 males, 30 females), with a mean age at diagnosis of 47 years. The most common country of origin was El Salvador (n = 24) and the most common detection method was by routine blood donor screening (n = 21). Screening family members of newly diagnosed patients identified 10 additional cases representing 4 families. Nearly half of the patients were asymptomatic. Of symptomatic patients, 40% had cardiac symptoms and 23% had gastrointestinal symptoms. The most common ECG abnormality was right bundle branch block, and the most common echocardiogram abnormalities were tricuspid regurgitation and ventricular wall motion abnormalities. Twenty-seven patients were treated with either benznidazole or nifurtimox, of whom 7 did not complete therapy due to side effects or were lost to follow up. Six patients underwent heart transplantation, and 2 experienced reactivation after transplant. Based on our experience, we recommend that targeted screening be used to identify at risk, asymptomatic patients before progression to clinical disease. Family members of newly diagnosed patients should be offered screening as well. Evaluation should include an ECG, echocardiogram, and chest x-ray; gastrointestinal imaging can be pursued if relevant symptoms are present. Patients should be treated if appropriate, but providers should monitor for adverse effects that may prevent treatment completion. Patients with advanced heart disease who are not candidates for medical treatment may require advanced therapies or heart transplantation.

1328

THE TRANSCRIPTION FACTORS BLIMP-1 AND HOBIT DETERMINE THE PATHOGENIC PHENOTYPE CD8 T CELLS EXHIBIT IN LEISHMANIAL LESIONS

Fernanda O. Novais, Phillip Scott

University of Pennsylvania, Philadelphia, PA, United States

Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania* and the most common form of the disease is cutaneous leishmaniasis (CL). Importantly, treatment for CL is frequently ineffective, in part because the disease is driven not only by the parasite load but also by immunopathologic responses. Therefore, a fundamental question in CL is what regulates the development of severe disease, information that is critical in order to develop host-directed therapies to ameliorate disease. In a series of studies utilizing murine models coupled with analysis of samples from *Leishmania braziliensis*-infected patients, we demonstrated CD8 T cell-dependent cytotoxicity as the main inducer of

immunopathology in CL. This result was unexpected since IFN-γ production by CD8 T cells plays a protective role by promoting pathogen elimination. To resolve this paradox, we studied the CD8 T cells in different anatomic sites, and found that the effector function of CD8 T cells in CL depends on their location: while CD8 T cells are cytotoxic and produce little IFN-γ in leishmanial lesions, CD8 T cells in the draining lymph nodes (dLN) have the opposite profile. Importantly, CD8 T cells in lesions and not dLN express the transcription factor Blimp-1. Our results show that Blimp-1 and Hobit (a homolog of Blimp-1) deficiency reduced the ability of CD8 T cells to express granzyme B in lesions. Importantly, we found that CD8 T cells from Blimp-1 and Hobit deficient mice are unable to cause severe pathology in *L. braziliensis* lesions. Together, our results indicate that Blimp-1 and Hobit are induced in *L. braziliensis* lesions and are required for CD8 T cell-mediated disease.

1329

MARKER FREE LIVE ATTENUATED *LEISHMANIA MAJOR* (*LMCEN-/-*) INDUCES STRONG HOST PROTECTIVE IMMUNE RESPONSE AGAINST VECTOR BITE TRANSMITTED VISCERAL LEISHMANIASIS

Ranadhir Dey[1], Subir Karmakar[1], Nevien Ismail[1], Fabiano Oliveira[2], Wenwei Zhang[3], Shinjiro Hamano[4], Greg Matlashewski[3], Shaden Kamhawi[2], Abhay Satoskar[5], Jesus G. Valenzuela[2], Hira L. Nakhasi[1]

[1]*CBER/Food and Drug Administration, Silver Spring, MD, United States,* [2]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States,* [3]*McGill University, Montreal, QC, Canada,* [4]*Nagasaki University, Nagasaki, Japan,* [5]*Ohio State University, Ohio, MD, United States*

Leishmaniasis are vector-borne parasitic diseases and there is no licensed vaccine available against human leishmaniasis. It has been shown that low dose of dermatotrophic *Leishmania major* infection (leishmanization) confers protection against cutaneous leishmaniasis (CL). However, such a method of immunization is not practical because of the greater risk of infection in a naïve population. Therefore, genetically modified live attenuated parasites that are non-pathogenic might induce the same protective immunity as leishmanization. We have developed centrin-gene deficient *Leishmania major (LmCen-/-)* using CRISPR-Cas methodology and evaluated the safety, immunogenicity as well as cross-protective efficacy against *L. donovani* challenge in hamster model. Although, intradermal immunization of golden Syrian hamsters with *LmCen-/-* did not develop any visible lesion, but it induced a strong pro-inflammatory immune response compared to wild type *L. major* infection as measured by Real time quantitative PCR. Immunized hamsters were challenged with *L. donovani* either by intradermal needle injection or by infected sand flies as natural mode of infection. In both sets of experiments, twelve months post-challenge, non-immunized challenged hamsters developed severe pathology of VL, while immunized hamsters were protected. We also evaluated the cellular immune response in immunized hamsters after challenge with the wild type parasites and compared with non-immunized and challenged hamsters. Spleen cells from *LmCen-/-* immunized and challenged hamsters produced significantly more Th1-associated cytokines including IFN-γ and TNF-α, and significantly reduced expression of the anti-inflammatory cytokines IL-10 and IL-21, compared to non-immunized and challenged animals. The enhanced pro-inflammatory immune response correlates with the control of parasitemia in immunized animals. Our studies demonstrate that the *LmCen-/-* mutant parasite are safe as an immunogen and have potential to be an effective vaccine against VL and can proceed to be tested in humans.

1330

PARASITE LOAD AND HOST CYTOTOXICITY-RELATED GENE EXPRESSION ARE POTENTIAL BIOMARKERS FOR TREATMENT OUTCOME

Camila Farias Amorim[1], Fernanda Novais[1], Ba Nguyen[1], Ana M. Misic[1], Lucas P. Carvalho[2], Edgar M. Carvalho[2], Daniel Beiting[1], Phillip Scott[1]

[1]*University of Pennsylvania, Philadelphia, PA, United States,* [2]*Universidade Federal da Bahia, Salvador, Brazil*

Gene expression in the skin, particularly inflammasome activation and cytotoxicity, drives pathology in cutaneous leishmaniasis (CL), yet the factors that initiate and sustain these programs have yet to be identified. It is unclear whether patients that exhibit stronger induction of these pathways go on to a worst clinical outcome. Here, in an unbiased approach we investigated whether differences in the expression levels of genes that are highly variable in lesions might influence disease outcome. We obtained biopsies of cutaneous lesions from *L. braziliensis* patients prior to drug treatment, and then monitored patients to determine who failed treatment. A set of genes were identified whose expression is highly variable between patients and which were strongly correlated with treatment outcome. Amongst the most variable genes were components of the cytolytic pathway, and expression of these genes appears to be driven by parasite load in the skin. This is the first demonstration that the outcome of disease in patients, in this case treatment failure, can be directly linked to the cytolytic pathway activated during leishmania infection. Using this host-pathogen biomarker profile, we show that treatment outcome can be predicted with high sensitivity and specificity before the start of treatment. These findings raise the possibility of point-of-care diagnostic screening to identify patients at high risk of treatment failure, and provide a rationale for a precision medicine approach to drug selection in cutaneous leishmaniasis.

1331

DISSEMINATED LEISHMANIASIS IS ASSOCIATED WITH THE ABILITY OF *LEISHMANIA BRAZILIENSIS* TO SURVIVE IN NEUTROPHILS AND MONOCYTES AND IN DOWN MODULATE PHAGOCYTES FUNCTION

Edgar M. Carvalho[1], Olívia Bacellar[1], Thiago Marconi[2], Andreza Dórea[1], Walker Nonato[1], Lucas P. Carvalho[1], Albert Schriefer[1], Paulo Machado[1], Luiz Henrique Guimarães[3]

[1]*Federal University of Bahia, Salvador, Brazil,* [2]*Gonçalo Moniz Institute (IGM), Fiocruz, Bahia, Salvador, Brazil,* [3]*Federal University of the South of Bahia, Itabuna, Brazil*

Disseminated leishmaniasis (DL) is an emergent form of *Leishmania braziliensis* infection characterized by 10 up to thousand papular acneiform and ulcerated lesions. There is no impairment in T cell response in DL, but isolates that cause DL are genotypically different from isolates that cause cutaneous leishmaniasis (CL). Soluble leishmania antigen (SLA) from *L. braziliensis* that cause DL induces a higher inflammatory response in comparison with SLA from CL. Here, we evaluate neutrophils and monocytes function after infection with isolates of *L. braziliensis* from DL or CL patients. In all cases DNA of *L. braziliensis* was detected by PCR. Participants were 30 healthy subjects (HS), 12 patients with CL and 12 with DL. *L. braziliensis* isolates from CL or DL were cultivated in Schneider media supplemented with serum. Neutrophils and mononuclear cells were isolated by density gradient and infection was performed at the ratio of 5 parasites:1 cell. The percentage of infected cells and number of amastigotes / 100 cells were measured by optical microscopy. The neutrophils' activation markers were evaluated by flow cytometry (FACS). The respiratory burst and expression of toll like receptors (TLR) in neutrophils and monocytes were evaluated by FACS. The percentage of infected neutrophils and the parasite load of HS polymorph nuclear cells infected with isolates of DL was higher than that observed in cells infected with isolates of CL ($P<0.01$). The expression of dihidrorodhamine (DHR) and CD66b were lower in neutrophils infected with isolates of DL

than CL (P<0.05). Regarding monocytes, while after 2 hours of infection there was no difference in the degree of infection in CL, DL and HS monocytes infected with DL or CL parasites, after 48 hours the number of amastigotes were higher in cells infected with DL isolate in comparison with CL isolate (P<0.01). After infection, the expression of TLR2 and TLR4 were higher in monocytes infected with isolate from DL than CL (P<0.05). These data indicate that evasion from killing mediated by phagocytes and modulation of phagocyte function by isolates from DL patients play a major role in the pathogenesis of DL.

1332

EVALUATION OF PROTECTIVE IMMUNITY INDUCED BY *LDCEN-/-* IN PRESENCE OF PRE-EXISTING *PLASMODIUM YOELII* INFECTION

Visceral leishmaniasis and malaria are two major parasitic diseases with overlapping geographic distribution and may also coexist in the same individuals. Studies of co-infection of virulent *Plasmodium* and *Leishmania* have shown activation of distinct immunological pathways that might lead to either exacerbation or control of pathogenesis depending on the parasite species. Towards developing a prophylactic vaccine against visceral leishmaniasis, we have tested centrin deleted live attenuated *Leishmania donovani* parasites (*LdCen-/-*) as potential vaccines. However, vaccination studies using *LdCen*$^{-/-}$ were performed mainly in naïve rodent models. The immunological correlates of protection thus derived might have limited applicability in conditions where the immunized host has prior exposure to *Plasmodium* infection. In addition, the most likely recipients of the vaccines in *Leishmania* endemic areas are likely to be asymptomatic carriers of *Plasmodium* infection due to co-endemicity of these pathogens. Towards examining the impact of prior exposure to *Plasmodium* on the *LdCen-/-* induced protection, we performed *LdCen-/-* immunization in mice that were infected with PyNL, and following clearance. Results showed that the PyNL infection shows no impact on the *LdCen*$^{-/-}$ specific CD4^{+} T cell responses including memory responses and polyfunctional responses. However, polyfunctional CD8^{+} T cell responses were impaired in presence of PyNL. Upon challenge with virulent *L. donovani*, reduction in splenic parasite burdens was impaired in mice pre-exposed to PyNL compared to those that received *LdCen-/-* alone suggesting that prior exposure to PyNL impacts the protective immunity induced by *LdCen-/-* parasites. Immune mechanisms impacting the protective immunity induced by *LdCen*$^{-/-}$ parasites due to PyNL will be discussed.

1333

THE 'PREMONITION PROVING GROUND': A SIMULATED FIELD ENVIRONMENT FOR DEVELOPING NOVEL MOSQUITO SURVEILLANCE TOOLS

Isaiah Hoyer[1], Michael R. Reddy[1], Douglas E. Norris[2], Nicolas Villar[1], Marcel Gavriliu[1], Ethan K. Jackson[1]

[1]*Microsoft Research, Redmond, WA, United States,* [2]*Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States*

Evaluation of novel mosquito surveillance devices in field settings may be hampered by seasonal fluctuations in mosquito abundance, local climatic conditions and other abiotic factors such as light regime that are difficult to control under natural conditions. Although field deployments serve as the ultimate test of surveillance device efficacy, research conducted prior to going to the field may reduce the cost and time of prototype development. Microsoft's Project Premonition has erected a custom instrumented, cloud-connected, ACL-2 compliant laboratory specifically designed to support testing of arthropod surveillance technologies in a simulated field setting. The "Premonition Proving Ground" replicates climate and solar cycles to mimic the temperature, humidity and ambient light characteristics of temperate and tropical venues for any given time of year. For instance, this allowed us to simulate and validate previous field trials showing that robotic collection devices can detect and distinguish wild *Culex* and *Aedes* mosquitoes to genus with 91% and 90% accuracy respectively based on digital phenotypes generated using infrared detection of wing beat frequency. We also used the Proving Ground to generate digital phenotypes from other sensors on wild mosquitoes that were field-collected as eggs and shipped to the Proving Ground for rearing. Adult mosquitoes were morphologically identified to species and a variety of digital phenotypes were recorded, producing a library of signatures used to train prototype devices to recognize and distinguish mosquito species. Flight trajectory data is captured using the Proving Ground's custom video tracking system indicating the willingness of mosquitoes to enter trappable areas. Such high-fidelity data allows us to quickly compare the relative efficacy of different device designs under controlled conditions. This process of controlled testing and evaluation greatly facilitates the training of our algorithms and rapid prototyping before transitioning to more costly field evaluations.

1334

ARBOMAP: SOFTWARE INTEGRATING ENVIRONMENTAL MONITORING WITH PUBLIC HEALTH SURVEILLANCE FOR ARBOVIRUS FORECASTING

Michael C. Wimberly[1], Justin K. Davis[1], Michael C. Hildreth[2], Joshua Clayton[3]

[1]*University of Oklahoma, Norman, OK, United States,* [2]*South Dakota State University, Brookings, SD, United States,* [3]*South Dakota Department of Health, Pierre, SD, United States*

West Nile virus (WNV) remains a persistent public health hazard in many parts of the U.S., and there is need for knowledge of when and where WNV outbreaks will occur to target public health responses. To address this need, we developed and implemented the Arbovirus Monitoring and Prediction (ArboMAP) system in South Dakota, the U.S. state with the highest incidence of WNV. ArboMAP produces weekly, county-level forecasts of WNV risk using remotely-sensed environmental data combined with entomological and epidemiological surveillance data. We made prospective WNV forecasts in South Dakota during the WNV seasons of 2016, 2017, and 2018. ArboMAP uses a data-driven approach in which human WNV cases are modeled as a function of meteorological variables and mosquito infection status. Gridded environmental data, including air temperature, vapor pressure deficit, and precipitation, were derived from NASA's North American Land Data Assimilation System. Mosquito surveillance were obtained from local trapping programs throughout the state and were pooled and tested for the prevalence of WNV on a weekly basis. We used distributed lags to model the delayed effects of environmental fluctuations, and a log-linear function to model growth of the infection rate in the early transmission season. ArboMAP is implemented using the R software environment for data processing, modeling, and reporting combined with a Google Earth Engine application for environmental data access. Forecasts of WNV transmission risk were made on a weekly basis using observed meteorological conditions and mosquito infection status during the current year. We were able to predict interannual variation in the incidence of WNV cases, as well as seasonal shifts in the start of the transmission season. We also predicted a change in the usual geographic pattern of WNV cases that occurred in 2017. These forecasts fill an important information gap in the early season, when vector abundance is not an effective predictor of risk and most human cases are still unreported. Current work on ArboMAP is focused on evaluating the model in new locations with different transmission cycles.

1335

NEW INSIGHTS ON DIAPAUSE CUES: INVASIVE *AEDES ALBOPICTUS* USE A BET-HEDGING STRATEGY ALONG THEIR NORTHEASTERN UNITED STATES EDGE

Talya Shragai, Laura Harrington

Cornell University, Ithaca, NY, United States

Aedes albopictus is an important vector of arboviruses impacting human health and the world's most rapidly invading mosquito. Since a single introduction in Texas, populations have expanded across the eastern United States (US) and established as far north as New York (NY) State and

Connecticut. Temperate *Ae. albopictus* overwinter through egg diapause; adult females lay diapausing eggs primarily in response to decreasing photoperiod and supported by low temperatures. Laboratory studies have shown that beyond a critical photoperiod, *Ae. albopictus* diapause incidence rapidly reaches 100%, and that populations have evolved diapause responses earlier in the season along a US-wide latitudinal cline. Only one published study has characterized diapause in the field and none have tested it across a fine-scale geographic range, making it difficult to accurately predict future invasions and population dynamics. We tested the hypothesis that diapause phenology varies between proximate cities in southern NY based on each site's microclimate. We collected eggs weekly between August and November using ovitraps with temperature loggers, and determined percent in diapause. We confirmed that beyond a threshold photoperiod, diapause incidence increased; however, it did not rapidly reach 100% as expected. Instead, diapause incidence fluctuated across the collection period in response to temperature, increasing as temperatures decreased, and stabilizing around 50% if temperature was sufficiently high. To investigate this further, we conducted a laboratory experiment using low generation *Ae. albopictus* from our NY field sites and more southern US states. Each population was held at a range of temperatures with NY's predicted critical photoperiod and tested for diapause incidence. Our results suggest, for the first time, that *Ae. albopictus* adopt flexible diapause cues and use a bet-hedging strategy in response to an unpredictable environment. This contrasts the current paradigm of diapause induction in *Ae. albopictus* and highlights the need to study disease vectors on regional geographic scales to understand current human transmission risk.

1336

SEASONAL TRANSCRIPTIONAL CHANGES OF *ANOPHELES COLUZZII* MOSQUITOES DURING DRY SEASON AESTIVATION

Benjamin J. Krajacich[1], Adama Dao[2], Alpha Yaro[2], Moussa Diallo[2], Djibril Samake[2], Zana Lamissa[2], Ousmane Yossi[2], Margery Sullivan[1], Roy Faiman[1], Jose Ribeiro[1], Tovi Lehmann[1]

[1]*National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [2]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali*

For *Anopheles* mosquitoes in sub-Saharan Mali, the 7-month dry season poses a significant challenge to survival. Mark-release-recapture studies have shown that mosquitoes transition between relatively short wet seasons that provide ample breeding habitat via aestivation (a dry season hibernation). However, this long-lived state remains poorly characterized, with mechanisms, behavior, and implications towards malaria competence remaining unknown. Using whole-body mosquito transcriptomes from four time points during the year (early/late dry season and early/late wet season), we found unique expression patterns in immunity, longevity, and metabolic gene expression. First, while these were wild caught, whole-body samples, samplings groups clustered closely to each other, indicating homogeneity in mosquito transcriptional state per season, with differences primarily between seasons. Comparing late dry season to early wet season mosquitoes, we see dry season mosquitoes have significantly higher expression of HSP70 and alpha-crystallin beta chain (7.60, 7.48 log2FC, qval < 0.01). These proteins have been shown to increase in diapause (hibernation) in *Culex* mosquitoes and increase survival under dehydration. This may indicate diapause and aestivation have similar stress response mechanisms and could open alternative climatic conditions to induce aestivation in the lab. In contrast to diapause in other insects, we found many mosquito immune genes (TEP1, TEP4, Defensin, CLIP-B3 and A8 serine proteases) were downregulated in dry season samples (Log2 fold changes of: -2.71, -3.10, -2.33, -2.54 and -2.33, q-value < 0.05). This may point to limited blood-feeding or limited pathogen presence during this period. Finally, when comparing KEGG functional pathways, we see differences in fatty acid metabolism and longevity regulating pathways which may be indicative of aestivation mechanisms. Future work will look to correlate genetic background (karyotype) with aestivation phenotype, and to verify if transcriptional profiles of laboratory mosquitoes under known diapause inducing conditions mimic those from wild aestivators.

1337

ANALYSIS OF THE GENETIC AND NON-GENETIC FACTORS THAT INFLUENCE THE MICROBIAL COMPOSITION OF MOSQUITOES

Haikel N. Bogale[1], Matthew Cannon[1], Kalil Keita[2], Denka Camara[2], Yaya Barry[2], Moussa Keita[2], Drissa Coulibaly[3], Abdoulaye Kone[3], Ogobara Doumbo[4], Mahamadou Thera[3], Christopher Plowe[5], Mark Travassos[1], Seth Irish[6], David Serre[1]

[1]*University of Maryland, Baltimore, Baltimore, MD, United States,* [2]*National Malaria Control Program, Conakry, Guinea,* [3]*Malaria Research and Training Center, Bamako, Mali,* [4]*Malaria Research Training Center, Bamako, Mali,* [5]*Duke Global Health Institute, Durham, NC, United States,* [6]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Mosquitoes are one of the most important insects in terms of public health, with their ability to transmit numerous pathogenic eukaryotic parasites and viruses that are responsible for millions of deaths worldwide. Several studies have shown that the mosquito commensal microbiota can influence parasitic and viral infection of the mosquito as well as impact the mosquito's development and the transmission of pathogens to humans. These discoveries have highlighted the potential of targeting the microbiota for alternative entomological control approaches. However, despite the clear importance of the mosquito-microbiota interactions, little is known about the relative importance of genetic and non-genetic factors in shaping the bacterial communities of mosquitoes. Here, we use a novel genomic assay to comprehensively characterize the bacterial populations, eukaryotic parasites and arboviruses carried by 665 individual *Anopheles* mosquitoes collected in several locations in Guinea and Mali. We identified two Apicomplexan, four Microsporidian and six nematode species as well as two Flaviviruses, infecting in total 127 mosquitoes. In addition, we use our assay to simultaneously characterize each mosquito's species, insecticide resistance alleles and the composition of its blood meal. Overall, we identified that 665 mosquitoes belonged to five *Anopheles* species, harbored varying frequencies of the *kdr-w* mutation, and were able to characterize the source of the blood meal for 133 mosquitoes. We then tested how these factors - geography, mosquito species, insecticide resistance genotypes, presence of pathogens, and mammalian blood-meal diet – influence the composition of the mosquito bacterial microbiota. We show that geographical location is the main driver of the microbial composition and diversity in wild-caught mosquitoes, with other factors having marginal effects. This study offers a comprehensive approach to disentangle the simultaneous influence of multiple factors in shaping the microbiota of *Anopheles* mosquito in nature.

1338

CHARACTERIZING VECTORS OF *PLASMODIUM FALCIPARUM* RESIDUAL TRANSMISSION IN AN ELIMINATION SETTING IN CHOMA DISTRICT, ZAMBIA

Mary E. Gebhardt[1], Kelly M. Searle[2], Tamaki Kobayashi[1], Timothy M. Shields[1], Harry Hamapumbu[3], Limonty Simubali[3], Twig Mudenda[3], Philip E. Thuma[3], Jennifer C. Stevenson[1], William J. Moss[1], Douglas E. Norris[1]

[1]*Johns Hopkins Bloomburg School of Public Health, Baltimore, MD, United States,* [2]*Division of Epidemiology and Community Health, Minneapolis, MN, United States,* [3]*Macha Research Trust, Macha, Zambia*

Macha, in Choma District in southern Zambia, is working toward a national goal to eliminate malaria by 2021. Malaria prevalence in Macha decreased from 9% in 2008 to 1% in 2013 after insecticide-treated bed net (ITN) campaigns, however residual malaria transmission still occurs with yearly infection prevalence from 1-3% under active case surveillance. As vector control strategies are successfully implemented, identification of the vectors of residual transmission remains challenging. While ITNs reduce the threat from predominately endophagic and endophilic vectors, other opportunistic foraging species may contribute as secondary vectors in residual transmission settings. In 2013, the Zambian government implemented a reactive test-and-treat strategy in Choma District to pursue

their goal of elimination. When an index case was identified positive for *P. falciparum* by rapid diagnostic test (RDT) at a local health facility, a healthcare worker visited their household and every household within 140 meters. In this study, the radius was extended to 250 meters and all individuals within the radius were screened for *P. falciparum* using a RDT and treated with ACT if positive. This procedure was repeated 30 and 90 days after the initial visit. At every visit, entomological samples were collected by placing CDC light traps indoors next to a person sleeping under a bed net and outdoors near animal pens in index and neighboring households. Each anopheline was morphologically and molecularly identified to species level by PCR, analyzed for recent blood meal content via PCR, and evaluated for sporozoite prevalence using a *P. falciparum* ELISA. A negative binomial regression will be performed to determine association of anopheline counts with risk factors including roof type, open house eaves, animal pens, and proportion of people sleeping under ITNs. Spatial analyses will also be performed to ascertain if proximity to index households influences the composition of anopheline species present. This study will provide information about vector dynamics in Macha and influence recommendations for future interventions to target residual malaria vectors.

1339

PREDICTING SPILLOVER OF YELLOW FEVER VIRUS TO HUMANS USING VECTOR AND PRIMATE ECOLOGY

Marissa L. Childs, Nicole Nova, Justine Colvin, Erin A. Mordecai

Stanford University, Stanford, CA, United States

Yellow fever is a (re)emerging disease that largely occurs in humans following virus spillover from non-human primates via mosquitoes. To predict spillover and better understand the conditions limiting spillover in the Americas, we develop a model based on the mechanisms of spillover including the ecology of mosquito vectors and non-human primate hosts, human susceptibility, and human density. We estimate four risk metrics based on hypotheses about the factors limiting spillover. We then test these hypotheses by comparing the risk metrics to the spatial and temporal occurrences of human cases of yellow fever in Brazil from 2001 to 2016. We find that environmental risk is the best predictor of spillover independent of human case data. Unexpectedly, incorporating information about human vaccine coverage and density did not improve prediction. This finding suggests that environmental suitability may be more limiting for spillover than availability of susceptible human hosts. More generally, this work points to the potential for ecological interactions to inform prediction of pathogen spillover.

1340

EVALUATION OF IMMUNE RESPONSES TO O-SPECIFIC POLYSACCHARIDE (OSP) IN NORTH AMERICAN HEALTHY ADULTS CHALLENGED WITH *VIBRIO CHOLERAE* O1 INABA

Motaher Hossain[1], Kamrul Islam[1], Meagan Kelly[2], Leslie M. Mayo-Smith[2], Richelle C. Charles[2], Ana Weil[2], Taufiqur R. Bhuiyan[1], Pavol Kovac[3], Peng Xu[4], Regina C. LaRocque[2], Stephen B. Calderwood[2], Jakub K. Simon[5], Wilbur H. Chen[6], Douglas Haney[7], Michael Lock[7], Caroline E. Lyon[8], Beth D. Kirkpatrick[8], Mitchell Cohen[9], Myron M. Levine[6], Marc Gurwith[7], Daniel T. Leung[10], Andrew Azman[11], Jason B. Harris[12], Firdausi Qadri[1], **Edward T. Ryan**[2]

[1]*Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Division of Infectious Diseases, Massachusetts General Hospital - Harvard Medical School, Boston, MA, United States,* [3]*National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), Laboratory of Bioorganic Chemistry (LBC), National Institutes of Health, Bethesda, MD, United States,* [4]*National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), Laboratory of Bioorganic Chemistry (LBC), National Institutes of Health, Baltimore, MD, United States,* [5]*Merck & Co., Inc., Kenilworth, NJ, United States,* [6]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [7]*PaxVax, Inc., Redwood City, CA, United States,* [8]*Vaccine Testing Center, Department of Medicine, University of Vermont College of Medicine, Burlington, VT, United States,* [9]*Cincinnati Children's Hospital Medical Center, and the Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States,* [10]*Division of Infectious Diseases, University of Utah School of Medicine, Salt Lake City, UT, United States,* [11]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [12]*Division of Infectious Diseases, Massachusetts General Hospital - Department of Pediatrics, Harvard Medical School, Boston, MA, United States*

There is a growing body of evidence that immune responses targeting O-specific polysaccharide (OSP) of *Vibrio cholerae* are involved in mediating protection against cholera; however, little is known about this immune response in immunologically naïve humans exposed to *V. cholerae*. To address this, we measured serum anti-OSP (Inaba and Ogawa) antibody responses in adult North American volunteers experimentally challenged with *V. cholerae* O1 Inaba El Tor N16961. We also measured vibriocidal and anti-cholera toxin B subunit (CtxB) responses, and compared responses to those induced in age, gender, blood group, and serotype matched *V. cholerae* O1-infected cholera patients in Dhaka, Bangladesh, an area endemic for cholera. We found that prominent anti-OSP responses develop following initial cholera infection, these response are largely IgM and IgA isotypes, are highest to infecting serotype but have significant cross-serotype reactivity, peak soon after infection but remain elevated over baseline for at least 6 months, correlate with vibriocidal responses, and that anti-OSP IgA responses are lower in blood group O cholera-naïve individuals than in non-O individuals. We also found significant differences in immune responses between naïve and endemic zone cohorts, presumably reflecting previous exposure in the latter.

1341

IMPACT OF BACKGROUND EXPOSURE DOSE ON DIRECT AND INDIRECT EFFECT OF KILLED ORAL CHOLERA VACCINES

Qifang Bi, Andrew S. Azman, Justin Lessler

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Previously published meta-analyses of the direct protection of killed whole-cell oral cholera vaccines (kOCVs) reported lower direct protection to people living in cholera-endemic Asian countries than African countries. Direct protection conferred by kOCV is also lower in children younger than 5 years than in those 5 years or older. Studies in India and Bangladesh showed that kOCVs provided stronger indirect protection to the unvaccinated than the vaccinated; specifically, the incidence among unvaccinated people was greatly reduced as community vaccine coverage increased with almost no corresponding incidence reduction among vaccinated people. Understanding the underlying mechanisms leading to these differences in direct and indirect protection across populations is important for forecasting disease dynamics and designing more effective vaccination strategies. Recently published studies have reported reduced vaccine efficacy at higher exposure dose for a range of pathogens, and these studies have shown that non-linear increase in probability of infection with increasing exposure dose could be an important contributing factor. If similar dose-response relationship is observed for cholera, we hypothesize that this dose-response relationship can explain the differences in the direct and indirect protection across populations. Using mathematical models incorporating dose-response relationships generated from historic human challenge studies of cholera, we show that increases in background exposure dose to *Vibrio cholerae* can lead to decreases in estimated direct vaccine efficacy. We also show that reductions in the bacterial exposure dose in the community as a result of vaccination coverage can explain the stronger indirect protection among unvaccinated compared to vaccinated individuals.

1342

LARGEST PRE-EMPTIVE VACCINATION WITH ORAL CHOLERA VACCINE PREVENTS CHOLERA OUTBREAKS AMONG THE ROHINGYA PEOPLE IN COX'S BAZAR: A 360 DEGREES OF LEARNING

Firdausi Qadri

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Bangladesh, a country with endemic cholera, faced a major challenge of epidemic potential in the middle of 2017 with the influx of 700,000 people from the Rakhine state in Myanmar (referred to as Forcibly Displaced Myanmar Nationals). The prevailing conditions of the stranded people made them prone to high risk for cholera. The Government of Bangladesh together with the support of icddr,b and partner organizations conducted four mass campaigns with the oral cholera vaccine (OCV) between October 2017 to December 2018 in Cox's Bazar in this fragile population. In the campaigns, a single dose of OCV was given to all FDMNs while a second dose was given to ≤5 years in a dose-sparing attempt since OCV was limited in supply. Subsequently, two doses were offered to all of FDMNs including the host population. An important implementation system emerged from the mass vaccination such that in the first 3 rounds, OCV was delivered in a campaign design in 150 vaccination sites, however in the final campaign conducted between November to December 2018, vaccine was delivered through the 780 routine EPI fixed and outreach sites by 65 outreach mobile teams as per national EPI program. In the four campaigns, a total of 2,143,920 OCV doses were administered. In the campaigns, the icddr,b, OCV staff were involved in vaccination at the field and in training, campaign planning, monitoring and problem-solving issues together with the EPI and others heralding another unique strength of icddr,b to partner with the government in this effort. Coverage evaluation was carried out which confirmed high rates of OCV coverage. Along with the campaigns, surveillance for *V. cholerae* is continuing for evaluation of the effectiveness of OCV in this fragile population in a test-negative case-control design and also to develop an early warning system for control of epidemics. Until now, no cholera epidemics have occurred among the FDMNs or host population. The successful OCV campaign in Cox's Bazar has become a showcase for Bangladesh on use of OCV for control of future endemic cholera using the existing EPI system of Bangladesh. Further evaluation of data will be presented.

1343

LONG READ SEQUENCING OF *VIBRIO CHOLERAE* REVEALS REGIONAL TRANSMISSION PATTERNS IN MALAWI

Shirlee Wohl[1], Watipaso Kasambara[2], Innocent Chibwe[2], Amanda Debes[1], David Mohr[3], Justin Lessler[1], Andrew Azman[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Public Health Institute of Malawi, Ministry of Health, Lilongwe, Malawi,* [3]*McKusick Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States*

Whole genome sequencing has recently been used to understand the movement of seventh pandemic El Tor (7PET) *Vibrio cholerae* strains into and out of Africa. However, much remains unknown about the spread and evolution of cholera at the local and regional levels. New highly portable long read sequencing technology (such as Oxford Nanopore (ONT)) has the potential to bring sequencing closer to the places where cholera outbreaks occur, potentially providing near real-time actionable data. Long read sequencing also has the potential to more clearly resolve long repeats and regions with structural variation than sequencing short reads. We performed whole genome sequencing using the ONT platform on cholera isolates collected throughout Malawi between 2015 and 2018 to identify the strains underlying recent outbreaks and to understand cholera dynamics within the region. We performed a phylogenetic analysis of these sequences and identified multiple distinct cholera sublineages within the larger 7PET lineage circulating within Malawi from 2015-2018. Additionally, we found that some, but not all, of the isolates contain a multidrug resistant IncA/C conjugative plasmid, in line with recent studies that identified acquired IncA/C plasmids in certain cholera lineages, including in isolates collected in East and West Africa. We compared these genomes to those sequenced on the Illumina platform (short reads) and to the results of commonly-used typing methods such as multi-locus variant analysis (MLVA). More generally, because of the potential for ONT sequencing to quickly provide genomic data important to a local public health response, we developed and implemented a pipeline for rapid lineage identification and antimicrobial resistance screening of cholera and discuss the potential added value of in-country real-time cholera sequencing during an outbreak.

1344

VIBRIO CHOLERAE TRANSMISSION IN BANGLADESH: INSIGHTS FROM A NATIONAL SEROSURVEY

Andrew S. Azman[1], Justin Lessler[1], Daniel Leung[2], Francisco J. Luquero[3], Jason Harris[4], Stephen Lauer[1], Fatema Khaton[5], Kishor K. Paul[5], Md. Taufiqur Rahman Bhuiyan[5], Henrik Salje[6], Firdausi Qadri[5], Emily S. Gurley[1]

[1]*Johns Hopkins School of Public Health, Baltimore, MD, United States,* [2]*University of Utah, Salt Lake City, UT, United States,* [3]*Epicentre, Paris, France,* [4]*Massachusetts General Hospital, Boston, MA, United States,* [5]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [6]*Institut Pasteur, Paris, France*

Despite years of cholera research in Bangladesh little is known about the geographic distribution and magnitude of pandemic *Vibrio cholerae* O1 (VC) transmission across the country as most surveillance data come from just a few sites. We aim to use recent advances in cholera seroepidemiology to describe cholera infection risk across Bangladesh. We conducted a nationally representative serosurvey across Bangladesh of 2,778 individuals from 70 randomly selected communities and tested serum samples using six assays quantifying different VC-specific antibodies. Using 6 biomarkers (vibriocidal Inaba, vibriocidal Ogawa, anti-CTB IgG, anti-CTB IgA, anti-LPS IgG and anti-LPS IgA), age and sex profiles from participants, we found that 20.7% of the population had evidence of VC infection in the previous year using a recently validated machine learning model (range: 0-73% across communities). We explored a number of potential individual- household- and community level risk factors and found that only age (risk increasing with age) and being female were significantly associated with increased cholera infection risk. We created high-resolution maps of VC infection risk using Bayesian model-based geostatistical methods and found that while the absolute infection risk is generally high throughout Bangladesh, there are clear areas with evidence of elevated transmission ('hotspots') that are qualitatively similar to clinical case maps based national diarrhea data and cholera sentinel surveillance data from the same time period. Our preliminary estimates suggest that as many as 35.4 million infections (95% CrI 29.5-41.5 million) occurred across Bangladesh in the year preceding the survey, although this estimate includes infections across the severity spectrum from asymptomatic to severe cholera gravis.These results illustrate how serosurveillance provides an avenue for identifying areas of high VC transmission and exploring key risk factors for infection across geographic scales. Future work combining serologic studies and estimates of clinical disease rates can help improve the interpretation of cross-sectional serosurvey results.

1345

PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF NOROVIRUS AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA IN THREE SUB-SAHARAN AFRICAN COUNTRIES: PRELIMINARY FINDINGS FROM THE VACCINE IMPACT ON DIARRHEA IN AFRICA (VIDA) STUDY, 2015 -2018

Richard Omore[1], Anna Roose[2], Samba Sow[3], Sanogo Doh[3], M. Jahangir Hossain[4], Benjamin Ochieng[1], Joquina Chiquita Jones[4], Syed M. Zaman[4], Henry Badji[4], Sharon M. Tennant[2], Irene Kasumba[2], Helen Powell[2], Dilruba Nasrin[2], Jie Liu[5], James Platts-Mills[5], Martin Antonio[4], Eric D. Mintz[6], Jacqueline E. Tate[7], Jennifer R. Verani[8], Marc-Alain Widdowson[8], Eric Houpt[5], Umesh D. Parashar[7], Karen L. Kotloff[2]

[1]*Kenya Medical Research Institute, Center for Global Health Research [KEMRI-CGHR], Kisumu, Kenya,* [2]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Center for Vaccine Development, Bamako, Mali,* [4]*Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [5]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States,* [6]*Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [7]*Division of Viral Diseases, US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [8]*Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya*

Norovirus is an important enteric viral agent causing acute gastroenteritis in children worldwide. Using data from Vaccine Impact on Diarrhea in Africa (VIDA), a prospective, health center and community-based case-control study from May 2015 - July 2018, we describe the epidemiology of norovirus among children <5 years old in The Gambia, Kenya, and Mali. An MSD case was defined as a child 0-59 months old, passing ≥3 loose stools in the previous 24 hours with ≥1 of the following: sunken eyes, poor skin turgor, dysentery, IV rehydration, or hospitalization within 7 days of diarrhea onset. Diarrhea-free controls matched for gender, age, time, and community were enrolled at home. Stools collected from cases and matching controls at enrolment were tested for a panel of enteropathogens, including norovirus genotype I (NVI) and II (NVII), using TaqMan Array Card (TAC). A TAC cut-off value of <35 Cq was considered positive. Adjusted attributable fractions (AF) for each pathogen significantly associated with MSD were derived for each site and age stratum using multiple conditional logistic regression (CLR). Using LR, adjusting for age, blood in stool, and study site, we evaluated factors associated with norovirus MSD. VIDA included 4, 779 MSD cases (221 NVI-positive and 550 NVII-positive), and 4750 controls (234 NVI-positive and 513 NVII-positive). Whereas NVI was not significantly associated with MSD, NVII was significantly associated with MSD in all sites in infants 0-11 months old (The Gambia AF 5.0, 95% CI [2.0-9.9]; Mali AF 7.9, 95% CI [3.7-12.4]; and Kenya AF 10.3, 95% CI [6.9-14.7]), and in Kenya among those 12-23 months (AF 5.6; 95% CI [3.0-9.6]). Compared to non-NVII MSD cases, NVII patients were more likely to be infants (284 [51.6%] vs. 1413 [33.4%], aOR 3.09, 95% CI 2.38-4.01) and to present with vomiting (308 [56%] vs. 1927 [45.6%], aOR=1.40, 95% CI 1.16-1.69), but were less likely to present with blood in stool (54 [9.8%] vs. 637 [15.1%], aOR =0.72, 95% CI 0.53-0.98). We found that infants bear the greatest burden of MSD associated with norovirus GII.

1346

USING KERNEL DENSITY ESTIMATES IN LIKELIHOOD RATIOS TO OPTIMIZE ETIOLOGICAL PREDICTIONS OF INFECTIOUS DIARRHEA IN RESOURCE-LIMITED SETTINGS

Benjamin J. Brintz[1], Joel Howard[1], Benjamin Haaland[1], Andrew Pavia[1], Tom Greene[1], Dennis Chao[2], Joshua Proctor[2], Adam Levine[3], Karen Kotloff[4], James Platts-Mills[5], Daniel Leung[1]

[1]*University of Utah, Salt Lake City, UT, United States,* [2]*Institute of Disease Modeling, Seattle, WA, United States,* [3]*Brown University, Providence, RI, United States,* [4]*University of Maryland, College Park, MD, United States,* [5]*University of Virginia, Charlottesville, VA, United States*

Non-laboratory methods to more accurately assess etiology are needed for appropriate management of pediatric diarrhea in low and middle income countries (LMICs). In LMICs, etiological diagnosis is rarely made, and a large number (up to 70%) of diarrheal patients are prescribed antibiotics empirically. Our goal is to build an electronic clinical decision support system (eCDSS) with multiple data sources appropriate for use in LMICs. We use clinical and quantitative molecular etiologic data from the Global Enteric Multicenter Study (GEMS) to develop predictive models for viral-only diarrheal etiology in young children, given the lack of efficacy of antibiotics for viral-only infections. We use the post-test odds (PTO) formulation which allows for prior knowledge as well as flexible inclusion or omission of one or more "tests", or information sources, for evaluating the likelihood of an etiology. In addition to using current patient clinical predictors, other sources of data we consider are clinical data from previous patients and local climate data. For climate, we calculate a rolling weighted average of temperature and rain data from NOAA weather stations in order to capture weather pattern trends, and we calculate a weighted average of recent patient data to account for influxes of a particular etiologies. We build a parsimonious set of prediction variables from the full set of GEMS survey responses via random forest variable importance screening. Variables predictive of viral etiology include age, vomiting, BMI, bloody diarrhea, breastfeeding. We address uncertainty in predictions by constructing Gaussian kernel density estimates of likelihood ratios for each test based on training predictions. Cross-validation shows that use of PTO improves average AUC by up to 0.04 (from 0.81 to 0.85) by including recent weather patterns in addition to clinical predictors. Overall, our post-test odds model's performance suggests that incorporating additional data sources can improve the performance of a clinical prediction rule for etiological prediction of diarrhea in LMICs over what can be achieved with clinical information alone.

1347

MEMORY B AND FUNCTIONAL ANTIBODY RESPONSES TO PAMVAC VACCINE IN BENINESE NULLIGRAVID WOMEN DURING PHASE IB CLINICAL TRIAL

Tatiana Sandrine Hountohotegbe[1], Déo-Gracias Berry[2], Wina Hasang[3], Elizabeth Aitken[3], Komi Bienvenu Gbedande[1], Firmine Viwami[1], Florentin Auussenac[4], Saadou Issifou[5], Euripide Avokpaho[5], Morten Nielsen[6], Benjamin Mordmüller[7], Odile Leroy[8], Achille Massougbodji[2], Nadine Fievet[4], Stephen Rogerson[3], Adrian Luty[4]

[1]*Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'enfance (CERPAGE), UMR*[261]*, Cotonou, Benin,* [2]*Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'enfance (CERPAGE), Cotonou, Benin,* [3]*Peter Doherty Institute, Laboratory of Malaria, Department of Immunology and Microbiology, University of Melbourne, Melbourne, Australia,* [4]*UMR*[261]*, Laboratoire de Parasitologie, Université Paris Descartes, Faculté de Pharmacie, Paris, France,* [5]*Institut de Recherche Clinique du Bénin, Fondation pour la Recherche Scientifique, Calavi, Benin,* [6]*Department of Immunology and Microbiology, University*

of Copenhagen, Copenhagen, Denmark, [7]Institut für Tropenmedizin, Eberhard Karls Universität Tübingen, Tübingen, Germany, [8]European Vaccine Initiative, Heidelberg, Germany

Placental Malaria (PM) pathogenesis is caused by interactions between the parasite protein VAR2CSA and chondroitin sulfate A (CSA) in the human placenta. The PAMVAC vaccine is a VAR2CSA protein-based vaccine, aiming to protect fetus and mother against the adverse effects of PM. After a safe Phase Ib clinical trial conducted in Benin from November 2016 to September 2017, PAMVAC immunogenicity was explored in 21 healthy nulligravid adult females randomized in 3 groups (9 Alhydrogel, 9 GLA-SE, 3 Placebo). They received 3 doses of 50 μg of adjuvanted PAMVAC or Placebo 28 days apart (Day 0, 28 and 56). Plasma samples and PBMC harvested at Days (D) -1, 56, 84, 168, 252 were stored respectively at -20°C and in liquid nitrogen for immunoassays. Memory B cells were quantified by ELISpot and functional antibody properties assessed for Variant Surface Antigen (VSA) recognition and opsonic phagocytosis using FACS. PAMVAC vaccination induced a memory B response that increased noticeably after the 2nd dose of vaccine (D56) and persisted over time at levels substantially higher than those we have previously measured in multigravid Beninese. The frequency of PAMVAC-specific antibody-secreting B cells was notably higher with GLA-SE than with alum, and responses were stable between D56 and D84. PAMVAC-specific B cell responses were undetectable in the Placebo group. PAMVAC immunization also induced antibody-mediated homologous VSA recognition and opsonic phagocytosis activities. IgG1 and IgG3 responses to VSA were higher in both adjuvant groups compared with Placebo, and opsonic phagocytosis of infected red blood cells was observable from D56 in the vaccinated groups. PAMVAC with alum induced stronger opsonic phagocytosis than with GLA-SE. In conclusion, the combination of PAMVAC with GLA-SE rather than alum was associated with the strongest B cell responses, whilst the combination with alum was associated with stronger functional antibody responses in women of childbearing age before their first pregnancy.

1348

HUMAN MAB BLOCKS MALARIA TRANSMISSION IN *PLASMODIUM*-INFECTED MOSQUITOES

Camila H. Coelho[1], Marty Butkhardt[1], Issaka Sagara[2], Jacob D. Galson[3], Thiago A. Silva[4], Justin Taylor[5], Miranda Byrne-Steele[6], Nichole Salinas[1], David Narum[1], Niraj Tolia[1], Jonathan Renn[1], Patrick E. Duffy[1]

[1]*Laboratory of Malaria Immunology and Vaccinology/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]University of Bamako, Bamako, Mali, [3]Kymab, UK, United Kingdom, [4]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [5]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [6]iRepertoire, Huntsville, AL, United States*

Malaria transmission blocking vaccines (TBVs) target sexual stage proteins of *Plasmodium falciparum* parasite in the mosquito vector, thereby reducing transmission. To improve the design of transmission-blocking therapeutic approaches, functional antibodies generated against these proteins need to be better characterized. Here, we generated functional human mAbs against the protein Pfs230, a surface antigen of *P. falciparum* gametes. Pfs230-specific single B cells generated by the fourth dose of Pfs230D1-EPA/Alhydrogel® in Malian adults were sorted after labelling with antigen-tetramers, and both heavy and light antibody chains were sequenced. Paired BCR sequences were selected from two vaccinees, based on repeated frequency among sorted cells and high mutation rates, and two BCR sequences were chosen to generate fully human recombinant IgG1 by expression in HEK cells. These antibodies LMIV230-01 and LMIV230-02, bound to Pfs230D1 and to protein extract of female gametocytes. LMIV230-01 and LMIV230-02 demonstrated high and similar binding affinities to recombinant Pfs230D1 antigen. However, only LMIV230-01 strongly bound to the surface of live female gametes while LMIV230-02 reacted minimally. LMIV230-01 also bound to gametocytes and zygotes, but did not bind to ookinetes, as expected. LMIV230-01 reduced *P. falciparum* oocyst burden in mosquitoes by 99% at 1000 μg/mL while LMIV230-02 reduced by 60% at the same concentration, although activity of both antibodies was determined to be complement-dependent. Competition assays suggested that the two antibodies react to different epitopes, and their CDR3 in the heavy chain shares no similarity. These functional mAbs provide the basis for the rational design of an improved Pfs230 vaccine or of antibody-based interventions against mosquito-stage proteins that prevent malaria transmission through parasite neutralization.

1349

UNDERSTANDING THE PROCESSES GOVERNING THE POPULATION-LEVEL IMPACT OF A TRANSMISSION BLOCKING VACCINE AGAINST MALARIA IN FIELD TRIAL SETTINGS

Joseph D. Challenger[1], Issaka Sagara[2], Daniela Olivera[1], Sara A. Healy[3], Mahamadoun H. Assadou[2], Abdoulaye Katile[2], Olga Muratova[3], Patrick E. Duffy[3], Thomas S. Churcher[1]

[1]*Imperial College London, London, United Kingdom, [2]University of Science, Techniques and Technologies of Bamako, Bamako, Mali, [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States*

The transmission of malaria parasites from humans to the mosquito vector involves relativity small numbers of parasites and has long been viewed as a target for interrupting transmission. With this aim in mind, a number of vaccines are currently under development. We have developed a mathematical description of a vaccine for *Plasmodium falciparum*, targeting antigen *Pfs*230, based on trial data collected in Mali. Incorporating the vaccine into a widely-used model of malaria transmission allows us to estimate the population-level impact of the vaccine, as well as identify the key demographics to target in a vaccination campaign. As transmission-blocking interventions are commonly measured by a standard membrane feeding assay, we consider how best to translate transmission blocking and reducing activities into different measures of efficacy in clinical trial settings. Unlike conventional vaccines, a transmission blocking vaccine confers no direct individual-level protection against bites from infectious Anopheles mosquitoes so vaccine effectiveness will depend on clinical incidence in both vaccinated and unvaccinated individuals and their relative spatial distribution. A high degree of mixing between these sub-populations will lead to the vaccine-derived benefits being shared throughout the entire population. In contrast, in a scenario in which transmission is focal and vaccinated subjects cluster together, the impact will be focused upon the vaccinated population. Such effects, which we estimate with our model, must be appreciated to accurately assess the full impact of transmission blocking interventions.

1350

NEUTRALIZATION OF *PLASMODIUM VIVAX* BY NATURALLY-ACQUIRED HUMAN ANTIBODIES THAT TARGET THE DUFFY BINDING PROTEIN

Darya Urusova[1], Lenore Carias[2], Yining Huang[3], Vanessa C. Nicolete[4], Jean Popovici[5], Camille Roesch[5], Nichole D. Salinas[6], Benoit Witkowski[5], Marcelo U. Ferreira[4], John H. Adams[7], Michael L. Gross[1], Christopher L. King[2], **Niraj Harish Tolia**[6]

[1]*Washington University in St. Louis, St. Louis, MO, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Eli Lilly and Company, Indianapolis, IN, United States, [4]University of Sao Paulo, Sao Paulo, Brazil, [5]Institute Pasteur in Cambodia, Phnom Penh, Cambodia, [6]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [7]University of South Florida, Tampa, FL, United States*

The *Plasmodium vivax* Duffy binding protein (DBP) is a prime target of the protective immune response and a promising vaccine candidate for *P. vivax* malaria. Naturally acquired immunity (NAI) protects against malaria in adults residing in infection-endemic regions, and the passive transfer of malarial immunity confers protection. A vaccine that replicates NAI will effectively prevent disease. Here, we report the structures of

DBP region II in complex with human-derived, neutralizing monoclonal antibodies obtained from an individual in a malaria-endemic area with naturally acquired immunity. We identified protective epitopes by X-ray crystallography, hydrogen-deuterium exchange mass spectrometry, mutational mapping, and *P. vivax* invasion studies. These approaches reveal that naturally-acquired human antibodies neutralize *P. vivax* by targeting the DARC–binding site and dimer interface of *P. vivax* DBP. Antibody binding is unaffected by polymorphisms in the vicinity of epitopes, suggesting the antibodies have evolved to engage multiple polymorphic variants of DBP. The human antibody epitopes are broadly conserved and are distinct from previously defined epitopes for broadly conserved murine mAbs. A library of globally conserved epitopes of neutralizing human antibodies opens new horizons for rational design of strain-transcending DBP-based vaccines and therapeutics against *P. vivax*.

1351

PRIME-TARGET IMMUNISATION WITH LIVER-STAGE MALARIA VACCINES: A FIRST-IN-HUMAN CHALLENGE TRIAL

Mehreen Datoo[1], Daniel Jenkin[1], Fernando Ramos-Lopez[1], Megan Baker[1], Amy Flaxman[1], Duncan Bellamy[1], Nick J. Edwards[1], Rebecca Makinson[1], Andres Noe[1], Pedro Folegatti[1], Ian Poulton[1], Daniel Silman[1], David Lewis[2], Saul Faust[3], Rachel Roberts[1], Alison M. Lawrie[1], Alexandra J. Spencer[1], Mohammad Ali Husainy[4], Katie J. Ewer[1], Adrian V. Hill[1]

[1]*Jenner Institute, University of Oxford, Oxford, United Kingdom,* [2]*NIHR Imperial CRF, London, United Kingdom,* [3]*Southampton NIHR Wellcome Trust Clinical Research Facility, Southampton, United Kingdom,* [4]*Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom*

Malaria could be the first disease against which any subunit vaccine working through $CD8^+$ T cells is licensed. Such a vaccine would need to target the liver-stage malaria parasite. ChAd63 and MVA ME-TRAP, two leading vectored vaccine candidates for this indication, induce high numbers of circulating $CD8^+$ T-cells, but only result in modest (20-25%) sterile efficacy against sporozoite challenge, although a small field trial in Kenya identified higher efficacy in semi-immune adults. Preclinical experiments demonstrated that changing the immunisation regimen, to include a final booster dose intravenously, substantially improved sterile efficacy against transgenic *P. berghei* challenge. Use of such a *prime-target* strategy, in which one or more intramuscular doses of a CD8 T cell-inducing viral vector is followed by an intravenous dose of the same or another viral vector with the same transgene, conferred 100% efficacy for numerous liver-stage antigens in mice. This strategy induces high numbers of antigen- specific tissue-resident memory T-cells (T_{RM}), in the liver, and their causal protective role was demonstrated by adoptive transfer studies. The first Phase I vaccine trial (VAC064) of any intravenous use of vaccine vectors demonstrated adequate safety and strong immunogenicity of single-dose intravenous ChAd63 and MVA ME-TRAP. We have now undertaken a Phase I/IIa (VAC066) CHMI trial to evaluate the *prime-target* strategy for the first time. This trial has enrolled 37 volunteers, 31 of which were vaccinated, according to four different immunization schedules, using ChAd63 and MVA encoding ME-TRAP. Fine needle aspirates of the liver in some volunteers allowed direct measurement of intrahepatic antigen-specific T cells.We will report the safety, immunogenicity and efficacy measured in this first clinical trial of a novel immunisation strategy, which could provide a rapid two-dose approach to inducing liver-stage immunity to malaria.

1352

NOVEL METHODS TO DETERMINE LIVER-STAGE MALARIA VACCINE CORRELATES OF PROTECTION: KINETICS, DEEP IMMUNE PHENOTYPING AND TRANSCRIPTOMICS

Andrés Noé, Duncan Bellamy, Amy Flaxman, Mehreen Datoo, Daniel Jenkin, Ali Husainy, Katie J. Ewer, Adrian V. Hill, Alexandra J. Spencer

The Jenner Institute, University of Oxford, Oxford, United Kingdom

Protection from liver-stage malaria requires high numbers of CD8+ T cells to find and kill *Plasmodium*-infected cells. A new malaria vaccine strategy, Prime-Target Vaccination (PTV), involves sequential viral-vectored vaccination by intramuscular and intravenous routes to target cellular immunity to the liver. The efficacy of leading liver-stage malaria vaccine candidates in mice can be enhanced with this approach from 0-30% to 100%. PTV substantially increases the number of antigen-specific tissue-resident memory CD8+ T cells (TRMs) in the liver of mice. The first human phase I/IIa clinical trial of PTV efficacy against controlled human malaria infection is ongoing. We hypothesise that protection correlates to the magnitude of the TRM response elicited in the liver by vaccination. We have seen that cells with a TRM phenotype transiently appear in the blood of IV vaccinated individuals, which is consistent with data in animal models. Herein, we track the kinetics of these blood-derived TRMs with the expectation of identifying a correlate of vaccine protection against CHMI. Directly sampling the human liver safely is difficult but possible through liver fine needle aspiration (FNA). We safely obtained FNAs from 13 volunteers. Our project is the first-ever to perform deep immune phenotyping and transcriptomic evaluation of the intrahepatic effect of a new and promising liver-stage malaria vaccine strategy. As a means to identify correlates of liver-stage malaria protection, we obtained time-matched PBMC samples from the same volunteers who underwent FNA. We used flow cytometry and single-cell RNA sequencing on lymphocytes from the liver and blood to identify differentially expressed genes. This work provides clinical proof-of-concept of multiple novel methods to investigate pre-erythrocytic malaria vaccines, immunological evaluation of a malaria vaccine strategy by CHMI, and insights into correlates of protection after vaccination. A simple and reproducible correlate of protection would be particularly helpful in trials of liver-stage malaria vaccines as they progress to phase III, large-scale testing in African infants.

1353

DEVELOPMENT OF MULTI-STRAIN AND MULTI-STRAIN HYBRID *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINES

BKL Sim[1], Ashley M. Vaughan[2], Tao Li[3], Christiane Urgena[3], Asha Patil[3], Yonas Abebe[3], Adam Frock[3], Lauren Smith[3], Ayyappan Rathakrishnan[3], Felix Ikanzo[3], Tanima Mallik[3], Abraham G. Eappen[3], Donald Ward III[3], Sumana Chakravarty[3], Minglin Li[1], Eric R. James[3], Stefan H. I. Kappe[2], Stephen L. Hoffman[3]

[1]*Protein Potential LLC, Rockville, MD, United States,* [2]*Seattle Children's Research Institute, Seattle, WA, United States,* [3]*Sanaria Inc., Rockville, MD, United States*

Title: Development of multi-strain and multi-strain hybrid *Plasmodium falciparum* sporozoite vaccines. Sanaria® PfSPZ Vaccine is composed of radiation attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ). Based on safety and vaccine efficacy (VE) results, Phase 3 clinical trials are planned for 2020. It has been hypothesized that broader, more potent VE can be obtained by immunizing with PfSPZ from diverse geographic origins. PfSPZ Vaccine is produced from the NF54 strain of Pf, thought to be from West Africa. Pf NF54 is an excellent producer of PfSPZ. On the other hand, PfNHP4026, a Thai isolate (F. Nosten, Thailand), is an exceptionally infectious to hepatocytes. This suggests that this parasite may be a more potent vaccine immunogen than PfNF54, because infection of the liver by the vaccine is a prerequisite for protective T cell responses. We are thus developing PfNHP4026 as a vaccine strain. In order to achieve diversity we used genetic crossing between the two Pf strains to create hybrid progeny

parasite strains that combine Pf antigens from different geographic areas. Use of hybrid Pf strains that produce more PfSPZ/mosquito and are more infectious to hepatocytes than PfNF54 would constitute a simple and elegant solution to increasing and broadening vaccine-induced protection. We have successfully crossed PfNF54 and the Thai isolate, PfNHP4026, and produced F1 progeny (F1 Hybrids). We are developing the F1 Hybrids to select a vaccine candidate. Our selection includes high PfSPZ productivity, high hepatocyte infectivity and high predicted proteomic (T cell epitope) diversity. We will report on optimization of aseptic production of PfSPZ from PfNHP4026 and selected PfHybrids, and characterization of their infectivity to hepatocytes *in vitro* and human liver-chimeric mice *in vivo*.

1354

USING MALARIA STRATIFICATION TO IMPROVE PROGRAM INTERVENTION TARGETING IN ZAMBIA

Hannah Slater[1], John M Miller[2], Busiku Hamainza[3], Kammerle Schneider[1], Laurence Slutsker[1], Duncan Earle[2], Jeff Bernson[4]

[1]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States,* [2]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [3]*National Malaria Elimination Centre, Lusaka, Zambia,* [4]*PATH, Seattle, WA, United States*

The Zambian National Malaria Elimination Centre have set ambitious targets for achieving malaria elimination. Key to achieving this is the efficient and optimal deployment of resources to maximize impact. The basis of this "optimal deployment" is multifaceted and will depend on the intervention or combination of interventions under consideration, as well as the epidemiological features of the transmission setting. In this study we develop stratification maps specific for each intervention, whereby targeting is based not only on the level of malaria transmission, but also a range of geospatial covariates including access to treatment based on the travel time to the nearest healthcare provider, level of rurality, duration of malaria transmission season, existing intervention coverage, and population density. The stratification map for each intervention is also tailored to the spatial scale at which the intervention is delivered. Furthermore, we use a range of data sources to track changes in malaria transmission to identify "breakpoints" where the optimal intervention package for a region should change. Traditionally, these decisions have been made using malaria incidence data from routine surveillance. However, improvements in surveillance and response as well as access to treatment have resulted in an increasing number of both passively and proactively identified cases, making interpretation of case totals and thus transmission more difficult. We circumvent this problem by employing a range of data sources, including test positivity rate and the proportion of cases in under-5-year-olds, to better elucidate underlying trends in transmission. Data-driven methods to inform stratification and intervention targeting, and to understand trends in transmission from routine data, have the potential to enhance elimination efforts in Zambia. Here we present an example of this approach and discuss how these methods can be adopted by national malaria control programs.

1355

CHOOSING THE RIGHT TOOL FOR THE JOB: ESTIMATING EFFECT SIZES FOR MULTIPLE OVERLAPPING INTERVENTIONS IN SOUTHERN PROVINCE, ZAMBIA

Joshua G. Suresh[1], Jaline Gerardin[2], John Miller[3], Busiku Haimanza[4], Thom Eisele[5], Edward A. Wenger[1], Caitlin A. Bever[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Northwestern University Feinberg School of Medicine, Chicago, IL, United States,* [3]*PATH, Lusaka, Zambia,* [4]*National Malaria Elimination Centre, Lusaka, Zambia,* [5]*Tulane University, New Orleans, LA, United States*

Modern anti-malarial campaigns often layer multiple interventions, such as indoor residual spraying (IRS), distribution of insecticide-treated bed nets (ITN), mass drug campaigns (MDA), and deployment of community health workers (CHWs). However, when these interventions are implemented together with spatially varying coverages and timings, it can be difficult to disentangle intervention effect sizes and identify which interventions were most impactful in a given region. This issue becomes critical when comparing regions which, on the surface, received the same interventions but arrived at disparate clinical endpoints. Such is the case with the Lake Kariba region of Southern Zambia, an area which has benefited from IRS and ITN campaigns as well as a multi-armed MDA trial and deployment of CHWs from 2014 onwards. The different health facility catchment areas, though receiving similar intervention packages, experienced a wide range of clinical burden reduction levels. We address the question of disparate impact using detailed spatial simulations of the entire MDA trial area, calibrated to all available data sources. Drawing from survey data, we implement the timing and coverage for each intervention on a per-square-kilometer basis. We also implement data-informed migration patterns, treatment-seeking, and reactive case detection. Relative abundances of the two primary vectors, An. arabiensis and An. funestus, are determined by calibrating to observed parasite prevalence and clinical incidence using entomological data as a prior. We are able to attribute effect sizes to each intervention employed in each catchment, and draw systematic conclusions about how intervention impact varies with key environmental covariates. We also explore hypothetical scenarios to identify the optimal package to yield a high probability of interruption in different risk strata, including with new proposed interventions that target outdoor biting such as endectocides and ATSBs. Finally, we introduce a stratification scheme, with optimal intervention packages targeted towards clinical burden reduction suggested for each risk stratum.

1356

MASS DRUG ADMINISTRATION FOR MALARIA: AN UPDATED COCHRANE SYSTEMATIC REVIEW

Monica Shah[1], Jimee Hwang[2], Leslie Choi[3], S. Patrick Kachur[4], Meghna Desai[1]

[1]*Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*U.S. President's Malaria Initiative, Malaria Branch, Centers for Disease Control and Prevention; Global Health Group, University of California San Francisco, San Francisco, CA, United States,* [3]*Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [4]*Malaria Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; Heilbrunn Department of Population and Family Health, Columbia University, Mailman School of Public Health, New York, NY, United States*

There has been renewed interest in mass drug administration (MDA) with antimalarial drugs to accelerate progress towards malaria elimination. We assessed the effect of MDA compared to no MDA on the interruption of transmission in settings of very low-to-low endemicity and on the reduction in malaria transmission in settings of moderate-to-high endemicity. We searched multiple databases for relevant studies comparing the effect of MDA against no MDA on *Plasmodium falciparum* parasitaemia prevalence. Studies with balanced co-interventions across arms and at least two sites per arm were included. For cluster randomized-controlled trials (cRCTs), we estimated risk ratios (RR) and we adjusted 95% confidence intervals (CIs) for clustering. Analyses were stratified by malaria endemicity. We assessed the certainty of evidence using the Grading of Recommendations Assessment, Development and Evaluation approach. Nine cRCTs were included. Two trials in moderate-to-high transmission areas showed that MDA probably leads to little or no reduction in parasitaemia prevalence through 6 months post-MDA (RR 1.76, 95% CI 0.58-5.36 at 1-3 months post-MDA and RR 1.18, 95% CI 0.89-1.56 at 4-6 months; moderate certainty evidence). Due to very low certainty evidence from seven trials in very low-to-low transmission areas, we do not know if MDA has an effect on parasitaemia prevalence within 12 months post-MDA; the effects ranged from RR 0.12 (95% CI 0.03-0.52) at < 1 month post-MDA, RR 0.06 (95% CI 0.01-0.45) to 1.34 (95% CI 0.50-5.92) at 1-3 months, RR 0.07 (95% CI 0-1.31) to 0.89 (95% CI 0.27-2.95) at 4-6 months, and RR 0.08 (95% CI 0-1.36) to 1.55 (95% CI 0.77-3.13) at 7-12 months. In very low-to-low transmission settings, MDA initially had strong but heterogeneous effect on parasitaemia prevalence that waned over time, but we found no evidence of interruption of

transmission. MDA did not reduce malaria transmission in moderate-to-high transmission settings at any post-MDA time point. Additional results from non-randomized studies, for other primary outcomes, and for *P. vivax* will be presented.

1357

RESULTS OF A PILOT OF TARGETED MASS DRUG ADMINISTRATION WITH SULFADOXINE-PYRIMETHAMINE AND PRIMAQUINE AS A COMPONENT OF A MALARIA ELIMINATION PACKAGE IN HAITI

Michelle A. Chang[1], Daniel Impoinvil[1], Karen E. Hamre[1], Alain Javel[2], Paul-Emile Dalexis[2], Jean-Baptiste Mérilien[3], Amber M. Dismer[4], Bernadette Fouché[1], Emilie Pothin[5], Katherine Battle[6], Ewan Cameron[6], Kathleen Holmes[1], Luccene Desir[7], Gregory Noland[7], Alyssa Young[8], Justin Cohen[8], Willy Lafortune[3], Lotus van den Hoogen[9], Gillian Stresman[9], Chris Drakeley[9], Eric Rogier[1], Ruth Ashton[10], Thomas Druetz[10], Thomas P. Eisele[10], Jean Frantz Lemoine[3]

[1]*Malaria and Entomology Branches, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*IMA Worldhealth, Port-au-Prince, Haiti,* [3]*Programme National de la Contrôle de Malaria, Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti,* [4]*Emergency Response and Recovery Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [5]*Swiss Tropical Public Health Institute, Basel, Switzerland,* [6]*Malaria Atlas Project, Oxford, United Kingdom,* [7]*The Carter Center, Atlanta, GA, United States,* [8]*Clinton Health Access Initiative, Boston, MA, United States,* [9]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [10]*Center for Applied Malaria Research and Evaluation, Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

In Haiti, the prevalence of *Plasmodium falciparum (Pf)* malaria is <1%. Malaria Zero, an alliance of partners led by the Haitian National Malaria Control Program, has piloted a package of interventions to accelerate progress toward malaria elimination. The package includes: expanded access to malaria case management (CM), strengthened case-based surveillance (CBS), indoor residual spraying (IRS), and targeted mass drug administration (tMDA). After deploying CM and CBS in 5 pilot communes, we conducted tMDA and IRS in 12 high-transmission malaria *foci* at the start of the rainy season in 2018 (15 October-5 November). For tMDA, sulfadoxine-pyrimethamine (SP) and single low dose primaquine (PQ) were administered with direct observation to all eligible residents during household visits. Pharmacovigilance of adverse events (AEs) from SP/PQ occurred for 30 days following the tMDA campaign; 54 AE reports were completed with 0 reports of clinical hemolysis or Stevens-Johnson Syndrome. The IRS campaign used the insecticide pirimiphos-methyl capsule suspension. A baseline cross-sectional household survey was done prior to the campaign; post-campaign surveys are planned at 10 weeks, 6 and 12 months to assess acceptability and changes in parasite prevalence and serological markers of malaria exposure. The tMDA effective coverage was 89.5% (36,388 people treated of 40,618). Preliminary results from the 10 week post-campaign survey found that among 1,317 respondents, 134 people did not take the tMDA medications; the most common reasons were absenteeism, refusals, and pregnancy (58.0%, 14.2%, and 8.9%, respectively). Of 1,183 respondents who took the tMDA medicines, almost all (99.6%) were willing to take the medicines in future campaigns. *Pf* prevalence measured by a conventional and a highly sensitive rapid diagnostic test was lower at 10 weeks post-campaign (0.95% after rainy season, n=1,365) than at baseline (1.7% pre-rainy season, n=1,196). The pilot demonstrated the feasibility of achieving high coverage for tMDA with SP/PQ in a single round. Additional results for IRS, access to care, and serological changes will be presented.

1358

THE *MAGUDE PROJECT:* DRASTIC REDUCTION OF MALARIA BURDEN AND SUSTAINED GAINS AFTER A MALARIA ELIMINATION PROJECT IN SOUTHERN MOZAMBIQUE

Beatriz Galatas[1], Helena Martí-Soler[1], Caterina Guinovart[1], Lidia Nhamussua[2], Wilson Simone[2], Humberto Munguambe[2], Arlindo Chidimatembue[2], Júlia Montañà[1], Fabião Luis[2], Krijn Paaijmans[1], Quique Bassat[1], Alfredo Mayor[1], Clara Menéndez[1], Baltazar Candrinho[3], Regina Rabinovich[1], Pedro Alonso[1], Francisco Saúte[2], Pedro Aide[2]

[1]*ISGlobal, Barcelona, Spain,* [2]*CISM, Manhiça, Mozambique,* [3]*National Malaria Control Programme, Ministry of Health, Maputo, Mozambique*

The feasibility of malaria elimination using currently available tools in low transmission areas of sub-Saharan Africa remains unknown. A before-after study was conducted in Magude district, southern Mozambique to evaluate the impact of a package of interventions on malaria morbidity and inpatient admissions and deaths. Two years of two mass drug administration (MDA) rounds per year (Phase I, August 2015-17) followed by one year of reactive focal drug administrations (rfMDA) at household level (Phase II, September 2017 to June of 2018) with dihydroartemisinin-piperaquine were deployed in combination with annual rounds of indoor residual spraying (IRS), programmatically distributed bednets and standard case management. Parasite prevalence was monitored through yearly cross-sectional surveys. Routine malaria data were used to depict case incidence and inpatient admissions and mortality trends. An interrupted time series analysis using a generalized estimated equation with Poisson was used to estimate the level and trend change in malaria cases associated with the project interventions, and the number of cases averted. The four MDA rounds covered 58%-72.3% of the population, and annual IRS campaigns covered >70% of households. All-age parasite prevalence by rapid diagnostic test declined from 9.1% (95%CI 7.0-11.8) to 2.6% (95%CI 2.0-3.4) (71% reduction) after phase I, and to 1.5% (95%CI 1.0-2.4) after phase II (83% overall reduction). Case incidence fell from 195 cases per 1000 to 77 cases per 1000 after phase I (65% reduction), and to 31 per 1000 after phase II (84.1% overall reduction). Phase I interventions were associated with a significant level reduction (Adjß=0.31, 95%CI 0.23-0.43), and an estimated 76.7% (95%CI 76.4% -77.1%) of expected cases averted throughout the project. In conclusiom, MDA and vector control served as an accelerator to rapidly reduce malaria transmission, and rfMDA sustained the gains up to one year, but malaria was not eliminated.

1359

A *FOCI* COHORT ANALYSIS TO ASSESS MALARIA ELIMINATION IN THAILAND

Prayuth Sudathip[1], Julien Zwang[2], Rungrawee Tipmontree[1], Suravadee Kitcharkarn[1], Thannikar Thongrad[1], Felicity Young[3], Richard Reithinger[3], Jui A. Shah[2], David Sintasath[4], Preecha Prempree[1], Darin Areechokchai[1], Jersuda Kajanasuwan[1], Cheewanan Lertpiriyasuwat[1]

[1]*Bureau of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand,* [2]*Inform Asia: USAID's Health Research Program, RTI International, Bangkok, Thailand,* [3]*RTI International, Washington, DC, United States,* [4]*U.S. President's Malaria Initiative, Regional Development Mission for Asia, United States Agency for International Development, Bangkok, Thailand*

Thailand's malaria elimination strategy is based on the identification and classification of malaria cases, including by place of residence or geographical locality. Active *foci* are geographical localities with ongoing malaria transmission, compared to reverted *foci* that have recorded no malaria cases in the previous 12 months. The objective of this study was to assess the effectiveness of Thailand's *foci*-based malaria elimination strategy by following a cohort of active *foci* from 2014 through 2018. Reversion was assessed by Kaplan-Meier survival analysis, and the predictors of *foci* reversion were measured as adjusted hazard ratios

using Cox regression models. At the start of the study period, 3,425 active *foci* reporting 24,558 malaria cases were identified. From 2014 to 2017, the reactive case detection ratio increased by 73.0%, from 15.2 to 26.3 people screened per case. Foci and case investigation rates remained high, at over 90.0%. Coverage of vector control interventions, including insecticide-treated nets and indoor residual spraying, showed minimal increases. The number of active *foci* dropped to just 435 in 2018, representing an 87.3% decrease during the study period. Similarly, the number of confirmed malaria cases dropped to 3,107 in 2018. Multivariate analysis showed that smaller *foci* (defined as <10 cases per year) were more likely to revert compared to larger *foci* (93.6% versus 50.7%), as well as *foci* with smaller population size (defined as <100 people) and *foci* with a greater proportion of *P. falciparum* cases. Foci located in Sisaket, Tak, and Yala provinces were less likely to revert compared to other provinces. Thailand's *foci*-based malaria strategy, comprising strong national treatment guidelines, high investigation rates, and an increased reactive case detection ratio, is showing success in decreasing active *foci* and cases. Thailand's strategy and these encouraging results may be a useful example for other countries in the Greater Mekong sub-region as they progress towards malaria elimination.

1360

OVERCOMING BARRIERS TO LOW MALARIA TESTING RATES IN AN ELIMINATION CONTEXT: RESULTS FROM MIXED METHODS RESEARCH IN LAO PDR

Samuel Haddad[1], Malaykham Duangdara[1], Rebecca Potter[2], Saysana Phanalasy[1]

[1]*Population Services International, Vientiane, Lao People's Democratic Republic,* [2]*Population Services International, Laos, Lao People's Democratic Republic*

Lao PDR aims to eliminate malaria by 2030; yet, at 4.1%, the annual blood examination rate (ABER) falls short of the 10% benchmark set by WHO. The NMCP revised national treatment guidelines in 2018 with an aim to boost testing rates. PSI Laos supports a network of 525 private sector providers, which detected 15% of the national malaria caseload in 2018. PSI conducted mixed methods research to better understand barriers to malaria testing among private providers. Qualitative and quantitative research was used to generate evidence about provider motivation, and a mystery client survey was conducted to document observations about provider behaviors and adherence to guidelines. These data were used to develop strategies for improving adherence to national guidelines. Routine surveillance data were analyzed pre- and post-intervention to estimate the impact on testing rates. Mystery client survey data showed that ~35% of clients presenting to a private provider with fever symptoms received an RDT, compared to 99% of suspected cases reported to receive RDTs through routine data. Qualitative analysis of provider motivation data revealed that private providers valued professional development opportunities, being part of a community of peers, and contributing to malaria elimination in their communities. These insights were integrated into provider behavior change communication (PBCC) interventions carried out by field officers providing monthly supportive supervision beginning in May 2018. Prior to the roll-out of new testing guidelines and PBCC intervention, private sector providers in PSI's network tested an average of 6.01 clients per month. Post-intervention, providers conducted an average of 10.29 tests per month. Sustaining provider motivation to maintain testing in low-burden environments remains a significant challenge for malaria elimination. Understanding provider motivations and developing strategies based on insights can improve testing rates. Complementary research on provider behaviors in public sector channels is needed to inform national strategies for improving provider adherence to national guidelines.

1361

EVALUATION OF EXTENDED EFFICACY OF TETRAVALENT CHIMERIC YELLOW FEVER-DENGUE (CYD) VACCINE AGAINST SYMPTOMATIC AND SUBCLINICAL DENGUE VIRUS INFECTIONS AMONG FILIPINO CHILDREN

Alan L. Rothman[1], Mary N. Chua[2], Mary T. Alera[3], Henrik Salje[4], Damon Ellison[5], Anon Srikiatkhachorn[1], Richard G. Jarman[5], In-Kyu Yoon[6], Louis R. Macareo[6]

[1]*University of Rhode Island, Providence, RI, United States,* [2]*Chong Hua Hospital, Cebu City, Philippines,* [3]*Philippines-Armed Forces Institute of Medical Sciences Virology Research Unit, Cebu City, Philippines,* [4]*Institut Pasteur, Paris, France,* [5]*Walter Reed Army Institute of Research, Washington, DC, United States,* [6]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

Dengvaxia, the tetravalent chimeric yellow fever-dengue (CYD) live attenuated virus vaccine, has been licensed for the prevention of symptomatic dengue in several dengue-endemic countries. However, vaccine implementation has been limited due to safety concerns and uncertainty about long-term benefit. In the pivotal phase III trials of CYD, active surveillance for symptomatic dengue was limited to 25 months, and only a subset of the cohort was evaluated for subclinical dengue virus (DENV) infections. We recruited 611 participants in the CYD trial in Cebu, the Philippines, into an ancillary study to further evaluate vaccine immunogenicity and efficacy. Ancillary study subjects were followed by active surveillance for over 5 yrs, with annual blood sampling. Symptomatic DENV infections were detected by RT-PCR and serology on acute/convalescent blood samples from febrile illnesses, and subclinical infections were detected based on a 4-fold or greater rise in neutralizing antibody titer in annual blood samples (from month 25 onward). During the study period, DENV2 was the predominant serotype in placebo recipients, followed by DENV1, DENV3, and DENV4. As observed in the overall trial, CYD vaccine had ~50% efficacy in the ancillary study cohort during the first 2 yr (up to 13 months post-vaccination). From year 3 on, CYD vaccine showed no efficacy against symptomatic dengue. Vaccine efficacy against subclinical infection was ~45% in year 2 but decreased in years 3 and 4. These data suggest that CYD vaccine efficacy may be relatively short-lived, even in a cohort with high baseline DENV seropositivity. The small sample size and predominance of DENV2 limit the interpretation of our data, however. Extended evaluations of the efficacy of this and other dengue vaccines will be important to guide appropriate utilization.

1362

EFFICACY OF A TETRAVALENT DENGUE VACCINE IN HEALTHY 4 TO 16 YEAR-OLD CHILDREN

Shibadas Biswal, TIDES Study Group

Takeda Vaccines Pte Ltd, Singapore, Singapore

Dengue, a mosquito-borne viral disease, is a WHO top ten threat to global health in 2019. There is an important unmet medical need for a safe and effective vaccine, particularly for dengue-naïve individuals. We present key efficacy data from Parts 1 and 2 of an ongoing phase 3 randomized trial of a tetravalent dengue vaccine candidate (TAK-003) in 8 endemic countries of Asia and Latin America. Healthy 4-16 year-old children were randomized 2:1 (stratified by age range and region) to receive two doses of vaccine or placebo three months apart. Participants presenting with febrile illness were tested for virologically-confirmed dengue (VCD) by serotype-specific RT-PCR. The primary endpoint was efficacy in preventing VCD induced by any dengue serotype occurring from 30 days post-second vaccination until the end of Part 1. The secondary efficacy endpoints were efficacy against individual serotypes, by baseline serostatus, and efficacy in prevention of dengue leading to hospitalization and severe dengue after end of Part 2. Part 1 was complete once 120 VCD cases were confirmed for primary endpoint analysis and participants had a minimum follow-up of 12 months post-second vaccination. Part 2 lasted for another 6 months. Of 20,071 children given at least one dose of vaccine or placebo, 19,021

were included in the per protocol analysis. The primary and secondary efficacy endpoints will be presented along with safety and immunogenicity data.

1363

PREEXISTING IMMUNITY ENHANCES RECRUITMENT AND INFECTION OF MYELOID CELLS WITH DENGUE AND ZIKA VIRUSES IN HUMAN SKIN

Priscila Mayrelle da Silva Castanha, Parichat Duangkhae, Geza Erdos, Simon C. Watkins, Louis D. Falo Jr, Ernesto T. Marques Jr, Simon M. Barratt-Boyes

University of Pittsburgh, Pittsburgh, PA, United States

Dengue (DENV) and Zika virus (ZIKV) infections in humans are likely subject to antibody-dependent enhancement (ADE), but the precise mechanism of ADE *in vivo* remains to be determined. Human skin is the primary site of virus replication after inoculation by an infected mosquito, making skin a potentially important site for ADE of infection in immune individuals. We used full-thickness human skin explants to determine whether preexisting immunity to one DENV serotype enhanced infection of a different DENV serotype or ZIKV. Monotypic DENV-immune sera were introduced into skin using microneedle arrays before inoculation with homologous or heterologous DENV or ZIKV. Skin explants were analyzed by confocal microscopy using antibodies to cell-specific markers and NS3 protein. As expected, inoculation of DENV-3 into skin pretreated with DENV-3 immune sera resulted in inhibition of virus replication in epidermis and dermis. In contrast, inoculation of skin with DENV-2 pretreated with DENV-3 immune sera resulted in a dose-dependent increase in infected cells in the dermis, reaching a 3-fold boost at the highest concentration of immune sera. When compared to infection of skin pretreated with naïve sera, 1,000 times more virus was needed to compensate for the effect of immune sera on the density of infected cells in dermis. Real-time quantitative PCR confirmed increased viral RNA copies/mg of tissue in the dermis of skin inoculated in the presence of immune relative to naïve sera. Within the dermis, ADE was mediated by increased recruitment, infection and migration of Langerhans cells, macrophages and dermal dendritic cells. Simultaneous blockage of FcγR CD32 and CD64 inhibited ADE in the dermis. Inoculation of ZIKV into skin pretreated with DENV-3 immune sera also lead to increased virus replication in the dermis, which was primarily mediated by increased infection of Langerhans cells and dermal dendritic cells, with minimal effect of macrophages. Collectively, our findings reveal that ADE readily occurs in human skin, primarily within the dermis, and highlights the importance of this site in initiating and supporting ADE of DENV and ZIKV infection.

1364

PREVIOUS ZIKA EXPOSURE IMPROVES IMMUNE RESPONSE AGAINST DENGUE WITHOUT PATHOGENESIS ENHANCEMENT IN RHESUS MACAQUES

Erick X. Pérez-Guzmán[1], Petraleigh Pantoja[1], Crisanta Serrano-Collazo[1], Mariah A. Hassert[2], Alexandra Ortiz[3], Idia V. Rodriguez[1], Luis Giavedoni[4], Vida Hodara[4], Laura Parodi[4], Lorna Cruz[1], Teresa Arana[1], Laura White[5], Melween I. Martinez[1], Daniela Weiskopf[6], James D. Brien[2], Aravinda de Silva[5], Amelia Pinto[2], Carlos A. Sariol[1]

[1]*University of Puerto Rico Medical Sciences Campus, San Juan, PR, United States,* [2]*St. Louis University School of Medicine, St. Louis, MO, United States,* [3]*University of Puerto Rico Rio Piedras Campus, San Juan, PR, United States,* [4]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [5]*University of North Carolina, Chapel Hill, NC, United States,* [6]*La Jolla Institute for Immunology, La Jolla, CA, United States*

The role of a previous Zika virus (ZIKV) immunity on subsequent Dengue virus (DENV) infections is poorly understood. This is relevant to anticipate the dynamics of forthcoming DENV epidemics in areas with previous ZIKV exposure. It is still uncertain if the immunity conferred by the recent ZIKV epidemic may contribute to protection or worsening DENV cases severity. To test this, three cohorts of rhesus macaques were infected with DENV-2 after no exposure (naïve group), 2 month (early-convalescence) or 10 month (middle-convalescence) after exposure to different contemporary ZIKV strains. We assessed DENV viremia, cytokine profile, neutralizing antibody response against multiple ZIKV strains and all DENV serotypes, and the activation and polyfunctional response of T cell subpopulations up to 90 days after DENV infection. Our results showed that a subsequent DENV infection in animals with early- and middle-convalescent periods to ZIKV do not promote an increase in DENV viremia nor pro-inflammatory status. We found that previous ZIKV exposure improves the neutralizing antibody and T cell responses against DENV and that the time interval between infections impacts the magnitude and durability—more efficient after longer ZIKV pre-exposure—of the immune response. Furthermore, our data suggest that the elicited immune modulation between both ZIKV-immune groups after DENV infection are more influenced by the time elapsed between ZIKV and DENV infections and the maturation of the cross-reactive immune memory, rather than a possible effect due to ZIKV strain variation. Collectively, our findings provide evidence of a non-detrimental effect of ZIKV immunity in a subsequent DENV infection. This supports the implementation of ZIKV vaccines that could also improve immunity against future DENV epidemics.

1365

CROSSREACTIVITY OF CD8+T CELL RESPONSES AMONG FLAVIVIRUSES AFTER DENV OR YF VACCINATION

Alba Grifoni[1], Hannah Voic[1], Aruna D. de Silva[2], Anna Durbin[3], Stephen Whitehead[4], Sean A. Diehl[5], Eva Harris[6], Alessandro Sette[1], Daniela Weiskopf[1]

[1]*La Jolla Institute for Immunology, La Jolla, CA, United States,* [2]*Kotelawala Defense University, Ratmalana, Sri Lanka,* [3]*Johns Hopkins University, Baltimore, MD, United States,* [4]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [5]*University of Vermont, School of Medicine, Burlington, VT, United States,* [6]*University of California, Division of Infectious Diseases and Vaccinology, School of Public Health, Berkeley, CA, United States*

Several flaviviruses, including Dengue Virus (DENV), Zika Virus (ZIKV), Japanese Encephalitis Virus (JEV), West Nile Virus (WNV), and Yellow Fever Virus (YFV), share significant sequence homology and often circulate in the same geographical regions. Significant levels of cross-reactivity could in turn result in pre-existing T cell immunity modulating T cell responses to subsequent flavivirus infections before or after vaccination. Whether and to what extent cross-reactivity at the level of CD8 responses is detected is currently unclear. To address this question, we stimulated PBMC of individuals vaccinated with DENV or YF with designed pools of epitopes and predicted HLA binding peptides derived from the different flavivirus (DENV, ZIKV, JEV, WNV and YFV) to assess their potential to recall antigen specific CD8 memory T cell response in Intracellular Cytokine Staining (ICS) assay. Significant cross-reactivity of CD8 T cell responses against several of the pools was observed both in the case of DENV and YF vaccinees, but the extent of cross-reactivity varied as a function of the flavivirus species considered, and the cross-reactive responses were significantly lower than the responses to the autologous virus. Phenotypic analyses showed a suboptimal expression of activation markers in cross-reactive responses. When the cross-reactive responses are characterized at the single epitope level in monovalent DENV vaccinees, the T cells are able to cross-react mainly across different DENV serotypes (and occasionally ZIKV) but not with other flavivirus included YFV. We are in the process to characterized crossreactivity at the single epitope level in YFV vaccinees, being YFV backbone frequently used to develop novel Flavivirus vaccination. Characterization of the extent and functionality of CD8 cross-reaction across different flaviviruses will contribute to the understanding of immunity in the natural infection, and has particular implications for vaccine design, efficacy and safety in endemic settings.

1366

VACCINATION AND PRIOR DENGUE EXPOSURE CORRELATE WITH VIREMIA LEVEL AMONG SYMPTOMATIC DENGUE 1 AND DENGUE 2 INFECTIONS IN COHORT SUBJECTS IN THE PHILIPPINES

Simon Pollett[1], Maria Alera[2], Wiriya Rutvisuttinunt[1], Anon Srikiatkhachorn[3], Abhinaya Srikanth[1], In-Kyu Yoon[4], Irina Maljkovic Berry[1], Damon Ellison[5], Louis Macareo[5], Alan L. Rothman[3], Richard G. Jarman[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Armed Forces Institute of Medical Sciences Virology Research Unit, Cebu, Philippines,* [3]*University of Rhode Island, Providence, RI, United States,* [4]*International Vaccine Institute, Seoul, Republic of Korea,* [5]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

The chimeric yellow fever-dengue vaccine (CYD, Dengvaxia) reduces the risk of symptomatic dengue in DENV-experienced hosts, but its effect on breakthrough infection viral load is unclear. We leveraged an ancillary study cohort of 584 subjects from Cebu, the Philippines, who had received placebo or CYD in the phase III CYD vaccine trial. Ancillary study subjects underwent active surveillance for symptomatic dengue for >5 yrs after the first vaccine dose. Confirmed DENV cases underwent viremia quantification by qPCR to determine any association between CYD receipt and DENV viral load. Baseline serostatus was determined by DENV PRNT and/or NS1 ELISA. There were 17 DENV-1, 40 DENV-2, 6 DENV-3, and 6 DENV-4 infections sampled. In the CYD arm there were 10 DENV-1 and 25 DENV-2 confirmed infections, of which 3 and 12 were DENV seropositive at baseline, respectively. In the placebo group there were 7 DENV-1 and 15 DENV-2 infections, of which 6 and 14 were DENV seropositive at baseline, respectively. The remaining DENV-1 and DENV-2 cases were seronegative or seroindeterminate at baseline. CYD receipt was associated with 1.16 log10 GE/mL (p=0.21) and 0.34 log10 GE/mL (p=0.55) lower geometric mean DENV-1 and DENV-2 viremia levels, respectively, when compared to placebo-recipients. Among those with prior dengue exposure, there was a 2.19 log10 GE/mL lower mean DENV-1 viremia level (p=0.19) and a 1.09 log10 GE/mL (p=0.1) lower mean DENV-2 viremia level in CYD vs. placebo recipients. Among CYD-recipients who experienced breakthrough infections, there was a 1.86 log10 GE/mL higher mean DENV-1 viremia level in those with no history of dengue exposure compared to those seropositive at baseline (p = 0.17), and a 1.63 log10 GE/mL higher mean DENV-2 viremia level in subjects with no history of prior DENV exposure compared to those with baseline DENV seropositivity (p = 0.03). Collectively our findings suggest that Dengvaxia receipt may reduce the infecting viral load of breakthrough dengue infections, but that this association is likely modified by host dengue exposure history. These findings prompt further analyses of a larger sample size pending from this cohort.

1367

PRIOR VACCINATION WITH CYD-TDV DID NOT POTENTIATE SYMPTOMATIC ZIKA IN DENGUE ENDEMIC AREAS OF LATIN AMERICA

Betzana Zambrano[1], Doris Maribel Rivera[2], José Luis Arredondo[3], Humberto Reynales[4], Kleber Luz[5], Carmen Deseda[6], Matthew Bonaparte[7], Edith Langevin[8], Yukun Wu[7], Margarita Cortes[9], **Gustavo H. Dayan**[7], Carlos Diaz Granados[7], Stephen Savarino[7], Fernando Noriega[7]

[1]*Sanofi Pasteur, Montevideo, Uruguay,* [2]*Inversiones en Investigación Médica, Tegucigalpa, Honduras,* [3]*Instituto Nacional de Pediatría, Mexico City, Mexico,* [4]*Centro de Atención e Investigación Médica, Bogota, Colombia,* [5]*Universidade Federal do Rio Grande do Norte, Natal, Brazil,* [6]*Caribbean Travel Medicine Clinic, San Juan, Puerto Rico,* [7]*Sanofi Pasteur, Swiftwater, PA, United States,* [8]*Sanofi Pasteur, Marcy L'Etoile, France,* [9]*Sanofi Pasteur, Bogota, Colombia*

An unprecedented Zika outbreak began in Latin America in 2015, including in the countries where Sanofi Pasteur's Phase III Dengue efficacy trial (CYD15, NCT01374516) was being conducted. The current analysis describes data on virologically-confirmed Zika (VCZ) episodes by study group in CYD15. This observational objective, added as an amendment to the CYD15 protocol, assessed the impact of dengue vaccination on the occurrence and clinical features of Zika disease in trial participants aged 9-16 years recruited in Brazil, Puerto Rico, Mexico, Honduras and Colombia and still enrolled in the study over the November 2015-March 2018 period (end of the trial). Acute serum samples from febrile (38°C for 2 consecutive days) subjects that had been collected for Dengue virological confirmation were also tested for Zika RNA (RT-PCR) detection. Dengue Anti-NS1-IgG ELISA was performed in all subjects from a sub-cohort (10% of the entire cohort) and in VCZ cases, 1 month after last trial injection to assess Dengue basal serostatus. Relative risk (RR) was calculated as the ratio of the VCZ episodes between CYD-TDV (Dengvaxia®) and Control groups (randomized in a 2:1 CYD-TDV:placebo ratio). When the analysis was stratified by dengue serostatus, a case-cohort design was used to estimate the Hazard Ratio (HR) from a weighted Cox regression. A total of 10,157 acute febrile samples were tested, 239 (2.4%) samples showed Zika virological-confirmation, 152 (2.3%) episodes from the CYD-TDV group (one subject had 2 episodes) and 87 (2.4%) from the Control group. Results indicated no increased risk in the CYD-TDV group as a whole (RR 0.86 [95%CI 0.65;1.13]). When focusing on Dengue seropositive subjects at baseline, 106 VCZ cases were observed in the CYD-TDV group and 68 in the control group (HR 0.72 [95%CI 0.52;1.00]). Our data indicate that prior vaccination with CYD-TDV does not increase the risk of symptomatic Zika and may protect dengue seropositive vaccinees from subsequent symptomatic Zika infection.

1368

TRACKING MOSQUITOES OVER TIME: TESTING THE ROLE OF AESTIVATION IN DRY SEASON PERSISTENCE

Roy Faiman[1], Adama Dao[2], Alpha S. Yaro[2], Moussa Diallo[2], Djibril Samake[2], Zana L. Sonogo[2], Yossi Ousmane[2], Margery Sullivan[1], Laura Veru[1], Benjamin J. Krajacich[1], Joy Matthews[3], Christine A. France[4], Gabriel Hamer[5], Leland Graber[1], Tovi Lehmann[1]

[1]*National Institutes of Health, Rockville, MD, United States,* [2]*Malaria Research and Training Center, Faculty of Medicine, Bamako, Mali,* [3]*University of California Stable Isotope Facility, Davis, CA, United States,* [4]*Smithsonian Institution Museum Support Center, Suitland, MD, United States,* [5]*Texas A&M University, College Station, TX, United States*

Despite its recognized importance, tracking mosquitos over extended time has been beyond medical entomology's tool kit. Stable isotope enrichment of mosquito breeding sites enables marking of mosquitoes without human handling. Mosquito larvae developing in [^{2}H]- enriched water are structurally marked for life, providing a unique opportunity to test the hypothesis that *Anopheles coluzzii*, but not *A. gambiae* s.s and *A. arabiensis*, locally persists in the Sahel through the dry season via aestivation. A large-scale experiment to test this hypothesis began in September 2017 in two Sahelian villages in Mali. We aimed to estimate the contribution of aestivation to persistence of mosquitoes through the 7-month long dry season by marking at least 50% of the *A. gambiae* s.l. adults by the end of the wet season and assess the proportion of marked adults through the dry season, and immediately after the first rain in June 2018. If aestivation is the only way *A. coluzzii* persists, the frequency of marked mosquitoes should be similar throughout. Finding no marked mosquitoes would be evidence against aestivation. Twenty-four natural larval sites were enriched from late September. The marked adult proportion by the end of the wet season was above 60%. Five months later a similar frequency of marked adults was detected. Notably, after the first rain, nearly 8 months after the onset of the dry season, 6% showed clear enrichment. All the marked mosquitoes detected after the onset of the DS were *A. coluzzii*, in accord with aestivation in this species alone. Because ^{2}H-marking in mosquitoes has steadily weakened over time, we suspect some of the seemingly un-enriched mosquitoes had lost their marking. Accordingly, we find that the distribution of ^{2}H in mosquitoes after the first rain exhibits an exceptionally heavy right tail, indicating that in addition to naturally un-enriched mosquitoes there are many enriched

mosquitoes that lost much of their enrichment but are clumped in that zone. Our results provide, for the first time, hard evidence of population-wide aestivation in *A. coluzzii* alone, supporting annual malaria resurgence shortly after the wet season onset.

1369

INVESTIGATING THE MOLECULAR PLASTICITY OF *AEDES ALBOPICTUS* IN RESPONSE TO ZIKA VIRUS INFECTION UNDER INCREASED TEMPERATURES

Maria G. Onyango, Sean Bialosuknia, Anne Payne, Mathias Nicholas, Lilli Kuo, Alexander Ciota, Laura D. Kramer

Wadsworth Centre, New York State Department of Health, Slingerlands, NY, United States

A rapid and significant range expansion of both Zika virus (ZIKV) and its *Aedes* vector has resultedin the virus being declared a global health threat. In spite of this, significant knowledge gaps exist on the interactions of ZIKV and *Aedes*. Mean temperatures are projected to increase globally, likely resulting in alterations of the transmission potential of mosquito-borne pathogens. Consequently, it is crucial to understand how future increase in temperature will alter ZIKV transmission. To investigate how ZIKV impacts its host physiology, especially under increased heat conditions, cDNA libraries were generated from midguts of ZIKV-infected female *Ae. albopictus*that were hatched and reared at Day 30°C; Night 26°C or Day 28°C; Night 24°C temperature regimes. The libraries were created from individuals held at both temperature regimes for 7 days after infectious or non-infectious blood meals. The resulting Illumina sequence reads were mapped to the *Ae. albopictus*genome, from which we detected the expression of 805 known genes and additional 1263 clusters not located within the predicted genes. Individuals infected with ZIKV presented a strong differential expression in genes and pathways involved in odorant reception [Log fold change 6; FDR P(7.19E-03)], signal transduction [Log fold change -3; FDR P(1.76E-03)] and sex-limited genes [Log fold change 2; FDR P(1.83E-04)] when compared to non-infected insects. Individuals exposed to higher temperatures showed a significant differential expression in genes related to chitin metabolism [Log fold change -8; FDR P(6.09E-12)] when compared to individuals reared at the lower temperatures. Individuals that were exposed to both higher temperatures and ZIKV infection presented a differential expression of genes important for virus transmission and those which are associated with host seeking biology of the mosquito. These gene sets form a relevant pool of candidates that could be further investigated as novel biological control tools.

1370

THERMAL ECOLOGY OF MALARIA TRANSMISSION AND THE POTENTIAL IMPACT OF BEHAVIORAL RESISTANCE

Eunho Suh[1], Marissa K. Grossman[1], Jessica L. Waite[1], Ellie Sherrard-Smith[2], Thomas S. Churcher[2], Matthew B. Thomas[1]

[1]*Pennsylvania State University, University Park, PA, United States,* [2]*Imperial College London, London, United Kingdom*

A number of studies report changes in the biting time of malaria mosquitoes following the introduction of long-lasting insecticide-treated bed nets (LLINs). Here, we explored whether timing of blood feeding interacts with environmental temperature to influence the vector competence of *Anopheles* mosquitoes for the human malaria parasite, *Plasmodium falciparum*. We found no effect of biting time on the proportion of mosquitoes that became infectious at constant temperature. However, the addition of realistic daily temperature fluctuation reduced the vector competence of mosquitoes feeding in the morning and increased the competence of those feeding in the early evening. A transmission dynamics model illustrates that such changes could have important implications for the epidemiological impact of "behavioral resistance". A shift in mosquito biting to the morning could reduce the transmission probability, and so poses little epidemiological risk. However, an increase in early evening biting could increase transmission not only because people are unprotected by bed nets, but also because there is a higher chance of blood-feeding mosquito becoming infectious.

1371

A NEW BIO-ASSAY TO MEASURE MOSQUITO MORTALITY AND OUTDOOR BITE PREVENTION STRATEGIES UNDER SEMI FIELD CONDITIONS

Mgeni M. Tambwe, Sarah Moore, Jason Moore, Hassan Chilumba, Adam Saddler

Ifakara Health Institute, Bagamoyo,Tanzania, United Republic of Tanzania

Aedes aegypti is the primary vector of several Arboviral diseases and is primarily controlled through larval source reduction, and space spraying, which are costly and logistically difficult to implement. Previous studies demonstrated that the volatile pyrethroids transfluthrin and metofluthrin are effective in preventing *Aedes aegypti* bites. We developed a long-acting transfluthrin passive emanator and evaluated its effect to prevent bites and kill *Aedes aegypti* mosquitoes over six months in an outdoor environment. Two evaluations were performed 1) in a large cage semi-field system (SFS) and 2) in the Large Ifakara-Ambient Chamber Test (LI-ACT). Both experiments were conducted for three hours using laboratory-reared *Aedes aegypti*. Two emanators (each treated with 3g of transfluthrin) were placed 6 m apart and human volunteer sat between them. The SFS experiment was conducted at 0, 3 and 6 months in a 21 x 9 x 4.5 m compartment to measure bite prevention by human landing catches (HLC). The LI-ACT measured feeding inhibition and mosquito mortality inside a large 20 x 4 x 4 m contiguous chamber that allows recapture of all released mosquitoes. In this experiment, HLC wasn't performed but a volunteer rested in the chamber. All mosquitoes were recovered scored as alive/dead, fed/unfed after 24 hours holding. In the SFS, significantly fewer *Aedes aegypti* were captured landing in the treatment compared to the control at month 0 and 6. In the LI-ACT, mosquitoes were inhibited from feeding and were more likely to die 24 hours later compared to the control. LI-ACT experiments are ongoing to measure the duration of effect. This is the first time that Transfluthrin passive emanators have been demonstrated to provide both personal and community protection against *Aedes aegypti* mosquitoes for up to six months. The devices reduce the ability of mosquitoes to feed and cause delayed mortality. These devices show promise as a public health intervention for outdoor and day-biting *Aedes aegypti* mosquitoes in the urban environment.

1372

VECTOR CHIP: A MINIATURIZED PLATFORM FOR HIGH-THROUGHPUT INTERROGATION OF MOSQUITO-PATHOGEN DYNAMICS

Shailabh Kumar, Felix Hol, Manu Prakash

Stanford University, Stanford, CA, United States

Curbing the spread of mosquito-borne diseases requires intimate knowledge of the dynamics of mosquitoes and the pathogens they transmit. There is a pressing need to develop field-deployable platforms which can provide this information in a geographical and temporal context in a high-throughput manner. Current laboratory and field approaches to interrogate mosquito-pathogen communities, are severely limited in throughput preventing a detailed understanding of mosquito-borne disease dynamics. To overcome current limitations, we exploit the fact that biting mosquitoes transmit pathogens by expectorating saliva, to autonomously collect saliva droplets resulting from single mosquito bites. We have developed a miniaturized platform which enables on-chip arrayed analysis of individual saliva droplets for high-throughput dissection-free characterization of the genetic make-up of the biting mosquito, and the pathogens it transmits. We demonstrate a lab-based proof of concept to screen populations of *Aedes aegypti* mosquitoes using this strategy, and are currently optimizing the platform to enable large-scale field deployment across several geographical locations. The vector chip

platform holds the potential to reveal yet unknown mosquito-pathogen interactions, serve as a local early warning system for mosquito-borne diseases, and can be optimized for other biting insects.

1373

ACTIVATION OF MOSQUITO IMMUNITY BLOCKS THE DEVELOPMENT OF TRANSMISSION-STAGE FILARIAL NEMATODES

Abigail R. McCrea, Elizabeth B. Edgerton, Corbett T. Berry, Yukwah Kwok, Brittany Watson, Letitia K. Thompson, Thomas J. Nolan, James B. Lok, **Michael Povelones**

University of Pennsylvania, Philadelphia, PA, United States

Mosquito-borne helminth infections are responsible for a significant worldwide disease burden in both humans and animals. Accordingly, development of novel strategies to reduce disease transmission by targeting these pathogens in the vector are of paramount importance. We found that a strain of *Aedes aegypti* that is refractory to infection by *Dirofilaria immitis*, the agent of canine heartworm disease, mounts a stronger immune response during infection than does a susceptible strain. Based on this finding, we hypothesized that activating a stronger immune response in the susceptible strain would render them refractory. Using a novel assay that we developed to measure the number of transmission-stage parasites capable of emerging from individual mosquitoes, we tested the effect of activating the Toll and IMD immune pathways using RNAi silencing of inhibitory proteins. Excitingly, we found that activation of the Toll immune signaling pathway in the susceptible strain arrests larval development, thereby decreasing the number of transmission-stage larvae. Co-silencing Rel1, a transcription factor that mediates Toll signaling, rescued development of transmission-stage larvae showing that the effect was specific. Importantly, this strategy also blocks transmission stage *Brugia malayi*, an agent of human lymphatic filariasis. Our data show that mosquito immunity can play a pivotal role in restricting filarial nematodes and suggest that genetically engineering mosquitoes with enhanced immunity will help reduce disease transmission.

1374

DIFFERENTIAL CONTRIBUTION OF *ANOPHELES* VECTORS TO MALARIA TRANSMISSION IN TWO NEIGHBORING VILLAGES IN THE RURAL COMMUNE OF ANDRIBA, MADAGASCAR

Jessy Marlene Goupeyou-Youmsi[1], Tsiriniaina Rakotondranaivo[2], Mandaniaina Radotiana Andriamiarimanana[2], Tsikiniaina Rasoloharimanana[2], Nicolas Puchot[2], Rado Lalaina Rakotoarison[2], Emma Rakotomalala[2], Romain Girod[2], Mamadou Ousmane Ndiath[2], Ines Vigan-Womas[2], Catherine Bourgouin[3]

[1]*University of Malawi College of Medicine, Blantyre, Malawi,* [2]*Institut Pasteur de Madagascar, Antananarivo, Madagascar,* [3]*Institut Pasteur, Paris, France*

In a region of moderate to high malaria transmission, the prevalence of malaria cases in two nearby villages with apparent similar pattern was significantly different. To try deciphering the reason for this difference, a multidisciplinary study including entomology, parasitology and immunology was conducted. Human and mosquito populations were targeted in order to evaluate *Plasmodium* carriage using real-time PCR. Rapid diagnostic tests (RDTs) and microscopy were also used for rapid assessment of *Plasmodium* carriage in the human population. In addition, prevalence and specificity of antibodies against *Plasmodium* antigens were determined using a multiplex bead-based serological assay. The overall human malaria prevalence among 590 samples was 8.0% by RDT, 4.8% by microscopy and 12.2% by PCR. Malaria infection cases were due to *P. falciparum* (84.3%), *P. vivax* (5.7%) and *P. malariae* (1.4%), with 8.6% of mixed infections. Serological makers for parasite and vector exposure perfectly matched with the human malaria prevalence variations in both villages (no significant difference), with an increase at the middle of the transmission season. Among 1553 anopheline mosquitoes collected by Human Landing Catches, three anopheline species were found carrying *Plasmodium* sporozoites by Taqman qPCR: *An. funestus* (n=3), *An. coustani* (n=7) and *An. arabiensis* (n=3). No significant difference on *Plasmodium* carriage in mosquitoes was observed between the two villages (SI=0.82% in the village of Ambohitromby and SI=1.15% in the village of Miarinarivo). However, the Entomological inoculation rate (EIR) shows that *An. arabiensis* had the major contribution in malaria transmission in Ambohitromby (EIR=0.10 infective bite/man/night), while it was *An. coustani* (EIR=0.17 ib/m/n) that contributed more in Miarinarivo. Different contribution of vectors in malaria transmission between the two villages has been observed despite the similarity of human malaria prevalence. This study demonstrates the variability of *Anopheles* vector dynamics that can be useful to better describe epidemiology and malaria transmission in Madagascar.

1375

CHILD DEATHS CAUSED BY *KLEBSIELLA PNEUMONIAE* IN KENYA: FINDINGS FROM THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS)

Jennifer R. Verani[1], Victor Akelo[2], Dianna M. Blau[3], Aggrey Igunza[4], Gunturu Revathi[5], Florence Murila[6], Magdalene Kuria[1], Emily Rogena[6], Paul Mitei[7], Emily Zielinski-Gutierrez[1], Bernard Ogony[4], Elizabeth Oele[8], Samuel Omondi[9], Pratima Raghunathan[10], Clayton Onyango[2], Marc-Alain Widdowson[1], Beth A. Tippett Barr[2], Dickens Onyango[8], Robert F. Breiman[11]

[1]*Centers for Disease Control and Prevention, Nairobi, Kenya,* [2]*Centers for Disease Control and Prevention, Kisumu, Kenya,* [3]*Centers for Disease Control and Prevention and Emory Global Health Institute, Emory University, Atlanta, GA, United States,* [4]*Kenya Medical Research Institute, Kisumu, Kenya,* [5]*Aka Khan University, Nairobi, Kenya,* [6]*University of Nairobi, Nairobi, Kenya,* [7]*Kisumu Specialists Hospital, Kisumu, Kenya,* [8]*Kisumu County Department of Health, Kisumu, Kenya,* [9]*Siaya County Department of Health, Siaya, Kenya,* [10]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [11]*Emory Global Health Institute, Emory University, Atlanta, GA, United States*

Klebsiella pneumoniae (Kp) is as an important cause of nosocomial infections, characterized by increasing antimicrobial resistance and high case fatality. Limited data are available on illness and death due to nosocomial or community-acquired Kp, particularly in resource-poor settings. We analyzed data from the Child Health and Mortality Prevention Surveillance (CHAMPS) in Kenya to characterize child deaths attributed to Kp. CHAMPS actively identifies deaths among children aged <5 years in an urban and rural area in western Kenya. Parents of deceased are interviewed and medical records reviewed. Minimally invasive tissue sampling is conducted, followed by extensive etiologic testing (microbiologic, molecular, and histologic), and review by two pathology teams. Cases are reviewed by an expert panel to determine immediate, comorbid, and underlying causes of death. Among 142 analyzed deaths from 05/2017 to 03/2018, Kp contributed to 21 (15%), including 18 (86%) as the immediate cause of death, and 3 (14%) as comorbid condition; other immediate causes of death were cerebral malaria (n=1), pneumococcal sepsis (n=1) and head injury (n=1). The most common underlying cause in Kp deaths was malnutrition (n=7, 33%). Kp was isolated from blood and/or cerebrospinal fluid of 11 (52%) cases; the remainder had molecular and/or immunohistochemical evidence of infection in tissues. The median age of Kp deaths was 4 months (range 1 day to 31 months), and 36% were male. Death occurred in a health facility for 13 (62%); median duration of admission before death was 5 days (range 0 to 38 days). Among facility deaths, 12 (92%) received antibiotics. Of those, 11 (92%) received penicillin and gentamicin, including 3 who also received a cephalosporin. Among 8 community deaths, none were hospitalized during the course of illness and 5 (62%) had received outpatient care; all received antibiotics (amoxicillin n=4 and cotrimoxazole n=1). Kp plays an important role in child death in Kenya, and infections seem to be both nosocomial and community-acquired. Commonly used antibiotics may not adequately treat Kp; current data on Kp susceptibility in Kenya are urgently needed.

1376

STREPTOCOCCUS PNEUMONIAE ASSOCIATED CHILD MORTALITY IN THE PNEUMOCOCCAL CONJUGATE VACCINE ERA

Adriana C. Gibby[1], Dianna M. Blau[2], Shabir Madhi[3], Richard Chawana[4], Dickens Onyango[5], Inacio Mandomando[6], Samba O. Sow[7], Shams El Arifeen[8], Emily Gurley[9], Beth A. Tippett Barr[10], Victor Akelo[10], Karen Kotloff[11], Quique Bassat[12], Robert F. Breiman[1]

[1]*Emory Global Health Institute, Emory University, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa,* [4]*Department of Science/National Research Foundation: Vaccine Preventable Diseases, University of Witwatersrand, Faculty of Health Sciences, Johannesburg, South Africa,* [5]*Kisumu County Public Health Department, Kisumo, Kenya,* [6]*Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique,* [7]*Center for Vaccine Development, Bamako, Mali,* [8]*icddr,b (International Centre for Diarrhoeal Disease Research, Bangladesh), Dhaka, Bangladesh,* [9]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [10]*US Centers for Disease Control and Prevention-Kenya, Kisumu, Kenya,* [11]*University of Maryland School of Medicine, Baltimore, MD, United States,* [12]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain*

Worldwide, an estimated 500,000 children die annually from pneumococcal disease. Introduction of pneumococcal conjugate vaccine (PCV) into the infant immunization programs in low- and middle-income countries could reduce child mortality. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network aims to improve understanding about causes of child mortality through longitudinal surveillance and etiologic investigation of under-five deaths and stillbirths by combining postmortem minimally invasive tissue sampling (MITS), molecular, microbiologic, and histopathologic testing, clinical records, and verbal autopsy. Such a surveillance provides a platform for more refined population-based estimates of cause-specific mortality fractions in Sub-Saharan Africa (Kenya, Mali, Sierra Leone, Ethiopia, Mozambique, South Africa) and South Asia (Bangladesh). Underlying, co-morbid, and immediate causes of death (causal chain) are ascribed according to ICD-10 guidelines by local panels of experts reviewing all data on each case. Of cases undergoing panel review thus far (n=594), *S. pneumoniae*-associated conditions were listed in the causal chain in 46 (7.7%) of 594 children under 5 years of age, ranging from 1.4 % of neonates, 13.2 % of infants, and 20.2% of children 12 months to 5 years; associated conditions included lower respiratory infections (81.8%), meningitis (15.9%), and sepsis (36.8%). Of the pneumococcal deaths, 12.8% were in HIV-infected children. Preliminary findings support a continued focus on prevention of invasive pneumococcal disease. Next steps are to assess: a) proportion of pneumococcal deaths due to vaccine serotypes; b) PCV coverage rates and occurrence of death among adequately vaccinated children c) and factors for under-immunization.

1377

NEAR UNIVERSAL MULTIDRUG RESISTANCE OF BACTERIAL INFECTIONS AMONG STILLBIRTHS, NEONATES AND CHILDREN UNDER FIVE YEARS OF AGE AT A TERTIARY CARE HOSPITAL IN BANGLADESH

Muntasir Alam[1], Dilruba Ahmed[1], Mariya Kibtiya Sumiya[1], Kyu Han Lee[2], Mohammed Ziaur Rahman[1], Jannatul Rafeya[1], Farzana Islam[1], Afruna Rahman[1], Shahana Parveen[1], Sanwarul Bari[1], Dianna M. Blau[3], Robert F. Breiman[4], Emily S. Gurley[2], Shams El Arifeen[1], Mustafizur Rahman[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Emory Global Health Institute, Atlanta, GA, United States*

Bacterial infections are life threatening in fetuses and newborns; timely, appropriate antimicrobial therapy is often pivotal for survival. However, increasing rates of multidrug resistance (MDR) has created therapeutic challenges. Therefore, determining drug susceptibility patterns of local pathogens is a crucial component for appropriate empiric antibacterial treatment. We isolated bacterial pathogens from blood of deceased and ill children in a tertiary care facility in Faridpur, Bangladesh through the Child Health and Mortality Prevention Surveillance (CHAMPS) project. Blood samples were collected from 35 CHAMPS (deceased: 16 stillbirth, 18 neonates and one 1-year-old child) and 41 non-CHAMPS (ill: 36 neonates and five children under 5 years of age) between October 2017 and December 2018. Bacteria were identified by blood culture and susceptibility was tested using the disk diffusion method. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Fifty one (67%) specimens were culture positive with lower isolation rate in CHAMPS (57%) than non-CHAMPS (76%) cases although the difference was not statistically significant (p-value= 0.096). Predominant isolates were *Klebsiella* spp. (29%) followed by coagulase-negative Staphylococci (16%), *Acinetobacter* spp. (7%) and *Enterococcus faecalis* (4%). Among 54 bacterial isolates (three cases had two isolates) recovered, 92.5% were categorized as MDR to routinely prescribed antibiotics in Bangladesh for pediatric bacterial infections, such as ampicillin (93%), gentamicin (75%) and cephalosporins (63%). Similar rate in MDR was observed when comparing CHAMPS (93%) and non-CHAMPS (92%) cases. *Klebsiella* spp. showed 67% resistance against carbapenem group antibiotics; susceptibility was greatest to vancomycin (100%), tigecycline (95%) and colistin (78%), thus, a consideration for empiric therapy of severe pediatric infections in this setting. Isolation of MDR bacteria warrants primary infection control strategy as well as proper diagnosis and administering antibiotics appropriately to treat pediatric infections.

1378

RISK FACTORS ASSOCIATED WITH NOSOCOMIAL BACTEREMIA AMONG NEONATES AT UNIVERSITY TEACHING HOSPITAL LUSAKA, ZAMBIA

Lukman Abdurrahim[1], Carter L. Cowden[2], Lawrence Mwanayanda[3], James Mwansa[4], Chilese Lukwesa-Musyani[5], Cassandra Pierre[6], Russell Localio[7], Davidson Hamer[8], Susan E. Coffin[2]

[1]*Boston Children's Hospital, Boston, MA, United States,* [2]*Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, PA, United States,* [3]*Right to Care Zambia, Lusaka, Zambia,* [4]*Department of Pathology and Microbiology, Lusaka Apex Medical University, Lusaka, Zambia,* [5]*Department of Pathology and Microbiology, University Teaching Hospital, Lusaka, Zambia,* [6]*Section of Infectious Diseases, Department of Medicine, Boston University School of Medicine, Boston, MA, United States,* [7]*Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States,* [8]*Department of Global Health, Boston University School of Public Health, Boston, MA, United States*

In low- and middle-income countries (LMIC), nearly 41% of all under 5 deaths occur in the neonatal period; one third of which are due to infections. As compared to industrialized countries, the rates of neonatal bloodstream infections (BSI) in LMIC are 3-20 times higher. With increasing facility-based deliveries, the risk of hospital-acquired infections rises. We aimed to identify maternal and neonatal risk factors for hospital-associated BSI (HA-BSI) among neonates at a sub-Saharan tertiary care facility. We performed a prospective observational cohort study of neonates admitted to the neonatal intensive care unit (NICU) at the University Teaching Hospital (UTH) Lusaka, Zambia. Blood cultures were performed in neonates with suspected sepsis based on established clinical criteria. Only blood cultures that grew a pathogen were consider positive. Cultures

were designated as either early-onset (EO-BSI) if they occurred within 3 days of birth; otherwise they were designated as late-onset BSI (LO-BSI). Risk factors were compared between newborns with confirmed BSI with those with suspected sepsis that was culture negative or whose culture yielded contaminants. Of the 3316 neonates hospitalized in the NICU, 407 (12%) had at least one BSI, including 228 EO-BSI and 179 LO-BSI. When compared to those who did not have any BSIs with a pathogen, factors associated with EO-BSI were low maternal education, prolonged rupture of membranes, and fewer than two antenatal visits (all p<0.001). Inborn (birth at UTH), maternal HIV infection, and C-section delivery were found to be protective against LO-BSI (p<0.01, p=0.04, p=0.01 respectively). Maternal and neonatal characteristics can increase the risk of pathogen-associated EO- or LO-BSI. Further study is needed to elucidate the impact of HIV status on neonatal BSI. Development and implementation of policies that will improve antenatal visit attendance and female education may help to reduce BSI-associated neonatal morbidity and mortality.

1379

THE ROLE OF NEONATAL SEPSIS IN THE OVERALL BURDEN OF ANTIMICROBIAL RESISTANCE IN NOSOCOMIAL PATHOGENS

Sulochana Manandhar[1], Sabina Dongol[1], Suchita Joshi[2], Shreejana Shrestha[2], Sameer Mani Dixit[3], Buddha Basnet[1], Stephen Baker[4], Abhilasha Karkey[1]

[1]*Oxford University Clinical Research Unit, Patan Academy of Health Sciences, Kathmandu, Nepal,* [2]*Patan Academy of Health Sciences, Kathmandu, Nepal,* [3]*Center for Molecular Dynamics Nepal, Kathmandu, Nepal,* [4]*The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam*

Antimicrobial resistance (AMR) in multidrug resistant Gram negative bacilli (MDR-GNB) is a global public health problem. South Asian countries including Nepal are considered as epicenter for this. The contributory role of neonatal sepsis in overall burden of nosocomial AMR is substantial. The difficulty in its clinical diagnosis and probable serious consequences in case of delayed treatment compel for the empirical use of antimicrobials without microbiological justification. Such increased empirical use of last resort drugs such as extended spectrum Cephalosporins and Carbapenems in neonatal intensive care unit (NICU), contribute in selective evolution of MDR-GNB. A six years (2012-2018) retrospective study on microbiological culture results from Patan hospital, a tertiary government hospital of Nepal, showed that 47%, 1254/2667 of the commonest GNB (*Klebsiella pneumoniae, Enterobacter* sps*, E. coli*, *Acinetobacter* sps) were MDR, 64%, 1381/2153 being ESBL producers and 22%, 474/2153 had reduced susceptibility against Carbapenems. Over half (54%, 372/691) of all inpatients' bacterial isolates were derived just from neonates (NICU and nurseries) with 61% (208/342) of the top four neonatal GNB being MDR, 55% (178/323) being ESBL producers and 34% (109/323) showing resistance against Carbapenems, possessing bla_{NDM-1}, bla_{KPC} or bla_{OXA} genes. Specifically 83% (52/63) of neonatal *Acinetobacter* sps were resistant to Carabapenem, possessing bla_{OXA51} (73%) and bla_{NDM-1} (54%) genes. A prospective NICU cohort study, showing a high incidence of neonatal sepsis (50%,71/141), revealed that 91% (128/141) of the cases received Ampicillin-Amikacin, though only 30% (22/141) yielded positive blood cultures. Over half of cases were given Cephotaxime, a quarter received Carbapenem, 17% had Colistin, all being the last resort drugs. Our study presents an evidence of alarming situation of AMR in nosocomial GNR and increased use of antimicrobials in NICU. Improved diagnostic tools, judicious use of antimicrobials and strong infection control/prevention policies are indispensable for combating this burgeoning problem of AMR.

1380

PREVALENCE OF BACTEREMIA AND ANTIMICROBIAL RESISTANCE IN KENYAN CHILDREN FROM A HOLOENDEMIC *PLASMODIUM FALCIPARUM* TRANSMISSION REGION

Tessa LeCuyer[1], Vincent Otieno[2], Nicholas Kondiek[2], Collins Ouma[3], Benjamin H. McMahon[4], Philip Seidenberg[5], Douglas J. Perkins[1]

[1]*University of New Mexico Center for Global Health, Albuquerque, NM, United States,* [2]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya,* [3]*Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Kisumu, Kenya,* [4]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [5]*University of New Mexico Center for Global Health and Depatment of Emegency Medicine, Albuquerque, NM, United States*

In endemic regions, clinical malaria frequently presents with concurrent bacteremia in children. Co-infection promotes poor health outcomes; we have previously shown that bacteremic children in rural western Kenya with malaria have higher rates of mortality and severe anemia than non-bacteremic children. High levels of antimicrobial resistance (AMR) further complicate treatment of bacteremia in many parts of sub-Saharan Africa. As such, we examined the prevalence of bacteremia and AMR patterns in children (1-36 months of age; n=1,639) in a *Plasmodium falciparum* holoendemic malaria transmission region of western Kenya (2004-2012). There was a decreasing trend in the prevalence of bacteremia in malaria(+) children over this time period: 9.8% from 2004-2006 versus 4.7% from 2009-2012 (p=0.05). The most common pathogen was non-typhoidal Salmonella (NTS), accounting for 56.8% (2004-2006) and 58.8% (2009-2012) of bacteremia cases, respectively. AMR patterns for NTS remained relatively constant between 2004-2012, except for 3rd generation cephalosporin resistance which increased from 0% (2004-2006) to 15% (2009-2012). A history of antimicrobial administration in the seven days prior to blood culture was observed for 17% of the children with NTS bacteremia and resistance rates for SXT were high (85.7% non-susceptible). However, a history of SXT administration in the past week was not associated with SXT resistant NTS infections. High rates of AMR in NTS to other commonly used antimicrobials were also observed: chloramphenicol (70.1% non-susceptible) and amoxicillin-clavulanic acid (73.2% non-susceptible). Bacteremia was associated with increased mortality: 9.6% in bacteremic children compared to 1.1% in non-bacteremic children (p=0.01). Results here demonstrate a consistently high prevalence of AMR to commonly used antimicrobials in invasive NTS since the early 2000s.

1381

EFFECTS OF SULFADOXINE-PYRIMETHAMINE INTERMITTENT PREVENTIVE THERAPY IN PREGNANCY ON MATERNAL CARRIAGE OF ENTEROPATHOGENS AND GUT MICROBIOMES AND INFANT BIRTH OUTCOMES

Andreea Waltmann[1], Jobiba Chinkhumba[2], Megumi Itoh[3], Fatsani Gadama[2], Enala Mzembe[2], Michael Kayange[4], Sydney M. Puerto-Meredith[5], Elizabeth T. Rogawski McQuade[6], Darwin J. Operario[6], Jeffrey Roach[7], Don P. Mathanga[2], Ian Carroll[8], Julie R. Gutman[3], Steven R. Meshnick[9]

[1]*Institute for Global Health and Infectious Diseases, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Malaria Alert Centre (MAC), University of Malawi College of Medicine, Blantyre, Malawi,* [3]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*National Malaria Control Program, Lilongwe, Malawi,* [5]*Undergraduate Biology Program, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [6]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States,* [7]*Microbiome Core Facility, University of North Carolina at Chapel Hill, Chapel Hill, NC, United*

States, [8]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [9]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Bacterial infections, including enteropathogens, are a leading cause of morbidity and mortality among pregnant women and their infants, particularly in low-resource countries. Sulfadoxine-pyrimethamine (SP) has been used in intermittent preventive treatment of malaria in pregnancy (IPTp). It has been postulated that, as a combination antibiotic and antifolate, SP may clear other pathogens, and that it may have an effect on the gut microbiota. We assessed whether monthly SP-IPTp has an effect on the carriage of common intestinal pathogens in expectant mothers and whether it associates with changes in the gut microbiome. We used stool specimens from 98 pregnant Malawian women enrolled in a randomized controlled study comparing the efficacy of SP to dihydroartemisinin-piperaquine (DP). Unlike SP, DP is an antimalarial with no known antibacterial effect. We screened for: five *Escherichia coli* strains, *Shigella spp.*,*Vibrio cholerae*, *Salmonella*, *Campylobacter coli/jejuni*, and three protozoa (*Giardia spp.*, *Entamoeba histolytica*, and *Cryptosporidium spp.*). We characterized the gut bacterial microbiota by deep sequencing the 16S ribosomal RNA gene and analyzing the data with the established bioinformatic pipeline QIIME2. Samples were assessed for pathogens and microbiome patterns at enrollment prior to study drug administration and at each subsequent antenatal visit, when study drug was administered. The lab remains blinded to study drug allocation. Initial results show that 51% of expectant mothers in our study (50/98 women) carry at least one gut pathogen prior to first drug dose, with the most common being enteropathogenic and enteroaggregative *E. coli*, detected in 51% and 48% of women, respectively. Evaluation of pathogen carriage rates and microbiome structure in additional samples after receipt of drug is ongoing and data will be analyzed by drug arm. With these results, we will show whether SP has a malaria-independent effect on the gut. We will discuss the significance of our results in the context of proposed discontinuation of SP-IPTp and explore implications the use of SP against other pathogens may have in sub-Saharan Africa.

1382

EXTENSIVE TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OCCUR DURING THE MATURATION OF MALARIA PARASITE SPOROZOITES

Scott E. Lindner[1], Kristian E. Swearingen[2], Melanie Shears[3], Michael P. Walker[1], Erin N. Vrana[1], Kevin J. Hart[1], Allen M. Minns[1], Photini Sinnis[4], Robert L. Moritz[2], Stefan H. Kappe[5]

[1]*Pennsylvania State University, University Park, PA, United States,* [2]*Institute for Systems Biology, Seattle, WA, United States,* [3]*University of Washington, Seattle, WA, United States,* [4]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [5]*Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA, United States*

Plasmodium sporozoites are transmitted from an infected mosquito to mammals in which they infect the liver. The infectivity profile of sporozoites changes as they egress from oocysts on the mosquito midgut into the hemocoel, and then invade the salivary glands, where they maintain a poised and infectious state until transmission occurs. Upon transmission, the sporozoite must then navigate the host skin, vasculature, and liver. All of these feats require distinct repertoires of proteins and capabilities that are coordinated in an appropriate temporal manner. Here, we report the comprehensive and dynamic transcriptomes and proteomes of both oocyst sporozoite and salivary gland sporozoite stages in both rodent-infectious *Plasmodium yoelii* parasites and human-infectious *Plasmodium falciparum* parasites. These data robustly define mRNAs and proteins that are Upregulated in Oocyst Sporozoites (UOS) or Upregulated in Infectious Sporozoites (UIS), which include critical gene products for sporozoite functions, as well as many of unknown importance that are similarly regulated. Moreover, we found that *Plasmodium* uses two overlapping, extensive, and independent programs of translational repression across sporozoite maturation to temporally regulate specific genes necessary to successfully navigate the mosquito vector and mammalian host environments. Finally, gene-specific validation experiments of selected, translationally repressed transcripts in *P. yoelii* confirmed the interpretations of the global transcriptomic and proteomic datasets. Together, these data indicate that two waves of translational repression are implemented and relieved at different times in sporozoite maturation to promote its successful life cycle progression.

1383

PLASMEPSIN V IS ESSENTIAL IN *PLASMODIUM* LIVER STAGES AND DIRECTS EXPORT TO THE INFECTED HEPATOCYTE

Pravin Rajasekaran, Ryan Steel, Matthew O'Neill, Bethany Davey, Annie Yang, Brad Sleebs, Alan Cowman, **Justin Boddey**

Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Central to the pathogenesis of malaria is the *Plasmodium* parasite's ability to remodel erythrocytes by exporting hundreds of proteins into the host cell. Export of effector cargo involves proteolytic cleavage of the PEXEL motif by the protease plasmepsin V prior to translocation across the parasitophorous vacuole membrane by the PTEX translocon. Prior to blood stage infection, malaria sporozoites deposited by an infected mosquito home to the liver and invade hepatocytes within a parasitophorous vacuole. The invaded parasites need to evade host innate responses in order to survive and grow. Long-standing questions in the field are whether liver stages export proteins into infected hepatocytes using the PEXEL machinery, and if so, why? Here, we conditionally knockdown Plasmepsin V expression in liver stages using the Flp/FRT site-specific recombination system. Dissection of *Plasmepsin V* knockdown sporozoites from mosquitoes and injection into mice resulted in normal liver infections at early time points but clearance of exoerythrocytic forms from mice by 24 hours, no patent malaria infections and production of protective immunity against challenge with a lethal dose of wild-type sporozoites. Immunofluorescence microscopy reveals that export of a PEXEL-containing effector protein to the hepatocyte is disrupted by plasmepsin V knockdown and immunoblotting reveals accumulation of uncleaved PEXEL precursors in PMV knockdown parasites. This study shows that plasmepsin V is essential in liver stages owing to a conserved role in cleavage of proteins for export to the host cell. Identification of further hepatocyte exported proteins is underway. Plasmepsin V thus represents a multi-stage, pan-*Plasmodium* target for a new class of antimalarial drugs, which are under development in collaboration with Merck and the Wellcome Trust.

1384

PHOSPHORYLATION OF THE VAR2CSA EXTRACELLULAR REGION IS ASSOCIATED WITH ENHANCED ADHESIVE PROPERTIES TO THE PLACENTAL RECEPTOR CSA

Dominique Dorin-Semblat[1], Marilou tetard[1], Aurélie Claës[2], Jean-Philippe Semblat[1], Sébastien Dechavanne[1], Zeineb Fourati[1], Romain Hamelin[3], Florence Armand[3], Graziella Matesic[1], Sofia Nunes-Silva[1], Anand Srivastava[1], Stéphane Gangnard[1], Jose-Juan Lopez-Rubio[4], Marc Moniatte[3], Christian Doerig[5], Artur Scherf[2], **Benoit Gamain**[1]

[1]*INSERM, Paris, France,* [2]*Institut Pasteur, Paris, France,* [3]*EPFL, Lausanne, Switzerland,* [4]*MIVEGEC, Montpellier, France,* [5]*Monash University, Melbourne, Australia*

Plasmodium falciparum is the main cause of disease and death from malaria. *P. falciparum* virulence resides in the ability of infected erythrocytes (IEs) to adhere to specific receptors in various tissues, causing significant clogging of capillaries leading to severe malaria. The parasite adhesion molecule *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), encoded by the *var* gene family, mediates adhesion to various host receptors expressed along blood vessels in different organs. However, tissue adhesion tropism of this pathogen remains poorly understood. Here we investigated the effect of phosphorylation on PfEMP1 adhesive properties on placental sequestration, which is mediated through the interaction between VAR2CSA, a member of the PfEMP1 family, and chondroitin sulfate A (CSA) on the placental syncytium. We show that

phosphatase treatment of intact IEs impairs cytoadhesion to CSA. In line with this observation, we demonstrate that the extracellular region of VAR2CSA is phosphorylated in regions known to interact with CSA. Mass spectrometry analysis of recombinant VAR2CSA phosphosites prior to and after phosphatase treatment, as well as mutation of these phosphoresidues to alanine in recombinant VAR2CSA, identified critical phosphoresidues associated with CSA binding. Furthermore, using CRISPR/Cas9, we generated a parasite line in which a phosphoresidue is changed to alanine and showed that this mutation strongly impairs IEs cytoadhesion to CSA under both static and flow conditions. Taken together, these results inform that phosphorylation plays a major role in infected erythrocytes cytoadhesion to CSA and provide new molecular insights for vaccine and therapeutic strategies aiming to reduce the morbidity and mortality of placental malaria.

1385

IN VITRO AND *IN VIVO* EVIDENCE THAT GDV1 REGULATES SEXUAL DIFFERENTIATION UPSTREAM OF *AP2-G*

Miho Usui[1], Surendra K. Prajapati[1], Ruth Ayanful-Torgby[2], Festus K. Acquah[2], Elizabeth Cudjoe[2], Courage Kakaney[2], Jones A. Amponsah[2], Evans Obboh[3], Deepti K. Reddy[1], Michelle C. Barbeau[1], Lacy M. Simons[4], Beata Czesny[4], Sorana Raiciulescu[1], Cara Olsen[1], Benjamin K. Abuaku[2], Linda E. Amoah[2], Kim C. Williamson[1]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana,* [3]*School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana,* [4]*Loyola University Chicago, Chicago, IL, United States*

The development of sexual stage parasites is critical for malaria transmission, yet much remains unknown about how this process is regulated. Recently, epigenetic repression and expression of the transcription factor AP2-G have been shown to play important roles in sexual commitment. However, the mechanisms that release repression and consequently allow gametocyte production are still a mystery. Our previous work demonstrated that *Pfgdv1* is required for gametocyte production. Here, we assess gametocytogenesis *in vitro* and demonstrate that GDV1 protein expression during schizogony is critical for gametocyte commitment and modulates transcript levels of *ap2-g*, a transcription factor associated with gametocytogenesis, as well as *msrp1*. In the next generation of ring stage parasites, *ap2-g* and *msrp1* RNA levels remain elevated and there is a GDV1-dependent increase in transcript levels of *gexp5*, an *ap2-g* independent gene. Ring stage RNA levels of all three GDV1-regulated genes, *ap2-g, msrp1* and *gexp5* correlated positively with gametocyte differentiation *in vitro* (R^2 = 0.45 to 0.92). Transcript levels for these genes were also significantly higher in field samples collected in Ghana during two malaria seasons, July-August 2016 and 2017, that had high ex-vivo gametocyte conversion rates (GCR) (Day4 GCR > 4.9 %; n=20) than those with low to undetectable ex-vivo GCR (n=20). Interestingly, GDV1 mutant allele (Histidine^{217}) which predominated in an area of limited seasonal malaria in the Gambia, has a high frequency only in high GCR samples (Fisher exact test, p=0.022) while *msp2* alleles are randomly distributed in both groups. Together the data suggests GDV1 acts as a rheostat for sexual differentiation upstream of *ap2-g in vitro* and plays a regulatory role *in vivo*.

1386

GEOGRAPHIC ACCESSIBILITY AND FACILITY-BASED HEALTHCARE IN ZAMBIA: A GEOSTATISTICAL MAPPING STUDY

Roy Burstein[1], Felix Masiye[2], Nancy Fullman[3], Simon Hay[4]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*University of Zambia, Lusaka, Zambia,* [3]*University of Washington, Seattle, WA, United States,* [4]*Institute for Health Metrics and Evaluation, Seattle, WA, United States*

Understanding who accesses healthcare, why, and for what type of services is critical for planning health systems, which ultimately ought to be responsive and available to all potential users. Several studies have attempted to exploit the strong association observed between distance to health services and utilization in order to predict utilization rates across large geographic areas with sparse data. Efforts to quantify the relationship between distance and utilization and then predict utilization rates onto gridded geospatial surfaces have been subject to considerable data and methodological limitations. These include: limited data on health facility locations, 'jittering' of household locations in public datasets, unrealistic simplifying assumptions used in measuring geographic accessibility, focus on only one illness or age group, ignoring model and data uncertainty, and omission of important covariates. In this study, we sought to overcome limitations in accessibility and utilization mapping using a triangulation of health facility, household, and geospatial data sources from Zambia to answer four primary questions: What is the status of geographic accessibility to health facilities in Zambia? How does travel time to nearest health facility affect utilization rates? And finally, can we make a reasonable map of utilization rates using a geostatistical model? How does this approach compare to previous methods? We found that a large proportion of the Zambian population lives relatively close to facility care, but that small differences in travel time within the first hour from a health facility seem to impact utilization rates more than those same differences in distances for households that are further away from health facilities. Our approach showed considerable predictive improvement over existing methods. Furthermore, in accounting for uncertainty we found that spatially resolved estimates of utilization are difficult to achieve with any practical degree of certainty. We conclude that, by ignoring uncertainty, previous spatial utilization studies may have overstated the strength of evidence they produced.

1387

DATA ACCESS COMMITTEES: WHAT SHOULD THEIR ROLES AND RESPONSIBILITIES BE AND WHO SHOULD BE ON THEM?

Phaik Yeong Cheah[1], Jan Piasecki[2]

[1]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*Jagiellonian University Medical College, Krakow, Poland*

Sharing individual-level health data has many benefits, however there are also some potential harms, such as misuse of data and breaching of participants' confidentiality. One way to promote benefits of sharing while ameliorating its potential harms is through the adoption of a managed access approach where data requests are channeled through a Data Access Committee (DAC), rather than making data openly available without restrictions. A DAC, a formal or informal group of individuals have the responsibility of reviewing and assessing data access requests. Many individual groups, consortium, and institutional DACs have been set up but there is currently no widely accepted framework for their organization and functioning. Based on literature review, ethical analysis, and three years' experience coordinating a DAC (PYC), we propose that DACs, should have both the role of promotion of data sharing and protection of data subjects, their communities, data producers and their institutions. We suggest that data access should be granted by DACs as long as the data reuse has some potential social value and provided there is low risk of foreseeable harms. To promote data sharing, DACs could encourage secondary uses that are consistent with the interests of primary researchers and their institutions. Given the suggested roles of DACs, there should

be transparent, simple and clear application procedures for data access. DACs should be established within institutional and legal frameworks with clear lines of accountability, terms of reference and membership. In order to fulfill its functions, a DAC should consist of a reasonable number of members, each with multiple relevant areas of expertise. Ideally there should be members representing senior management, data management, ethics, relevant research areas and potentially a data sharing advocate. It is also desirable to have independent members.

1388

BEACON: A TOOL FOR EVALUATING MHEALTH READINESS IN GLOBAL HEALTH

Thomas F. Scherr[1], Carson Moore[1], Saidon Mbambara[2], Philip Thuma[2], David Wright[1]

[1]*Vanderbilt University, Nashville, TN, United States,* [2]*Macha Research Trust, Macha, Zambia*

Mobile health interventions are increasing in popularity, due in part to the ubiquity of mobile phones and data-rich possibilities. The growth of mobile phones has been a global trend, yet uncertainty remains amongst key stakeholders and decision makers that these interventions could successfully be used in global health. These skepticisms may be merited in some cases, however, in others, mHealth could be used to reduce geographic, financial, and social barriers to quality healthcare. Overall, there remains hesitation to use mHealth due to a lack of tools that can effectively evaluate a site's readiness to implement an intervention. This reduces the debate to an argument between empirical observations and dated facts, or worse, opinions. To this end, we have developed Beacon - a tool for evaluating mHealth readiness in global health. Beacon is an automated mobile phone application that repeatedly collects spatiotemporal data on network performance. Each data point is tagged with a timestamp and GPS coordinates, along with ping/download/upload latency and duration. Results can be collected, even in the absence of network connectivity, and uploaded to REDCap (a web-based electronic research database) for further analysis. As a demonstration of its utility, we present results collected in and around Macha, Zambia over a 4-week time period in 2019 (over 10,000 data points). These results show times of day when cellular networks experience a heavy load and slow down, compare performance of different cellular networks, and find geographic "dead zones" with limited or no cellular service. Beacon was developed to measure performance as it relates to our work in disease surveillance through point-of-care diagnostics, but it could be readily tailored to evaluate other mHealth interventions. We envision Beacon as a tool that could be used by 1) organizations that are considering an mHealth intervention in a low- and middle-income country, but are questioning its feasibility - including infrastructure and cost, and 2) organizations that have an existing mHealth intervention and are looking to optimize its delivery with improved logistics management.

1389

TRANSLATING CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) RESULTS INTO ACTION AT THE DISTRICT LEVEL: THE EXPERIENCE OF MOZAMBIQUE

Inaco Mandomando[1], Pio Victorino[1], Saquina Cossa[1], Maria Maixenchs[2], Bento Nhancale[1], Estevao Mucavele[1], Madalena Ripinga[1], Rosauro Varo[2], Jaume Ordi[2], Elizabeth O'Mara[3], John Blevens[3], Navit Salzberg[3], Robert Breiman[3], Dianna Blau[3], Carla Carrilho[4], Quique Bassat[2], Khatia Munguambe[1]

[1]*Manhica Health Research Centre, Maputo, Mozambique,* [2]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [3]*Emory Global Health Institute, Atlanta, GA, United States,* [4]*Hospital Central de Maputo, Maputo, Mozambique*

CHAMPS was established in rural Southern Mozambique to ascertain causes of child death (CoD), inform policy and public health action. Minimally Invasive Tissue Sampling (MITS) for histopathology and microbiological CoD assessment, together with a comprehensive demographic surveillance for detecting childhood deaths are on-going at the Manhiça District. Families of deceased under-5 children from the catchment area are approached for consenting to MITS, and individual CoD results returned to the family while aggregate data are fed back to the District authorities. We describe the process of translating CHAMPS results into action at the District level to improve health care quality. From December 2016 to March 2019, 161 (63%) MITS of the 255 approached cases were performed and CoD assigned to the first 100 cases, and results returned to 88 families within ~6 months. CHAMPS results may have a significant impact at the household level, as they entail the possibility of treating conditions (in household members) that have been detected in the deceased case. Clinical follow-up needs were identified for 15 families of which some of their members presented health problems that received additional care at the hospitals in follow-up to the CHAMPS feedback visit (e.g. HIV infected mothers who had abandoned the antiretroviral treatment; syphilis cases and the mother was found pregnant during the feedback results, and she was recommended to promptly attend the antenatal clinic until the successful delivery; mothers with cardiomegaly, endocrine and hematological disorders). While the individual-level tests are critical to timely reporting of communicable emerging diseases or unexpectedly important pathogens (e.g. cytomegalovirus), action was not limited to individual or household level support. Results feedback process triggered immediate action by the District Health Authorities to establish follow up visits for mothers referred by CHAMPS to the referral hospitals. In addition, the community death component has led to transporting, severe patients from the community to the hospital, a service that was previously restricted to pregnant women.

1390

EXPANDING GLOBAL PARTNERSHIPS TO STRENGTHEN PATHOLOGY-BASED MORTALITY SURVEILLANCE

Tia R. Paganelli[1], Norman J. Goco[2], Lindsay M. Parlberg[2], Elizabeth M. McClure[2]

[1]*RTI International, Seattle, WA, United States,* [2]*RTI International, Research Triangle Park, NC, United States*

The Minimally Invasive Tissue Sampling (MITS) Surveillance Alliance is a global, multidisciplinary consortium which aims to improve the quality of global mortality data, specifically cause of death classification through the expansion of the use of the MITS technique. MITS, a less-invasive alternative compared to complete diagnostic autopsies, targets only specific organs and can be performed by pathology technicians with specialized training. Initial studies have shown that MITS can generate high quality mortality data in low- and low-middle-income countries. The MITS Surveillance Alliance provides a forum for knowledge management, standardization of procedures and training, and sharing best practices for tissue processing and cause of death classification. Members include a collaborative body of clinical researchers, epidemiologists, pathologists, microbiologists, social scientists, and specialists interested in, or already implementing MITS. Through the support of the Secretariat the MITS Surveillance Alliance has organized dynamic Communities of Practice which serve as the cornerstone for knowledge sharing. With insight from members of various sized projects and geographic contexts, the Communities of Practice have collaboratively developed several resources; including guidance for introducing and conducting post-mortem research studies in vulnerable populations, tools for ensuring quality control in specimen collection. Additionally, the Communities of Practice have provided input on streamlined guidelines for the minimum data sources required for determining cause of death in different populations. By fostering global partnerships and advocating for high quality data through the expansion of MITS, the MITS Alliance can help governments, institutions, and stakeholders strengthen mortality surveillance and prioritize policies and interventions.

1391

ELECTRONIC DATA MANAGEMENT FOR GLOBAL HEALTH FIELD RESEARCH PROJECTS

Katiuscia K. O'Brian[1], Amy Rigney[2], Gary J. Weil[1]

[1]*Washington University School of Medicine, St Louis, MO, United States,* [2]*SPRI Clinical Trials, Pittsboro, NC, United States*

It can be challenging for global health research projects to collect and store data with tools and systems that are affordable and compliant with global standards. Special approaches are needed for collection of high quality, secure data in resource-poor settings with limited internet connectivity and on-site technical expertise. Electronic data management, coordinated with a strong data management plan (DMP) to ensure high quality data for analysis, is a useful approach. The benefits of electronic data management is the ability to collect data offline in areas with variable internet access, in near real-time, with remote monitoring and management, and producing high data quality. Data management can be all electronic (eSource) or a mixed system that includes both paper and electronic data capture (EDC) systems. DMPs should contain the following components: careful database design and development; data collection and capture procedures; data security; data quality and validation checks; data reporting, database closure, and archiving. It is important for researchers to discover if their projects needs to be 21 CFR Part 11 compliant, as this will dictate which EDC system would be appropriate. Compliance requires software controls including system validations, audit trails, electronic signatures, and documentation for software used to collect and process data in a manner that is trustworthy, reliable, and equivalent to paper records. Additional steps that we suggest for a successful and comprehensive global health data management plan should include: extensive user acceptability testing of EDC system; training teams for site initiation; technology assessment; on-site data manager; and frequent data cleaning during the study period so that errors can be corrected while participants are still available. Our poster will summarize lessons learned in multiple locations previously considered off the digital track using RedCAP (webportal EDC), EpiInfo (EDC App on Android BLU phone device syncing with Azure cloud server), and a proprietary system (EDC App on iOS iPad device syncing with Amazon cloud server) that is 21 CFR Part 11 compliant.

1392

STRENGTHENING THE VACCINE SAFETY SYSTEM IN KENYA: ASSESSMENT OF BEST PRACTICES FOR VACCINE SAFETY AMONG HEALTHCARE WORKERS IN KENYA

Zunera Gilani[1], Dorothy C. Koech[2], Lucy Mecca[3], Christabel Khaemba[4], Martha Mandale[4], Wilbrod Mwanje[5], Laura Conklin[1], Tabu Collins[3], Jane Gidudu[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*African Field Epidemiology Network, Nairobi, Kenya,* [3]*Kenya National Vaccines and Immunization Program, Nairobi, Kenya,* [4]*Kenya Pharmacy and Poisons Board, Nairobi, Kenya,* [5]*African Epidemiology Network, Kampala, Uganda*

A functional vaccine safety system is a key component for sustaining confidence in an immunization program, yet these systems are sub-optimal in many African countries including Kenya. This study's objectives were to: (1) assess healthcare worker (HCW) adverse event following immunization (AEFI) knowledge and practice; and (2) identify gaps, challenges and strategies to improve the AEFI reporting system. We selected 68 health facilities (HF) in six counties in Kenya; 61 were selected randomly and seven purposely as the largest facilities in the county/sub-county. At each HF, HCW that provided vaccination and/or managed AEFI were administered a structured questionnaire. In addition, we conducted key informant interviews (KII) with HCW involved in vaccine safety leadership, monitoring and reporting. Among 72 HCW surveyed, nearly all (n=68; 94.4%) knew the country's AEFI definition and most (n=61; 84.7%) knew the AEFI Reporting Form. Most HCW had not encountered an AEFI in the past two years (n=53; 73.6%). For those that had encountered an AEFI, half (n=7; 50%) believed they did not need to report non-serious AEFI and 57.1 % (n=8) believed they did not need to report AEFI they could manage. Only 63.3% (n=38) of HCW reported having an AEFI Reporting Form at their workstation. Nearly all HCW indicated that they provide AEFI information to caregivers of children they immunize (n=71; 98.6%). Finally, most HCW indicated they had not been trained on AEFI (n=63; 87.5%). Forty-five HCW participated in KII. Gaps and challenges identified centered on capacity, reporting and data. Strategies to address these gaps include: 1) HCW training to improve awareness of reporting processes; 2) improved availability of AEFI reporting forms, 3) development of job aids to encourage reporting all AEFI; and 4) strengthening data use and AEFI data systems. There have been few assessments of HCW vaccine safety knowledge and practice in the African region. Our study is one of the first to utilize a qualitative component. We identified good vaccine safety knowledge and practice, as well as gaps and strategies, with potential for applicability in similar geographical settings.

1393

SOCIAL ACCEPTABILITY OF COMPLETE DIAGNOSTIC AUTOPSY AND MINIMALLY INVASIVE TISSUE SAMPLING IN THE KILIMANJARO REGION OF NORTHERN TANZANIA

Francis P. Karia[1], Martha O. Mwanga[2], Elizabeth F. Msoka[2], Venance P. Maro[1], John A. Crump[3], Matthew P. Rubach[3], Lauren S. Blum[4]

[1]*Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania,* [2]*Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania,* [3]*Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, NC, United States,* [4]*Consultant, Duke University, Durham, NC, United States*

Lack of reliable information on cause of death (CoD) in low- and middle-income countries (LMICs) is recognized as a constraint to global health and development. While complete diagnostic autopsy (CDA) is considered the gold standard to determine CoD, researchers often claim that CDA procedures are incongruent with cultural and religious beliefs and therefore less acceptable in LMICs. Minimally invasive tissue sampling (MITS), a useful but less comprehensive means to establish CoD, has been assumed to be more socially acceptable in LMICs. We conducted research from May 2016 through April 2019 to assess autopsy acceptability in two referral hospitals in the Kilimanjaro Region of Tanzania where CDA and MITS are being offered for in-patient febrile deaths. Families approached for authorization were first offered CDA; if they refused, MITS was offered. In-depth interviews were conducted longitudinally across the study time span and involved 24 families who accepted autopsy, including six accepting MITS, and 24 families who refused CDA or MITS to assess reasons for acceptance and refusal. Of 407 families approached, 117 (29%) accepted autopsy procedures including 95 (81.2%) authorizing CDA. Of 312 refusing CDA, 22 (7%) authorized MITS. Reasons for CDA acceptance included uncertainty about CoD, benefits to family and science, and whether witchcraft contributed to the CoD. Reasons families accepted MITS were that it is less invasive and to conceal procedures from other members. Reasons for refusal of autopsy procedures included the CoD had already been determined, results would not benefit the deceased, inadequate information, or inappropriate timing of authorization. Reluctance to have the body cut was reported by comparatively fewer families. Results show that acceptance and refusal of autopsy is primarily related to the perceived benefits of learning the CoD and conduct of authorization, and less about the type of procedure. In northern Tanzania, families desiring to learn the CoD will choose CDA. Counter to assumptions about CDA acceptability in LMICs, CDA acceptance was relatively high and uptake of MITS by families who refused CDA marginal.

1394

THE ROLE OF TRUST IN EMERGENCY PREPAREDNESS: INSIGHTS FROM A QUALITATIVE STUDY ON ZOONOTIC DISEASES IN CÔTE D'IVOIRE

Natalie Tibbels[1], Danielle Naugle[1], Abdul Dosso[2], William Benié[2], Walter Kra[3], Corinne Fordham[1], Mieko McKay[2], Valère Konan[4], Jeanne Brou[5], Jocelyne Nebre[5], Adaman Kouadio[4], Zandra Andre[6], Diarra Kamara[2], Stella Babalola[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Johns Hopkins University, Abidjan, Côte D'Ivoire,* [3]*Alassane Ouattara University, Bouaké, Côte D'Ivoire,* [4]*Department of Veterinarian Services Ministry of Animal Ressources and Fisheries, Abidjan, Côte D'Ivoire,* [5]*National Institute of Public Hygiene, Abidjan, Côte D'Ivoire,* [6]*U.S. Agency for International Development, Abidjan, Côte D'Ivoire*

Recent outbreaks of zoonotic diseases in West Africa, such as the 2014-2016 Ebola epidemic, have drawn attention to the need for stronger trust between communities and the health system. Trust has been linked to increased healthcare-seeking practices, and lack of trust can hinder compliance with emergency response efforts. Previous research has identified specific types of trust that operate in a health systems setting (e.g. interpersonal, institutional), as well as determinants (such as perceived competence or honesty of the provider or agency). However, gaps remain in our understanding of public trust during zoonotic epidemics. A qualitative study was implemented in early 2019 to understand individual, social, and cultural determinants of risk, prevention, and response behaviors related to five priority zoonotic disease groups in Côte d'Ivoire. The study involved 32 focus group discussions, 33 in-depth interviews, 20 observations, and 20 community mapping activities engaging a total of 234 adult men and women across four urban sites. The salience of trust was a key finding from the study. Trust was linked to relational proximity, with peers and local leaders more trusted than actors operating outside of or above the local level. Participants at times questioned even the existence of zoonotic diseases and described suspicions related to specific authorities, such as veterinarians, health providers, public officials, and international entities. Participants who engaged in animal husbandry as their main economic activity expressed distrust of authorities due to an insufficient compensation system for slaughtered sick animals. Fear of losing their animals and livelihoods fostered distrust and impeded open and expedient communication with authorities concerning sick animals. Herders and poultrymen described high risk behaviors that may lead to zoonotic outbreaks. These findings point to the need for community engagement around livelihood protection and prevention of zoonotic disease transmission as well as programs that fairly compensate animal husbandry workers, to build trust toward epidemic prevention and response.

1395

IMPLEMENTATION OF THE SMITHSONIAN'S *OUTBREAK DIY* TOOLKIT FOR LOCAL COMMUNITIES OF LAIKIPIA, KENYA: SUCCESSES, CHALLENGES AND LESSONS LEARNED

Jennifer H. Yu[1], Sabrina Sholts[2], Dawn Zimmerman[1], Joseph Kamau[3], Elizabeth Ashby[1], Dino Martins[4], Fardosa Hassan[4], Suzan Murray[1], Kerri Dean[5]

[1]*Global Health Program, Smithsonian's National Zoo and Conservation Biology Institute, Washington, DC, United States,* [2]*Department of Anthropology, Smithsonian National Museum of Natural History, Washington, DC, United States,* [3]*Molecular Biology Unit, Institute of Primate Research, Karen-Nairobi, Kenya,* [4]*Mpala Research Centre and Wildlife Foundation, Laikipia, Kenya,* [5]*Department of Exhibitions, Smithsonian National Museum of Natural History, Washington, DC, United States*

In 2018, the Smithsonian's National Museum of Natural History launched *Outbreak DIY*, a free, multilingual, and customizable toolkit for public health education in communities worldwide. Intended to generate discussion and raise public awareness about zoonotic threats, the *Outbreak DIY* materials include digital files for panels, videos, and games that communicate the role of animals in public health, anthropogenic factors that drive their emergence, and risk mitigation strategies. While the *Outbreak DIY* toolkit has been used more than 85 times in 29 countries to date, here we present its use for public displays in Kenya as part of the larger USAID Emerging Pandemic Threats PREDICT project, with overall goals of strengthening health security as part of the Global Health Security Agenda. In addition to emphasizing the overarching message of the benefits of the One Health perspective, panels were customized to: 1) target regional priority diseases; 2) depict endemic and regional species of interest; 3) emphasize country-specific One Health policy, institutional bodies, and interventions; and 4) highlight key individuals and partners working in One Health. In communities of Laikipia, Kenya, *Outbreak DIY* achieved some initial objectives of its users, improving knowledge of the role animals play in disease transmission and ways to live safely with wildlife. The display prompted discussion of diseases, associated clinical signs, species that carry them, and behavioral practices that increase risk. Another success was the engagement of Kenyan graduate students in the communication of the exhibit's content, allowing for further tailoring of the subject matter to community contexts. These experiences show that the *Outbreak DIY* toolkit can be easily adapted for local communities, but to maximize its educational value, further consideration of additional concerns is recommended by its users, including: 1) facilitating engagement of local actors and health workers in the presentation; 2) further customization of the content to local communities for context on a finer scale; and 3) consideration of diverse or varied audiences in messaging.

1396

IMPACT OF A FACILITY-BASED INTERVENTION ON PROVIDERS' CASE-MANAGEMENT SKILLS RELATED TO CHILDHOOD DIARRHEA: LEARNING FROM A QUASI-EXPERIMENTAL STUDY CONDUCTED IN UTTAR PRADESH, INDIA

Lopamudra Ray Saraswati, Prince Bhandari, Animesh Rai, Ambrish Chandan, Ashutosh Mishra

RTI International India, New Delhi, India

Globally, India ranks first in terms of childhood diarrheal deaths, most of which are preventable with correct diagnosis and treatment. We conducted a quasi-experimental study to assess the effectiveness of an intervention aimed at reducing child mortality through capacity building of facility-based health providers toward appropriate case management of childhood diarrhea. This paper measures the impact of intervention using difference-in-differences (DID) analysis. We observed 331 suspected diarrhea cases (treatment: 191; comparison: 140) pre-intervention, and 306 cases (treatment: 160; comparison: 146) post-intervention, receiving care at the facilities in three intervention and three comparison districts. Appropriate case management was defined as the suspected diarrhea cases being diagnosed with diarrhea, assessment being done using World Health Organization's facility-based integrated management of neonatal and childhood illnesses (F-IMNCI) checklist, and prescription of correct treatment, that is ORS and Zinc for diarrhea with no or some dehydration, intravenous fluids for diarrhea with severe dehydration, and Ciprofloxacin or Ceftriaxone for dysentery. The intervention proved to be effective in strengthening providers' skills of diagnosis [DID coefficient: 0.26; 95% confidence interval (CI): 0.10 - 0.41] and treatment [DID: 0.17; 95%CI: 0.07 - 0.26]. However, it remained ineffective in improving their adherence to F-IMNCI guidelines for assessment, an important component of fidelity to evidence-based clinical practice. These findings indicate that interventions adopting a conventional approach can only partially improve the case management skills of providers and fails to make an impact on their practicing skill which is associated with attitudes and beliefs. Hence future child survival programs should focus on providers' behavioral aspect and explore innovative strategies to improve their practices for assessment of childhood diarrhea, besides continuing working on skill development for appropriate diagnosis and treatment.

1397

TRAVEL HEALTH PERCEPTIONS AND BEHAVIOR AMONGST PARTICIPANTS IN A TROPICAL MEDICINE DIPLOMA COURSE

Kyle Denison Martin[1], Sophie Dunin de Skrzynno[2], Yvonne Wekesa[3], Alex Fleming-Nouri[4], Stephanie Laura Wilson[5], Adam Boggon[2], Tembe Carveth-Johnson[2], Maria Henriksson[2], Joy Jones[2], Christopher Sanford[6]

[1]*Brown University, Providence, RI, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Tenwek Mission Hospital, Bomet, Kenya,* [4]*Oxford University Hospitals, Oxford, United Kingdom,* [5]*University College of London Hospital, London, United Kingdom,* [6]*University of Washington, Seattle, WA, United States*

In an increasingly interconnected world, travelers face new risks to their health. Previous studies have explored travel health perceptions and behavior amongst the general population of international travelers. However, few studies have examined the perceptions and behaviors of health care professionals. This information could be used to identify knowledge gaps and improve safety for health care professionals who travel abroad. This study aimed to examine travel health perceptions and behaviors amongst participants in a tropical medicine diploma course. This study featured a survey presented to participants in the East African Diploma in Tropical Medicine and Hygiene course offered by the London School of Hygiene & Tropical Medicine in 2018. The response rate for the survey was 71% (n=52). The most common sources of travel health information were the internet (59%), Travel Health specialists (51%) and participants themselves (37%). Participants identified road traffic accidents as the primary threat to their health (78%). Nonetheless, 59% of participants admitted to riding on a motorcycle taxi during their travel and only one-third of respondents noted wearing a helmet with >90% frequency. In fact, 8% confessed to wearing a helmet <10% of the time when using a motorcycle taxi. 68% of survey participants received a prescription for malaria prophylaxis. Of this group, only 71% took the medication as directed. The most common reason for not receiving a prescription for malaria prophylaxis was that participants did not feel prophylaxis was necessary. The health care professionals in this study relied heavily on the internet and their own personal knowledge for travel health information. Participants identified road traffic accidents as the primary threat to their health. Nonetheless, the majority of respondents confessed to high-risk behaviors such as using motorcycle taxis, often without a helmet. Only half of participants were taking malaria prophylaxis as directed. The information from this study can be used to develop travel health education for health care professionals to improve knowledge and decrease high-risk behavior.

1398

MANAGEMENT OF COMMUNITY-DIRECT TREATMENT WITH IVERMECTIN (CDTI) CAMPAIGN DURING INSECURITY IN THE SOUTHWEST REGION OF CAMEROON

Georges Nko'Ayissi[1], Victor Mbome NJie[1], Nde Bea[1], Biholong Benjamin Didier[1], Julie Akame[2], Patrick Mbia[2], Yaobi Zhang[3], Steven D. Reid[4], Ismael Teta[2]

[1]*Ministry of Public Health, NTD Coordination Unit, Yaoundé, Cameroon,* [2]*Helen Keller International, Yaoundé, Cameroon,* [3]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [4]*Helen Keller International, New York, NY, United States*

The 2018 community-direct treatment with ivermectin (CDTI) for onchocerciasis in the South West and North West regions of Cameroon was carried out amidst socio-political unrest. At the time of the CDTI some districts like Buea, Tiko and Ekondo-titi were affected by a mass population exodus from health districts (HDs) when rumors of a possible lockdown of the region were circulated. Since the census was completed the month prior to the CDTI, the target population was not the same during the CDTI. To ensure reaching those targeted for treatment, the ministry of health (MOH) developed several strategies that would allow the CDTI to continue during insecurity but obtain sufficient program coverage. To conduct health personnel training prior to the CDTI, pooled trainings in more secure health districts (HDs) in the region were developed as well as training sites established in neighboring HDs of other regions. Community drug distributor (CDD) training was organized according to the local context, for example, chiefs of health areas carried out training for CDDs in their safe locations. The 'Hit & Run' strategy was used to deliver the mass drug administration (MDA). The census and treatment were completed simultaneously in all HDs except one and the MDA was done in farms and camps. The HDs which could not begin the campaign the same time as the rest of the region were advised to begin whenever possible. Insecurity in some areas made it difficult to carry out MDA. In some areas there were rumors of poisoned medications being distributed so MOH staff used in-person discussions and opinion leaders to reassure population that IVM has been distributed safely for over 20 years. Despite a reduction in population of 37.9% from the 2018 MDA census data, these new approaches enabled a programmatic coverage of 74.2% of those who remained in the regions. To improve this performance during the next MDA campaign, the regional Delegation of Public Health agreed to inform the population beforehand of the distribution period, to carry out census and treatment simultaneously in all HDs in these regions and to intensify team work at all levels.

1399

NEMO: AN OPEN SOURCE SOFTWARE FOR SURVEY FORM DESIGN AND AGGREGATION FOR USE WITH OPEN DATA KIT COLLECT

Dottie Hunt, Andrew W. Nute, Gregory S. Noland, Emily Griswold, Lindsay J. Rakers, Brianna Poovey, Avery Davis-Roberts, Seseni Nu, Adam J. Weiss, E. Kelly Callahan, Scott D. Nash

The Carter Center, Atlanta, GA, United States

Collection of electronic data for global health and neglected tropical diseases (NTDs) in particular, is becoming the norm, and in some cases a requirement, by various stakeholders. However, most software available have high operating costs and create reliance on external parties for technical support. Beginning in 2011, The Carter Center began to create an open source data collection software for election monitoring called ELMO to be used in conjunction with the Android application, Open Data Kit Collect. The Center eventually developed the next generation of the software, now called NEMO, to facilitate the survey capabilities needed by its other programs. To date, NEMO has been used to design and deploy surveys for monitoring and evaluation efforts related to the NTDs guinea worm, trachoma, lymphatic filariasis (LF), onchocerciasis, schistosomiasis, soil-transmitted helminthiasis, and loiasis. Defined as a specific program/initiative in a given country, 75 missions have been established in 10 languages across 25 countries of which 12 (16%) missions in six countries are related to NTDs or global health. Data collection for election monitoring has involved as many as 25,000 enumerators and have produced as much as 1.3 gigabytes of survey data for a single mission. Common NTD assessments facilitated in NEMO include Trachoma Impact Surveys, Transmission Assessment Surveys for LF, and coverage surveys for mass drug administration of a variety of preventive chemotherapeutic drugs. Other programs at The Center have used NEMO for survey use cases investigating progress in human rights and access to information internationally. Through low skill requirements, NEMO empowers the user with holistic ownership of the data collection process resulting in better control of the data produced. By doing this, NEMO has proven the feasibility of high-quality data collection at scale without presenting the drawbacks that are typically associated with similar survey management software.

1400

A MULTI-SECTOR ENGAGEMENT APPROACH TO DEVELOPING A PLATFORM FOR ETIOLOGICAL DIAGNOSIS OF FEBRILE ILLNESSES IN WEST AFRICA

Edward O. Nyarko[1], Andrew Letizia[2], William Asiedu[1], Patricia Adams[1], Mihret F. Amare[3], Jayda Jones[3], Suzanne Mate[4], Kara Lombardi[3], Leigh Ann Eller[3], Inger-Marie Vilcins[3], Zahra Parker[3], Abdulwasiu B. Tiamiyu[5], Edward Akinwale[5], Amy Castellano[3], Ayesha Rashid[3], Mark Milazzo[3], Heather Lieu[3], Jarrett Headley[3], Michael Iroezindu[5], Joseph Diclaro[6], Paul Scott[4], Merlin Robb[3], Nelson Michael[4], Julie Ake[4], Kayvon Modjarrad[4]

[137] *Military Hospital, Accra, Ghana,* [2]*Navy Medical Research Unit-3 Ghana Detachment, Accra, Ghana,* [3]*Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States,* [4]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [5]*Henry M. Jackson Foundation Medical Research International, Abuja, Nigeria,* [6]*Navy Entomological Center of Excellence, Jacksonville, FL, United States*

The 2014 West African Ebola virus disease (EVD) outbreak demonstrated the threat of infectious diseases and highlighted the need for improved surveillance of high-risk pathogens. The Joint West Africa Research Group (JWARG) is a military and academic collaboration between Ghana, Liberia, Nigeria and the United States. JWARG's goal is to improve partner nation capacity while looking to identify the etiology of febrile illness in West Africa. Since 2017, individuals presenting to select military treatment facilities in Ghana, Liberia, and Nigeria with signs and symptoms consistent with a fever of unknown origin were eligible. Those who consented provided clinical and laboratory data upon enrollment and subsequently at a convalescent encounter 28 days later. Although the testing methodology includes molecular, serologic and genomic analysis, preliminary results from rapid diagnostic tests (RDTs) provide an initial insight into the pathogens causing febrile illness. As of April 2019, 379 participants, 55% female, averaging 37-years old have been enrolled with 74% from Nigeria, 25% from Ghana and 1% from Liberia. At enrollment, 56 (14%) participants were positive for malaria by RDT and 49 (12%) thick/thin smear by microscopy with about 30% discordance. A total of 48 (12%) participants had two concordant positive HIV RDTs and 5 cases were positive for tuberculosis though identification of acid-fast bacilli or by GeneXpert testing. Schistosomisasis RDT was positive in 30 participants. Additionally, 112 participants samples have tested negative for viral hemorrhagic fever as well as flaviviruses and alphaviruses. JWARG improves biopreparedness by establishing a shared research platform for rapidly diagnosing various etiologies of febrile illnesses in West Africa. While still pending confirmatory testing, multiple pathogens of public health concern including HIV, malaria, TB and schistosomiasis have been identified. Further analysis with more specific testing is ongoing and will provide valuable insight into the epidemiology of febrile illness in Ghana, Liberia, and Nigeria.

1401

BUILDING AN EBOLA-READY WORKFORCE: LESSONS LEARNED ON STRENGTHENING THE GLOBAL WORKFORCE THROUGH UNIVERSITY NETWORKS

Katey Pelican[1], Saul Tzipori[2], **Jeff Bender**[3]

[1]*University of Minnesota, St. Paul, MN, United States,* [2]*Tufts University, North Grafton, MA, United States,* [3]*University of Minnesota, Minneapolis, MN, United States*

Recent and on-going threats around the world have underscored a critical need for health workers that are prepared to manage diseases that cross human, animal, and environmental health sectors. The 2013 emergence of the Ebola virus in West Africa posed a global threat to human and animal health, as well as national security and economic prosperity. The epidemic called for a workforce that had the technical skills and competencies to work well within disciplines and sectors, but also possessed the skills to work across sectors and disciplines to promote coordination and communication among all the stakeholders necessary for effective and efficient control of an infectious disease outbreak at this global scale. The USAID One Health Workforce (OHW) Project, launched in 2014, aimed to achieve a workforce that is better prepared to prevent, detect, and respond to emerging infectious disease threats. Focusing on two infectious disease hot spot regions in Africa and Asia, OHW strengthens training and educational programs in universities in 12 countries to create a skilled workforce in using a transdisciplinary approach known as One Health. The focus of the work is on multi-sectoral engagement, education and training, and strengthening of Universities. Accomplishments to date include 140 community outreach activities including 15 outbreak investigations. 12 countries have conducted 14 national health workforce assessments. The individuals trained include over 3,600 students, 1,200 in-service professionals, and 29 fellows placed in health-related organizations. To support University institutions, which includes over 162 Schools/faculties across 84 Universities, professional development to 3,500 faculty was provided and 24 new academic and professional programs (i.e. Wildlife Health and Epidemiology Programs in DRC and Senegal) were established. This One Health effort leveraged the resources from established University networks creating a sustainable transformation among the health workforce in Africa and Southeast Asia.

1402

CAPITALIZING ON A COLLABORATIVE MODEL TO STRENGTHEN INSTITUTIONAL HEALTH SERVICE DELIVERY: A SUCCESSFUL PARTNERSHIP BETWEEN THE AUSTERE ENVIRONMENTS CONSORTIUM FOR ENHANCED SEPSIS OUTCOMES (ACESO) AND A GHANAIAN TERTIARY HOSPITAL

George Oduro[1], Chris Oppong[1], Alex Owusu-Ofori[2], Daniel Ansong[3], Anne Fox[4], Andrew Letizia[4], Josh Chenoweth[5], Charmagne Beckett[6], Benjamin Espinosa[6], Danielle Clark[5]

[1]*Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [2]*Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [3]*Department of Child Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [4]*Naval Medical Research Unit Number 3, Accra, Ghana,* [5]*Henry M Jackson Foundation, Austere Environments Consortium for Enhanced Sepsis Outcomes, Bethesda, MD, United States,* [6]*Naval Medical Research Center, Frederick, Austere Environments Consortium for Enhanced Sepsis Outcomes, Frederick, MD, United States*

We describe how a collaborative study with The Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO) has positively strengthened health service delivery and research capacity in a Ghanaian tertiary hospital. ACESO is a consortium of military medical and academic research institutes aiming to improve early recognition, diagnosis, and treatment of sepsis in low-resource settings. KATH sees patients from the northern half of Ghana and three neighbouring West African countries. From July 2016, demographic and clinical data from 220 subjects with suspected sepsis were collected and analyzed. In addition to CBC, biochemistry, and blood cultures, RDT panels were run for malaria, Dengue, Chikungunya, Hepatitis A, B, and C, and HIV. Patients with sepsis had a mortality rate of 40%. Benefits to the host institution include ergonomic redesign of the microbiology laboratory, training of laboratory personnel in technologies such as DNA extraction and utilization of chromogenic bacterial agar, reliable access to ATTC quality control strains of bacteria, development of local expertise in bacterial species identification, improvement in antimicrobial susceptibility testing, and expansion of research infrastructure and skills. Patients benefited from quick turnaround of laboratory tests. The collaboration has enabled international educational exchanges. For example, KATH has hosted US military physicians with an interest in tropical diseases annually. Personnel from KATH have had training in Nigeria on malaria diagnosis and laboratory assays. These exchanges provide opportunities to gain knowledge in international best practices and improve biosurveillance capabilities for infectious disease threats in the subregion. In conclusion, the collaboration has facilitated training for clinical and laboratory personnel, and provided functional and infrastructural development to the host institution. We contend that this delivers a firm base for future

collaboration involving intervention studies, with prospects for developing tests to aid early recognition, diagnosis and treatment of sepsis in low-resource settings.

1403

CHOLERA IN INTERNALLY DISPLACED PERSONS CAMPS IN BORNO STATE—NIGERIA, 2017: A QUALITATIVE STUDY OF THE MULTI-SECTORIAL EMERGENCY RESPONSE TO STOP THE SPREAD OF THE OUTBREAK

Moise C. Ngwa[1], Alemu Wondimagegnehu[2], Ifeanyi Okudo[3], Collins Owili[4], Uzoma Ugochukwu[3], Clement Peter[3], Isabelle Devaux[5], Lorenzo Pezzoli[6], Chikwe Ihekweazu[7], David A. Sack[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*International Health Consultancy, LLC, Atlanta, GA, United States,* [3]*World Health Organization Country Office, Nigeria, Abuja, Nigeria,* [4]*World Health Organization Country Office, Nigeria, Abuja, Nigeria,* [5]*World Health Organization Country Office, Borno State, Nigeria, Maiduguri, Nigeria,* [6]*World Health Organization, Geneva, Switzerland,* [7]*Nigeria Centre for Disease Control, Nigeria, Abuja, Nigeria*

In August 2017, a cholera outbreak started in Muna Garage camp for internally displaced persons, Borno State-Nigeria, and circa 5000 cases occurred in six local government areas. This qualitative study evaluated perspectives about the emergency response to this outbreak. We conducted 39 key informant interviews, focused group discussions, and reviewed 21 documents linked with surveillance, water-sanitation-hygiene, case management, immunization, communications, logistics, and coordination. Data analysis used thematic techniques comprising key-words-in-context, and word-repetition. Authorities were alerted quickly of outbreak, but declaration took 12 days due to a 10 day delay in laboratory confirmation. Investigation revealed several transmission channels including a leaking latrine around the index cases' house. Families refused chlorine use due to rumors that it would sterilize women. This could have been avoided with improved community consultation. Initially, communication was in Hausa, although 'Kanuri' was the main language; later this was corrected. Preparedness plans lacked exercise drills to identify weaknesses. Response by the Rural Water Supply and Sanitation Agency was perceived to be slow and increased risk from Eid El Jabir festival with increased movement and food sharing was not recognized. Treatment centers provided case management, but some partners were concerned that their work was recognized asking, "who gets the glory and the data?" Oral cholera vaccine was provided to nearly one million people using a robust polio vaccine structure; however, peripheral staff payment needed resolution. Initial coordination of multi-sectoral response activities by Borno Ministry of Health was slow, but improved by activating an Emergency Operations Centre and Incident Management System. The synergy between partners and government improved when each recognized the government's leadership role. Despite a timely alert of the outbreak, the delayed declaration led to a slowed initial response, which improved during the course of the outbreak. Improvements in camp and laboratory capacities are urgently needed.

1404

OBSTETRICS-GYNECOLOGY GRAND ROUNDS AS A MEANS TO EVALUATE AND IMPROVE PROVIDER KNOWLEDGE OF CONGENITAL CHAGAS DISEASE

Erica L. Crosley, Federico Palacio-Bedoya

Emory University School of Medicine, Atlanta, GA, United States

Chagas disease, a vector-borne parasitic infection by *Trypanosoma cruzi*, affects over 340,000 people in the U.S. Vertical transmission perpetuates Chagas disease burden in non-endemic countries, and early detection decreases morbidity. A 2010 survey by the American College of Obstetricians and Gynecologists found limited knowledge of Chagas vertical transmission among obstetrician-gynecologists and suggested more provider education. Grady Memorial Hospital (GMH) in Atlanta, GA is a large component of Emory School of Medicine's academic medical training. 20% of GMH deliveries are to Hispanic females largely from endemic countries. This study seeks to replicate the ACOG survey among obstetrician-gynecologists from Emory satellite clinics and three main university hospitals. It also evaluates the effect of Grand Rounds on provider congenital Chagas knowledge. Faculty attendings, fellows and resident physicians from obstetrics-gynecology were present, along with medical students. 31 surveys were completed-38.7% by attending faculty, 19.4% by residents, 32.3% by medical students, and 9.7% by fellows/other. Before Grand Rounds, 93.5% rated their Chagas knowledge as 'limited' or lower, 83.8% reported 'never' or 'rarely' considering Chagas in patients from endemic areas, 80.6% incorrectly answered possible maternal infection timing in relation to vertical transmission, and 80.6% incorrectly answered vertical transmission risk in chronic Chagas. After Grand Rounds, there was a significant difference (α 0.05) in the percent reporting limited Chagas knowledge (64.5%), the percent incorrect regarding maternal infection timing and vertical transmission (19.35%), and the percent incorrect regarding vertical transmission risk of chronic Chagas (29.0%). Education is an important tool for Chagas awareness, and there is limited knowledge even at high-volume academic centers seeing at-risk patients. The study highlights the need for targeted provider education regarding Chagas screening. This educational activity resulted in the creation of a prenatal Chagas screening program at GMH by an interdisciplinary Emory team.

1405

PRECOSAN: CONCERTED RESEARCH PROGRAM IN HEALTH ECONOMICS AT KINSHASA SCHOOL OF PUBLIC HEALTH

Aimée Lulebo Mampasi[1], Serge Mayaka Manitu[1], Patrick Suykerbuyk[2], Diana De Graeve[2]

[1]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo,* [2]*University of Antwerp, Antwerp, Belgium*

The Democratic Republic of the Congo (DRC) lacks necessary resources to provide efficient and equitable health services. Reinforcing local capacity is essential to constructively evaluate all health-care financing alternatives, to demonstrate the economic burden of health-related conditions, and to meaningfully determine the value of proposed programs and interventions, appropriate within the Congolese context. To provide efficient and equitable health services for improving the health of the Congolese population, we developed a seed-grant funded project including the following two specific objectives (SO): SO1. Strongly reinforce a concerted research program in health economics (HE) at Kinshasa School of Public Health (KSPH). To achieve this goal, we organized and facilitated both interactive consultations and needs assessment with KSPH staff members and governmental, non-governmental, private, academic and international stakeholders. The main outcome of these bottom-up sessions is the development and implementation of a strategic sustainability plan for a HE research program at KSPH (PRECOSAN: Programme de Recherche Congolais en Economie de la Santé) that is both need and demand driven. Furthermore, we will increase the visibility of PRECOSAN through the development and implementation of a dissemination and outreach plan (e.g., website, networking, meetings and conferences). SO2. Strengthen the research and training capacities in HE at KSPH. To achieve this goal, we mentor and supervise two health economists (PhD-level) to improve their capacities to successfully navigate the academic ladder (both scientifically and management) as part of the capacity retention at KSPH. Furthermore, we offered three scholarships for a master training in HE at KSPH and will award four additional research grants (#2 per year) for master's thesis research in HE. The selected students will be offered research opportunities in the newly developed HE research program (PRECOSAN). Here we will present the main outcomes of the needs and demand assessment in HE conducted in 2018-2019 among the key health stakeholders in DRC.

1406

GEOSPATIAL ANALYSIS REVEALS SUBNATIONAL VARIATION IN CHILD MORTALITY SURVEILLANCE COMPLETENESS ACROSS CENTRAL AND SOUTH AMERICA

Nathaniel Henry[1], Roy Burstein[2], Michael Collison[1]

[1]*Institute for Health Metrics and Evaluation, Seattle, WA, United States,* [2]*Institute for Disease Modeling, Bellevue, WA, United States*

Renewed emphasis on strengthening the capacity of district health programs and new technologies for storing and disseminating health records have fostered growth in both the quantity and quality of local health surveillance data in countries across the Global South. Given that national health agencies often use routine surveillance data to inform decisions about health funding priorities, the global health community must develop a suite of sophisticated and appropriate statistical tools for analyzing local health status and health program performance based on these data. However, despite analytical and policy advantages, spatially-resolved analyses of national health surveillance data have historically been limited due to the challenge of correcting for varying data incompleteness across space and time. This research develops a method for incorporating government surveillance data and household surveys into a unified model of child mortality at the sub-national level. The method integrates these two data types in a joint model that produces two space-time structured surfaces, one estimating child mortality rate, and the other estimating reporting completeness for surveillance data. The model is applied to produce estimates of both child mortality and vital registration incompleteness at the district level across 12 countries in Latin America, revealing local variation and inequality that are masked by national estimates.

1407

INCREASED ACCESS TO ESSENTIAL HEALTH COMMODITIES THROUGH SUPPLY CHAIN AND INFORMATION SYSTEM INTEGRATION IN LAO PDR

Bounxou Keohavong[1], Many Thammavong[2], Lauren Theis[3], Dalavone Sengamphay[3]

[1]*Ministry of Health Food and Drug Department, Vientiane, Lao People's Democratic Republic,* [2]*Ministry of Health Medical Product Supply Center, Vientiane, Lao People's Democratic Republic,* [3]*Clinton Health Access Initiative, Vientiane, Lao People's Democratic Republic*

Vertical supply chain systems lead to inefficient stock management, surveillance, and administration, causing patients in Lao People's Democratic Republic (Lao PDR) to face inconsistent access to essential health commodities due to stock-out and expiry. In 2014, 31% and 25% of high-burden health facilities reported a malaria rapid diagnostic test (RDT) or artemisinin-based combination therapy (ACT) stock-out, respectively. Between 2013 and 2015, more than $400,000 of anti-retroviral (ARV) drugs expired, equivalent to 70% of the country's annual ARV budget needs. In 2015, the Lao PDR Ministry of Health (MoH) began implementing significant reforms to address these issues, largely through the integration of multiple, vertical supply chains managed by programs into one supply chain system managed by the Food and Drug Department (FDD). These reforms include the adoption of an electronic logistics management information system, mSupply, at national, provincial, and district levels, which has led to efficiencies in stock forecasting, procurement, management, and distribution. In 2018, fewer than 15% of high-burden facilities in Lao PDR experienced an ACT or RDT stock out. In 2019, modifications enable interoperability between the national mSupply and DHIS2 surveillance systems so that health actors across all levels can view and analyze commodity and epidemiological data together in a single system. The Lao PDR MoH's supply chain and information system integration has created a robust surveillance system that transforms the country's ability to make data-informed decisions and increase access to essential health commodities and services.

1408

MANAGING OPERATIONAL AND FUNDAMENTAL RESEARCH TO CREATE SYNERGIES IN PUBLIC AND MILITARY HEALTH EFFORTS ADDRESSING MALARIA

Nicole Y. Zdrojewski, Thi Phuong Hoa Nguyen, John W. Fallon

Vysnova Partners, Inc., Landover, MD, United States

Operational malaria research is conducted to reduce the impact of malaria in civilian and military populations. A primary challenge associated with its design and execution is the presence of many actors carrying out research under malaria elimination agendas. Research and communications gaps are inherent in these multi-governmental and multi-agency programs. Such gaps are missed opportunities to harmonize research agendas and leverage resources. Developing shared research program management and knowledge management capabilities would enable funders, national malaria control programs (NMCPs), and militaries to enhance the impact of limited research funds on salient issues in the fight against malaria. Evaluative reports on complex foreign assistance programming (e.g., Global Fund or the US foreign assistance portfolio) have identified coordination challenges among funders with similar missions and goals. While supporting operational malaria research in Vietnam, we noted programmatic challenges in planning and executing (e.g., identifying duplicative efforts, cementing trusting partnerships, executing time-constrained funds, assuring quality) as well as systematized knowledge management (KM) within and among teams and institutions. We developed an adaptive program management model, including knowledge management, for operational research. Our cooperative approach engages partners and stakeholders, provides real-time analysis and manages interlocking projects with an evolving array of desired endpoints. Based on lessons learned from collaborations in Vietnam, we recommend program activities be developed considering the following critical elements: 1) situational awareness, 2) relationships (in place or in progress), 3) management infrastructure, 4) scientific infrastructure and 5) desired research endpoints. Instituting a research-focused program management office complemented with a KM platform for operational malaria research would facilitate more strategic protocol design and study execution that furthers funders' and NMCPs' goals among critical military and civilian populations.

1409

PORTABLE SCREENING DEVICES TO ASSESS MEDICINES QUALITY FOR NATIONAL MEDICINES REGULATORY AUTHORITIES

Céline Caillet, Paul N. Newton

Lao-Oxford Mahosot Hospital Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic

The World Health Organization estimated that ~10% of medical products circulating in low- and middle-income countries are either substandard or falsified (SF). SF risk increased morbidity and mortality, economic losses and diminished public confidence in health systems. SF antimicrobials, particularly those containing reduced quantities of Active Pharmaceutical Ingredients (APIs), may be key but neglected drivers of antimicrobial resistance. Medicines quality screening devices hold great promise to detect SF during post-market surveillance (PMS) by Medicines Regulatory Authorities. We will give insight into the advantages/limitations of diverse screening devices in the hands of end-users observed in a field setting evaluation in Laos. Most portable devices are accurate to detect zero and wrong API medicines. However, the screening of low % API remains a significant gap, as there are major concerns about their availability in diverse supply chains. Indeed, substandard medicines have been found in most of the recent large surveys. The abilities and feasibility of using screening devices to quantitate APIs in pharmaceutical products are explored. Our evaluation also suggested that policy makers wishing to implement devices in PMS should be aware that overconfidence in the

devices may cause harm by reducing inspectors' investment in visual inspection. The latest developments of portable and handheld devices will also be presented.

1410

UNDERWEIGHT AND STUNTING AMONG BANGLADESHI FEMALE ADOLESCENTS: FINDINGS OF A NATIONALLY REPRESENTATIVE SURVEY

Kazi Istiaque Sanin, Ahshanul Haque, Mansura Khanam, Gulshan Ara, Tahmeed Ahmed

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Optimum nutritional status of an adolescent girl is not only vital for herself but also for the future generation. Nutritional status of the adolescents are often under-reported in resource-poor countries like Bangladesh. Bangladesh Integrated Household Survey (BIHS) contains data on nutrition status of every household member, individual food consumption and other potential determinants of nutrition. We analyzed the BIHS 2015 round data to explore the nutritional status of the rural adolescent girls aged 10-19 years and to investigate associated factors. We collected data on households those are nationally representative (rural only). We extracted data for girls aged 10-19 years, their anthropometric measurements, 24-hour dietary recall data and additional information. We had information of 1,835 adolescent girls with an average age of 14.1±2.8 years. Majority of the girls (89.9%) were never married and attending school during the survey period (82.2%). Among the menstruating girls, only 10.4% used disposable sanitary pad. The average height was 146.4±9.4 cm and weight was 38.4± 9.7 kg. Among the girls, 14.8% had BMI-for-age Z score <-2SD (thinness) and 5.4% were overweight or obese. About 26.5% adolescent girls were stunted (height-for-age Z score<-2SD). The major sources of animal protein for these girls were fish (71.7%), dairy products (32.1%) and egg (22.5%). In multivariable regression, only age and education level of these adolescents were significantly associated with both underweight and stunting. Age was a protective factor in underweight (AOR=0.91, 95% CI 0.85-0.98) and girls having only primary or less education had 64% higher odds (AOR=1.64, 95% CI 1.16-2.34) of being underweight compared to those with secondary education. Age (AOR=1.23, 95% CI 1.17-1.30), primary or less education (AOR=1.94, 95% CI 1.48-2.55) and unhygienic toilet (AOR=1.29, 95% CI 1.03-1.63) were the factors associated with stunting in these girls. The prevalence of underweight and stunting was high among adolescent Bangladeshi girls. Policymakers must focus on this group, as they are the key to break the intergenerational cycle of undernutrition.

1411

MENTAL ILLNESS AND HOMELESSNESS FROM THE FEMALE PERSPECTIVE: INSIGHTS FROM LOS ANGELES COUNTY

Hannah L. Stewart

University of Southern California, Los Angeles, CA, United States

Abstract Research has consistently shown that individuals with mental illness are at increased risk of life events that may lead to homelessness, such as low income, poor coping skills, and incarceration. But little is known about how previous life events and mental illness may affect individuals once homeless. What is known about homelessness in urban environments is largely based on samples that are predominantly male. However, housing inadequacy in the United States is increasingly affecting women and female-headed families. This study analyses a large sample (n = 1351) of women experiencing homelessness in Los Angeles County. The cross-sectional data provides insights into the demographic makeup of unsheltered women in the county, including indicators of mental health. The mean age of the sample was 46 years of, with most the women identifying as either white (41%) or black (34%). Nearly half (48.7%) of the sample reported a history of depression, post-traumatic stress disorder, and/or another serious and persistent mental illness. More than half (55.9%) of these women reported a history of depression. The prevalence of post-traumatic stress disorder in this sample was 37.8% and rates of drug and alcohol abuse were 28% and 21% respectively. Chi-squared analyses showed that adults who were both mentally ill and homeless were more likely than homeless adults without mental illness to report prior domestic violence, human trafficking, and incarceration ($p < 0.01$). A t-test for independent samples revealed that mental illness was also statistically significantly associated with longer periods of homelessness ($p < 0.01$). This study provides support for the notion that traditional methods of alleviating homelessness, like job training programs, will do little to treat a one of the major drivers of chronic homelessness. The data presented here alludes to the fact that supportive housing with integrated mental health services and trauma informed services will be necessary to deal with the growing number of homeless women in Los Angeles.

1412

COMPARATIVE BEHAVIORAL RESPONSES OF *AEDES AEGYPTI*, *AEDES ALBOPICTUS* AND *CULEX QUEQUINFASCIATUS* (DIPTERA: CULICIDAE) TO PLANTS BASE REPELLENT OF VETIVER COMPOUNDS

Jirod Nararak[1], Sylvie Manguin[2], Theeraphap Chareonviriyaphap[1]

[1]*Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand,* [2]*HydroSciences Montpellier (HSM), Institut de Recherche pour le Développement (IRD), CNRS, Université Montpellier, Montpellier, France*

There are many reports revealed that some plant-derived are considered non-toxic, alternative insect repellents for humans. Vetiver compounds were assessed as repellent mosquito vectors. *Aedes aegypti*, *Aedes albopictus* and *Culex quequinfasciatus* were testes behavior responses with vetiver compounds through the High Throughput Screening System (HITSS). As a result, the behavioral responses of mosquitoes to plant base of vetiver compounds indicated that some compounds had spatial repellent, contact irritant and/or toxic properties. The study provides informative data on the mosquito-repellent property of vetiver plant against *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus*. We conclude that the pure compounds from vetiver could potentially be developed as promising plant-based repellents fight against the bite of mosquitoes.

1413

ASSESSING THE IMPACT OF CLIMATE CHANGE ON SLEEPING SICKNESS IN ZIMBABWE USING A GEOSPATIAL MODEL OF TSETSE POPULATION DYNAMICS

Joshua Longbottom, Jennifer Lord, Stephen Torr

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Climate change influences the entomological and epidemiological components of vector-borne disease transmission. Rhodesian human African trypanosomiasis (rHAT), a zoonosis caused by trypanosomes transmitted by tsetse flies, occurs at localised *foci* across East and Southern Africa. At one focus in northern Zimbabwe, a long-term decline in the abundance of tsetse appeared to be correlated with recent increases in temperature. A mechanistic model of tsetse population dynamics suggests that this decline is due to the impact of temperature on tsetse mortality and development rates. We aimed to produce a geospatial model of tsetse population dynamics to explore the effect of climate change on the abundance of the rHAT vector *Glossina pallidipes* across all sites in Zimbabwe where rHAT has been reported. We compared remotely sensed MODIS temperature data to local weather station data previously used to fit a mechanistic model to longitudinal *G. pallidipes* abundance. We applied an adjustment to convert from land surface temperature to air temperature, and then used the adjusted data to estimate mortality and development rates in the existing ordinary differential equation (ODE) model, running simulations for each 1 km × 1 km cell in Northern Zimbabwe. The projection of this model throughout Northern Zimbabwe produced estimates of changes in tsetse abundance for locations currently lacking longitudinal data. Our model predicts decreasing abundance of tsetse within several low elevation areas in relation to increasing

temperature trends during 2000-2016. Conversely, we show that several high elevation areas, previously considered too cold to sustain tsetse, are now suitable climates, with viable population predictions. The model produced here details the first temperature-driven spatial projection of tsetse population dynamics within Zimbabwe, and suggests that tsetse abundance is decreasing across much of the Zambezi valley. Future work, including empirical studies to validate model predictions and forecasting in relation to future climate trends, is planned.

1414

RISK OF TRANSMISSION OF DENGUE, CHIKUNGUNYA AND ZIKA IN SOME YELLOW FEVER HOTSPOT AREAS IN NORTHERN GHANA

Joannitta Joannides[1], Mawuli Dzodzomenyo[2], Faustus Azerigyik[1], Esinam E. Agbosu[3], Deborah Pratt[3], Joseph H. Osei[1], Rebecca Pwalia[1], Godwin K. Amlalo[1], Maxwell A. Appawu[1], Hayashi Takashi[1], Andrea Buchwald[4], Rosemary Rochford[5], Daniel A. Boakye[1], Kwadwo A. Koram[6], Kofi Bonney[3], Samuel K. Dadzie[1]

[1]Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Department of Biological, Environmental and Occupational Health, School of Public Health, University of Ghana, Accra, Ghana, [3]Department of Virology, Noguchi Memorial Institute for Medical Research, Accra, Ghana, [4]Department of Environmental and Occupational Health, School of Public Health, University of Colorado, Denver, CO, United States, [5]Department of Immunology and Microbiology, University of Colorado, Anschutz Medical Campus, Denver, CO, United States, [6]Department of Epidemiology, School of Public Health, University of Ghana, Legon, Accra, Ghana

This study investigated the risk of transmission of Dengue (DENV), Chikungunya (CHKV) and Zika (ZIKV) viruses in Larabanga and Mole game reserve. Larabanga and Mole in the Northern part of Ghana have been hotspot areas for previous outbreaks of Yellow fever due to the presence of a high population of *Aedes (Ae.) aegypti*. We collected immature and adult stages of *Aedes* mosquitoes from Larabanga and Mole game reserve. A total of 1,930 *Aedes* mosquitoes were collected during the rainy season and morphologically identified. Of these, 1,915 (99.22%) were *Aedes aegypti* and 15 (0.22%) were *Aedes vittatus*. During the dry season, 27 *Aedes aegypti* mosquitoes were collected. The proportion of *Ae. aegypti* mosquitoes collected per area were 144 (7.46%) for Larabanga and 1,771(91.76%) for Mole in the rainy season. A total of 627 *Ae. aegypti* mosquitoes were molecularly identified to subspecies level and pools of 30 were examined by Reverse Transcriptase Polymerase Chain Reaction to detect Dengue (serotypes 1-4), Zika virus and Chikungunya. Both *Ae. aegypti aegypti and Ae aegypti formosus* exist in sympatry in the area. All the pools were negative for DENV, CHKV and ZIKV. Three Larval indices namely House Index, HI (percentage of houses positive for larvae or pupae), Container Index, CI (the percentage of containers positive for *Aedes* larvae or pupae) and Breteau Index, BI (the number of positive containers (with larvae and/or pupae per 100 inspected houses) were assessed. During the survey, a significantly ($P<0.05$) higher number of containers 70 (17.07%) were positive for *Aedes* larvae in the rainy season compared to the dry season 2 (0.92%). Larabanga had a HI, CI and BI of 36.4%, 15.5% and 67.6% respectively for the rainy season with the dry season recording an HI, CI and BI of 2.3%, 1.3% and 2.3% respectively. Mole had higher larval indices compared to Larabanga during the rainy season. The larval indices were low for both areas during the dry season but significantly ($P<0.005$) higher during the rainy season. The implications of these findings within the context of DEN, CHKV and ZIKV transmission in Ghana will be discussed.

1415

KENYAN LONG-TERM EXCLUSION EXPERIMENT REPLICATION STUDY INVESTIGATING POTENTIAL INFLUENCE OF CATTLE ACARICIDES ON ENVIRONMENTAL TICK DENSITIES

Sheryne Zeitoun[1], Rachel Morrison[1], Lindsey Shields[2], Dawn Zimmerman[2], Dino Martins[3], Duncan Kimuyu[3], Wilfred Odadi[4], **Michael E. von Fricken**[1]

[1]George Mason University, Fairfax, VA, United States, [2]Smithsonian Institution, Washington, DC, United States, [3]Mpala Research Center and Wildlife Foundation, Laikipia, Kenya, [4]Egerton University, Department of Natural Resources, Nakuru, Kenya

African savannas support some of the most biodiverse large mammal populations in the world, which in turn, lead to diverse tick populations. To reduce the economic impact of tick-borne diseases in Kenya, cattle are often treated with acaricides. However, the impact of acaricides on the overall density and community composition of ticks in savanna ecosystems warrants additional investigations. Building upon previous work by Keesing et al., a team of George Mason University students sampled for ticks in different types of enclosures to assess the effects of wild life and acaricide treated cattle have on tick densities. This work was carried out in the Mpala Research Centres Kenya Long-term, Exclusion Experiment (KLEE), as part of a larger project conducting training on One Health and emerging infectious diseases. Over two weeks in August 2018, nymphal and adult ticks were sampled from KLEE enclosures that controlled for the presence of cattle and for the presence of two categories of large wild mammals: megaherbivores, and all other large wild herbivores. A lower tick density was observed on plots that cattle had access to compared to plots which other large mammals, which is likely due to acaricides on the cattle. Three species of adult ticks were found in the plots, 90% of which were *Rhipicephalus pulchellus*, 6% were *Amblyomma gemma*, and 4% were *Hyalomma dromedarii*. Our results suggest that the presence of acaricide-treated cattle greatly reduced the number of host-seeking adult ticks, but did not significantly reduce the number of host-seeking nymphs. Further efforts are underway to investigate if infection rates vary by enclosure plot, with additional training planned for August 2019. Continued monitoring of ticks and the pathogens they transmit is critical for understanding transmission dynamics that can eventually be used to guide education and control efforts.

1416

TISSUE-CULTURE PLATE-BASED FECUNDITY AND FERTILITY ASSAY SYSTEM FOR *AEDES* MOSQUITOES

Hitoshi Tsujimoto, Zachary N. Adelman

Texas A&M University, College Station, TX, United States

As major arboviral vectors, control of *Aedes* mosquitoes represents a substantial component of public health strategies to reduce transmission. Many studies aim to reduce survivorship, fecundity (number of eggs produced) and fertility (number of hatched larvae) of *Aedes* mosquitoes. Conventional methods to assess fecundity and fertility use 50-mL tubes or fly culture tubes, which involve significant labor to find and count eggs and hatched larvae. We introduce a tissue-culture plate-based fecundity and fertility assay for *Aedes* mosquitoes. We found that a 24-well tissue culture plate provide sufficient area for single females to lay eggs, while simplifying counting of eggs and larvae by imaging the wells. This method saves space and potentially reduces the workload for this type of assay, increasing the throughput that such experiments can be conducted.

1417

COMPARATIVE EFFICACY OF CATTLE-BAITED NET TRAPS (CBNT), CDC LIGHT TRAPS (LT) AND BG SENTINEL TRAPS (BG) FOR COLLECTION OF SANDFLIES IN SELECTED FIELD SITES IN SRI LANKA

Sanath C. Senanayake[1], Raushan Siraj[1], Nissanka De Silva[2], Nadira Karunaweera[1]

[1]University of Colombo, Colombo 10, Sri Lanka, [2]University of Sri Jayawardenepura, Nugegoda, Sri Lanka

Sri Lanka is a tropical country with rising number of cases of cutaneous leishmaniasis (CL). A few cases of visceral and mucosal leishmaniasis were also reported in recent years. The probable vector is *Phlebotomus aregentipes var glaucus* (*Ph. glaucus*). The characteristics of the sandfly fauna in Sri Lanka is poorly understood, hence the need for studies that can aid formulation of control methods to reduce transmission of leishmaniasis in the local setting. This study was carried out in two selected sites in North-Western (Ambanpola of Kurunegala district) and Southern provinces (Dickwella of Matara district) of Sri Lanka. The main objective of the study was to compare the three widely used trapping methods to collect sandflies to study their effectiveness. Two (2) CBNTs, twenty (20) CDC light traps and two (2) BG Sentinel traps were used to collect sandflies monthly for a period of 13 months from Jan 2017 to Dec 2018 in the selected field sites. A total of 5,111 sandflies were collected form CBNT (2661 Amabanpola, 2450 from Dickwella) with 4,394 males and 717 females (male: female 6:1) with an average of 98 flies per trap per night. CDC light traps gave a collection of 211 sandflies with 132 males and 79 females (male:female 3:2). There were no sandflies trapped in BG sentinel traps. *Ph. glaucus* was the only species found in CBNTs. Only fifteen (15) *Sergentomyia* species sandflies were found in CDC light traps. The CBNT is the best method for sampling *Ph. glaucus* in Sri Lanka though a higher male ratio was found. CDC light taps gave the best proportion of female flies though numbers were small. It is also effective in trapping the *Sergentomyia spp*. sandflies though the numbers may reflect the abundance and distribution of sandflies in selected sites. BG sentinel traps were totally ineffective in collecting sandflies in Sri Lanka.

1418

COMPARISONS OF TEMPERATURE-STABILIZING MATERIALS FOR LIVING ARTHROPOD SHIPMENTS

Catherine M. Hunt[1], Mark Q. Benedict[1], C. Matilda Collins[2], Ellen M. Dotson[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centre for Environmental Policy, Imperial College London, London, United Kingdom

Shipments of living mosquitoes and other arthropods require temperatures that are stabilized within a range compatible with their health and survival. In addition to use of express shipping and insulated containers, materials are often included that either store heat (*i.e.* have thermal mass) or otherwise stabilize the temperature. We present the results of comparisons of thermal mass and phase change materials to stabilize the temperature under various conditions. We compared a rigid foam refrigerant and a number of phase change materials to bubble wrap for their capacity to moderate temperature change by measuring the temperatures in standard uninsulated shipping containers during exposure to high (37°C), cold (4°C) and freezing (-20°C) temperatures. We determined which of the tested materials maintain temperatures below 30 and 35°C at an ambient temperature of 37°C and above 5 and 10°C at cold and freezing temperatures. Under all conditions, one of the phase change materials (Phase 22™ Flex Packs by Cryopak) kept the temperature within these ranges for the longest period of time. The second best-performing material was the foam refrigerant, 'Re-Freez-R-Brix™.'

1419

MOLECULAR ANALYSIS OF ENGORGED SAND FLIES FOR IDENTIFICATION OF BLOOD MEAL SOURCES AND DETECTION OF *LEISHMANIA* AND *BARTONELLA* DNA

Marisa Lozano[1], Liz Espada[1], Victor Zorrilla[1], Michael Kosoy[2], Clifton McKee[2], Lynn Osikowicz[2], Heriberto Arevalo[3], Mario Troyes[4], Craig Stoops[1], **Gissella Vasquez**[1], Michael Fisher[1]

[1]US Naval Medical Research Unit-6, Callao, Peru, [2]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [3]Peruvian Ministry of Health – San Martin Regional Health Directorate, San Martin, Peru, [4]Peruvian Ministry of Health – Jaen Health Directorate, Cajamarca, Peru

Blood meal analysis in wild caught sand flies can provide insights into sand fly host preference patterns and potential reservoirs of infectious pathogens. The objectives of this study were to identify blood meal sources, and detect *Leishmania* and *Bartonella* in wild-caught, engorged female sand flies from endemic regions in Perú. Engorged sand flies from the states of Ancash (86 specimens, 1 species), Cajamarca (18 specimens, 3 species), Madre de Dios (9 specimens, 3 species and 1 subgenus) and San Martin (91 specimens, 6 species and 4 subgenera) were identified morphologically and processed for blood meal identification by cytB and COI PCR, and for *Bartonella* and *Leishmania* DNA detection by combined ITS/gltA PCR and kDNA PCR, respectively. Preliminary testing of a subset of sand flies from San Martin (2 species) identified human blood in *Lutzomyia nevesi* by cytB PCR. Moreover, *Bartonella* DNA was detected in *Lu. nevesi* (8 out of 55 specimens) with two specimens found infected with a genotype close to *Candidatus* B. rondoniensis, originally described in *Erathyrus mucronatus* from French Guyana. *Bartonella* DNA was detected in a subset of *Lu. verrucarum* (8 out of 32 specimens) from Ancash; sand flies from Cajamarca and Madre de Dios were negative. A sub-set of sand flies from San Martin (3 species) were screened for *Leishmania* DNA, no positives were found. *Leishmania* screening and blood meal identification on remaining specimens is ongoing. Our combined blood meal source identification and pathogen detection analysis of engorged sand flies proves useful for vector-borne pathogen surveillance, and can provide valuable information for identification of potential reservoirs of *Bartonella* and *Leishmania* in highly diverse, remote endemic areas in Peru..

1420

DISTRIBUTION OF TICK SPECIES COLLECTED FROM THREE WEST AFRICAN COUNTRIES

Shirley C. Nimo-Paintsil[1], Mba-Tihssommah Mosore[2], OgheneKaro Omodior[3], Seth O. Addo[2], Nermeen T. Fahmy[4], Reham Tageldin[4], Eric Behene[2], Arthur B. Kamuah[5], Andrew G. Letizia[1], Fatorma Bolay[6], Samuel Dadzie[2], Hanayo Arimoto[7], Joseph W. Diclaro II[8]

[1]Naval Medical Research Unit No. 3 Ghana Detachment, Accra, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]Indiana University Bloomington, School of Public Health, Bloomington, IN, United States, [4]Naval Medical Research Unit No. 3, Cairo, Egypt, [5]Central Agriculture Research Institute, Suakoko District, Bong County, Monrovia, Liberia, [6]Liberia Institute for Biomedical Research, Margibi County, Charlesville, Liberia, [7]Camp Pendleton, 1st Medical Battalion, Oceanside, CA, United States, [8]Navy Entomology Center for Excellence, Jacksonville, FL, United States

Ticks are blood sucking arthropods which serve as vectors and reservoirs for transmitting pathogens in both animals and humans. The blood meal activity of ticks depends on various ecological factors such as vegetation, host and temperature. There has been very little work done to understand the distribution of ticks in West Africa. Our study compares tick data from Ghana, Liberia and Nigeria. A total of 2,675 ticks were collected from domestic animals (cattle, dogs, goats, and sheep) between 2015 and 2016 and morphologically identified using taxonomical keys of African Ixodidae. Eleven tick species, including *Amblyomma variegatum* (64.1%), *Rhipicephalus sanguineus* (21.5%), *Hyalomma truncatum* (5.9%), *Boophilus annulatus* (3.8%), *Hyalomma rufipes* (1.9%), *Boophilus*

decoloratus (1.8%), and *Haemaphysalis leachi* (0.09%), were identified. *Amblyomma variegatum* was dominant in both Ghana (61%) and Nigeria (51%) whereas *Rhipicephalus* (92%) species was dominant in Liberia. Nearly 80% (N = 2,102) of the ticks were collected from cattle and the remaining 20% (N = 573) were collected from sheep, dogs and goats. There was a significant association between country and the type of tick species identified (P < 0.001). *Boophilus* and *Haemaphysalis* species were found only in Nigeria. The prevalence of *Amblyomma* and *Rhipicephalus* genera of ticks in these countries highlights the need for continuous tick surveillance to better understand the distribution and how ecology as well as the environment impact prevalence and transmission of tick-borne pathogens in West Africa to guide effective control measures.

1421

THE ROLE OF RELISH IN *RICKETTSIA RICKETTSII* INFECTION WITHIN THE AMERICAN DOG TICK

Chanida Fongsaran, Krit Jirakanwisal, Victoria I. Verhoeve, Kevin R. Macaluso

School of Vet Med, Louisiana State University, Baton Rouge, LA, United States

Rickettsia rickettsii is the causative agent of Rocky Mountain spotted fever (RMSF), which is considered the most severe of all tick-borne rickettsiosis. Several tick species are responsible for the spread of the disease, including *Dermacentor variabilis*, yet the contributing tick-derived factors associated with vector competence are not known. Relish-type nuclear factor-kappa B (NF-κB) molecules have been suggested to play a role in the immune response by functioning as an immune responsive transcription factor in *D. variabilis*. In this study, we characterized the regulation of Relish in *Dermacentor variabilis*. NF-κB transcription factors interacted with Rel homology domain (RHD), suggesting they play a role in tick immune response. Accordingly, to investigate *DvRelish* expression in ticks infected with *R. rickettsii* and to determine the effect of *DvRelish* gene knockdown on rickettsial infection we used RNA interference-mediated gene silencing in ticks to demonstrate that transcription of *DvRelish* was decreased after 24 hours post-injection of siRNA. Next, we will characterize the response of *D. variabilis* when exposed to spotted fever group *Rickettsia* in a species- or pathogen-specific manner. *D. variabilis* will be exposed to either *R. rickettsii* or *R. montanensis* and transcription of *DvRelish* will be determined by quantitative real-time PCR. Also, RNAi will be used to determine the effect of *DvRelish* gene knockdown on *R. rickettsii* acquisition and transmission. Therefore, *DvRelish* expression in *Dermacentor* tick could be functionally important for tick innate immunity to pathogens and the identification of the immune induction in ticks will lead to understanding the molecular determinants of vector competence.

1422

GALLERIA MELLONELLA (*LEPIDOPTERA*): A POTENTIAL *IN VIVO* MODEL FOR ASSESSING THE PATHOGENESIS OF GROUP B *STREPTOCOCCUS*

Maria del Pilar Crespo-Ortiz, Maria Elena Burbano-Torres, Mauricio Barreto-Parra

Universidad del Valle, Cali, Colombia

Group B *Streptococcus* (GBS) is a colonizing bacterium in the gastrointestinal tract and the urogenital tract particularly in women. GBS invasion leads to severe infections in neonates, pregnant women, immunocompromised patients and the elderly people worldwide. Nevertheless the mechanisms of pathogenesis and virulence factors involved in the GBS transition from commensal to pathogen and the immune host interactions remain to be fully elucidated. Moreover, studies on the pathogenesis of isolates from low and middle income countries are limited. As environmental determinants may regulate gene expression for GBS invasion, we have assessed the potential of the moth *Galleria mellonella* as an *in vivo* model to study invasive and noninvasive human GBS isolates from our population. The invertebrate model is rapid, low-cost, easy to adapt to laboratory conditions and it has an innate immune system similar to mammals. The survival of *G. mellonella* larvae inoculated with GBS strains (ATCC 12386 and ATCC 12403) was determined. Temperature, pH and bacterial competition effects were examined as well as the response of *Galleria* hemocytes to GBS infection. GBS strains were able to effectively grow and infect *G. mellonella* in a dose-dependent manner with a (half-lethal dose) LC_{50} $1x10^7$ CFU/larva after 24 h. Preliminary results showed depletion of the pool of hemocytes particularly for the most invasive strain (ATCC 12403, serotype III), however severe vacuolation in most hemocytes and melanization were observed in all infected larvae. The survival after 24 h improved in larvae inoculated with GBS strain ATCC 12403 and incubated at 27 °C whereas pre-incubation of GBS inocula at pH 5 and 6.4 seemed to have not effect on killing rates. These findings are similar to those obtained in other systems suggesting that *G. mellonella* may be used as an *in vivo* model to study the pathogenesis of GBS human isolates. While these are preliminary results, we will determine how representative may be this model by infecting *G. mellonella* larvae with invasive and noninvasive (colonizing) clinical isolates which have been fully characterized by DNA-based microarrays.

1423

LAND USE IN RELATION TO COMPOSITION AND ABUNDANCE OF PHLEBOTOMINES (DIPTERA: PSYCHODIDAE) IN FIVE FOCI OF DOMICILIARY TRANSMISSION OF CUTANEOUS LEISHMANIASIS IN THE ANDEAN REGION OF COLOMBIA

Mabel Moreno[1], Lina Guzmán-Rodríguez[1], Carlos Valderrama Ardila[2], Neal Alexander[1], **Clara B. Ocampo**[1]

[1]Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM), Cali, Colombia, [2]Universidad Icesi, Cali, Colombia

American cutaneous leishmaniasis is a public health concern in Colombia with incidence being sustained or focally increased principally due to the emergence of domestic transmission concomitantly with the adaptation of the phlebotomine vectors to habitat transformation around households. Critically, risk of leishmaniasis transmission varies even within individual townships and houses, hence the need to understand the factors favoring the presence and abundance of phlebotomines within and around houses. The study was carried out in five sites within the Andean region of Colombia having history of Leishmania transmission and differing land use. The peri-domestic habitat was evaluated using a rapid methodology along eight radial outdoor transects of 90m, and placement of 3 CDC light traps over two nights in both indoors and outdoors (10m from the house) settings. The study demonstrated site-specific dominance of one vector species associated principally with agricultural monoculture. Specifically, *Pintomyia* (*Pifanomyia*) *quasitownsendi* was associated with sugar cane in two sites; while *Pi.* (*Pif*) *longiflocosa* was associated with coffee plantations in other two sites. The fifth site had a more diverse array of land use and forest coverage, a lower number of specimens, but higher species diversity. In terms of distance from the house to a given land use, the abundance of *Pi.* (*Pif*) *quasitownsendi* was inversely related with distance to sugar cane (Spearman correlation coefficient, rho = -0.56, $p < 0.001$ for outdoors catches, and rho = -0.50, $p < 0.001$ indoors). A similar inverse relationship was observed for *Pi.* (*Pif*) *longiflocosa* with technified coffee (rho = -0.51, $p < 0.001$ outdoors and rho = -0.48, $p < 0.001$ indoors). This rapid characterization methodology could guide public heath decision makers in identifying houses at higher risk of domestic transmission, and also educate farmers to increase the distance between their crops and houses.

1424

INTERACTIONS AMONG CHAGAS DISEASE VECTORS AND NATURALLY INFECTED DOGS ALONG THE TEXAS-MEXICO BORDER

Alyssa C. Meyers, Lisa Auckland, Sujata Balasubramanian, Ashley Saunders, Sarah Hamer

Texas A&M University, College Station, TX, United States

In the southern US, triatomine vectors maintain *Trypanosoma cruzi* in sylvatic cycles with occasional spillover to humans and domestic animals. Infection with *T. cruzi* may be asymptomatic or lead to heart disease and death. With no vaccine, limited antiparasitic treatments for humans, and no approved animal treatments, reducing the burden of disease must focus on vector control. However, there is an incomplete understanding of vector-host interactions that impedes vector control efforts. In the epidemiological setting of south Texas, we previously identified widespread *T. cruzi* infection in working dogs along the US-Mexico border. Using these dogs as a model host system, our objective was to characterize the triatomine vector communities in environments where these dogs work and quantify the *T. cruzi* infection prevalence in the vector. To determine vector hosts we used Sanger sequencing and amplicon deep sequencing of a vertebrate gene from the vector's gut contents. Finally, we analyzed selected measures of cardiac health (from electrocardiograms, echocardiographs, and cardiac troponin I, a biomarker for cardiac injury) in infected vs. uninfected dogs with a goal of relating clinical status to the locally-circulating parasite DTUs. We found two triatomine species (*Triatoma gerstaeckeri* and *T. rubida*) (n=78) collected from canine environments, where 48.7% were infected with *T. cruzi* comprised of DTUs 'TcI' (52.9%), 'TcIV' (29.4%) and 17.6% mixed-DTU infections. Bloodmeal analysis based on Sanger sequencing (n=47) revealed vector feeding on dogs, wildlife, and humans. Ongoing analysis of amplicon deep sequencing data demonstrate the majority of these bugs feed on multiple host taxa. In our clinical investigation of 24 *T. cruzi*-infected and 24 uninfected dogs, we found only a single infected dog (4.2%) had *T. cruzi* PCR-positive blood ('TcI') and ECG abnormalities ($p<0.0001$) and cardiac troponin I levels ($p=0.044$) were higher in infected dogs. Ecological tracking of the host-vector-parasite interactions provides insight on the sylvatic maintenance and spillover risk of *T. cruzi* along the Texas-Mexico border.

1425

DEVELOPMENT OF A NOVEL APPLICATION FOR DIFFERENTIAL DIAGNOSIS OF TICK-BORNE DISEASES

Corey B. Meyer, Jaleal Sanjak, Audrey Cerles, Christian Garnier, Laurel MacMillan

Gryphon Scientific, Takoma Park, MD, United States

Timely diagnosis and treatment of tick-borne diseases (TBDs) is critical for mitigating their adverse health outcomes, but differential diagnosis of TBDs is challenging because many symptoms are non-specific and commonly used diagnostic assays have significant shortcomings. Further, although the local incidence of TBDs is recognized as an important factor in their clinical diagnosis, tools to help clinicians formally consider surveillance data in their diagnostic decision-making are not available. To address these gaps, Gryphon Scientific developed a differential diagnosis application (app) for TBDs that calculates a patient's likelihood of infection with specific TBDs based on their symptoms, risk factors, and state of suspected tick exposure. A differential diagnosis model for TBDs was developed using two types of data: (1) TBD symptom and risk factor prevalence in TBD patient populations, collected from clinical studies; and (2) human TBD incidence data from notifiable disease surveillance systems and tick infection prevalence data from reports and public databases, which were combined to develop an environmental risk measure. These data were used to build a Bayesian Belief Network (BBN) model that predicts TBD infection probabilities based on a patient's symptoms, risk factors, and state of suspected tick exposure, which was incorporated into an app developed using R-shiny, called TBD-DDx. A pilot application was developed that includes ten states (AR, CT, MA, ME, MN, MO, NH, RI, VT, and WI) and the eleven TBDs that are endemic to those states. Performance of the TBD-DDx model was validated using case studies from the biomedical literature. The model identified the correct disease within the top three predicted TBDs in 84% of cases, demonstrating that the TBD-DDx app is a promising tool for informing clinical diagnoses of TBDs to guide selection of diagnostic testing and treatment. Further, this study represents the first use of a BBN modeling approach that incorporates an environmental risk measure, and therefore could be adapted for differential diagnosis of other infectious diseases with environmental or other exposure risks.

1426

TICK-BORNE PATHOGENS IN TICKS OF HORSES, DOGS, AND MIGRATORY BIRDS IN THE REPUBLIC OF KOREA

Yun Sang Cho[1], Jinheong Noh[1], Mi-Sun Yoo[1], Hyun-Ji Seo[1], Keun Ho Kim[1], Yeojin Park[1], Hyunkyoung Lee[1], Jung-Won Park[1], Seunghee Lee[1], Soon-Seek Yoon[1], Heung-Chul Kim[2]

[1]Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea, [2]65th Medical Brigade, Pyeongtaek, Republic of Korea

Recently, tick-borne diseases have been increasing due to changes in environments such as global warming, increase in outdoor leisure activity, increase in deforestation by expansion of development area, increase in international movement and trade. Therefore, there is an urgent need for national monitoring in the situation where the risk of global epidemics of ticks and tick-borne diseases is increasing. In this study, ticks were collected from Korean horses, dogs, and migratory birds, and their pathogens were examined using genetic diagnosis. In horses, 9,813 ticks were collected, among which 99.9% (n = 9,807) of *Haemaphysalis longicornis* were identified, followed by 0.1% (n = 6) of *Ixodes nipponensis*. In dogs, of the 1,836 ticks collected, 98.69% (n = 1,812) of them were identified as *H. longicornis* and were dominant species as well as horses. Next, 0.82% (n = 15) of *I. nipponensis* and 0.44% (n = 8) of *H. flava* were identified. In migratory birds, 992 ticks were collected. Among them, 63.7% (n = 587) of *I. turdus* and 17.1% (n = 158) of *H. flava* were found. There were many differences, compared with horses and dogs. Collected tick pathogens were examined by PCR. *Anaplasma phagocytophilum, A. platys, Ehrlichia chaffeensis, E. canis, Borrelia* spp., *Babesia* spp. in the horse tick-borne pathogen test, *A. phagocytophilum* (numbe of pools = 5) was positive in 0.5% and *Borrelia burgdoferi* (number of pools = 4) was positive in 0.4%. In dogs, *A. phagocytophilum* (number of pools = 15) was found in 1.98%, and *Babesia* spp. (number of pools = 7) were positive in 0.92%. In migratory birds, *A. phagocytophilum* (1 pool of *I. nipponensis*) and *Borrelia* spp. (14 pools of *I. turdus*) were positive. The major pathogens in ticks were *A. phagocytophilum*, *B. burgdoferi*, and *Babesia* spp., but the positive rate were less than 2%. Monitoring of pathogens in ticks will provide important information for improving public health and livestock hygiene. Moreover, it is thought that DB construction related to domestic and overseas will be a useful information resource for prevention of tick-borne diseases, which is one of the obstacles to the improvement of One Health.

1427

STUDY OF FRANSAIELLESIS DISEASE AMONG CATTLE IN AZERBAIJAN IN 2016 - 2018

Adalat Talibov

Azerbaijan Food Safety Institute, Baku, Azerbaijan

Fransaiellesis is a blood-parasitic disease of cattle and small ruminants, characterized by fever, jaundice, hematobyuria and animal death. *Fransaiella colchica* and *F. caucasica* types belong to *Babesiidae* family are considered the causative agent of the fransaiellesis disease of cattle. *Ixodes ricinus* and *Boophilosis calcaratus* are the vectors of the abovementioned causative agents. The disease is widespread in the plains and foothills areas of Azerbaijan, in the Kura-Araz Lowland and Lankaran zone per the previous studies' data. The goal of this study is to define the spread of

fransaiellesis disease among cattle in Azerbaijan in 2016 - 2018. During 2016 - 2018 the Central Veterinary Laboratory (CVL) received pathological materials from dead cattle to examine for piroplasmosis from Absheron, Gobustan, Khizi, Lankaran, Bilasuvar, Gakh, Barda and Shamakhi. Smears from the samples (livers and lungs) were stained with Romanovski-Gimza and analyzed by microscope. 50 pathological material samples were received by CVL in 2016-2018. 38 samples (38 - livers, and 37 - spleens, 10 - kidneys) were positive for fransaiellesis. There were 4 positive samples from Absheron, 1 from Gobustan, 1 from Khizi, 12 from Lankaran, 1 from Bilasuvar, 18 from Gakh, 1 from Barda. The received results showed that the most positive samples were found in Lankaran (32%) and Gakh (47%). According to the literary data the tick vectors of the disease mostly spread in these two rayons and this fact could impact the amount of the positive cases. Moreover, the analysis of the received results showed that the mortality rate from fransaiellesis is higher among imported cattle (25 out of 38) than local animals (13 out of 38). It's important to study the ticks that spread fransaiellesis in Azerbaijan. However, there is no veterinary entomology specialty in the animal healthcare system of Azerbaijan. It is crucial to train the veterinary specialists on veterinary entomology by the international specialists and to introduce this training into the veterinarian education program.

1428

CLIMATE CHANGE COULD EXPOSE 1.3 BILLION NEW PEOPLE TO ZIKA VIRUS TRANSMISSION RISK BY 2050

Sadie Jane Ryan[1], Colin J. Carlson[2], Blanka Tesla[3], Matthew H. Bonds[4], Calistus N. Ngonghala[1], Erin A. Mordecai[5], Leah R. Johnson[6], Courtney C. Murdock[3]

[1]*University of Florida, Gainesville, FL, United States,* [2]*Georgetown University, Washington, DC, United States,* [3]*University of Georgia, Athens, GA, United States,* [4]*Harvard Medical School, Boston, MA, United States,* [5]*Stanford University, Stanford, CA, United States,* [6]*Virginia Polytechnic Institute and State University, Blacksburg, VA, United States*

In the aftermath of the 2015 pandemic of Zika virus, concerns over links between climate change and emerging arboviruses have become more pressing. Given the potential for much of the world to remain at risk from the virus, we use a new model of thermal bounds on Zika transmission to project climate change impacts on transmission risk by mid-century (a generation into the future). Using a mechanistic, temperature driven model of Zika virus transmission in *Aedes aegypti* mosquitoes, we projected transmission suitability under multiple global circulation models (GCMs), for emissions pathways (RCPs) in the recent IPCC5 report on climate. We present globally gridded data as maps, demonstrating how many months of the year are suitable for transmission, in each pixel, for each scenario. As *Aedes aegypti* are tenacious, urban-adapted, container breeding mosquitoes, potential habitat is tied to human population density patterns. Thus, we projected transmission suitability onto future global population, accounting for socioeconomic conditions - the shared socioeconomic pathways projections (SSPs) - to measure current and future risk, in terms of people, as the geography of suitability shifts across the face of our planet. In the worst-case scenario emissions pathway, 1.3 billion previously unexposed people could face suitable environmental conditions for Zika virus by 2050, with over 1.1 billion living in areas outside those affected by the 2015 epidemic. Given these projections, we suggest an increased priority on research establishing the immune history of vulnerable populations, modeling when the next Zika virus outbreak might occur, evaluating the efficacy of conventional and novel intervention measures, and increasing surveillance efforts to prevent further expansion of Zika. The next generation will face substantially increased Zika virus transmission suitability in North America and Europe, where naïve populations might be particularly vulnerable. Mitigating climate change could significantly reduce global expansion of climates suitable for Zika virus transmission, potentially protecting up to about half a billion people.

1429

ABILITY TO RECOGNIZE THE LYME DISEASE VECTOR BY THE GENERAL PUBLIC IN THE NORTHEAST AND MIDWEST UNITED STATES.

Gebbiena M. Bron[1], Maria del Pilar Fernandez[2], Jean I. Tsao[3], Maria A. Diuk-Wasser[2], Lyric C. Bartholomay[1], Susan M. Paskewitz[1]

[1]*University of Wisconsin - Madison, Madison, WI, United States,* [2]*Columbia Univerisity, New York, NY, United States,* [3]*Michigan State University, East Lansing, MI, United States*

"An ounce of prevention is worth a pound of cure." A statement that holds true today, but incomplete information will undermine these efforts. Public health messages on tick-borne diseases have focused on the use of personal protective measures, with "check yourself for ticks" being the most commonly recommended measure. However, this strategy is likely ineffective if the primary vector of Lyme disease, the nymphal blacklegged tick, is not recognized due to its small size. In 2018, we launched a smartphone app, The Tick App, to study behavioral factors associated with tick exposure. Approximately 80% of Tick App users reported having checked for ticks (n=1,463). Interestingly, users identified 15.4% of submitted tick images as nymphs, but only half actually were (7.6%). In the summer of 2019, we compared the ability of residents from two Lyme disease endemic areas in the US to recognize ticks, in particular nymphs. We hypothesized that Lyme vector identification would be more accurate in the Northeast compared to the upper Midwest, because Lyme disease has long received ample media and research attention in the Northeast. We used The Tick App to solicit participant information and images of ticks encountered, and used an in-person survey to assess residents' ability to identify nymph-stage ticks from a specimen tray with three tick species, including nymphs and adults of the blacklegged tick, and two additional insects sometimes misidentified as ticks. We expected that Northeasterners, outdoor enthusiasts, and well-informed participants would be better at recognizing the nymphal blacklegged tick. This difference in ability to recognize the Lyme vector illustrates that the adoption of personal prevention is influenced by knowledge, risk perception and the perceived self-efficacy to carry out the intervention, but may vary by region. Ultimately, the comparison of vector recognition and, by extension the success of personal prevention measures, between two highly endemic regions will help inform regionally tailored prevention tools and public health messaging.

1430

DYNAMICS OF POWASSAN VIRUS AND *BORRELIA BURGDORFERI* INTERACTIONS IN CO-INFECTED *IXODES SCAPULARIS* TICKS AND THEIR POTENTIAL IMPACT ON HUMAN CLINICAL OUTCOMES

Charles E. Hart, Saravanan Thangamani

Upstate Medical University, Syracuse, NY, United States

Powassan virus (POWV) is a neurotropic flavivirus endemic to North America. It causes neuroinvasive disease resulting in up to 15% fatality with long-term neurological sequelae in 55% of survivors. It is transmitted by *the Deer tick Ixodes scapularis* which is also the primary vector of *Borrelia burgdorferi*, the causative agent of Lyme disease. Coincidentally, the white footed mice, *Peromyscus leucopus*, acts as a reservoir for both *B.burgdorferi* and POWV, and the immature state of *I. scapularis* ticks primarily feed on *P. leucopus*. Thus there is a greater chance of *I. scapularis* to be co-infected with both *B. burgdorferi* and POWV. Here we describe the dynamics of POWV and *B. burgdorferi* Interactions in co-infected *I. scapularis* ticks and their potential impact on human clinical outcomes. To facilitate this study, we generated co-infected ticks via oral infection route and dissection midgut and salivary glands at 3 weeks post infection. Pathogen load was quantified using quantitative RT PCR. Our preliminary data indicates that a significant decrease in *B. burgdorferi* was observed in female co-infected ticks when compared to that of ticks infected with just *B. burgdorferi*. However, we didn't observe any change in the POWV load. Currently, we are investigating the differential tick immune responses of

co-infected tick compared to a tick infected with one pathogen by RNAseq analysis. Similarly, the tick metabolic responses will also be compared. We are also investigating the pathogen transmission dynamics by a coinfected tick. Our preliminary data suggest that POWV /*Borrelia* coinfection in ticks is plausible, and that humans bitten by such ticks may be at risk for the development of combinational disease should both pathogens successfully transmit. Reductions in *B. burgdorferi* concentrations by POWV suggest the possibility of subtle interactions within their sylvatic cycle, potentially altering coinfection dynamics in nature.

1431

EVALUATING THE EFFECTS OF HUMAN MOBILITY PATTERNS ON TICK EXPOSURE USING A SMARTPHONE APPLICATION, THE TICK APP

Maria P. Fernandez[1], Gebbiena M. Bron[2], Pallavi A. Kache[1], Jean I. Tsao[3], Lyric C. Bartholomay[2], Susan M. Paskewitz[2], Maria A. Diuk-Wasser[1]

[1]*Columbia University, New York, NY, United States,* [2]*University of Wisconsin, Madison, WI, United States,* [3]*Michigan State University, East Lansing, MI, United States*

Vector-borne disease transmission to humans depends on infected vector densities and human-vector contact rates. In the case of Lyme disease, the most commonly reported vector-borne disease in temperate regions, the risk will depend not only on the density and distribution of vector ticks, but also on human behaviors that influence where and how often human-tick encounters occur. Thus, there is a need to simultaneously assess ecological and behavioral risk factors, although the latter remain poorly understood in part because of methodological limitations. In this study, we use a novel smartphone application, The Tick App, to collect data on self-reported human-tick encounters and personal protection measures, while simultaneously collecting GPS trajectories using location services in smartphones. We hypothesize that the combination of time spent in risky environments and frequency and type of outdoor activities will increase the risk of human-tick encounters. During May-September of 2018 and 2019, we recruited participants to download The Tick App in the Upper Midwest and Northeast regions of the United States. During 2018, 40% of the total number of recurring users (n=734) allowed for location services. Users had equal gender distribution, the median age was 50 years old, and were mostly located in the upper Midwest (54%). We collected GPS coordinates every 15 min, which led to a median of 858 points per user in approximately a 9-day follow-up period. The 2018 and 2019 data were combined to classify mobility patterns; identify dynamic and static (i.e., significant places) behaviors; and assess the effect of mobility patterns on self-reported tick encounters. This information will advance our understanding of how the interactions between human behavior and tick densities determine Lyme disease risk.

1432

A NETWORK ANALYSIS FRAMEWORK FOR THE EVALUATION OF MOSQUITO ABATEMENT SERVICE DELIVERY IN MACHALA, ECUADOR

Catherine A. Lippi[1], Anna M. Stewart-Ibarra[2], Liang Mao[3], Sadie J. Ryan[1]

[1]*Department of Geography and Emerging Pathogens Institute, University of Florida, Gainesville, FL, United States,* [2]*Center for Global Health and Translational Science, Upstate Medical University, Syracuse, NY, United States,* [3]*Department of Geography, University of Florida, Gainesville, FL, United States*

Dengue fever places a high burden on the tropical coastal city of Machala, Ecuador (pop. 245,972), both in terms of human morbidity and economic strain on the community. Medical treatment for dengue mainly consists of palliative care, and consequently comprehensive vector control programs remain the primary method of controlling human disease outcomes through the reduction of mosquito populations. In Machala, vector control services are delivered through two deployment hubs managed by the Ecuadorian Ministry of Health. Currently, public health professionals in Machala face several logistical issues when delivering mosquito abatement services, namely applying limited resources in ways that will most effectively suppress dengue transmission. Mosquito control services, such as application of larvicides and truck-mounted ULV fogging, are not performed in a systematic manner. Using a transportation network analysis framework, we built models of service areas and optimized delivery routes based on distance-based costs associated with accessing census neighborhoods throughout the city. These models were compared to underlying risk factors and incidence for dengue fever in Machala, creating a visual tool to guide decision makers and maximize the efficiency of mosquito control programs. Using this framework, we identified different locations for targeting mosquito control efforts, dependent upon management goals and specified risk factors of interest, including human population and housing condition. Our models indicate that neighborhoods on the periphery of Machala, locations with the poorest housing conditions, are the most costly to access. Furthermore, we conclude that the current locations of mosquito control facilities in Machala are not ideal for reducing driving distances or maximizing populations served. This work represents a first step in creating a spatial tool for planning and critically evaluating the systematic delivery of mosquito control services in Machala.

1433

EXCITO-REPELLENCY ACTIVITY OF *CANANGA ODORATA* (MAGNOLIALES: ANNONACEAE) AGAINST DENGUE AND MALARIA VECTORS

Chutipong Sukkanon[1], Michael J. Bangs[2], Theeraphap Chareonviriyaphap[1]

[1]*Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand,* [2]*Public Health and Malaria Control Department, PT Freeport Indonesia/International SOS, Papua, Indonesia*

The essential oils of *Cananga odorata* (CO) flowers was evaluated for its noncontact repellency, contact excitation, and knockdown/toxicity response against three colonized mosquito vector species; *Aedes aegypti*, *Anopheles dirus* and *Anopheles minimus* using an excito-repellency assay system under laboratory-controlled conditions. The escape responses from noncontact and contact oil-treated papers inside chambers were observed at four concentrations (0.5-5.0% v/v). CO oil showed strong spatial repellency against *An. minimus* (97-99% escape response) at the 2.5-5.0% concentrations. At 2.5%, higher repellency for *An. dirus* (64.4% escape) and *Ae. aegypti* (39.3% escape) was observed. Contact excitation was more pronounced compared to repellency alone with significantly quicker and higher percent escape seen in *An. minimus* (83-100% escape), and more moderate effects in *Ae. aegypti* (51-59% escape) and *An. dirus* (40-50% escape). After adjustment for repellency, escape due to contact alone was seen at low concentration (0.5%), with the greatest effect seen in *An. minimus* (67.2% escape). Relatively low percent knockdown or mortality at 24-h post-exposure was only observed in *Anopheles* mosquitoes at 5.0% concentration. These findings indicate the CO oil is more active against anopheline mosquitoes tested, particularly *An. minimus*; a primary malaria vector in mainland SE Asia. This study demonstrates compelling evidence that CO oil performs primarily as a spatial repellent at 2.5% and 5.0% concentrations, while contact irritancy is a contributor at low concentrations (0.5-1.0%). Further study is needed on this oil extract as a potential active ingredient in repellent products against mosquitoes and other biting arthropods.

1434

PROSPECTS OF THE GARDEN ANT, *LASIUS NIGER*, EXTRACT AS NON-FLORAL SOURCE OF ANTI-MALARIAL DRUG AND MOSQUITO INSECTICIDAL LEAD AGENTS

Israel K. Olayemi[1], Kamoru A. Adeniyi[1], Oluwatosin K. Shittu[1], Adeolu T. Ande[2], Chioma N. Amajoh[3], Azubuike Christian Ukubuiwe[1]

[1]*Federal University of Technology, Minna, Nigeria,* [2]*University of Ilorin, Ilorin, Nigeria,* [3]*Community Vision Initiative, Abuja, Nigeria*

The present need and, hence, aggressive search for alternative antimalarial and mosquito-larvicidal drugs and insecticides, respectively, from natural products other than floral sources informed this study. Entomo-bioactive extracts were prepared from black garden ant (*Lasius niger*) and analysed for entomo-chemical composition, after which graded concentrations (0.1 to 1.2 mg/L) of the extract were assayed against *Plasmodium berghei* in infected mice and 4th instar larvae of *Anopheles gambiae* s. l mosquitoes, following standard procedures.The antimalarial activity of the insect extract was performed against established infection in *Plasmodium berghei* infected mice. The entomo-chemical screening revealed the presence of medicinal metabolites such as flavonoid, saponnins and terpenoids. The results further indicated significant mosquito-larvicidal activities of the insect crude extract; with its toxicity been time and concentration dependent. Probit regression analysis revealed LC50 of 0.75 mg/L and LC90, 1.17 mg/L. Acute oral toxicity revealed an LD50 extrapolated to be above 5000 mg/kg body weight (b. wt). The antiplasmodial bioassay results showed that the extract significantly reduced the level of parasitaemia in the mice, with peak activity recorded on the last day of observation especially, in the highest dose tested (600 mg/kg b. wt). The extract, also, elongated the survival time of *P. berghei* infected mice relative to the infected non-treated group. This study, therefore, validates the prospect of mosquito larvicidal and antiplasmodial activities of crude extract of the black garden ant. However, further investigations are necessary, for isolating the specific bioactive entomo-chemicals and elucidating their mechanisms of actions.

1435

AN ASSESSMENT OF COMMUNITY ACCEPTANCE OF YEAST INTERFERING RNA BAITED OVITRAPS AS A BIORATIONAL MEANS OF CONTROL FOR *AEDES* MOSQUITOES IN TRINIDAD

Nikhella Winter[1], Akilah Stewart[1], Limb K. Hapairai[2], Jessica Igiede[3], Azad Mohammed[1], David W. Severson[3], Molly Duman-Scheel[2]

[1]*The University of the West Indies at St. Augustine, St. Augustine, Trinidad and Tobago,* [2]*Indiana University School of Medicine, South Bend, IN, United States,* [3]*The University of Notre Dame, Notre Dame, IN, United States*

Zika, dengue, chikungunya and yellow fever viruses are a major public health concern in Trinidad and throughout the Caribbean region. *Aedes* mosquitoes, the primary vectors for these viral pathogens, lay eggs in water-filled containers located in close proximity to humans and their dwellings. This preference for container breeding sites provides opportunities for surveillance and control of *Aedes* mosquitoes with larval lethal ovitraps, dark colored containers filled with water, attractants to lure gravid females, and larvicides to kill resultant larvae that hatch from eggs laid in the traps. Insecticide resistance and concerns for the impacts of pesticides on non-target organisms threaten the sustainability of this intervention. To address this, we are developing lure-and-kill interfering RNA ovitraps. A yeast-mediated interfering RNA delivery system employed in these traps is of particular interest due to combined attraction of gravid females and effective killing of mosquito larvae by the yeast. Prior to field evaluation of these ovitraps, paper surveys and community engagement forums were used to assess stakeholders living in prospective field site communities in the St. Augustine, Trinidad region. These assessment tools facilitated evaluation of study participants' general knowledge of mosquitoes, mosquito control practices, and feelings about the new technology. A majority of the paper survey respondents have good working knowledge of mosquitoes, practice some means of larval mosquito control, and were willing to use the new ovitrap intervention if it were shown to be safe and effective. After gaining more information about ovitraps and interfering RNA technology, forum participants voiced support for the new ovitraps and offered useful advice concerning product design, distribution, and pricing. The results of this investigation suggest that the study participants would be willing to both host field trials in their communities and to consider integrating yeast-based ovitraps into treatment regimes around their homes.

1436

A NON-LIVE INSECTICIDE DEVELOPED FROM THE BACTERIUM *CHROMOBACTERIUM SP.* PANAMA (*CSP_P*) EFFECTIVELY KILLS MOSQUITOES UNDER LABORATORY AND SEMI-FIELD CONDITIONS

Eric P. Caragata, Luisa Otero, George Dimopoulos

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Mosquitoes are the vectors of many clinically important human diseases including malaria, dengue, chikungunya and yellow fever. The human cost of these diseases remains high, and the development of novel insecticides is required to target mosquito populations that are becoming increasingly resistant to commonly used mosquitocidal chemicals. We have developed an insecticide based on crude, dried extracts of the bacterium *Chromobacterium sp.* Panama (*Csp_P*). Our *Csp_P* insecticide is non-live and highly effective at killing the larvae of prominent mosquito vectors including *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*. It also effectively kills mosquito larvae that have developed genetic resistance to pyrethroids, suggesting that it could prove to be a useful tool to supplement current mosquito control programs. The non-live *Csp_P* insecticide has proven to be highly effective at killing mosquitoes under more natural environmental conditions at our field site in Gurabo, Puerto Rico, where we have translocated breeding sites from the field. Optimized *Csp_P* culturing regimes and larval and adult bait formulations give this product great potential as a tool for mosquito control.

1437

THE WEST AFRICAN *KNOCK DOWN RESISTANCE* MUTATION DETECTED IN THE PRIMARY MALARIA VECTOR *ANOPHELES ARABIENSIS* (DIPTERA: CULICIDAE) IN ETHIOPIA

Esayas Kinfe Woldesilasse[1], Habte T. Tekie[2], Irish R. Seth[3]

[1]*Ethiopian Public Health Institution, Adiss Ababa, Ethiopia,* [2]*Addis Ababa University, Addis Ababa, Ethiopia,* [3]*US President's Malaria Initiative and Entomology Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Ethiopia has a long history of using indoor residual spraying, beginning with DDT. When DDT resistance was found to be widespread, malathion, deltamethrin was used as an IRS treatment. Quite quickly, deltamethrin resistance led to use of bendiocarb and propoxur in recent years. Insecticide resistance is an important concern for national malaria control programs in Ethiopia. Monthly larvae and adult *Anopheles* mosquitoes collections were undertaken from the two study sites Jolie and Gogete selected by using multistage sampling methods. A standard susceptibility tests were done following the WHO procedure and kit. To detect the presence of the L1014S (East African) and the L1014F (West African) *knock down resistance* (*kdr*) mutation a sub-sample of *An. gambiae* were tested using adapted versions of the allele-specific polymerase chain reaction. The L1014F *kdr* mutation in *An. arabiensis* was detected at a relatively high frequency 41.7% in Gogete and 35.9% in Jolie study site. The L1014S *kdr* alleles were not found in the study sites. The *kdr* mutation was related with high levels of both pyrethroid and DDT resistance; the phenotypic and genotypic resistance shows the needs to

urgently implement resistance management strategies such as bi-treated nets, combination of IRS and LLINs with different insecticides, or other strategies.

1438

DOES VEGETABLE FARMING CONTRIBUTE TO INSECTICIDE RESISTANCE SELECTION IN THE MALARIA VECTOR *ANOPHELES COLUZZII*?

Rousseau Djouaka

IITA- Cotonou, Benin, West Africa, Cotonou, Benin

This research analysed the contribution of vegetable production in insecticide resistance selection in malaria vectors in Benin. A KAP-study was undertaken with vegetable farmers to identify the most commonly used insecticides. The susceptibility profile of anopheles was assessed in surveyed farms and the presence of insecticide residues and heavy metals in their breeding sites was determined. λ-Cyhalothrin constituted the main insecticide used in surveyed vegetable farms. An.coluzzii was the main species found in surveyed vegetable farms. High resistance levels to λ-Cyhalothrin were recorded in sampled An. coluzzii. However, few of the examined breeding sites were found to be contaminated by λ-Cyhalothrin residues. Interestingly, a positive correlation was recorded between the presence of copper in breeding sites and the λ-cyhalothrin resistance profiles of sampled anopheles populations.In conclusion, These results suggest the presence in breeding sites of non-insecticidal compounds such as copper which likely contributes to insecticide resistance selection in An. coluzzii.

1439

SPATIO TEMPORAL DISTRIBUTION AND INSECTICIDE RESISTANCE STATUS OF *AEDES* MOSQUITOES IN GHANA

Christopher M. Owusu-Asenso[1], Julius A. Mingle[1], David Weetmann[2], Yaw Asare Afrane[1]

[1]*University of Ghana, Accra, Ghana,* [2]*Liverpool School of Tropical Medicine and Hygiene, Liverpool, United Kingdom*

The *Aedes* has been transmitting Yellow fever and dengue fever viruses and other unknown arboviruses in Ghana. This study investigated the spatio-temporal distribution and insecticide resistance status of *Aedes* mosquitoes in Ghana. This study was carried out in three ecological landscapes of Ghana. Indoor and outdoor sampling was done with BG traps, human landing catch (HLC) and prokopack aspirator (PPK) during the dry and rainy seasons of 2017/2018 to determine spatio-temporary distribution. Phenotypic insecticide resistance status of *Aedes* was determined using the WHO susceptibility bioassay. Host blood meal sources was determined by PCR. A total of 2193 adult *Aedes* mosquitos were collected comprising; *Aedes aegypti* (97.3%), *Aedes africanus* (2.2%) and *Aedes luteocephalus* (0.05%). The dry and rainy season had 73.5 (42.1%) and 103.95 (57.9%) Aedes respectively. HLC had the highest densities of 210.9 (77%) followed by PPK 74 (17.8%) and BG trap 15.5 (5.2%). The test results showed that *Aedes* mosquito populations from all study sites were resistant to DDT (0 - 84%). Vectors showed resistance to deltamethrin in Tema (68%) and patchy resistance in the other sites. Vectors showed resistance to permethrin in Accra (40.0%) and Larabanga (88.8%), suspected resistance in Konongo (90%), Navrongo (90%) and Paga (96%). *Aedes* mosquitoes showed resistance to bendiocarb in Larabanga. *Aedes* mosquitoes were susceptible to organophosphates at all sites. Blood meal analysis showed that the *Aedes* mosquitoes were mostly anthropophilic with HBI of 0.9. The development of resistance by Aedes mosquitoes to DDT, pyrethroids and carbamates may have an operational impact on the efficacy of insecticides on vector control interventions

1440

THE FORGOTTEN VECTORS IN INSECTICIDE RESISTANCE MONITORING OF MALARIA VECTORS

Duncan K. Athinya[1], Melinda P. Hadi[1], Seline A. Omondi[2], Eric O. Ochomo[2]

[1]*Vestergaard, Nairobi, Kenya,* [2]*Kenya Medical Research Institute, Kisumu, Kenya*

The reduction in global malaria cases since 2000 has been largely attributed to vector control with long lasting insecticidal nets and indoor residue spraying. This steady decline has stagnated, and the World Malaria Report 2018 highlighted an increase in malaria cases between 2016 and 2017. Insecticide resistance in malaria vector is one of the challenges identified. Monitoring of insecticide resistance in malaria vectors is essential for successful vector control campaigns. Visualizing such data in time and space provides an indication where insecticide resistance may play a role in persisting malaria burden. IR Mapper (www.irmapper.com) was launched in 2012 and continues to be updated monthly to geospatially display data from published literature on insecticide resistance in *Anopheles* species. As of March 2019, IR Mapper presented data from 20,953 tests from 430 published articles and reports of both phenotypic resistance and insecticide resistance mechanisms globally. 85.4% of this data is from Africa. *Anopheles gambiae s.l.* and *An. funestus s.l.* accounts for 90.1% and 9.5% of the tests, respectively. *An. coustani*, *An. pharoensis* and *An. labranchiae* together account for 0.4% of the tests. Among the phenotypic resistance tests between 2010 and 2018 using pyrethroids, 61.5% of the tests on *An. gambiae s.l.* reported confirmed resistance while 76.1% of tests on *An. funestus s.l* reported confirmed resistance. *Kdr* mutations were detected only in *An. gambiae s.l.* Most insecticide resistance monitoring efforts in Africa focus on *An. gambiae s.l.* Secondary vectors such as *An. coustani* and *An. pharoensis* are rarely tested. Such data gaps can present challenges for malaria control. It is essential for researchers to test relevant *Anopheles* species especially where they occur in sympatry in high malaria transmission zones and where possible, secondary malaria vectors. IR Mapper is useful for identifying such data gaps.

1441

EFFICACY OF A NEW INDOOR RESIDUAL INSECTICIDAL COMBINATION CONTAINING CLOTHIANIDIN AND DELTAMETHRIN ("FLUDORA® FUSION") IN SIMPLE HUT TRIALS AGAINST *ANOPHELES ARABIENSIS*

Abebe Animut[1], Meshesha Balkew[2]

[1]*Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia,* [2]*Abt Associates Inc., VectorLink Project Ethiopia Office, President's Malaria Initiative, Addis Ababa, Ethiopia*

The spread of insecticide resistant malaria vectors prompted a need for new insecticides. Residual efficacy of Fludora Fusion WP-SB56.25 ('Fludora'), Clothianidin WG70 and Ficam® was evaluated against *Anopheles arabiensis* in simple huts in Oromia Regional State, Ethiopia. Each insecticide was sprayed in three huts and the remaining hut was sprayed with water (control). The wall of each house was divided into four equal sprayable surface types (painted, dung, smooth mud and rough mud) and checked for insecticidal activity prior to the spray. Three cones were placed at different heights of each surface type. Ten unfed, 2-4 days old female *Anopheles arabiensis* of insectary colony DebreZeit (susceptible to all current public health insecticides), were transferred to each cone using mouth aspirator and exposed for 30 minutes. After 30 minutes, the mosquitoes were transferred to clean holding cages and supplied with sugar solution by moistening a pad of cotton. Knockdown effect was recorded at 60 minutes and mortality at 24 hours, 48 hours and 72 hours holding time post-exposure. The tests began one month after spraying and thereafter at monthly intervals for 12months. The Fludora combination caused the highest residual insecticidal activity at 24 hours, 48 hours and 72 hours holing time post-exposure until 12 months after treatment (MAT) compared to Ficam and Clothianidin WG70. Its activity

was 100% mortality on all wall surface types until 3 MAT. It caused ≥98% mortality on dung and smooth mud surfaces at 4 MAT, 5 MAT and 6 MAT, >90% on rough mud surfaces from 4 MAT to 12 MAT (except 11 MAT) > 80% from 4 MAT to 12 MAT on painted surfaces (except at 7 MAT and 11 MAT) at 24 hours post-exposure. The measured residual activity of Fludora increased when the holding time of exposed mosquitoes was extended from 24 hours to 48 hours and 72 hours. Ficam revealed the highest residual insecticidal activity on painted surfaces at 24 hours holding time post-exposure resulting in 100% efficacy until 4 MAT, 92% at 5 MAT and 74% at 6 MAT and 7% at 7 MAT and lost its efficacy thereafter. Fludora can be used as alternative insecticide in indoor residual spraying programs in Ethiopia.

1442

ACCREDITATION FOR ENHANCE VECTOR CONTROL IN MEXICO AND THE AMERICAS

Pablo Manrique-Saide, Anuar Medina-Barreiro, Abdiel Martin-Park, Yamili J. Contreras-Perera, Azael Che-Mendoza, Gabriela González-Olvera

Collaborative Unit for Entomological Bioassays, Mérida, Yucatán, Mexico

The roll out of good laboratory practice (GLP) in vector control led by the World Health Organization (WHO), aims at enabling consistent, reliable and repeatable data to be generated from vector control studies. The Collaborative Unit for Entomological Bioassays (UCBE by its initials in Spanish) from the University of Yucatan in Mexico, is an independent non-profit academic unit, currently in the last stage of WHO-GLP accreditation. Since its creation in 2014, UCBE has contributed to the generation of technical and scientific evidence to enhance and innovate the surveillance and control of vector-borne diseases, with an emphasis in *Aedes aegypti*, the main vector of dengue, chikungunya and Zika. Currently UCBE is recognized by the Federal Ministry of Health of Mexico as a reference unit for monitoring susceptibility of insecticides, evaluation of formulations and application equipment used for vector control. Transitioning UCBE to GLP status required restructuring personnel, with the establishment of roles and positions for trial performance and quality control; full-time members will be part of the unit, with additional temporary personnel hired for insecticide evaluations. Facilities were improved, thanks to funding from WHO, to add insectary space, bioassay laboratory space and administrative space. Standard Operation Procedures were developed in Spanish for unit administration, procedures and experiments as well as database management. Key UCBE personnel participated in WHO-sponsored training workshops aimed at transferring knowledge and skills. While UCBE-GLP is in the latest stage of accreditation, this poster will provide description of the challenges and opportunities of achieving GLP status. This accreditation represent a regional effort to promote health research and infrastructure development, to support local, regional and international evidence-based decision making for Control Programmes of ABD in developing countries.

1443

OPINIONS OF COMMUNITY MEMBERS TOWARDS TRADITIONAL AND NOVEL *AEDES AEGYPTI* CONTROL METHODS IN PONCE, PUERTO RICO

Carmen L. Pérez-Guerra[1], Sue A. Ramos-Díaz[1], Coral Rosado-Santiago[1], Karla M. Marrero-Santos[1], Angela F. Harris[1], Liliana Sánchez-González[1], Adriana Romero[2], Mary H. Hayden[3], Gabriela Paz-Bailey[1]

[1]*Centers for Disease Control and Prevention/CCID/NZVED/, San Juan, PR, United States,* [2]*Centers for Disease Control and Prevention/CSTLTS/PHAP, San Juan, PR, United States,* [3]*University of Colorado/Trauma, Health and Hazards Center, Colorado Springs, CO, United States*

Communities Organized for the Prevention of Arboviruses (COPA) is a project developed to evaluate *Aedes aegypti* control strategies to reduce morbidity and mortality associated with mosquito-borne diseases in Puerto Rico. Previous group discussions (GDs) in 2017 with community members from Ponce indicated that mosquitoes, debris, and containers with water were common problems, which increased their concerns for the transmission of arboviral diseases. The purpose of the present study was to elicit community opinions of traditional versus new methods to control *Aedes aegypti*, the mosquito vector of Zika, dengue and Chikungunya viruses. We conducted four GDs from April-May 2018 with leaders and residents selected through a snowball technique. Discussions were led using a guide and a slide set with mosquito-control method descriptions and illustrations. MAXQDA 12 software was used for content analysis. Thirty-two people from eight clusters participated. All participants supported Autocidal Gravid Ovitraps (AGOs) and source reduction, while almost all supported hand-applied larvicide and truck-mounted larvicide spraying. More than half would support the release of male mosquitoes with Wolbachia and genetically modified (GMO) mosquitoes because these methods would reduce the number of adult mosquitoes. Half would support the release of male and female mosquitoes with Wolbachia to block virus transmission although it would not reduce the number of mosquito bites. All requested information about the effectiveness, adverse effects on health, financial cost, and study results from other countries to support its implementation in their communities. Best practices to disseminate information included educational and promotional campaigns with experts and trained community leaders, using traditional and social media. AGOs, truck-mounted larvicide spraying, male mosquitoes with Wolbachia and GMO mosquitoes were the vector control methods supported by participants. Implementation of these methods is feasible if comprehensive education is provided to the community and government funding is allocated to ensure sustainability.

1444

EXPLORING RISK FOR MALARIA INFECTIONS ASSOCIATED WITH NOCTURNAL FISHING IN RUSINGA ISLAND, WESTERN KENYA

Evelyn Adhiambo Olanga[1], Wolfgang Richard Mukabana[2], Lucy Wachuhi Irungu[3]

[1]*Malaria Alert Centre of the College of Medicine, Malawi, Blantyre, Malawi,* [2]*University of Nairobi, Nairobi, Kenya,* [3]*Machakos University, Machakos, Kenya*

Malaria vectors traditionally bite humans indoors at night which led to the development of indoor-based interventions. Fishermen of Rusinga Island in Western Kenya work outdoors at night and are beyond the protective range of these interventions. In this study, spatial and temporal exposure to malaria mosquitoes and infection in relation to capture fishing activities was investigated. Hourly mosquito biting and human fishing activities were recorded on Kolunga fishing beach from 6pm till 7 am for 73 nights in 2012. Variation in mosquito numbers and prevalence of malaria among residents found within a transect measuring 1km by 2km from the shoreline was determined in Sienga village. A health facility-based malaria survey was carried out to determine association between occupation and malaria. A total of 1,577 female mosquitoes were captured in Kolunga of which 37 were *An. gambiae* s.l., 231 were *An. funestus* s.l. and 1,299 were non-malaria vectors. Two peak periods of biting activity were observed in *An. gambiae* s.l. between 10-11 pm and 4-5 am while *An. funestus* peaked between 10-11pm and 5-6 am. Similar peaks were noted for fishing activities between 9-11pm and 3-7am. A total of 4,338 female mosquitoes were captured in Sienga village of which 15 were *An. gambiae* s.l., and 10 were *An. funestus* s.l. Compared to malaria mosquitoes captured at close proximity to the shoreline, there was no significant difference in densities of *An. gambiae* s.l. (p=0.742) and *An. funestus* (p=0.424) caught at 1,800 metres from the shoreline. No association between malaria infection and distance of 1,800 metres from the shoreline (p=0.312) was observed in this study. Among patients, malaria was significantly associated with individuals involved in fishing (p=0.031) and farming (p=0.003) activities. The study demonstrated an association between malaria cases and individuals involved in fishing and related activities. The risk of malaria on Rusinga Island appears to be associated

with the time that individuals are found outdoors at night suggesting the need to develop complementary vector control tools to protect individuals engaged in nocturnal outdoor activities.

1445

CHARACTERIZING THE MOSQUITO POPULATIONS ON THE ISLAND OF MAUI, HAWAII

Priscilla Seabourn[1], Helen Spafford[1], Lee Goff[2]

[1]*University of Hawaii at Manoa, Honolulu, HI, United States,* [2]*Chaminade University, Honolulu, HI, United States*

Mosquito-borne diseases present a global threat to public health and wildlife conservation. The introduction of mosquitoes to the Hawaiian Islands has supported several dengue virus outbreaks, with the most recent occurring in 2015 on Hawai'i island, and provides the capacity for arbovirus transmission for the future. Currently, there are six species of mosquitoes that have been reported in the state of Hawaii, *Culex quinquefasciatus*, *Aedes albopictus*, *Aedes aegypti*, *Wyeomyia mitchellii*, and *Aedes japonicus*, with all but *W. mitchellii* capable of transmitting human and zoonotic diseases. Hawaii is under constant threat of new species incursions due high levels of tourism and cargo shipments. To inform vector control strategies, the development of comprehensive distribution maps is critical but lacking for. some of the islands of Hawaii, like Maui, To address this gap, this project aimed to characterize the current distribution of mosquitoes (Diptera: Culicidae) on the island of Maui in Hawaii by deploying ovipositional and BG-sentinel traps around the island of Maui for one year at various elevations and in different habitats. *A. albopictus* is abundant and widely distributed around Maui but was not collected in some areas. *C. quinquefasciatus* was also found to be abundant,but had a more limited distribution as compared to *A. albopictus*. *A. vexans* and *W. mitchellii* were also infrequently collected. *A. japonicus* has been reported on Maui but was not sampled in our study. The unique landscape, high biodiversity, and year-round suitable climate in Hawaii make it an ideal environment for a diverse community of mosquitoes and reservoir for disease. Establishing the existing distribution or mosquitoes and continuous monitoring for new incursions is essential for mitigation of mosquito-borne disease.

1446

BITES, BLOOD AND BEHAVIOR: BIOPHYSICAL APPROACHES TO UNDERSTANDING MOSQUITO BLOOD-FEEDING BEHAVIOR

Felix J. Hol[1], Louis Lambrechts[2], Manu Prakash[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*Insect-Virus Interactions Unit, Institut Pasteur, Paris, France*

Mosquito-borne pathogens are transmitted during blood feeding, yet despite its crucial role in pathogen transmission, blood-feeding behavior remains ill understood. In particular, the sensory integration of physical and chemical cues on the skin and below its surface is poorly characterized and it is unclear how pathogen infections influence this behavior. These knowledge gaps are largely due to a lack of quantitative tools to study mosquito behavior. To overcome these limitations, we leverage machine vision and an engineered human skin mimic to create high-throughput assays to study *Aedes aegypti* blood feeding. Deep learning based analysis of mosquitoes feeding on a transparent skin mimic enables the characterization of the behavioral trajectory leading to blood feeding and the dynamics of arbovirus transmission. To unravel the effects of a mosquito›s physiological state on blood feeding, we quantitatively characterize how distinct physiological states (e.g. arbovirus infection, nutrition, age) shape blood-feeding behavior. This strategy will provide a deep understanding of the behavioral drivers of blood feeding in mosquitoes. Furthermore, we anticipate that the open availability of the developed tools will enable researchers to dissect the behaviors that make mosquitoes, and other insects, such efficient vectors of human pathogens.

1447

MALARIA VECTOR POPULATION DENSITIES AND MALARIA TRANSMISSION IN A HOLOENDEMIC AREA OF WESTERN KENYA

Andrew A. Obala[1], Judy Mangeni[2], Emma Kimachas Kimachas[3], Kelsy M. Sumner[4], Steve M. Taylor[5], Lucy Abel[3], Wendy P. O'Meara[6]

[1]*School of Medicine, College of Health Sciences, Moi University, Eldoret, Kenya,* [2]*School of Nursing, College of Health Sciences, Moi university, Eldoret, Kenya,* [3]*Academic Model Providing Access to Healthcare, Eldoret, Kenya,* [4]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, United States,* [5]*Division of Infectious Diseases, Duke University School of Medicine, Durham, NC, United States,* [6]*Duke Global Health Institute, Duke University School of Medicine, Durham, NC, United States*

The population density and behaviour of malaria vectors determine the number of infective bites received by human hosts in high malaria transmission areas. In sub-Saharan Africa, daily exposure to infective bites is enormous due to high densities of the vector populations. We report malaria vector populations and transmission capacities in an area of high insecticide treated nets (ITNs) coverage. We used longitudinal cohort design to collect data. Malaria vectors were captured from three (3) sites, namely; Kinesamo, Maruti and Sitabicha villages in the Webuye Health and Demographic Surveillance System (HDSS) area. The malaria vectors were captured once a week on separate days for each site between 6am to 9am from June 2017 to July 2018. We used Prokopack technique to collect mosquitoes. *Anopheles gambaie* sl, *Anopheles funestus* and other anopheles species were identified morphologically and their abdominal status recorded after which each female mosquitoes were dissected, and head and abdomen stored, each of which were tested using real-time PCR to detect both human and *P. falciparum* DNA. Of the 1136 anopheles mosquitoes identified, 90% were *An. gambiae* sl while *An. funestus* comprised only 6.2%. Other anopheles species captured were *An. pretoriensis* (1.1%), *An. demeilloni* (1.1%), *An. coustani* (0.5%), *An. dancalicus* (0.4%), *An. salbii* (0.4%), *An. maculipalpis* (0.3%) and *An. rufipes* (0.2%). Indoor resting, which comprised half gravid and gravid components, was at 40%, with a human blood index of 54% suggests these mosquitoes predominantly bite and rest indoors in spite of high ITNs coverage. We report high entomological inoculation rate (EIR) of 13.97, however, malaria transmission intensity between the villages differed significantly (Fisher's exact test; p=0,029). For instance, sporozoite rate for Kinesamo was 10% compared to 9% and 7% for Maruti and Sitabicha respectively. Both high EIR and sporozoite rates reported here suggest the current control strategies are unlikely to lower malaria transmission in malaria endemic areas of western Kenya.

1448

METAGENOMIC SEQUENCING AND ANALYSIS OF THE VIROME OF *HAEMAGOGUS JANTHINOMYS*: A MAYARO VIRUS VECTOR IN TRINIDAD

Renee Ali[1], Azad Mohammed[1], Jayaraj Jayaraman[1], Chinnaraja Chinnadurai[1], Christine Carrington[1], Dave W. Severson[2], Adesh Ramsubhag[1]

[1]*University of the West Indies, St. Augustine, Trinidad and Tobago,* [2]*University of Notre Dame, Notre Dame, IN, United States*

In the last decade, several arboviral diseases including alphaviruses have significantly affected the Americas. The Mayaro virus, an emerging alphavirus that is endemic to the South American continent is raising concerns, with reports of sporadic outbreaks and imported cases to non-endemic regions. It is primarily transmitted by the mosquito vector *Haemagogus janthinomys* in its sylvatic cycle. The true burden of the Mayaro virus is not fully understood and the role of its mosquito vector in the transmission cycle has not been well investigated. The *Haemagogus* mosquito is also the sylvan vector for the yellow fever virus and may be a vector for other arboviral diseases; which needs to be further explored. In this study, a metagenomic approach using RNA sequencing was used to

characterize the viromes of field caught *Hag. janthinomys* from forested areas in Trinidad, West Indies. Here we report on the RNA metavirome of *Hag. janthinomys* collected from the Caroni Swamp during the rainy season (June to December). The characterized viruses that dominated were Phasi Charoen-like, Humaita-Tubiacanga and Semiliki Forest Complex virus. This study is the first report of metavirome work done on the *Haemagogus* mosquito vector and can contribute knowledge required to prevent and control emerging arboviruses for this region.

1449

ENVIRONMENTAL AND DEMOGRAPHIC RISK FACTORS FOR *AEDES AEGYPTI* VECTOR PERSISTENCE IN URBAN AND RURAL KENYA

Sindiso Nyathi[1], Harun N. Ngugi[2], Amy Krystosik[3], Bryson Ndenga[4], Donal Bisanzio[5], Uriel Kitron[6], Erin Mordecai[7], Desiree LaBeaud[3], Francis Mutuku[8]

[1]*Department of Health Research and Policy, School of Medicine, Stanford University, Stanford, CA, United States,* [2]*School of Biological Sciences, Department of Zoology, University of Nairobi, Nairobi, Kenya,* [3]*Department of Pediatrics, Division of Infectious Diseases, School of Medicine, Stanford University, Stanford, CA, United States,* [4]*Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [5]*RTI International, Washington, DC, United States,* [6]*Department of Environmental Sciences, Emory University, Atlanta, GA, United States,* [7]*Department of Biology, Stanford University, Stanford, CA, United States,* [8]*Environment and Health Sciences Department, Technical University of Mombasa, Mombasa, Kenya*

Aedes aegypti is the vector for several disease pathogens, including the agents of emerging and re-emerging tropical diseases such as dengue, chikungunya and zika. Although vaccine development for these vector-transmitted diseases is still ongoing, vector control interventions at the household level remain the most effective option for disease control. In order to design and implement efficient vector control strategies, the ecology of *A. aegypti* must be well understood. We use household, demographic and environmental data to evaluate factors that contribute to pupal persistence in rural and urban sites in Kenya. Data were collected monthly from 89 households in 2 urban and 2 rural sites over a 4-year period. Pupae count data was collected via monthly survey sampling of potential breeding containers inside households and in the peri-domestic area. Demographic and household data were collected via written surveys and household/compound inspection. Locally collected hobo-logger data and remotely sensed environmental data were used to determine temperature, humidity and land use variables. We hypothesize that certain households have a high pupal persistence and consistently produce high pupae counts. We used a Kendall's W statistic to examine the consistency of pupal production of households across time. We then used Generalized Additive Models (GAMs) to examine the environmental and demographic risk factors for high pupal persistence, while accounting for temporal and spatial correlation, and non-linear effects. In our initial results, the total pupal count across 89 households in 4 sites is 6 221 (Kisumu – 1 197, Chulaimbo – 1 276, Ukunda – 1 680, Msambweni – 2 068). The average 4-year household pupae count is 69.90, with 33% of households falling above the mean (30% in Kisumu, 32% in Chulaimbo, 45% in Ukunda, and 26% in Msambweni). Our initial results suggest that control interventions targeting high persistence households could make vector control more efficient.

1450

BEHAVIORAL INTERACTION OF MOSQUITOES WITH TOPICAL REPELLENTS: A 3D FLIGHT TRAJECTORY ANALYSIS

Mathurin Fatou[1], Steven Nicholas Fry[2], Pie Müller[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*SciTrackS GmbH, Bertschikon, Switzerland*

Topical repellents are important tools for personal protection against mosquito bites and are available as lotions, sprays, gels, roll-ons, etc. that may contain a range of active ingredients. Though several active ingredients may provide protection over several hours we have little concept as to how these topical repellents impact mosquito behaviour. A better understanding of how mosquitoes interact with topical repellents may, however, lead the way to improved formulations or designs of more realistic assays for product evaluation. We measured the behaviour of host-seeking mosquitoes combining the standard arm-in-cage test with Trackit 3D, allowing for measuring the 3D flight trajectories of several mosquitoes with 90 position data points in real time. The real time functionality allowed us to monitor the measurement during the experiments and write the position data on disk without the need to store large amounts of video data, and process these off line. Filming under near-infra-red lighting allowed us to measure the natural behaviour of both day active *Aedes* and night active *Anopheles* mosquitoes under naturalistic lighting conditions. We are able to measure the 3D flight trajectories of several mosquitoes in parallel and in real time in the vicinity of topical repellents using a novel approach, and we will present our latest results.

1451

WOLBACHIA AFFECTS *AEDES AEGYPTI* VECTOR COMPETENCE FOR MAYARO VIRUS

Sujit K. Pujhari, Marco Brustolin, Cory Henderson, Jason L. Rasgon

Pennsylvania State University, State College, PA, United States

Wolbachia is a gram-negative endosymbiont that can infect a broad range of hosts and can often alter the ability for mosquitoes to transmit pathogens. Recently, *Aedes aegypti* artificially infected with Wolbachia have been released at multiple geographic locations to control Dengue virus. Mayaro virus (MAYV) is an emerging alphavirus expanding its presence in the Americas. Here, we have investigated MAYV blocking by wMel and wAlbB strains of Wolbachia in *Aedes aegypti*. Our data indicate that both Wolbachia stains have suppressive effects on MAYV.

1452

ANTHROPOGENIC LANDSCAPES AND THE SPATIAL DYNAMICS OF VECTOR-BORNE DISEASE EMERGENCE IN COSTA RICA

Brett R. Bayles[1], Carlos Faerron Guzmán[2], Gabriellah Agar[1], Bobin Chen[1], Keira Dagy[1], Tyler Hummel[1], Emma Kelly[1], Kira Kuwada[1], Alec Murrer[1], Andria Rusk[3]

[1]*Dominican University of California, San Rafael, CA, United States,* [2]*Interamerican Center for Global Health (CISG), San Vito, Costa Rica,* [3]*Florida International University, Miami, FL, United States*

In the neotropics, agricultural intensification has resulted in large-scale changes to forest ecosystems. Such anthropogenic landscapes can significantly impact the transmission dynamics of vector-borne diseases (VBD), resulting in new or altered focal points of risk. We explored the spatial and temporal trends of three distinct categories of VBD in Costa Rica, including: 1) emerging flaviviruses (Zika virus disease and Dengue fever), 2) neglected tropical diseases (cutaneous leishmaniasis and Chagas disease), and 3) a recently declared eradicated disease (malaria). District-level incidence data was collected between 2006-2017 and spatial statistics were used to identify hotspots of statistically elevated risk. We then quantified the associations between types of human-altered land use (e.g. deforestation rates and agricultural land use) and both the presence (i.e. hotspot or not) and persistence (i.e. number of years as a hotspot) of elevated transmission risk over time. We detected clear patterns of non-random disease risk across each of the three categories of VBD, with Zika and Dengue exhibiting the most spatial overlap in the Caribbean and North Pacific regions. Districts with medium-high rates of deforestation were statistically significantly associated with the increased presence of Zika (OR=6.02, $p<0.001$), Dengue (OR=2.64, $p<0.05$), and leishmaniasis (OR=8.94, $p<0.001$) hotspots. Districts with the highest proportion of crop cover were also statistically significantly associated with the presence of hotspots for Zika (OR=15.19, $p<0.001$), Dengue (OR=13.00, $p<0.001$), leishmaniasis (OR=4.46, $p<0.01$), Chagas (OR=3.09, $p<0.05$), and Malaria

(OR=8.40, p<0.01). The association between land-use change and the persistence of hotspots over time was less clear. In summary, we identified hotspots of VBD of global public health significance and show that these focal points of disease risk may be attributable to human-altered landscapes. Additional research is needed to better understand the role that socioeconomic factors play in the geographic distribution of VBD risk.

1453

RAPID INDUCTION OF APOPTOSIS AS A DEFENSIVE MECHANISM IN THE MIDGUT OF *AEDES AEGYPTI* FOLLOWING FLAVIVIRUS INFECTION

Jasmine B. Ayers, Seokyoung Kang, Rhoel R. Dinglasan, Lei Zhou

University of Florida, Gainesville, FL, United States

Arbovirus transmission via a mosquito between mammalian hosts requires obligatory infection of the mosquito midgut. We hypothesize that following ingestion of virus, the mosquito attempts to clear infected cells via apoptosis to avoid productive infection of the midgut and subsequent dissemination. Apoptotic cell death has been considered as an immune pathway against viral replication in vertebrates, but the role of apoptosis had remained unclear in mosquitoes. We have shown that resistance of *Aedes aegypti* larvae to a mosquito baculovirus is mediated by rapid induction (less than 4 hours post-infection (hpi)) of apoptosis via Inhibitor of Apoptosis (IAP) antagonist *michelob-x* (*mx*). Caspase inhibitor treatment to delay induction of apoptosis negates the resistance phenotype and the mosquitos succumb to infection, suggesting that rapid cell death is vital to arresting viral spread before viral amplification occurs. *mx* is induced within 3 hours in adult *A. aegypti* mosquitos following feeding on blood containing dengue virus serotype 2 (DENV2), and the *mx* mRNA levels are 2.5-fold higher in the dengue-resistant MOYO-R strain than the susceptible MOYO-S strain of *A. aegypti*, supporting the hypothesis that rapid induction of apoptosis affects mosquito susceptibility to medically relevant flaviviruses. Current work involves characterization of the rapid induction of apoptosis following DENV2 and Zika virus (ZIKV) infection of the midgut both *in vivo* after blood feeding and in an *ex vivo* system where the midgut is maintained briefly in tissue culture. Induction of apoptosis has been observed by DNA fragmentation (Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling assay) and active caspase assay at less than 2 hpi with DENV2 and ZIKV in both systems. Preliminary data shows inhibition of apoptosis in the midgut by treatment with aqueous Zn^{2+} (inhibitor of caspase), which is used to explore the function of the apoptotic response in suppressing viral replication. RNA *in situ* hybridization will examine earlier stages of apoptosis alongside virion labelling to clarify what role virus attachment/entry plays in midgut cell death.

1454

DISPERSAL DYNAMICS OF THE ASIAN TIGER MOSQUITO

Laura Vavassori[1], Ann-Christin Honnen[1], Adam Saddler[2], Pie Müller[1]

[1]*Swiss Tropical and Public Health Insitute; University of Basel, Basel, Switzerland,* [2]*Swiss Tropical and Public Health Insitute; University of Basel; Ifakara Health Institute, Bagamoyo, United Republic of Tanzania*

The Asian tiger mosquito (*Aedes albopictus*) is one of the most invasive insect species globally and is of considerable public and veterinary health relevance as a disease vector. While human-aided transport is clearly a key driver, it is still not clear to what extent the mosquito is passively dispersed and how far it colonises new areas through active flight. Understanding the dispersal dynamics at regional scale is, however, fundamental to improve vector surveillance and to target vector control efforts. In order to measure active dispersal we deployed devices by which field-caught mosquitoes are self-marked with a fluorescent dye, avoiding the negative impacts of traditional marking methods. To measure passive dispersal we sampled mosquitoes from Switzerland and neighboring countries and sequenced these using ddRAD next-generation sequencing, generating a vast number of Single Nucleotide Polymorphisms (SNPs). On the basis of these SNPs and the mark-release-recapture study we reconstruct the invasion of the Asian tiger mosquito in newly colonized areas in Switzerland, and we will present our latest results.

1455

AEDES AEGYPTI BLOOD AND SUGAR-FEEDING PATTERNS ARE ASSOCIATED WITH HOUSING QUALITY AND HUMAN BEHAVIOR IN LOS ANGELES, CALIFORNIA

Marisa A. Donnelly[1], Christopher M. Barker[1], Bradley Main[1], Susanne Kluh[2]

[1]*University of California Davis, Davis, CA, United States,* [2]*Greater Los Angeles County Vector Control District, Santa Fe Springs, CA, United States*

Since 2015, more than 1,000 travel-related human infections with Zika and dengue viruses (ZIKV and DENV) have been detected in California. Local transmission has not been reported, but many detections have occurred in areas infested with *Aedes aegypti*, which was first detected in California in 2013. Los Angeles County (LA) is California's most populous county, and accounts for more than 20% of the state's travel-related infections of ZIKV and DENV. In addition, *Ae. aegypti* has continued to spread in LA, with 82 cities reporting infestations. In the U.S., some studies suggest that improved housing quality reduces exposure to *Ae. aegypti* biting; however, no study has investigated how household characteristics and human behaviors (e.g. air-conditioner usage) may modify feeding patterns. To better understand the relationships between household risk factors and *Ae. aegypti* biting, we identified bloodmeal hosts, quantified sugar-feeding prevalence, and identified household predictors for blood and sugar feeding in LA. During summer 2017, we surveyed 163 households across six cities in LA. We administered a survey in English or Spanish to collect data on *Ae. aegypti*-relevant human behaviors and household characteristics, and collected adult mosquitoes indoors and outdoors. In total, we collected 185 adult female *Ae. aegypti*; 47 were blood-engorged and 138 were non-blood-engorged. We identified host species by sequencing 16S and cytochrome B mitochondrial *loci* using Illumina sequencing, and quantified the blood and sugar-feeding prevalence of non-blood-engorged mosquitoes using anthrone and vanillin assays. The anthrone assay quantifies fructose and glycogen and the vanillin assay quantifies lipids, together reflecting the balance of a mosquito's metabolic activities and nutritional inputs and storage from feeding on blood and sugar. Our study enhances our understanding of *Aedes*-borne virus epidemiology in relation to measurable socio-demographic factors, which is important for targeting vector control in the arid southwestern U.S. where travel-related infections are common and *Ae. aegypti* and other vectors continue to spread.

1456

IMPACTS OF CHEMOSENSORY ORGAN ABLATION ON HOST-SEEKING ACTIVITY IN THE MALARIA VECTOR *ANOPHELES COLUZZII*

Zachary R. Popkin-Hall, Michel A. Slotman

Texas A&M University, College Station, TX, United States

Host-seeking in mosquitoes is modulated by chemosensory organs, which include the antennae, maxillary palps, and labellum of the proboscis. The maxillary palps express CO_2 receptors, which are thought to activate the host-seeking process, and both the maxillary palps and labella express olfactory receptors. We conducted a series of ablation experiments in the highly anthropophilic malaria vector *Anopheles coluzzii* to determine the respective role of chemosensory organs in both host-seeking and host preference. We tested five treatment groups: unablated control, injury effect control (leg-ablated), antenna-ablated, maxillary palp-ablated, and labellum-ablated. Injury effect control mosquitoes had one metathoracic leg removed with fine forceps, while the chemosensory organs were ablated in pairs. Experiments were conducted in a two-port olfactometer at 28 °C and 80% humidity two hours after the start of the dark cycle. Mosquitoes were exposed to an air current containing 5% CO_2 and ports

containing either human or cow odor. Airflow was maintained at ~0.4 m/s. We measured response rates to both odors, as well as activation (i.e. leaving the release cage). Not surprisingly, preliminary results show the highest activation level in control mosquitoes, which entered the olfactometer 86% of the time. Antenna-ablated mosquitoes showed the lowest activation, at only a 35% rate, while 55% of labellum-ablated mosquitoes and 77% of palp-ablated mosquitoes entered the olfactometer. While injury effect may explain some reduction in activation, 67% of leg-ablated mosquitoes were activated, nearly double the rate of the antenna-ablated group. Total odor response rates show a similar trend: 27% of control mosquitoes entered an odor port, compared to 0% of antenna-ablated, 8% of labella-ablated, 11% of palp-ablated, and 8% of leg-ablated mosquitoes. This trend also holds true when considering only the response rates of activated mosquitoes. All experimental mosquitoes, and 99% of control mosquitoes that responded chose human odor over cow. However, these trends may change as sample sizes increase.

1457

TRACKING THE NATURAL DISPERSION OF ISOTOPICALLY MARKED *AEDES AEGYPTI* IN DONNA, SOUTH TEXAS

Selene M. Garcia-Luna[1], Jose G. Juarez[1], Edwin A. Valdez[1], Ester Carbajal[1], Courtney J. Avila[2], Wendy Tang[1], Luis F. Chaves[3], Estelle Martin[1], Ismael E. Badillo-Vargas[2], Gabriel Hamer[1]

[1]*Texas A&M University, College Station, TX, United States,* [2]*Texas A&M AgriLife Research, Weslaco, TX, United States,* [3]*Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud, Tres Ríos, Cartago, Costa Rica*

The movement of the mosquito *Aedes aegypti*, the primary vector of dengue, chikungunya and Zika viruses, has important implications for the transmission of the diseases the mosquito might carry and also to the development of effective vector population management. Studies of mosquito dispersal have used mark-release-recapture (MRR) designs with laboratory reared or field caught mosquitoes and mostly by marking the mosquitoes with fluorescent dusts. These approaches are subject to concerns regarding low marker retention or altered mosquito behavior. More recently, the marking of mosquitoes with stable isotopes has emerged with applications to other mosquito species. In this study, we utilized a stable isotope mark-capture design to identify movement and dispersal behavior of *Ae. aegypti* in South Texas. Isotopic enrichment with ^{13}C and ^{15}N was conducted in discarded containers and tires containing naturally occurring immature mosquitoes along a canal adjacent to a community of about 104 homes in Donna, Texas. We captured the mosquitoes using BG-Sentinel 2 traps located at different quadrants and at different distances from the larval source habitat. Mean distance traveled by unfed females, gravid females, and males was analyzed by equations calculating Mean Dispersal Distance (MDD) and a logistic mixed model. We documented that male *Ae. aegypti* traveled farther (MDD = 165m) than unfed and gravid females (MDD=105 and 123 m, respectively) for mosquitoes marked with ^{15}N. Similarly, for male mosquitoes marked with ^{13}C had a MDD= 243 m whilst unfed and gravid females (MDD= 179 and 131 m, respectively). Isotopically marked individuals collected in the community on the opposite side of the canal document successful flight across a canal, which is about 10 m in width. This study provides evidence that the stable isotope mark-capture approach is appropriate for studies of *Ae. aegypti* and will provide locally-relevant vector biology to inform vector management.

1458

ECOLOGICAL METACOMMUNITY DYNAMICS OF THE MOSQUITO MICROBIOME

Matthew C. Medeiros, Priscilla S. Seabourn, Helen Spafford

University of Hawaii at Manoa, Honolulu, HI, United States

The mosquito microbiome (an ecological community of microbial symbionts that live within a mosquito) is a major modulator of a host's capacity to sustain disease transmission. Paratransgenesis aims to suppress mosquito borne disease by exploiting these effects through the manipulation of the mosquito-symbiont metagenome, principally by adjusting the microbiome's composition. A common paratransgenic technique involves the manipulation of a natural symbiont's genome to express an effect molecule that depresses vectorial capacity, and the introduction of the modified microbial platform to natural vector populations that sustain disease transmission. Efficacy of this technique is dependent on the transmission efficiency of the transformed symbiont among individual mosquitoes. We hypothesize that the transmission and persistence of these new colonizing microorganisms is a function of ecological and stochastic forces associated with ecological metacommunity assembly, including niche effects (differential fitness across environments), dispersal, and ecological drift (stochastic changes in the demographic rates of community members). Here, we analyze a dataset of 116 *Aedes albopictus* microbiotas collected across the environmentally-heterogeneous island of Maui, Hawai'i. Specifically, we test how both niche effects associated with ecological (cosymbiont abundance, host sex) and environmental (temperature and rainfall) variables, and dispersal limitation influence the distribution of symbiont taxa that are under-development as paratransgenic platforms. The results of this research are expected to guide the development and implementation of paratransgenesis to reduce mosquito-borne diseases that threaten public health.

1459

WHAT IS THE ROLE OF AESTIVATING MOSQUITOES IN MAINTAINING *PLASMODIUM* BETWEEN WET SEASONS?

Samake Djibril[1], Zana Sanogo[1], Adama Dao[1], Alpha S. Yaro[1], Moussa Diallo[1], Ben Krajacich[2], Roy Faiman[2], Tovi Lehmann[2]

[1]*Malaria Research and Training Center (MRTC)/Faculty of Medicine, Pharmacy and Odonto-stomatology, Bamako, Mali,* [2]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States*

Mosquitoes are the engine of malaria transmission and for over a century, have been the most effective target for disease control. The dry season biology of the African malaria mosquito and its consequences for disease transmission are poorly understood. Recent findings showed that aestivating mosquitoes survive during the 7 month-long dry season. Whether these mosquitoes transmit malaria during the dry season, albeit in low levels, and whether they act as a reservoir of the parasite besides humans remains unknown. If so, aestivating mosquitoes will initiate disease transmission after the first rains, even if all humans were successfully cleared of their infection, complicating elimination efforts. We measured mosquito infection rate and malaria case load during the dry season in the Sahel. If mosquitoes act as a plasmodium reservoir, we predict that i) mosquito infection rate during the dry season will be moderate to high, and ii) 2-3 weeks after the late dry-season peak in mosquito density (mid-March—early April) malaria case load will rise and so will happen after the first rain (June). On the other hand, if aestivating mosquitoes are not infectious, the malaria case load will not change after the late dry season peak or shortly after the rain. Mosquito sporozoite infection, determined by CSP ELISA on ~10,000 *Anopheles coluzzii* females collected in the village Ballabougou between 2012 and 2016 showed that contrary to our prediction, infection rate during the dry season was very low (<1%), suggesting that these mosquitoes had minimal exposure to plasmodium, or have lost their infection. Likewise, malaria cases in the regional clinic 5 km from Ballabougou was low and stable throughout the dry season, with minimal case load after the high biting frenzy during the late dry-season peak. Our preliminary analysis provides evidence that aestivating mosquitoes do not act as a reservoir for *Plasmodium* during the dry season and that transmission rate by aestivating mosquitoes is minimal.

1460

ABUZZ : A MOBILE PHONE BASED CITIZEN SCIENCE PLATFORM FOR CROWDSOURCING ECOLOGICAL DATA FOR MOSQUITO SURVEILLANCE

Haripriya Mukundarajan[1], Rebecca Konte[1], Felix J. Hol[1], Hazel Soto-Montoya[1], Ansley Murphy[2], Benjamin McKenzie[2], Sam Abernethy[1], Doyeon Park[2], Sarah Zohdy[2], Manu Prakash[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*Auburn University, Auburn, AL, United States*

The creation of large-scale, high-resolution, ecological datasets on the abundance, distributions, and behavior of mosquito species is essential to lay the foundation for the successful application of promising new technologies for mosquito-borne disease management, such as genetic engineering and predictive modeling. However, the high-throughput collection, processing, standardization, and dissemination of reliable observations from the field remains the limiting factor in creating such datasets. In earlier work, we developed a method capable of crowdsourcing mosquito observations from the general public, by using simple mobile phones to record their species-specific wingbeat sounds for automated species identification together with metadata on the time and location of data collection, enabling low-cost, high-throughput spatio-temporal mapping of mosquito populations. Based on this concept, here we present ABUZZ - a citizen science initiative to collect acoustic data from mosquitoes using a custom mobile app, together with an associated web framework to organize, analyze, and visualize this data. Data recorded by mobile phones from users across the world is routed to a dedicated server, where our algorithms process the sound to identify the species and map the observation. We envision several use cases for this platform - (1) by researchers, as part of individual projects associated with answering scientific questions in mosquito-borne disease ecology, (2) by entomologists and public health workers, for creating and expanding the baseline for ecological mapping in a region, (3) by educators, as a teaching tool about mosquito-borne disease and mosquito biology for children and communities, and (4) by the lay public, to generate large quantities of mosquito surveillance data. We further outline our experience training local communities in various countries to use our tools, laying out a vision for multi-stage expansion of this platform, with applications in monitoring insect biodiversity, invasive species, and disease outbreaks.

1461

CRYOPRESERVATION OF MOSQUITO (*ANOPHELES STEPHENSI*) EGGS

Eric Robert James, Yingda Wen, Kristen Pluchino, James Overby, Abraham G. Eappen, Stephen L. Hoffman, Peter F. Billingsley

Sanaria, Rockville, MD, United States

Research on mosquitoes, and in particular on *Anopheles* spp, is constrained by the lack of a method for the long-term preservation of stocks of the large number of wild type species, isolates, laboratory-bred strains, and genetically altered lines. Cryopreservation would provide a powerful tool for mosquito research. Several insect species have been successfully cryopreserved including some genera of dipterans, but to date, despite considerable effort, no method has been developed for mosquitoes - until now. We present a method for the cryopreservation of *Anopheles* spp. eggs that reproducibly yields a hatch rate of ~25%. The eggs are stable in liquid nitrogen vapor phase (LNVP) below -150 ^{o}C for >4 years, the longest storage time tested to date. Hatched larvae develop normally through to adults and the females blood feed normally, mate, and produce viable second generation eggs that also develop normally. Vector competency of adult mosquitoes obtained from cryopreserved eggs was demonstrated by infection with the malaria parasite, *Plasmodium falciparum,* and the development of salivary gland sporozoites in numbers identical to those produced in controls. Critical components of the cryopreservation technique are the time of egg harvest after oviposition, the cryoprotectant additive (CPA), the temperature and duration of exposure to the CPA, cooling and thawing rates, and dilution. The technique can easily cryopreserve small or large (>100,000) batches of eggs, adequate for banking species and strains, and to date has been used to bank *A. stephensi* SDA500, the mosquito used in manufacture of Sanaria® PfSPZ Vaccine, PfSPZ Challenge, and PfSPZ-GA1, several genetically altered lines of *A. stephensi*, and *A. gambiae*.

1462

EVALUATION OF LONG LASTING NEUTRALIZING ANTIBODY AND ITS PROTECTIVE EFFICACY INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE CANDIDATE, KD-382 IN DENGUE PRE-IMMUNIZED CYNOMOLGUS MONKEYS

Masaya Yoshimura[1], Kazuhisa Kameyama[1], Yasuhiko Shinmura[1], Kengo Sonoda[1], Sutee Yoksan[2], Kazuhiko Kimachi[1]

[1]*KM Biologics CO., Ltd., Development Department, Kumamoto, Japan,* [2]*Mahidol University, Center for Vaccine Development, Institute of Molecular Biosciences, Nakhon Pathom, Thailand*

One of challenges in dengue vaccine development, there being four serotypes of dengue virus, is that a vaccine has to induce long lasting neutralizing antibody (Nab) response against all four serotypes simultaneously. Our tetravalent dengue vaccine candidate KD-382, currently under development, is a live attenuated vaccine using a classical host range mutation strategy and thus, expected to induce strong and comprehensive immune response like dengue natural infection. To date, we have revealed that cynomolgus monkeys administered a single dose of KD-382 were seroconverted for all four serotypes (written as Tetra-Nab response from now on) regardless of the presence or absence of existing immunity for dengue. In this study, under pre-existing immunity induced by a monovalent dengue virus immunization, we evaluated the trend of Nab titer after single dose KD-382 administration and protective efficacy against secondary heterologous parental wild-type dengue virus challenged after immunization with KD-382. KD-382 (5 Log_{10} FFU/dose for each serotype) was injected to 12 cynomolgus monkeys pre-immunized with one of four serotypes of parental wild-type strains (DENV-3: 6 Log_{10} PFU/dose,DENV-1,2,4:5 Log_{10} PFU/dose). After KD-382 vaccination, Nab titer against each parental wild-type virus was evaluated by a focus reduction neutralization assay at intervals for over two years. As a result, regardless of the pre-immunized serotype, all the monkeys showed Tetra-Nab response and it maintained for over two years. The monkeys were then challenged with a heterologous serotype of parental wild-type virus 27 months after KD-382 administration, and it was revealed that the viral RNA in serum was under the lowest limitation of quantification, which indicates complete protection against the secondary heterologous challenge. Consequently, KD-382 successfully induced long-lasting and protective Tetra-Nab response in the dengue pre-immunized monkeys, suggesting that KD-382 is a promising vaccine candidate for both dengue seronegative and seropositive people.

1463

DENGUE ENDEMIC SYNCHRONY ACROSS THE AMERICAS

Talia M. Quandelacy[1], Rachel Lowe[2], Anna Stewart[3], Maria Vincenti[4], Esteban Ortiz Prado[5], Cesar V. Munayco[6], Mercy Borbor-Cordova[7], Leslie Rollock[8], Laura Figueroa[9], Rolando Masis[10], Dania M. Rodriguez[1], Maria Grillet[11], Gabriela Paz-Bailey[1], Steve Waterman[1], Isabel Rodriguez-Barraquer[12], Derek Cummings[13], Michael A. Johansson[1]

[1]*Centers for Disease Control and Prevention-Dengue Branch, San Juan, PR, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*State University of New York Upstate Medical University, Syracuse, NY, United States,* [4]*University of Groningen, Groningen, Netherlands,* [5]*OneHealth Research Group, Universidad de Las Americas, Quito, Ecuador,* [6]*Centro Nacional de Epidemiologia, Prevencion y Control de Enfermedades, Lima, Peru,* [7]*Escuela Superior Polytecnica del Litoral, Guayaquil, Ecuador,* [8]*Ministry of Health and Wellness, Saint Michael, Barbados,* [9]*Ministerio de Salud y Asistencia Social, Guatemala City,*

Guatemala, [10]Ministerio de Salud, San Salvador, El Salvador, [11]Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela, [12]University of California San Francisco, San Francisco, CA, United States, [13]University of Florida, Gainesville, FL, United States

Regional synchrony of dengue epidemics has been observed in Southeast Asia, yet few studies have examined the dynamics of dengue outbreaks across the Americas. We aimed to assess the synchrony of seasonal and major epidemics of dengue in Latin America & the Caribbean region. We applied wavelet decomposition to isolate seasonal (8-16 months) and multiannual (16-70 months) components of monthly dengue surveillance data from 220 provinces in 11 countries, spanning 22 years. We examined the coherence and phase differences between province pairs on both time scales to assess how coherence and phase differences relate to distance, country, and geographic location. Seasonal dengue patterns were strongly synchronized across most provinces, though timing of peaks varied with latitude. The multi-annual component showed that major epidemics occurred across many countries in the region in 2001, 2005, and 2009-2011. Overall mean coherence was similar across seasonal (0.48 [95% CI: 0.14 to 0.75]) and multiannual (0.46 [95% CI: 0.22 to 0.73]) components. Highest coherence and lowest phase differences occurred between nearby provinces, indicating higher synchrony. For the seasonal component, the average time lag increased linearly with increased distance. However, the multiannual component only increased up to approximately 1000 Km, after which the average phase difference remained approximately 7 months regardless of distance, indicating regional synchrony for major epidemics. These findings highlight important high-level regional dynamics. While individual provinces have seasonal patterns unique to their latitude and shared with nearby provinces, larger multiannual epidemics occur at a much larger scale with average time lags limited to only ~ 7 months even between faraway provinces. Ongoing work will further identify potential drivers of these patterns, such as climate variability, local transmission intensity, and serotype introductions. These findings can lead to a better understanding of the mechanisms leading to large epidemics and how to provide early warnings to mitigate their impact at a continental scale.

1464

USE OF DENGUE RAPID DIAGNOSTIC TESTS TO DETECT HISTORICAL DENGUE VIRUS INFECTIONS IN POPULATIONS WHERE DENGUE AND ZIKA VIRUSES CO-CIRCULATE

Leah Katzelnick[1], Sully Marquez[2], Sandra Vivero[3], William Cevallos[3], Angel Balmaseda[4], Eva Harris[1], William Messer[5], Joseph Eisenberg[6], Gabriel Trueba[2], Josefina Coloma[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [2]Instituto de Microbiologia, Universidad San Francisco de Quito, Quito, Ecuador, [3]Universidad Central, Quito, Ecuador, [4]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua, [5]Division of Infectious Diseases, School of Medicine, Oregon Health Sciences University, Portland, OR, United States, [6]School of Public Health, University of Michigan, Ann Arbor, MI, United States*

The licensed dengue vaccine, Dengvaxia, is protective in individuals who have experienced ≥1 previous dengue virus (DENV) infections but increases risk of severe dengue in those who have not been infected with DENV previously. The World Health Organization recommends use of a companion test such as the dengue rapid diagnostic test (RDT) to determine prior DENV infection history and thus vaccine suitability. We tested whether the SD BIOLINE Dengue IgM/IgG RDT detects historical flavivirus infections in healthy annual serum samples from 56 participants ages 2-60 years old in a dengue and Zika cohort study in Borbón, Ecuador (2018-2021). An earlier study in Borbón found 43% DENV seroprevalence for individuals age <5 years old, 74% for ages 5-13, 84% for ages 14-29, and 91% for ages ≥30. We developed a protocol to measure the IgG band in the dengue RDT using control serum samples. Images were taken of RDT strips at 15-20 minutes (as recommended by the manufacturer), 1, 2, and 3 hours. Pixel intensity was estimated in R by subtracting background intensity from each band (control, IgM, IgG). IgG positivity was also classified manually (negative, ambiguous, positive). Two dynamics in IgG pixel intensity were observed: flat with a small uptick at 3 hours and steadily increasing from 15 minutes to 3 hours. IgG pixel intensity was positively correlated with IgG positivity classified manually ($r=0.76$, $p<0.001$). At 15-20 minutes, none of those age <5 and 19-47% of those ages ≥5 were IgG-positive. by 1 and 2 hours, IgG positivity was observed for 22-33% of individuals age <5, 52-62% of those ages 5-13, 93-100% of those ages 14-29, and 100% of those ages ≥30. by 3 hours, IgG and IgM bands appeared in some otherwise negative samples, suggesting non-specific reactivity. Thus, IgG positivity measured with a dengue RDT increases with age in a DENV-endemic population. However, the RDT must be read later than current manufacturer recommendations. We will also estimate sensitivity and specificity using the Panbio IgG ELISA and test samples from individuals with known prior DENV and Zika virus infections in a pediatric cohort in Nicaragua and a traveler cohort in Oregon.

1465

HOW MUCH OF PASSIVE DENGUE SURVEILLANCE CASES ARE NON-SECOND, AND DOES IT MATTER?

Angkana Huang, Derek Cummings

University of Florida, Gainesville, FL, United States

To most effectively identify dengue intervention targets, measures of transmission intensity are needed. Force of infection (FoI) is the often used measure. While seroprevalence data is the gold standard for FoI estimations, its availability is globally limited. Inferring FoI from age-stratified case counts provides a feasible alternative but requires the risk of clinical disease upon infection at different ages and upon first, second, third or fourth infection to be estimated or assumed. Current methods widely assumed that third and fourth infections were minimally observed. However, it is unknown if their contributions are small enough to be neglected. Here, we relax the structure of catalytic models in the literature to allow unrestricted contributions of all dengue infections (first thru fourth), assuming life-long homotypic protection against each infecting serotype. Model parameters were estimated from 37 years (1981-2017) of provincial age-stratified dengue case data in Thailand for all 72 provinces using Bayesian Monte Carlo Markov Chain estimation. During the time, mean age of dengue cases dramatically increased in all provinces from 9.7 (inner 95% quantile 7.7, 17.5) in 1990 to 22.9 (inner 95% quantile 17.9, 31.9) in 2017. Our models fit this transition with changes in the FoI suggesting the impact of human demographic changes on the transmission of dengue. We estimate the fraction of infections that are observable for first, second, third and fourth infections as well. Simulation studies show that our models 97.5% of times capture pre-assumed fractions, giving confidence in our results. FoI estimated under the unrestricted models differed from when capturable infections were restricted to only second infections. Bayesian information criterion supports the unrestricted estimates. Our results stress the need to include detailed disease process in models and show that information can be extracted from passive surveillance datasets, particularly when analyzed longitudinally. Our approach also highlights the continuing age shift of dengue cases in Thailand from a pediatric disease to a disease of late to middle adulthood.

1466

INTER-HOUSEHOLD SPREAD OF DENV-1 AND DENV-2 IN SEMI-RURAL THAILAND IS STRONGLY SPATIALLY STRUCTURED AND TIME-DEPENDENT ON A LINEAR SCALE

Irina Maljkovic Berry[1], Melanie C. Melendrez[2], Simon Pollett[1], Chonticha Klungthong[3], Katherine Figueroa[1], Butsaya Thaisomboonsuk[3], Tao Li[1], Michael Panciera[1], Louis Macareo[3], Alan L. Rothman[4], In-Kyu Yoon[5], Stephen J. Thomas[6], Timothy Endy[6], Richard G. Jarman[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]St. Cloud State University, St. Cloud, MN, United States, [3]Armed Forces*

Research Institute of Medical Sciences, Bangkok, Thailand, [4]University of Rhode Island, Kingstown, RI, United States, [5]International Vaccine Institute, Seoul, Republic of Korea, [6]Upstate Medical University of New York, Syracuse, NY, United States

Dengue virus (DENV) causes an estimated 390 million infections a year with an estimated 3.9 billion people at risk for infection globally. Approximately 75% of at-risk individuals reside in the Asia-Pacific region, including Thailand. A prospective cluster investigation study in Kamphaeng Phet (KPP), Thailand, was conducted between 2009 and 2012. In order to analyze trends in the spatio-temporal dispersal of DENV, we sequenced and analyzed 410 full viral genomes from this study, from 16 sub-districts of KPP. Consistent with previous whole genome studies of DENV dispersal on national, city and suburban scales, our discrete analyses on the sub-district level indicated a center-out DENV spread in KPP, with early season DENV introductions into the central sub-districts, followed by late-season virus dispersal to the rural areas. In order to determine viral spread on a finer spatial scale, we extended our discrete trait analyses to reconstructions of house-to-house transmission events utilizing select DENV sub-lineages only. For additional phylogenomic resolution, within-host DENV-1 minor variant frequencies were mapped on the taxa of these time-scaled phylogenies to confirm possible inter-household transmissions. This approach confirmed 8 inter-household transmissions, with 6 households sharing two or more transmission chain-specific DENV minor variants. The individual transmission chain households were located within 20-3000m, with median distance of 490m (IQR 70m - 1800m). A regression of transmission-pair distances over sampling time showed that DENV disperses an average of 70 meters per day (95% CI 54 - 86 m/day) between households in these communities. This relationship was strikingly linear (r^2=0.91), although the skew of the data prompts further sampling and analysis to avoid model misspecification and to mitigate any possible sampling bias. Remarkably, our approach also resolved three transmission chains involving individuals residing in the same households, providing the first genomic fine-scale confirmation for models suggesting that dengue transmission and infections are generally occurring close to home.

1467

IDENTIFICATION OF TRANSCRIPTIONAL SIGNATURES OF DISEASE-INDUCED HOST RESPONSE AS PROSPECTIVE PREDICTORS OF SEVERITY AND VIRUS MICROEVOLUTION IN DENGUE PATIENTS

Elihu Aranday-Cortes[1], Brian Schwem[2], Ma. Jowina Galarion[2], Coleen Pangilinan[2], Phil Lewis[3], Connor Bamford[1], Lily Tong[1], Natasha Johnson[1], Ana Filipe[1], David Matthews[3], Carol Leitch[1], Raul Destura[2], Andrew Davidson[3], John McLauchlan[1]

[1]*MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom,* [2]*National Institutes of Health, University of the Philippines, Manila, Philippines,* [3]*School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom*

Dengue virus (DENV) is the most important arthropod-borne human virus associated with viral disease. Infection produces a variety of clinical presentations, but the factors contributing to differential disease severity are poorly understood. The lack of diagnostic tests to predict severe disease with haemorrhagic complications forces high levels of hospital admissions for safety purposes. Our objective, by adopting a liquid biopsy approach and integrating clinical and molecular data, is to correlate the spectrum of disease severity with DENV genetic diversity and host transcriptomic- changes observed in the periphery. This should enable identification of candidate virological and host biomarkers to develop diagnostics that predict progression to severe disease. We have developed pipelines to generate and analyse viral metagenomic and host transcriptomic data from prospectively-collected whole-blood and sera from DENV-infected patients. We assembled full-length DENV genomes without PCR-based enrichment and all four DENV serotypes were identified. Hotspots of intrahost codon diversity across the virus genome were found predominantly in a CD8 T cell epitope located in the NS5 RdRP domain. We defined whole-blood transcriptomic signatures of disease severity and identified genes that were deregulated in severe disease compared to dengue with/without warning signs. Network analysis revealed upregulated genes were involved in complement activation, coagulation and angiogenesis whereas downregulated genes were involved in the acute phase response and immune defence. Analysis of such patterns of gene abundance could lead to the identification of prospective biomarkers which can be tested further for their potential to discriminate DENV-mediated disease conditions

1468

NEUROLOGIC ILLNESS AMONG HOSPITALIZED PATIENTS WITH ARBOVIRUS INFECTION, PUERTO RICO, 2012-2018

Luisa I. Alvarado[1], Chelsea Major[2], Eva I. Gordian Rivera[1], Luzeida Vargas[1], Vanessa Rivera[3], Stephen Waterman[2], Gabriela Paz Bailey[2], Tyler Sharp[2]

[1]*Ponce Health Sciences University and Saint Luke's Episcopal Hospital Consortium, Ponce, PR, United States,* [2]*Division of Vector-borne Diseases, Centers for Disease Control and Prevention, San Juan, PR, United States,* [3]*Ponce Health Sciences University, Ponce, PR, United States*

Arboviruses transmitted by Aedes mosquitoes, including dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) have been associated with potentially fatal neurologic illness. Such manifestations may result from the direct effect of the virus, host immune responses to infection, or metabolic derangements presenting as encephalopathy. While the 2009 WHO case definition for severe dengue includes severe organ involvement of the central nervous system, better understanding of the burden and spectrum of neurologic illness caused by arboviruses is needed. Using data from the Sentinel Enhanced Dengue and Acute Febrile Illness (AFI) Surveillance System (SEDSS) in Ponce, Puerto Rico, we investigated the frequency of neurologic illness among hospitalized patients with arbovirus infection. The study population included patients hospitalized at the SEDSS clinical site during May 2012-December 2018 with evidence of DENV, CHIKV, and ZIKV infection by reverse transcription-polymerase chain reaction. Patients with neurologic illness were diagnosed with encephalitis/encephalopathy, seizures, aseptic meningitis, or acute paralysis. Chart abstractions are underway to further investigate clinical features. Of 599 hospitalized patients with arbovirus infection, six (2%) of 336 patients with DENV, 14 (6%) of 228 patients with CHIKV, and none of the 35 patients with ZIKV had neurologic illness. Median age of patients with neurologic illness was 3 years (range: 0-87), and 60% were male. Most (5, 83%) patients with DENV and 50% (7) of patients with CHIKV and neurologic illness had diagnoses of encephalitis/encephalopathy; 57% (8) of patients with CHIKV and neurologic illness had seizures. Among patients with DENV or CHIKV infection, those with neurologic illness were less likely to report joint and muscle pain (P<0.001), but were similar in reporting of other AFI symptoms, age and sex distribution, and length of hospitalization compared to those without. The frequency of neurologic illness among hospitalized DENV and CHIKV patients in Puerto Rico underscores the importance of arbovirus testing in patients with febrile neurologic syndromes.

1469

IDENTIFICATION OF GENES THAT ARE DIFFERENTIALLY EXPRESSED IN RESPONSE TO DENGUE, ZIKA OR CHIKUNGUNYA VIRUS INFECTION IN NICARAGUAN PATIENTS

Eunyoung Kim[1], Yan Che[1], Daniela Michlmayr[2], Steven Wolinksy[1], Eva Harris[2]

[1]*Division of Infectious Diseases, Northwestern University, Chicago, IL, United States,* [2]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States*

Dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) viruses cause mosquito-borne diseases endemic in many tropical and subtropical regions worldwide. These re-emerging arboviral diseases have similar epidemiology, transmission cycles and clinical symptoms, though complications differ significantly. Comparison of transcriptomes enables

identification of host genes that are differentially expressed in response to infection, providing important insights into the dynamics of the host-virus interaction. Here, we profiled the transcriptome in whole blood from children naturally infected with DENV, ZIKV or CHIKV. RNA-seq was used as an unbiased way to map host genes that are differentially expressed during acute and convalescent phases of infection. We cataloged transcript species (mRNAs, non-coding RNAs and small RNAs), resolved the transcriptional structure of genes (start sites, 5′ and 3′ ends, splicing patterns), and measured their abundance. Distinct transcriptomic signatures were associated with time of infection or symptom severity. Over 4,000 significant differentially expressed genes (DEGs) were shared among the 3 arboviruses (FDR<0.05), the most common being upregulated in the acute phase. Pathway and enrichment analyses showed the shared highly enriched (-log10(P) >20) DEGs that were upregulated involved defense responses to virus, interferon signalling, cytokine production, and regulation of innate immune response, whereas DEGs that were down-regulated involved eukaryotic translation elongation. There was an enrichment for blood transcriptome modules (BTMs) for monocytes, macrophages, NK cells, type-1 IFN, and cell cycle during the acute phase of infection, and B cells, T cells and Th2 cells during the convalescent phase of infection. Certain BTMs (e.g., T/NK cells, B cells) were enriched and others (e.g., neutrophils) were less enriched in CHIKV vs. DENV and ZIKV infections. Comparison of transcriptomes among DENV, ZIKV and CHIKV provide biological insights into the transcriptional and signaling networks in arboviral infections that can be used to resolve pathogenic mechanisms.

1470

A PHASE 1, RANDOMIZED, OPEN-LABEL, SINGLE-CENTER COMPARISON OF HETEROLOGOUS PRIME-BOOST VACCINATION SCHEDULES OF TETRAVALENT DENGUE VIRUS PURIFIED INACTIVATED VACCINE (PIV) AND TETRAVALENT DENGUE VIRUS LIVE ATTENUATED VACCINE (LAV) IN HEALTHY ADULTS IN A NONENDEMIC REGION THROUGH 28 DAYS POST VACCINATION

Michael Koren, Simon Pollett, Keisha Akerele, Christine Lee, Kristin Mills, James Moon, Paul Keiser, Jack Hutter, Melinda Hamer, Justin Curley, Nathaniel Copeland, Mark Sanborn, Wiriya Rutvisuttinunt, Rafael De La Barrera, Richard Jarman

Walter Reed Army Institute of Research, Silver Spring, MD, United States

A heterologous prime boost vaccination regimen may offer protection from dengue infection, and be particularly useful in dengue naïve individuals. It has been previously shown that a purified inactivated tetravalent dengue vaccine (PIV) followed by tetravalent dengue live attenuated vaccine (LAV) was highly immunogenic and safe, and that a PIV-LAV administration schedule of 0-6 months resulted in superior immunogenicity when compared to a 0-1 month schedule. In order to evaluate expedited prime-boost dosing schedules, we compared a 0-3 month PIV/LAV to a PIV/LAV 0-6 month regimen. 20 healthy dengue naïve adults 18-42 years of age were enrolled per group. Results through 28 days post final vaccination are presented. Both vaccination schedules were tolerated without any serious adverse events or potentially immune mediated events. Most adverse events reported were either mild or moderate in severity. There was a trend towards increased reactogenicity in the 0-3 month vaccination group following LAV. Rates of fever (32% versus 25%), headache (53% versus 38%), myalgia (42% versus 18%), arthralgia (31% versus 0%) and rash (32% versus 25%) were all higher in the 0-3 month compared to the 0-6 month group. Both regimens resulted in robust increases in measurable dengue neutralizing antibody at 28 days following LAV with a trend towards higher neutralizing antibody GMT per serotype in the 0-3 month group compared to the 0-6 month group. Geometric mean titers per serotype ranged from 2056(DENV-3) to 4254(DENV-2) for 0-3 month group and 1633(DENV-3) to 3188(DENV-2) in the 0-6 month group. Tetravalent seroconversion rates were 95% (95% CI 74%-99%) in the 0-3 month group and 100% (95% CI 79% - 100%) in the 0-6 month group. RNAemia was common in both groups, with dengue serotype 2, dengue serotype 4 and dengue serotype 1 detected. This interim analysis suggests that an expedited 0-3 month prime-boost DENV vaccination schedule is as immunogenic and safe when compared to a 0-6 schedule, but may be associated with relatively higher rates of reactogenicity. Further evaluation of heterologous prime boost strategies for protection from dengue is warranted.

1471

MICROSCALE SPATIOTEMPORAL TRANSMISSION DYNAMICS OF DENGUE IN PUERTO RICO, 2009-2013

Carlos A. Moreno[1], Kyra H. Grantz[1], Michael Johansson[2], Derek A. Cummings[1]

[1]*University of Florida, Gainesville, FL, United States,* [2]*Centers for Disease Control and Prevention (CDC) Dengue Branch, San Juan, PR, United States*

Puerto Rico has experienced constant dengue outbreaks since the 1960s, making it a prominent hub for dengue circulation in the Americas. Control efforts to reduce mosquito populations or exposure to mosquitoes are resource intensive, and difficult to deliver to all locations at once. An improved understanding of the spatiotemporal distribution of dengue could help public health authorities improve the targeting of intervention resources. While previous studies have demonstrated fine-scale transmission within cities and in rural areas, these studies have been primarily focused in Southeast Asia. In this study we analyzed the fine-scale spatiotemporal dependence of dengue in Puerto Rico. The data were collated from ongoing passive surveillance conducted by the US Centers for Disease Control and Prevention (CDC). A total of 6149 confirmed infections were serotyped and geolocated between 2009-2013. We characterized the spatial dependence of homotypic cases, $\tau(d_1, d_2)$, as the odds that cases occurring within twenty-one days and a specified spatial window (d_1 , d_2) are homotypic, relative to the odds that cases are homotypic within twenty-one days regardless of spatial location. This statistic is robust to variable reporting and other latent spatial heterogeneities (e.g., population density, environmental conditions). We constructed the 95% confidence intervals by using the 2.5% and 97.5% percentiles of 1,000 bootstrap samples. Our results suggest that cases within 100m have 4.63 (95% confidence interval [CI] 3.74, 5.81) times higher odds of being homotypic relative to all cases occurring within twenty-one days. This estimate falls to 1.32 (95% CI 1.22, 1.43) times higher odds for cases within 750m to 1-km, but continues to remain statistically significant up to 4.7 km. These findings are consistent with highly focal transmission of dengue at less than 1-km scales. This finding is also consistent with estimates from other urban areas endemic for dengue.

1472

ASSESSING RISK FOR DENGUE LIVE ATTENUATED VACCINE VIRUS ANTIBODY DEPENDENT ENHANCEMENT IN INDIVIDUALS PRIMED WITH A PURIFIED INACTIVATED DENGUE VACCINE

Natalie D. Collins[1], Mark Sanborn[1], Shannon Walls[2], Jun Hang[1], Greg Gromowski[1], Michael Koren[1], Richard Jarman[1]

[1]*Walter Reed Army Insitute of Research, Silver Spring, MD, United States,* [2]*Walter Reed Army Insitute of Research, USAMRU-G, Silver Spring, MD, United States*

There are four genetically distinct dengue viruses (DENV), termed DENV-1, -2, -3 and -4 that can cause a wide spectrum of disease manifestations from mild infections to severe. It is hypothesized that the more severe presentations of dengue disease, including hemorrhagic fever and shock syndrome, are the result of antibody dependent enhancement (ADE), where antibodies generated against one DENV serotype can enhance a subsequent infection with a heterologous serotype. Speculation that vaccination with Dengvaxia may have led to cases of severe dengue disease due to ADE, necessitates further research into ADE assay development and correlation with clinical outcomes. Typically, *in vitro* assays utilized to evaluate vaccine-related ADE involves use cells that are not permissive to DENV infection, such as the myeloid lineage K562 cells that support infection by Fc-receptor mediated uptake of the DENV-

antibody complex. However, this K562 based assay has several limitations, and the considerable dilution required to decrease serum toxicity of the cells restricts accurate depiction of the immune state of vaccine recipients. This was demonstrated previously when, subjects vaccinated with formalin inactivated DENV vaccine (DPIV) had undetectable DENV-2 ADE in the traditional K562 cell-based assay with diluted immune sera, despite 46% of subjects developing DENV-2 viremia following a live attenuated DENV vaccine (TDENV) boost at 6 months post DPIV vaccination. Therefore, an ADE assay was developed utilizing BHK cells expressing Fc γRIIA (BHK-Fc γRIIA) in order to evaluate *in vitro* ADE at very low serum dilutions. The DPIV primed, 6 month TDENV boosted subjects were re-evaluated utilizing the BHK-Fc γRIIA based ADE assay and greater than 50% of subjects had significant DENV-2 enhanced virus replication. Association of the BHK-Fc γRIIA based ADE assay resluts with viremia and symptoms in these vaccine recipients will be described. The BHK-Fc γRIIA *in vitro* ADE assay will help advance our understanding of the biological significance of ADE in humans.

1473

DISCOVERY OF EPITOPE BIOMARKERS FOR THE DIAGNOSIS OF DENGUE AND ZIKA VIRUS INFECTION

Volker Stadler[1], Felix Loeffler[2], Renate Sekul[1], **Kirsten Heiss**[1], Magelda Montoya Cruz[3], Josefina Coloma[3], Eva Harris[3]

[1]*PEPperPRINT GmbH, Heidelberg, Germany,* [2]*Max Planck Institute of Colloids and Interfaces, Potsdam, Germany,* [3]*University of California, Berkeley, CA, United States*

Infections with dengue (DENV) and Zika viruses (ZIKV), co-circulating in tropical and subtropical regions of the world, have become a thread to public health. Severe disease outcomes are associated with infections with all DENV serotypes and ZIKV, respectively. A precise and early diagnosis of these viruses, however, is hampered not only by similar clinical symptoms, but also by the serological cross-reactivity among ZIKV, DENV and flaviruses in general. Therefore, novel diagnostic tests are urgently needed to identify and discriminate co-circulating ZIKV and DENV particularly in the acute phase, and to predict severe disease outcomes. Instead of using full length proteins, we intended to screen for epitope biomarkers for the differentiation of antibody responses against flaviviruses, i.e. immunodominant ZIKV and DENV peptides that are not shared with other flaviviruses. We generated proteome-wide DENV and ZIKV peptide microarrays and screened them for IgG and IgM antibody responses with ~1000 plasma samples of patients with acute and convalescent infections with DENV serotypes 1, 2 and 3 and ZIKV. For DENV, we observed distinctive and shared antibody responses and applied machine learning to predict discriminative epitopes for acute and convalescent stage of infection, primary and secondary infection and subtypes, respectively. The proteome-wide ZIKV screening revealed a very strong and common epitope in NS2b in both the acute and convalescent phase of infection. A substitution analysis of this immunodominant ZIKV epitope highlighted discriminative amino acid positions that enable a specific diagnosis of ZIKV infections in the acute phase. The differentiation of ZIKV and DENV antibody responses on epitope instead of on protein level yielded a more comprehensive picture of DENV and ZIKV infections and peptide biomarkers for early ZIKV diagnosis as well as for DENV serotypes 1, 2 and 3. This work was supported by the U.S. Department of Homeland Security Science & Technology Directorate (Contract HSHQDC-15-C-B0010).

1474

ANALYSIS OF ANTIBODIES INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE IN CHILDREN WHO SUBSEQUENTLY EXPERIENCED DENGUE SEROTYPE 1 BREAKTHROUGH INFECTIONS

Sandra Henein[1], Matthew Boneparte[2], Ralph Baric[1], Aravinda de Silva[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Sanofi Pasteur, Allentown, PA, United States*

Dengue is the most significant arboviral infection of humans. The four dengue virus serotypes (DENV1-4) are estimated to infect several hundred million people each year. Sanofi Pasteur developed a live attenuated tetravalent dengue vaccine (Dengvaxia) that has been licensed in several countries. In clinical trials, Dengvaxia efficacy varied by DENV serotype and the baseline DENV immune status of subjects. In particular, overall vaccine efficacy was lower in children who were DENV-naïve at baseline compared to children who were DENV-immune. Here we present a comparative analysis of the level and quality of serum antibodies in 1) 16 baseline DENV seronegative subjects who were vaccinated and subsequently experienced DENV1 breakthrough infections; 2) 11 baseline DENV seronegative subjects who were vaccinated and did not experience DENV breakthrough infections; 3) 10 healthy individuals who had been previously exposed to primary DENV1 infections. DENV neutralizing antibodies after primary infection are strongly correlated with long-term protection against disease caused by the homologous serotype. We observed that Dengvaxia induced moderate levels of DENV1 neutralizing antibodies (NAbs) that were comparable in groups 1 and 2 and lower than the levels in group 3. The DENV1 NAbs in groups 1 and 2 were mainly driven by DENV-serotype cross-reactive Abs. The absence of DENV1 type-specific NAbs in the vaccine groups is indicative poor replication of the DENV1 vaccine component. On the other hand, individuals who had recovered from primary DENV1 natural infections mainly developed DENV1 serotype-specific NAbs. Our findings are applicable to understanding vaccine efficacy and for identifying immune correlates for guiding vaccine development.

1475

EVALUATION OF DUAL PLATFORM IMMUNIZATION APPROACH USING TETRAVALENT DENGUE DNA VACCINE AND TETRAVALENT INACTIVATED WHOLE VIRUS DENGUE VACCINES

Appavu K. Sundaram[1], Daniel Ewing[1], Zhaodong Liang[1], Maria Blevins[2], Josef Lassan[2], Jorge Requena[2], Kanakatte Raviprakash[1], Maya Williams[3], John W. Sanders[2], Kevin R. Porter[3]

[1]*Viral and Rickettsial Diseases Department, Naval Medical Research Center, Silver Spring, MD, United States,* [2]*Section on Infectious Diseases, Wake Forest School of Medicine, Winston-Salem, NC, United States,* [3]*Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, MD, United States*

Viral pathogens produce B- and T-cell responses through immune interactions with both pathogen-specific proteins and nucleic acid. The nucleic acid also works through interactions with Toll-like receptors. Therefore, we hypothesized that simultaneous administration of a DNA vaccine and a protein vaccine will elicit the broadest possible immune responses by stimulating different arms of the adaptive immune system. Hence, we evaluated the Dual Platform immunization (DuPl) approach in nonhuman primates by simultaneous administration of a tetravalent dengue DNA vaccine (TVDVs) and the chemically inactivated DENV vaccines. Highly purified (by chromatographic methods) monovalent vaccine lots of formalin-inactivated DENV 1-4 (FIVs) and psoralen-inactivated DENV 1-4 (PsIVs) were prepared and tetravalent FIV and PsIV vaccines were prepared by combining the respective monovalent vaccines. Mice were immunized twice with either monovalent or tetravalent vaccine formulation. Immunogenicity of the tetravalent dengue PsIVs and FIVs was also evaluated in nonhuman primates along with the evaluation

of the DuPI immunization approach, which involved the simultaneous administration of TVDVs with either the tetravalent dengue FIVs or PsIVs. In mice, the DENV PsIVs elicited higher titers of DENV neutralizing Abs than the FIVs and the Ab profiling of sera samples with DENV3 and DENV4 proteome microarrays (PEPperMAP) confirms the better neutralizing antibody response from PsIVs over the FIVs. The tetravalent vaccines produced the same neutralizing antibody pattern (PsIV > FIV) in nonhuman primates as well. Although addition of TVDV to FIV enhanced the neutralizing Ab response, addition of TVDV to PsIV did not improve the immune response. Interestingly, PsIV by itself elicited the best neutralizing Ab response of all the groups tested. These results suggest that psoralen-inactivation (acting at nucleic acid level) of DENV preserves the native conformations of surface proteins important for protective immune responses and is better suited for eliciting DENV-neutralizing Abs.

1476

SEQUENCING OF ZIKA VIRUS ISOLATE FROM THE AMNIOTIC FLUID OF A FETUS WITH MICROCEPHALY DURING AN OUTBREAK IN HONDURAS-2016

Leda Parham[1], Mónica García[2], Brett Pickett[3], Gene S. Tan[3], Nadia Fedorova[3], Paolo Amedeo[3], Kimberly García[1], Pilar Viedma[3], Alan Durbin[3], Torrey Williams[3], **Ivette Lorenzana**[1]

[1]*Centro de Investigaciones Genéticas, Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras,* [2]*Centro Hondureño de Medicina Fetal, San Pedro Sula, Honduras,* [3]*J. Craig Venter Institute, La Jolla, CA, United States*

Zika virus (ZIKV) infection became a worldwide public health concern due to its association with microcephaly and congenital malformations in infants born to infected mothers. After its introduction in 2015, Honduras had a dramatic increase of newborns with microcephaly. We describe a case where we detected/sequenced a viral isolated collected from the amniotic fluid of a fetus with microcephaly. The pregnant mother exhibit ZIKV-associated symptoms as myalgia, arthralgia, rash and joint inflammation at 7 weeks of gestation (January-2016). At the first-trimester of gestation, the ultrasonography was normal. At 22 weeks (May-2016), ultrasound revealed signs of cerebral malformation in the fetus with bilateral ventriculomegaly, microcephaly, rhomboencephalosynapsis and arthogryposis. At 23 weeks, amniotic fluid was collected. The fluid, which may contain viral particles and/or genetic material, was filtrated/concentrated. Viral-RNA was extracted at UNAH. The amniotic fluid was ZIKV positive by qRT-PCR (Lanciotti et al.-2008). Partial-genome-sequencing was done using targeted next-generation-sequencing with Ion-Torrent/Illumina technology at the J. Craig Venter Institute, La Jolla, CA, USA. The baby was born alive and diagnosed with congenital Zika syndrome. We detected ZIKV-RNA in the amniotic fluid. Subsequently, sequence-analysis revealed that this Honduran ZIKV isolate had the point-mutation S139N in the prM-region recently associated with fetal microcephaly by others. The signs of cerebral malformation in the fetus, could be expected to contribute to severe developmental difficulties in the baby. These findings strengthen the reports of the capability of ZIKV to cross the placental barrier and its association with neurological damage in fetus. Also highlights the value of molecular testing in the diagnosis of ZIKV during pregnancy. More studies are needed to determine the number of microcephaly cases that contain this point-mutation and its role in the severity in congenital Zika syndrome.

1477

AGARICUS BRASILIENSIS SULFATED POLYSACCHARIDE INHIBITS DENGUE VIRUS INFECTION AND DENGUE VIRUS NS1-MEDIATED PATHOGENESIS

Francielle Tramontini Gomes de Sousa[1], Camila Malta Romano[2], Ester Cerdeira Sabino[2], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Department of Infectious and Parasitic Diseases, Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil*

Dengue virus (DENV) severe infections are characterized by increased vascular permeability and hemorrhagic manifestations. Despite its substantial morbidity and mortality, no therapeutic agents exist for treatment of dengue, and the currently available vaccine does not confer full protection. Thus, development of therapeutic and/or preventive drugs is urgently needed. Nonstructural protein 1 (NS1) plays important roles in host immune evasion and viral pathogenesis by directly triggering endothelial barrier dysfunction and inducing inflammatory responses, contributing to vascular leak *in vivo*. Here we evaluated the *in vitro* and *in vivo* efficacy of the (1-6,1-3)-β-D-glucan isolated from *Agaricus brasiliensis* fruiting bodies (FR) and its sulfated derivative (FR-S) against DENV infection and DENV NS1-mediated pathogenesis. FR-S, but not FR, significantly inhibited DENV2 (strain N172-06) replication in human monocytic U937-DC-SIGN cells (EC_{50}=30.5 μg/mL) when added simultaneously with viral infection. No inhibitory effect was observed when FR or FR-S were added 1 hour post-infection, indicating viral entry inhibition as the main mechanism of action of FR-S. In an *in vitro* model of endothelial permeability, FR (0.25 ug/mL) significantly inhibited Trans-Endothelial Electrical Resistance (TEER) reduction (>50%) induced by DENV2 NS1 treatment of human pulmonary microvascular endothelial cells (HPMECs), while FR-S displayed 100% efficacy in blocking TEER reduction at 0.12 ug/mL. Confocal microscopy indicated 64 and 73% inhibition of DENV NS1 binding to HPMECs by treatment with 0.25ug/mL of FR and FR-S, respectively. Further, FR-S significantly reduced (42%, p=0.048) hyperpermeability in mouse skin induced by DENV2 NS1 injected intradermally into wild-type mice (C57BL/6). In summary, we demonstrate FR-S efficacy against DENV infection *in vitro*, as well as against NS1-induced endothelial disruption *in vitro* and permeability *in vivo*. The findings stimulate further exploration of FR-S and other glycan candidates for dengue treatment alone or as combination therapies with compounds with different mechanisms of action.

1478

ANTIBODY-DEPENDENT CELLULAR PHAGOCYTOSIS IS A CORRELATE OF PROTECTION AGAINST SYMPTOMATIC DENGUE VIRUS INFECTION

Magelda Montoya[1], Vicky Roy[2], Laura White[3], Antonio Gregorio Dias Junior[1], Parnal Narvekar[1], Leah Katzelnick[1], Sandra Henein[3], Premkumar Lakshmanane[3], Angel Balmaseda[4], Josefina Coloma[1], Aravinda de Silva[3], Galit Alter[2], **Eva Harris**[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard, Cambridge, MA, United States,* [3]*Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC, United States,* [4]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua*

For many currently licensed vaccines and natural infections, antigen-specific antibody (Ab) neutralization titers are used as a correlate of protection. In our long-standing cohort study of dengue in Nicaragua, we have shown that pre-infection dengue virus (DENV) neutralizing Abs play an important role in protection against symptomatic infection, while low pre-existing Ab titers can enhance dengue disease severity. Beyond antigen-specific Ab titers and neutralization, Abs can confer protection or mediate risk through multiple other mechanisms. The role of Fc effector

function in dengue has not been systematically investigated and is a critical gap in knowledge. We investigated antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent neutrophil activation/phagocytosis (ADNP) in pre-infection sera from 35 inapparent and 36 symptomatic DENV infections in our Nicaraguan pediatric cohort study. Fluorescent beads were labeled with DENV2 and DENV3 virions and recombinant envelope (recE), incubated with test sera and then THP-1 monocytic cells or primary neutrophils, and analyzed by flow cytometry. Significant differences between pre-inapparent and pre-symptomatic infections were observed in ADCP (p=0.0007) and ADNP (p=0.01) for DENV3 but not DENV2 recE-coated beads. When analysis was restricted to DENV3 inapparent (n=10) and symptomatic (n=30) infections, significant differences in ADCP were maintained (p<0.001). Thus, Fc-effector profiles indicate that differential monocyte phagocytosis tracks with inapparent DENV infection. Currently, pre-infection sera from symptomatic vs. inapparent DENV1 and DENV2 infections, as well as from DF vs. DHF/DSS cases, are being analyzed via multiplexed Fc biophysical profiling (e.g., subclass, glycosylation variants, interaction with innate immune Fc receptors, lectin-like molecules, and complement) and a larger panel of cell-based assays to measure Fc effector function. Antigens include Nicaraguan DENV viruses, recE, stabilized E homodimers, E domain III, and NS1 from DENV1-4 and Zika virus. This work addresses the critical role of Ab Fc functionality in dengue.

1479

ROBUST GENOMIC DETECTION OF VACCINE VIREMIA IN DENGUE NAÏVE AND IMMUNE SUBJECTS FROM A PHASE II TRIAL OF THE NIH TETRAVALENT DENGUE LIVE ATTENUATED VACCINE IN A DENGUE ENDEMIC SETTING DEMONSTRATES EFFECTIVE VACCINE VIRUS REPLICATION

Marya Carmolli[1], Connor Klopfer[1], Mary Claire Walsh[1], Sean Diehl[1], Kristen Pierce[1], Dorothy Dickson[1], Elisabeth R. Colgate[1], Benjamin McElvany[1], Mohammad Shafiul Alam[2], Sajia Afreen[2], Masud Alam[2], Mohammad Kibria Golam[2], Rashidul Haque[1], Anna Durbin[3], Steve Whitehead[4], Beth D. Kirkpatrick[1]

[1]*University of Vermont, Burlington, VT, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Clinical trials of vaccines and controlled Human Infection Models (CHIMs) play an important role in determining the safety and efficacy of future dengue vaccines. Viremia is used to evaluate safety, infectivity, and efficacy of a vaccine or challenge strain. It is measured by viral amplification in cell culture (VAC) or by a more sensitive assay, qRT-PCR. Our aim was to determine if vaccine viremia can be detected in volunteers with previous exposure to dengue. Since vaccination with the NIH live attenuated tetravalent (serotypes DENV1-4) vaccine causes low-level viremia in naïve volunteers by culture, we hypothesized that dengue-exposed volunteers would only have vaccine -induced viremia for the serotypes for which they had not been previously exposed. To address this, we evaluated the NIH TV005 dengue vaccine in a Phase II trial in Dhaka Bangladesh for post-vaccine viremia by VAC and real-time qRT-PCR at days 7 and 14. Dengue seropositivity was defined as a Plaque Reduction Neutralization Titer ($PRNT_{50}$) of ≥ 10 against any serotype from pre-vaccination serum. A serotype was determined dominant as follows: serotype was the only one with $PRNT_{50}$ ≥ 10 or $PRNT_{50}$ ≥ 4 fold higher than the next highest serotype titer. VAC-positive post-vaccination viremia for any serotype was low; detected in 4/72 (5.6%) subjects: 4/30 (13%) naïve and 0/30 dengue-exposed. by PCR, viremia was detected in 46/72 (65%) subjects, 23/30 (77%) naïve and 23/42 (55%) exposed. Only serotypes to which the volunteers did not have prior exposure were detected by PCR. Among subjects for whom DEN1 was the dominant previous exposure serotype, post-vaccination viremia was found for DEN3 1 of 2 (50%), and DEN4 2/2 (100%). Similarly, for DEN2 previous exposure, PCR was positive for DEN3 5/8(63%) and DEN4 4/8 (50%). For DEN3 as the dominant previous exposure, viremia was found for DEN2 1/6 (17%) and DEN4 3/6 (50%). In previously exposed volunteers, there was no evidence of enhanced viremia. Since PCR is more sensitive than VAC, these data support the addition of genomic methods to detect vaccine viremia in vaccine trials and as an appropriate efficacy trial endpoint in experimental challenge models.

1480

TYPE-SPECIFIC AND CROSS-REACTIVE B CELL RESPONSES ELICITED BY A LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE

Daniela Michlmayr[1], Paulina Andrade[1], Parnal Narvekar[1], Mayuri Sharmna[2], Hansi Dean[3], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [2]*Vaccines Business Unit, Takeda Pharmaceuticals Inc., Cambridge, MA, United States,* [3]*Vaccines Business Unit, Takeda Pharmaceuticals Inc., Cambridge, MA, United States*

Dengue is caused by four antigenically distinct serotypes of dengue virus (DENV-1-4). Takeda's live attenuated tetravalent dengue vaccine candidate (TAK-003) is composed of an attenuated DENV-2 (TDV-2) and chimeric viruses containing prM and E genes of DENV-1, -3 and -4 on the DENV-2 genomic backbone. An effective dengue vaccine should elicit immune responses to all four DENV serotypes. Binding and neutralizing antibodies to DENV-1-4 after natural infection or vaccination can be quantitated; however, these assays cannot distinguish between type-specific (TS) and cross-reactive (CR) antibodies. A Multi-Color FluoroSpot assay has been developed to enable quantitation of serotype specificity and cross-reactivity of individual memory B cells (MBCs) secreting DENV-specific antibodies. We determined the frequency of TS and CR MBCs in 15 randomly selected individuals who received a single TAK-003 vaccination in a Phase 2 trial in Singapore (DEN-205). Peripheral blood mononuclear cells collected on days 0, 30 and 180 post-single-dose TAK-003 vaccination were tested. Eight individuals were seronegative for dengue prior to vaccination, and 7 had evidence of prior immunity to at least one DENV serotype. Pre-existing MBC responses were only detected in pre-immune vaccinees at day 0. Following vaccination, both TS and CR MBCs to all four DENV serotypes were observed in all individuals, in whom the proportion of TS MBCs was higher than CR MBCs and remained stable between days 30 and 180 post-vaccination. The pattern of TS MBCs following TAK-003 vaccination was distinct from natural DENV infection; tetravalent vaccination elicited tetravalent TS MBCs, while primary natural DENV infection elicits monovalent MBCs, and the ratio of TS to CR MBCs was higher following vaccination. These results demonstrate that all four antigen components of TAK-003 contribute to the DENV-specific MBC response following vaccination.

1481

EFFECTS OF DENGUE PRE-IMMUNITY ON ANTIBODY EFFECTOR PROPERTIES FOLLOWING INFECTION WITH A SUBSEQUENT HETEROTYPIC DENGUE INFECTION

Ruklanthi de Alwis[1], Tom Agnero-Rigot[1], Koh Min Jie[1], Leong Yan Shan[1], Eng Eong Ooi[1], Tun Linn Thein[2], Katja Fink[3], Leo Yee Sin[2], Raphael Zellweger[1]

[1]*Duke-NUS Medical School, Singapore, Singapore,* [2]*National Center for Infectious Diseases, Singapore, Singapore,* [3]*Singapore Immunology Network, A*STAR, Singapore, Singapore*

Dengue virus (DENV) belongs to the flavivirus family of arboviruses, which have a long-standing history of causing human disease. There are several live-attenuated vaccines against DENV in phase 3 clinical trials and a recently licensed one. Unfortunately, the licensed DENV vaccine showed low efficacy in DENV-naïve individuals (and even enhancement of disease). Interestingly, the vaccine efficacy was markedly improved in the presence of cross-reactive flavivirus antibodies in pre-immune individuals. Hence, a better understanding of how pre-existing immunity modulates protective mechanisms is important to improve and assess the immunogenicity of vaccines currently under development. Pre-existing cross-reactive

antibodies have the potential to shape both antigen-recognition and effector functions of vaccination-induced humoral response through Fcγ-receptor (FcγR) mediated signaling. Therefore, to characterize the role of pre-existing DENV antibodies in modulating immune responses to a subsequent heterotypic DENV infection, our group recently enrolled both primary and secondary DENV infected patients (with serotype 2) from an adult Singaporean population. Patients were followed up for one year, with blood sampling at 4, 6 and 12 months. We then conducted a series of serological assays to profile both the antigen-recognition properties (such as IgG antibody titers, neutralization, avidity) and effector properties (IgG subclassed, antibody-dependent cellular phagocytosis and antibody-dependent cellular cytosis) of the polyclonal response to the most recent DENV infection, which in this study was DENV2. We observed that the presence of cross-reactive antibodies significantly affected both antigen-recognition properties (such as antigen binding, neutralization and affinity) as well as effector properties. Our current work provides additional insight into protective mechanisms following flaviviral vaccination, particularly in the presence of pre-existing immunity, and may improve current flaviviral vaccine regimens.

1482

EVOLUTION AND EPIDEMIOLOGIC DYNAMICS OF DENGUE VIRUS SEROTYPES IN NICARAGUA DURING THE EMERGENCE OF CHIKUNGUNYA AND ZIKA VIRUSES

Sean V. Edgerton[1], Chunling Wang[2], Panpim Thongsripong[1], Saira I. Saborio[3], Magelda Montoya[2], Josefina Coloma[2], Angel Balmaseda[3], Eva Harris[2], Shannon N. Bennett[1]

[1]*California Academy of Sciences, San Francisco, CA, United States,* [2]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [3]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua*

Arthropod-borne viruses (arboviruses) comprise a significant and ongoing threat to human health, infecting hundreds of millions annually. Three such arboviruses include the circumtropical dengue, Zika, and chikungunya viruses. All three viruses continue to emerge geographically along with their same urban domestic *Aedes* mosquito vector. Nicaragua has experienced endemic dengue virus (DENV) transmission involving multiple serotypes since the mid 1990s; chikungunya virus (CHIKV) was first reported in 2014, followed by Zika virus (ZIKV) first reported in 2016. In order to identify patterns of genetic variation and selection pressures shaping the evolution of co-circulating DENV serotypes preceding and in light of the arrival of CHIKV and ZIKV, the latter of which is a close relative and known to interact with DENV at a population level via the host immune response, we employed whole-genome sequencing on an Illumina MiSeq platform of random-amplified total RNA libraries to characterize 45 DENV isolates collected from viremic patients in Nicaragua between 2013 and 2016. Our approach also revealed clinically undetected co-infections with both CHIKV (8) and ZIKV (1). Of the three DENV serotypes (1, 2, and 3) co-circulating during our study, we uncovered distinct patterns of evolution using comparative phylogenetic inference. DENV-1 genetic variation was structured into two distinct co-circulating lineages with no evidence of positive selection in the origins of either lineage, suggesting that they are equally fit. In contrast, evolutionary history of DENV-2 was marked by positive selection, and a unique, divergent lineage correlated with high epidemic potential that emerged in 2015 and drove an outbreak in 2016. DENV-3 genetic variation remained unstructured into lineages throughout the period of study. This study reveals insights into evolutionary and epidemiologic trends exhibited prior to and during the circulation of multiple arboviruses in Nicaragua.

1483

SUSTAINABLE, HEALTHY CITIES: PROTOCOL OF A MIXED METHODS CLUSTER RANDOMIZED CONTROLLED TRIAL FOR *AEDES* CONTROL IN BRAZIL

Kate Zinszer[1], Andrea Caprara[2], Antonio Lima[3], Monica Zahreddine[1], Kellyanne Abreu[2], Mabel Carabali[4], Beatriz Parra[5], Beatriz Parra[5], Neil Andersson[4], Valery Ridde[6]

[1]*University of Montreal, Montreal, QC, Canada,* [2]*Universidade Estadual do Ceará, Fortaleza, Brazil,* [3]*Fortaleza Public Health Department, Fortaleza, Brazil,* [4]*McGill University, Montreal, QC, Canada,* [5]*Universidad del Valle, Cali, Colombia,* [6]*Research Institute for Sustainable Development, Paris, France*

Dengue is increasing in its global presence with an estimated 4 billion people at-risk of infection in at least 128 countries. The only preventive measure of dengue infection is through mosquito vector control and there is increasing resistance to insecticides and larvicides and growing evidence of their negative environmental and health impact. In a context of sustainable healthy cities promoted the sustainable development goals, environmentally friendly approaches to *Aedes* control are needed to attain permanent reductions in mosquito populations. Despite the promising results of EcoHealth and community mobilization approaches for *Aedes* reduction, more evidence of their efficacy on reducing dengue risk is needed. The principal research question is to determine if interventions based upon community mobilization reduce the risk of dengue virus infection among children 3 to 9 years old compared to usual dengue control practice in Fortaleza, Brazil. The present study is a pragmatic cluster RCT design with randomization at the census tract level with equal allocation to the two arms. There will be 34 clusters in each arm of 80 children between 3 to 9 year olds for an expected total of 5,440 children enrolled in the study. Household visits will occur every six months for a total of six visits over a 3 year period to cover both dry and rainy seasons. Following allocation, community mobilization activities will begin. There will be qualitative studies included within the cRCT to evaluate the process, acceptability, fidelity, and sustainability of the intervention. Baseline results will be available by November 2019. The results of our study will provide evidence on community mobilization as an intervention for dengue control. Our study contains several innovative aspects including embedded qualitative research and a biomarker of individual exposure to *Aedes* saliva. We anticipate that if community mobilization is effective in Fortaleza, through broad and active dissemination, the results of this study will help develop evidence-based vector control programs in Brazil, and also in other countries struggling with *Aedes*-transmitted diseases.

1484

ANALYSIS OF THE POST-VACCINATION ANTIBODY RESPONSE OF DENGUE SEROTYPE 2 BREAKTHROUGH INFECTIONS

Usha Nivarthi, Emily Gallichotte, Matt Delacruz, Mathew Boneparte, Ralph Baric, Aravinda de Silva

University of North Carolina, Chapel Hill, NC, United States

The four serotypes of dengue virus (DENV1-4) are estimated to cause 390 million infections annually, mostly in the tropical and subtropical regions of the world. Sanofi Pasteur developed a live attenuated tetravalent dengue vaccine, (Dengvaxia) that was tested in two large phase III trials (CYD14/15) in Asia and Latin America before it's licensure in 2015. Overall vaccine efficacy was 22% and 78% in children who were dengue seronegative and seropositive at baseline, respectively. Moreover, vaccine efficacy varied by DENV serotype and the lowest efficacy was observed against DENV2. Using methods to deplete specific populations of DENV binding antibodies as well as recombinant chimeric DENVs displaying epitopes of interest, we characterized the level and quality of vaccine-elicited serum antibodies in naïve and pre-immune individuals who subsequently experienced DENV2 breakthrough infections. Dengvaxia failed to induce DENV2 serotype-specific (TS) neutralizing antibodies in the majority of subjects who experienced breakthrough infections. The absence of DENV2 TS neutralizing antibodies is indicative of limited if

any replication of the DENV2 vaccine component in these individuals. As antibodies that develop after primary WT DENV2 infection are strongly correlated with long-term protection against DENV2, we compared vaccine induced antibodies in DENV2 breakthrough cases to antibodies in people who had recovered from primary DENV2 infections. Unlike in the case of vaccine breakthrough cases, all individuals exposed to primary WT DENV2 infections had high levels of DENV2 TS binding and neutralizing antibodies. We discuss the implications of our results for understanding vaccine efficacy and for identifying immune correlates to guide vaccine development.

1485

A COMBINATION OF INCIDENCE DATA AND MOBILITY PROXIES FROM SOCIAL MEDIA PREDICTS THE INTRA-URBAN SPREAD OF DENGUE IN YOGYAKARTA, INDONESIA

Aditya L. Ramadona[1], Yesim Tozan[2], Lutfan Lazuardi[3], Joacim Rocklöv[1]

[1]*Department of Public Health and Clinical Medicine, Section of Sustainable Health, Umeå University, Umeå, Sweden,* [2]*College of Global Public Health, New York University, New York, NY, United States,* [3]*Department of Health Policy and Management, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia*

Few studies have investigated the potential of using geotagged social media data for predicting the patterns of spatio-temporal spread of vector-borne diseases. We herein demonstrated the role of human mobility in the intra-urban spread of dengue by weighting local incidence data with geo-tagged Twitter data as a proxy for human mobility across 45 neighborhoods in Yogyakarta city, Indonesia. To estimate the dengue virus importation pressure in each study neighborhood monthly, we developed an algorithm to estimate a dynamic mobility-weighted incidence index (MI), which quantifies the level of exposure to virus importation in any given neighborhood. Using a Bayesian spatio-temporal regression model, we estimated the coefficients and predictiveness of the MI index for lags up to 6 months. We used a Poisson regression model with an unstructured spatial covariance matrix and compared the predictability of the MI index to that of the dengue incidence rate over the preceding months in the same neighborhood (autocorrelation) and that of the mobility information alone. We based our estimates on a volume of 1·302·405 geotagged tweets (from 118·114 unique users) and monthly dengue incidence data for all study neighborhoods in Yogyakarta city from August 2016 to June 2018. The MI index, as a standalone variable, had the highest explanatory power for predicting dengue risk in the study neighborhoods, with the greatest predictive ability at a 3-months lead time. The MI index was a better predictor of dengue risk in a neighborhood than the recent transmission patterns in the same neighborhood or the mobility patterns between neighborhoods. Our results suggest that human mobility is an important driver of dengue spread within cities when combined with information on local circulation of the dengue virus. The geotagged Twitter data can provide important information on human mobility patterns to improve our understanding of the direction and risk of spread of diseases, such as dengue. The proposed MI index together with traditional data sources can provide useful information for the development of more accurate and efficient early warning and response systems.

1486

IDENTIFYING DENGUE ILLNESS PHENOTYPES USING LATENT TRAJECTORY ANALYSIS

Robert C. Reiner[1], William Elson[2], Gonzalo Vazquez-Prokopec[3], John Elder[4], Valerie Paz-Soldan[5], Alan Rothman[6], Amy Morrison[7], Thomas Scott[7]

[1]*University of Washington, Seattle, WA, United States,* [2]*University of California Davis, Lima, Peru,* [3]*Emory, Atlanta, GA, United States,* [4]*San Diego State University, San Diego, CA, United States,* [5]*Tulane, New Orleans, LA, United States,* [6]*University of Rhode Island, Kingston, RI, United States,* [7]*University of California Davis, Davis, CA, United States*

Dengue is an acute febrile illness lasting 4-7 days typically accompanied by headache and musculoskeletal pain, which is caused by any of the four serotypes of dengue virus (DENV). It has been increasingly recognized that individuals infected by DENV display a much greater variation in clinical manifestations and evolution of symptoms over time than is captured by classical descriptions. However, efforts to classify this spectrum of illness have been limited by a lack of individual-level data over the course of illness. We leveraged a detailed dataset from Iquitos, Peru of 423 surveys of the intensity of 12 dengue-related symptoms on 79 subjects with PCR-confirmed DENV infections to identify distinct clinical phenotypes of dengue-related illness. Using a multidimensional, spline-based finite mixture model that accounted for a varying number of repeated observations within each individual, we identified 4 latent classes of dengue illness, each corresponding to a unique temporal trajectory across symptoms. In particular, one phenotype clustered all individuals who experienced a resurgence across numerous symptoms in the second week of illness. Beyond also identifying which symptoms are most predictive of overall illness phenotype, our modeling approach also identified how much data is needed before an individual's illness can be classified as one phenotype or another. A well described phenomenon associated with some moderate to severe dengue is a clinical deterioration at around 7 days despite an earlier ebb in symptoms; improving our ability to predict which individuals are most likely to develop severe dengue would afford health care personnel additional time to more adequately treat these patients. Moreover, though the detailed data we used in this analysis is relatively rare due to the resource-intense nature of its collection, our modeling approach should be applicable more broadly to instances where individual-level data on a number of symptoms is collected repeatedly across the duration of an illness.

1487

DENGUE AS A CAUSE OF NON-MALARIAL FEBRILE ILLNESS IN SOUTHWEST UGANDA

Ross Mathew Boyce[1], Matthew C. Collins[2], Rabbison Muhindo[3], Regina Nakakande[3], Emily Ciccone[1], Samantha Grounds[1], Matte Michael[3], Moses Ntaro[3], Dan Nyehange[4], Edgar Mulogo[3], Jonathan J. Juliano[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Emory University, Atlanta, GA, United States,* [3]*Mbarara University of Science and Technology, Mbarara, Uganda,* [4]*Epicentre, Mbarara, Uganda*

Despite being endemic in over 100 countries, there is little consensus regarding the incidence and geographic distribution of dengue in sub-Saharan Africa. However, the indirect evidence for the presence of endemic dengue transmission in many countries, including Uganda, is strong. Given the lack of reliable diagnostic tools and non-specific nature of symptoms, dengue fever may be misdiagnosed as malaria, contributing to unnecessary antimalarial administration and overestimation of the malaria burden. Therefore, we conducted a prospective cohort study to determine if dengue was unrecognized cause of febrile illness among pediatric outpatients (age <18 years) presenting to three health facilities located in areas of differing urbanicity and malaria transmission intensity in southwestern Uganda. After consent was obtained, eligible children were screened for malaria with a HRP-2 based rapid diagnostic test (RDT). Individuals with a positive result were excluded from further consideration, while those with a negative malaria test underwent additional testing with a dengue RDT (Dengue Duo, SD Bioline). Blood spots were collected for serological testing and participants at the referral hospital in Mbarara had serum collected for RT-PCR (Real Star, Altona Diagnostics). In total, we screened 1,705 children (median age 5 years, IQR 2-10). Overall, 371 (21.8%) had a positive malaria RDT result, which varied from 33.9% of children at the rural site in Bugoye to 3.7% of children at the urban site in Mbarara. Only 7 (0.04%) children had a positive dengue RDT, including 3 with a positive IgM band, 3 with a positive IgG band, and 1 with both

IgM and IgG bands. Serological testing identified 21 (3.3%) children with a reactive IgG antibodies. Notably, 15 of the 21 positives occurred at the urban site in Mbarara, where the prevalence was 7.7% (95% CI 4.7 - 12.2) of participants. All samples (n=196) tested negative for dengue virus by RT-PCR. These results suggest that dengue is not a common cause of febrile illness in southwestern Uganda. However, the relatively high seroprevalence at the urban site suggests that sustained, low-level transmission is occurring.

1488

DENGUE VIRUS DEFECTIVE INTERFERING PARTICLES IN MOSQUITOES

Leon E. Hugo[1], Melissa C. Graham[1], Malik Hussain[2], Kym Lowry[2], Gregor J. Devine[1], John G. Aaskov[2]

[1]*QIMR Berghofer Medical Research Institute, Herston, Australia,* [2]*Institute of Health and Biomedical Innovation, Queensland University of Technology, Herston, Australia*

Defective Interfering Particles (DIPs) are naturally occurring particles of RNA and DNA viruses that are missing large (90-99%) and essential portions of the parent virus genome. DIPs are non-pathogenic and can only be replicated in the presence of full-genome virus but can inhibit parent virus replication at high abundances. DIPs are therefore being developed as novel antivirals against RNA viruses, including Influenza. DIP genomes also naturally co-evolve with the parent virus, therefore DIP-based therapies would not be prone to the development of resistance. Dengue virus DIPs have been isolated from patient sera but not previously from mosquitoes and the role that mosquitoes would play in DIP therapies against dengue or other vector-borne diseases is unknown. Here, we present entomological investigations into the involvement of mosquitoes in a potential DIP therapy against dengue as part of the DARPA INTERfering and Co-Evolving Prevention and Therapy (INTERCEPT) program. We developed a DIP detection system in which mosquitoes are orally challenged with dengue virus via glass membrane feeders, incubated for 14 days and DIPs detected in bodies, legs and wings and saliva by RT-PCR, cloning and sequencing. We show that DIPs do infect mosquitoes and accumulate within mosquito tissues to form large populations with a high diversity of RNA sequence forms (30-58 variants identified per serotype for DENV-2 and DENV-3). We identified variants in mosquitoes that have been previously isolated from patient sera. Importantly, we also isolated DIPs from mosquito saliva, demonstrating that DIPs can be transmitted to new hosts. We present the results from systematic experiments testing the antiviral efficacy of specific DIPs by administering the DIP or DIP RNA to virus-challenged mosquitoes. Mosquitoes are likely to be active participants in DIP based therapeutic strategies against DENV and may even play a role in community dissemination of DIP-based antivirals.

1489

SEROLOGICAL SCREENING FOR INAPPARENT FLAVIVIRUS INFECTION IN U.S. TRAVELERS

Mariam Goreish, Lillian Chen, Daniel Espinoza, Yerun Zhu, Matthew H. Collins

Emory University, Decatur, GA, United States

Flaviviruses are a group of mosquito-borne viruses that can cause symptoms ranging from mild to severe. Dengue (DENV) and Zika (ZIKV) viruses are two medically important flaviviruses, and recent epidemics have demonstrated how viruses can be introduced into new populations. These introductory events are facilitated by marked rises in international travel in recent times. Additionally, travelers from the US and Europe are increasingly traveling to destinations in the tropics where many flaviviruses are endemic. Travelers constitute an important subpopulation for surveillance of emerging and global infections. Data on travel-related infections enable clinicians to properly advise travelers. Additionally, this population can serve as a sentinel for potential spread of infectious diseases into new areas. Finally, robust surveillance of travel-related infection can supplement public health activities in travel destination sites, where surveillance systems may not be well developed. For flaviviruses such as DENV, the majority of infections are inapparent. Thus, surveillance that focuses on symptomatic presentations will at best capture a minority of travel-related infections. We conducted a pilot study in a busy travel medicine clinic to recruit international travels and serologically assess for interval flavivirus infection by testing paired pre and post travel specimens. Fifty patients were enrolled. No subjects reported an acute febrile illness consistent with flavivirus infection during or within 1 month of travel. Eleven (22%) had detectable DENV IgG by antigen capture ELISA prior to travel. Most (87.5%) of DENV positive specimens were also ZIKV IgG positive. Of the flavivirus IgG positive subjects, 44% reported some history of vaccination. Two subjects exhibited seroconversion to DENV, one of which did not receive yellow fever vaccine and tested negative for ZIKV IgG. Our pilot suggests that the incidence DENV infection could be captured by serologic surveillance. A larger sample size and more specific assays could clarify the true epidemiology of inapparent flavivirus infection in travelers.

1490

DENGUE ENDEMICITY AND EMERGENCE OF OTHER ARBOVIRUSES IN PIEDECUESTA, COLOMBIA

Maria Isabel Estupiñan Cardenas[1], Anyela Lozano-Parra[1], Rosa Margarita Gelvez[1], Victor Mauricio Herrera[1], Jessica Vanhomwegen[2], Henrik Salje[2], Jean Claude Manuguerra[2], Derek A. Cummings[3], Maria Consuelo Miranda Montoya[1], Luis Angel Villar Centeno[1], Isabel Rodriguez-Barraquer[4]

[1]*Universidad Industrial de Santander, Bucaramanga, Colombia,* [2]*Institut Pasteur, Paris, France,* [3]*University of Florida, Gainesville, FL, United States,* [4]*University of California San Francisco, San Francisco, CA, United States*

Arthropod-borne viruses (arboviruses) are among the most rapidly emerging infectious diseases globally. However, quantifying their burden remains challenging due to the large proportion of asymptomatic infections. Here, we present preliminary findings from our population based studies conducted in Piedecuesta, Colombia between 2014 and 2017 around the time of chikungunya (CHIKV) and Zika (ZIKV) emergence. These studies included a baseline household-based cross-sectional serosurvey conducted among 1200 individuals in 2014, as well as a longitudinal cohort that enrolled 2400 participants between 2015-2017. The cohort study involved active fever surveillance (via a call center followed by medical care and blood sampling for febrile illnesses) as well as yearly visits in which a blood sample was collected. Serological testing was performed using a multiplex bead based immunoassay that simultaneously quantifies IgG against multiple arboviruses. This assay was validated using ~500 well characterized samples from the cohort study and shown to have good sensitivity and specificity for CHIKV, DENV and ZIKV. At baseline, 66.5% (95% CI= 64.6%-68.3%) of participants tested positive for IgG against dengue (1632/2453) and the age-specific seroprevalence was consistent with endemic circulation of this flavivirus. Throughout 4.983person-years of follow-up, there were 860 incident cases of febrile illness, including 16 cases of DENV, 18 cases of CHIKV, 87 cases of ZIKV and 19 cases of unspecified-flavivirus confirmed by PCR and/or serology. Preliminary results suggest that the attack rates of the CHIKV (2014/2015) and ZIKV (2016) outbreaks were 22% (95% CI= 18.7%-26.1%) and 34% (95%CI=30%-38%) respectively. The dengue seroconversion rate between 2016 to 2017 was 6% (95% CI=2.4%-11.9%). We estimate that 43% of chikungunya infections and 30% of Zika infections were symptomatic. Additional analyses will evaluate risk factors associated with infection and disease. In particular, we will explore whether prior exposure to DENV modified the risk and/or outcome of ZIKV infection during the 2016 outbreak, as has been suggested in other settings.

1491

INTEGRATED SURVEILLANCE QUICKLY FOLLOWED BY COMMUNITY EDUCATION SUCCESSFULLY PREVENTED LARGE-SCALE OUTBREAKS OF DENGUE IN SOUTHERN TAIWAN, 2016-2018

Chwan-Chuen King[1], TingChia Weng[2], Yi-Hua Pan[1], Tzong-Shiann Ho[3], Thomas C. Tsai[4], Marie Wu[4], Hui-Ying Ko[1], Chris Chin[1], Ping-Wei Kate Shih[1], Po-Yau Chen[1], Chih-Huan Chung[5], Chao-Ying Joe Pan[6], Liang-Yi Wang[7], Yi-Yeh Chen[8], Wu-Chun Tu[9], Chin-Gi Huang[10], Ta-Chien Chan[11], Kun-Hsien Tsai[12], Yen-Jen Oyang[13], Chia-Chi Ku[14]

[1]*College of Public Health, National Taiwan University, Taipei, Taiwan,* [2]*Dept of Occupational and Environmental Health, National Cheng-Kung University Hospital (NCKUH), Tainan, Taiwan,* [3]*Dept of Emergency Med., NCKUH and Institute of Microbiology and Immunology, College of Med., CKU, Tainan, Taiwan,* [4]*Dept of Med, NTU College of Med (NTU-CM)., Taipei, Taiwan (100), Taipei, Taiwan,* [5]*Kuo's General Hosp., Tainan, Taiwan (700), Tainan, Taiwan,* [6]*Kaohsiung City Dept of Health, Kaohsiung Taiwan (802), Kaohsiung, Taiwan,* [7]*Institute of Public Health, College of Med., CKU, Tainan, Taiwan (704), Tainan, Taiwan,* [8]*Taiwan Association for Promoting Public Health, Tainan, Taiwan (704), Tainan, Taiwan,* [9]*Department of Entomology, National Chung Hsing University, Taichung, Taiwan (402), Taichung, Taiwan,* [10]*National Mosquito-Borne Diseases Control Research Center, Miaoli, Taiwan (350), Miaoli, Taiwan,* [11]*Research Center for Humanities and Social Sciences, Academia Sinica, Taipei, Taiwan (115), Taipei, Taiwan,* [12]*Institute of Environmental Health, NTU-CPH, Taipei, Taiwan (100), Taipei, Taiwan,* [13]*Institute of Biomedical Electronics and Bioinformatics, College of Electrical Engineering and Computer Science, NTU, Taipei, Taiwan,* [14]*Institute of Immunology, NTU-CM, Taipei, Taiwan*

Epidemiological findings on longer duration per epidemic wave and higher transmission intensity were strongly associated with increasing cases of dengue hemorrhagic fever (DHF) in Tainan in 1998 and in Kaohsiung in 2001-2002. Additionally, genetic and phenotypic variants of dengue virus (DENV) with higher replication, transmissibility, and selection advantages were identified in dengue clusters during later periods of the epidemics. Therefore, geographic information system (GIS) has applied from surveillance though epidemiologically spatial-temporal data analyses and policy evaluation to minimize dengue clusters. As southern Taiwan with tropical climate facilitating dengue vectors of *Aedes aegypti* and *Aedes albopictus* had the largest and most severe epidemics of dengue in Tainan and Kaohsiung in 2015. Since then, a community-based integrated surveillance informatics system was designed involving clinical syndromic surveillance, virological surveillance, serological surveillance, mosquito surveillance, environment surveillance, and all important risk factors identified from epidemiological investigations (such as lower land levels associate with flood, past outbreak areas with mosquito-breeding sites, rainfall etc.). Once the surveillance indices or risk factors were above the thresholds or with warning signs, health education and community-based mosquito source reduction campaign were immediately implemented. As Taiwan had the oldest severe and fatal dengue cases caused by serotype 2 (DENV-2), syndromic groups of dengue using age-stratified data from a local regional hospital and a medical center in Tainan showed that elderly over 65 years of age did have lower body temperature of fever. In addition, the syndromic groups of elderly with different comorbidities also varied. In conclusion, our successful experiences in starting from integrated public health informatics plus timely prevention and control of dengue can extend to many dengue-epidemic or endemic countries/areas worldwide.

1492

FLAVIVIRUS ANTIBODY SCREENING ASSAY UTILIZATION FOR DIFFERENTIATION OF FLAVIVIRUS-NAÏVE AND EXPOSED SUBJECTS

Tim Powell[1], Melissa Zahralban-Steele[1], Ginger Young[1], Lydia Young[1], Kelly Bohning[1], Hetal Patel[1], Eric Shaw[1], Tim Betit[2], Laurie Stephen[3], Hansi Dean[1]

[1]*Takeda Vaccines, Cambridge, MA, United States,* [2]*Luminex Corp, Austin, TX, United States,* [3]*Ampersand Biosciences, Saranac Lake, NY, United States*

Flavivirus (FV) vaccine development is complicated by the continued spread of multiple flaviviruses (e.g. dengue, Zika, West Nile), and the complexity of determining flavivirus exposure history in clinical trials and preclinical studies. Here we describe the development and use of a simple, high throughput, rapid, and broadly reactive antibody screening assay to differentiate between flavivirus-naïve and previously exposed individuals. The FV antibody screening assay utilizes a multiplex Luminex® platform and includes envelope and/or NS1 antigens from dengue (all 4 serotypes), Zika, Japanese Encephalitis, West Nile, Yellow fever, St. Louis Encephalitis and Usutu viruses. The antigens were coupled to beads with unique fluorescent signals and the antigen sets optimized. The bead/antigen set are multiplexed and incubated with serum. IgG binding level to each antigen is assessed by the beads' fluorescent signal. Results from over 45 samples can be obtained in less than 5 hours. The FV screening assay was assessed using both non-human primate (NHP) and human samples with regard to background noise, naïve cutoff, and reactivity to known human positive serum. Naïve cutoff was determined using randomly selected human sera from Kansas. Assay positivity demonstrated the presence of flavivirus-reactive antibodies in a Midwestern population. Analysis of over 800 human and NHP samples revealed a wide spectrum of responses, and demonstrated that the FV assay is able to differentiate between pre-exposed and naïve subjects, and NHP responses to vaccination. Human samples were used to identify antigen cross-reactivity among the different flaviviruses. The FV screening assay demonstrated the capability of differentiating flavivirus-naïve from flavivirus-exposed subjects. Many antigens were shown to be cross-reactive, potentially allowing for a rapid, single test, and identification of a broad range of flavivirus exposures during pre-screening for both preclinical NHP studies and human vaccine and natural history studies.

1493

DECLINE IN MAGNITUDE OF ZIKA VIRUS-SPECIFIC LONG TERM MEMORY T-CELLS

Hannah Greig

University of Western Australia, Nedlands, Australia

The immune response to flavivirus infection may play roles in both protection and pathogenesis. Longer duration of pre-existing DENV immunity is associated with more severe disease outcomes. Recent studies indicate that long term DENV -specific T-cell immunity is associated with decline in proliferative and cytokine responses years to decades after infection. Prior flavivirus exposure is increasingly reported to also influence flavivirus-specific immunity, however most studies have focused on immune responses 1 month post-infection (MPI), and little is known about the effect months to years after infection. Using INFγ ELISpot and ZIKVPRVABC59-based peptides we measured ZIKV-specific T-cell responses in 8 individuals with monotypic ZIKV infection or ZIKV infection in the context of other flavivirus exposure (DENV infection; YFV vaccination), sampled between 9 and 42 MPI. Sequential samples were analysed in 4 individuals. A decline in magnitude of the ZIKV-specific IFNγ response was seen between 9 and 20 MPI (3 individuals) and 30 and 37 MPI (1 individual). A reduction in breadth of responses to ZIKV peptides was noted in 2 of the 4 individuals. For all 8 ZIKV-infected individuals the magnitude of the ZIKV-specific response was not associated with any high or low magnitude HLA alleles previously reported for DENV. ZIKV-infected subjects with prior flavivirus exposure had higher magnitude responses

to ZIKV peptides compared with ZIKV monotypic subjects, however the difference was not statistically significant. In summary, the frequency of ZIKV-specific IFNy-producing cells declines over time in the absence of re-exposure. Memory phenotype and cytokine profiles are currently being determined and will inform our understanding of long-term ZIKV-specific memory T-cell responses.

1494

ESTIMATING JAPANESE ENCEPHALITIS BURDEN AND IMPACT OF VACCINATION

Tran Minh Quan[1], Nguyen Manh Duy[2], Tran Minh Nhat[2], Tran Thi Nhu Thao[3], **Hannah Clapham**[4]

[1]*Notre Dame University, South Bend, IN, United States,* [2]*Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam,* [3]*University of Bern, Bern, Switzerland,* [4]*University of Oxford, Ho Chi Minh City, Vietnam*

Japanese Encephalitis (JE) has wide spread transmission across Asia. Though most infections are asymptomatic, of patients who have clinical symptoms, a large proportion die or experience long-term disabilities. Humans are dead-end hosts for JE, with transmission from animal reservoirs such as pigs, via mosquitoes. There are a number of effective vaccines for JE, with countries such as Japan with long running vaccination programs seeing a dramatic reduction in cases; however the price has meant vaccination is of reach for many countries. The recently developed cheap and Gavi funded vaccine means vaccination is now a possibility for many more. Therefore now is the optimal time to assess the impact vaccination has had, and to quantify the disease burden across Asia to provide recommendations for future vaccination. In order to make these estimates, we undertook systematic reviews to find papers and surveillance data with case data with age information and with numbers who died or experienced disabilities. We fit a catalytic model to the age-stratified case data to estimate the transmission intensity for JE in these study locations. We then extrapolated transmission intensity to other locations using WHO area grouping, regression and machine-learning models using relevant co-variates such as climate and pig density. by applying this extrapolated transmission intensity to populations across Asia, and including information on vaccination in the model we made estimates of the number of JE infections with and without vaccination. We then used the detailed case data to update estimates of the morbidity and mortality rates, estimating that mortality rates are in fact decreasing over time. These estimates provide an update to the previously cited estimates, and an evidence base for these numbers. Combining our morbidity and mortality rates with our infection number estimates we estimated the number of deaths and DALYs due to JE across Asia with and without vaccination, and therefore impact of vaccination. With our new estimates of burden we highlight areas of high transmission that should be future targets for vaccination.

1495

PRECLINICAL EVALUATION OF ZIKA VIRUS VACCINE CANDIDATES BASED ON COVALENTLY STABLE E DIMERS

Giuditta De Lorenzo[1], Jennifer Doig[1], Rapeepat Tandavanitj[1], Monica Poggianella[2], Ricardo Sanchez-Velazquez[1], Chayanee Setthapramote[1], Hannah Scales[3], Jose Luis Slon Campos[2], Alain Kohl[1], James Brewer[3], Oscar R. Burrone[2], Arvind Patel[1]

[1]*MRC - University of Glasgow Centre for Virus Research, Glasgow, United Kingdom,* [2]*International Centre for Genetic Ingeneering and Biotechnology, Trieste, Italy,* [3]*University of Glasgow, Glasgow, United Kingdom*

The recent outbreak of Zika virus (ZIKV) has highlighted the urgency of a vaccine but the close relationship with Dengue virus (DENV) can lead to immune cross-reactivity, with detrimental effects if it involves poorly neutralizing antibodies. In this work we developed and validated two vaccines based on a mutant E sequence that creates a disulphide bridge between DII of two interacting E proteins, stabilizing their dimeric conformation and abrogating trimerization. The generation of these covalent dimers (cvD) entails a double advantage: i) it reduces the exposure of immunodominant epitopes located on DI/DII, recognized by cross-reactive poorly neutralizing antibodies, and ii) it favors instead the display of Envelope Dimer Epitopes (EDE), hopefully overcoming the risk of Antibody-Dependent Enhancement (ADE) of other Flavivirus infection. We tested the immunogenicity of cvD both in form of soluble protein (sE-cvD) and incorporated in virus-like particles (VLP-cvD), in comparison with the wild-type counterparts. In both cases, the cvD mutation promoted the production of antibodies binding to E dimer with a good neutralizing capacity against ZIKV. Both vaccines fully protected the vaccinated mice from lethal challenge with no detectable viremia in blood and peripheral organs as brain, testis and ovaries. In vitro ADE assay showed that the cvD vaccines are less likely to enhance DENV infection compared to the WT. All together, these results suggest that ZIKV E covalent dimers are a promising element for an effective ZIKV vaccine.

1496

PREGNANCY AND INFANT OUTCOMES POST-ZIKA VIRUS INFECTION IN NICARAGUA

Anna Gajewski[1], Oscar Ortega[1], Liliam Llufrio[1], Douglas Elizondo[1], Magelda Montoya[2], Damaris Collado[1], Anna Urbina[1], William Rivas[1], Guillermina Kuan[3], Angel Balmaseda[4], Eva Harris[2]

[1]*Sustainable Sciences Institute, Managua, Nicaragua,* [2]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [3]*Centro de Salud Sócrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua,* [4]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua*

The rapid spread of Zika virus (ZIKV) throughout the Americas has spurred interest in screening, diagnosis, and treatment for microcephaly, congenital hearing loss, and congenital ophthalmological issues, as well as other birth defects associated with Congenital Zika Syndrome (CZS). ZIKV was first detected in Managua, Nicaragua, in January 2016 and was followed by an explosive epidemic that peaked between July and September 2016. A cross-sectional seroprevalence study of ZIKV-related birth outcomes was conducted in 3 districts of Managua. Women who were pregnant before June 15, 2016, and who had a due date after September 15, 2016 (including miscarriage or stillbirth) and their infants were eligible for participation in the study. Mothers and infants provided a blood sample upon enrollment. These samples were tested for evidence of previous ZIKV infection using the ZIKV NS1 blockade-of-binding (BOB) assay. Infants were followed at 12 and 24 (ongoing) months of age and were screened for microcephaly, vision problems, hearing loss, and neurodevelopmental delays. A total of 615 women were enrolled, including 3 miscarriages, 2 stillbirths and 7 women whose children died between birth and the study visit. ZIKV seroprevalence is different across the 3 districts of Managua and an overall seroprevalence of ~50%, with 80% of the samples processed. Of the 478 children with a completed neurodevelopmental screening at 12 months, 50 (10.5%) were outside of the normal range in one or more areas. Follow-up assessment using the Bayley-III test confirmed delays in 15 (34%) of the 44 children who completed follow-up. Thirteen children had cerebral circumferences that were below the 3rd percentile; of those, 9 (69%) had abnormal results on the neurodevelopmental screening and abnormal follow-up results with the Bayley III. Ten children had abnormal ophthalmological findings that are associated with CZS. Our study found evidence of CZS-related disorders in a Central American population 12 months after the Zika epidemic. Analysis of the associations between these disorders and ZIKV seroprevalence in the mother is ongoing and will be reported.

1497

DENGUE VIRUS IMMUNE STATUS AND ANTIBODY TITERS AMONG ASYMPTOMATIC PREGNANT WOMEN DURING THE ZIKA OUTBREAK IN SALVADOR, BRAZIL

Kaitlin Driesse[1], Wen-Yang Tsai[1], Carlos Brites[2], Celia Pedroso[2], Wei-Kung Wang[1]

[1]*John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States,* [2]*LAPI-Laboratório de Pesquisa em Infectologia-School of Medicine, Federal University of Bahia, Salvador, Brazil*

The outbreak of Zika virus (ZIKV) and associated congenital Zika syndrome in dengue virus (DENV)-endemic regions raised questions about DENV immunity and ZIKV infection. Although DENV antibodies were found to enhance ZIKV infection *in vitro and in vivo*, two non-human primate studies reported conflicting results. Recent studies showed that preexisting high DENV antibody titers are associated with reduced risk of symptomatic ZIKV infection. Herein we investigated DENV antibody titers in non-ZIKV-infected individuals during the ZIKV outbreak in Salvador, Brazil. 120 serum samples collected from asymptomatic parturient women between Nov. 2015 and Dec. 2016 that tested negative by Euroimmun ZIKV non-structural protein 1 (NS1) IgG kit were analyzed. We employed combined DENV and ZIKV NS1 ELISAs to examine DENV and ZIKV serostatus (Tsai et al. Clin Infect Dis 2017;65:1829) and virion IgG ELISA to determine ELISA titers. Of the 120 samples, 20 were from primary DENV, 8 secondary DENV, 73 DENV (unclassified primary/secondary) infections, 1 primary ZIKV infection, 5 ZIKV with previous DENV infection and 13 naive. The DENV seroprevalence was 88.3% (106/120). A linear relationship was found between DENV and ZIKV ELISA endpoint titers. Higher endpoint titers to DENV and ZIKV were found in secondary DENV than primary DENV panels. The DENV ELISA endpoint titers of samples collected during the second period (22 Mar. 2016 - Dec. 2016) were higher than those during the first period (Nov. 2015 - 21 Mar. 2016) (P<0.0001). Our findings suggest that higher DENV antibody titers among ZIKV uninfected individuals may contribute to the decline of the ZIKV outbreak, though the underlying causes and mechanisms remain to be investigated.

1498

SURVEY FOR CELL FUSING AGENT VIRUS (*FLAVIVIRUS*) IN *AEDES AEGYPTI* MOSQUITOES FROM TEXAS, USA AND THE INFLUENCE ON ZIKA VIRUS VECTOR COMPETENCE

Estelle Martin, Selene Garcia-Luna, Jose Juarez, Megan Wise de Valdez, Ismael Badillo-Vargas, Gabriel Hamer

Texas A&M, College Station, TX, United States

The emergence and re-emergence of mosquito-borne diseases such as Zika, chikungunya and dengue fever remain a global public health challenge that threatens many communities in tropical and subtropical regions of the world. These viruses are driven principally by *Aedes aegypti* mosquitoes and naturally-occurring microbes are intrinsic factors that can influence the ability of mosquitoes to transmit viruses. Viral symbionts (i.e. insect-specific viruses) are examples of microbes that are not only capable of influencing vector competence for other viruses but also offer opportunities for innovative vector control strategies such as the way in which *Wolbachia* plays a role in creating populations refractory to the transmission of certain arboviruses. We had previously identified the presence of cell fusing agent virus (CFAV) in *Ae. aegypti* population in Texas. Our objective was to better understand the prevalence of CFAV in natural *Ae. aegypti* population and to investigate its impact on Zika virus transmission in the Lower Rio Grande Valley, a region in South Texas where local transmission of Zika virus occurred in 2016-2017. Our preliminary results showed the year around presence of cell fusing agent virus (CFAV). Although variation was observed in the minimum infection rate (MIR) between month of the study (2017-2018), no clear pattern of seasonality was identified. Detection of the virus was done in male and females mosquitoes confirming the vertical transmission of this virus. Additionally, CFAV MIR was similar between specimens sampled using Autocidal Gravid Ovitraps (AGO) and BG Sentinel 2 traps which documents the utility of AGOs to function as not only surveillance of vector populations but also for arboviruses. Additionally, in an effort to understand the interaction of CFAV with Zika virus, we conducted sequential infection in cell culture. No impact was observed in the *in vitro* experiment using C6/36 cells. We are currently conducting *in vivo* experiment using naturally CFAV infected *Ae. aegypti* mosquitoes to confirm these results.

1499

ZIKA VIRUS SEROPREVALENCE ESTIMATES IN A U.S. MILITARY POPULATION INDICATE POSSIBLE CRYPTIC ZIKV TRANSMISSION IN PUERTO RICO BY EARLY 2015

Caitlin H. Kuklis[1], Simon D. Pollett[1], David A. Barvir[1], Richard G. Jarman[1], Brett M. Forshey[2], Gregory D. Gromowski[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Armed Forces Health Surveillance Branch, Silver Spring, MD, United States*

Accurate estimates of Zika virus (ZIKV) cryptic transmission during the recent ZIKV pandemic and assessments of serological cross-reactivity with dengue virus (DENV) are needed to inform risk mitigation efforts during future outbreaks. The first Puerto Rico ZIKV case was detected in November 2015, but it is unclear how long ZIKV may have been circulating before then. We therefore performed a ZIKV seroepidemiology study of 500 active duty military members who were assigned to Puerto Rico through 2014 - 2016, and who had paired sequential sera collected within-deployment or pre-deployment and then in January - June 2015. The mean age of this sample was 28.0 years and 84.2% were male. At least 69.0% were born in a DENV endemic area and the mean time in Puerto Rico prior to the second sample was 5.1 years. We employed a high-throughput, flow cytometry-based neutralization (FlowNT) assay to screen serum samples for ZIKV and DENV neutralizing antibody (NAb). Samples that demonstrated >80% neutralization of ZIKV infectivity at a 1:40 FlowNT screening dilution were subsequently screened at a 1:10 dilution using a standard plaque reduction neutralization (PRNT) assay. The PRNT assay appeared to have better specificity than FlowNT for detecting ZIKV-specific NAb in sera from individuals that also had DENV NAb. Only 1.4% (7/500) individuals had detectable ZIKV NAb by both FlowNT and PRNT screening assays by June 2015. The 50% neutralization titers were determined for these 7 paired sera in order to identify ZIKV seroconversions. Only one individual seroconverted to ZIKV during this time period, providing serological evidence that ZIKV may have been circulating in this country for at least 4 months before the first cases were recognized and reported. Our findings emphasize the value of serosurveillance in evaluating the threat of ZIKV to at-risk populations, and prompts further studies that leverage archived sera available from military members that were stationed in or deployed to ZIKV endemic locations.

1500

PRETREATMENT WITH PUTATIVE NOVEL ADJUVANTS MODULATE T FOLLICULAR HELPER AND B CELL RESPONSES TO ZIKV-E ANTIGEN

Brien K. Haun, Albert To, Teri Wong, Lishomwa Ndhlovu, Axel Lehrer

University of Hawaii, Honolulu, HI, United States

Emerging infectious viruses such as Zika virus (ZIKV) pose serious threats to human health. Currently there are no clinically available treatments or vaccines for ZIKV. Our lab has developed a vaccine candidate using recombinant envelope protein (E) of the ZIKV. Macaques immunized with this ZIKV-E antigen produced robust humoral response and neutralizing antibodies, which were determined to be protective factors. While eliciting humoral responses may benefit a large population of people, persons with select immune deficiencies may benefit from broader immune responses. It is known that prime-boost strategies can broaden immune response. Therefore, we investigated the immunological effects of administering novel adjuvants one day prior to recombinant antigen immunization in BALB/c mice. We observed an increase in CD4 T cells among the adjuvanted pretreatment groups, changes in overall T follicular helper

cell (Tfh) populations, and overall phenotypic changes in Tfh and B cell subsets. Based on our preliminary observations, we believe that pre-treatment with our novel adjuvant formulations prior to immunization may modulate the magnitude of humoral responses to recombinant antigen vaccination. The work to investigate these phenomena in more detail is ongoing.

1501

INTERFERON LAMBDA (IFNΛ1, IFNΛ2, AND IFNΛ3) ENHANCES ZIKA VIRUS REPLICATION IN GLIAL CELLS

William G. Valiant, **Joseph John Mattapallil**

Uniformed Services University, Bethesda, MD, United States

Zika virus (ZIKV) is an arbovirus that has emerged as a major public health threat worldwide. Infection with ZIKV has been associated with congenital birth defects such as microcephaly, eye abnormalities, and neurological sequelae. Using a glial cell line derived from the human eye, we show that ZIKV replicates efficiently in these cells leading to productive infection that was accompanied by complete suppression of IFNα responses. Interestingly, increased replication was associated with a significant increase in the mRNA and protein levels of all the 3 subtypes of Type III IFN namely, IFNλ1, IFNλ2, and IFNλ3. Production of Type III IFN was accompanied by a dramatic increase in the expression levels of Interferon Stimulated Genes (ISG). Blocking of IFNλ1, IFNλ2, and IFNλ3 with neutralizing antibodies significantly suppressed ZIKV replication and ISG expression as compared to untreated cells. Pretreatment of glial cells with recombinant IFNλ1, IFNλ2, and IFNλ3 was found to protect the cells from infection suggesting that once the cells are infected, secreted IFNλ1, IFNλ2, and IFNλ3 contribute to an amplification of ZIKV replication in these glial cells. Gene expression analysis of cells harvested 6 hours after infection with ZIKV using focused nanoString arrays in combination with pathway analysis identified a number of candidate genes that likely contribute to increased ZIKV replication in the presence of IFNλ1, IFNλ2, and IFNλ3. Studies are currently underway to characterize the role of these genes. Our results, for the first time demonstrates a pathogenic role for Type III IFN in ZIKV infection and identifies a potential innate immune mechanism that is subverted by ZIKV to efficiently replicate inside glial cells. These findings have significant implications for understanding the pathogenesis of ZIKV infection.

1502

ZIKA VIRUS MEMORY B CELL RESPONSES DIFFER IN DENGUE IMMUNE AND DENGUE NAIVE INDIVIDUALS WITHIN A US-BASED TRAVELER STUDY COHORT

Alena Janda Markmann[1], Huy Tu[2], Stephen Graham[1], Matthew Collins[3], Sean Diehl[2], Aravinda de Silva[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*University of Vermont, Burlington, VT, United States,* [3]*Emory University, Atlanta, GA, United States*

Zika virus memory B cell responses differ in Dengue immune and Dengue naïve individuals within a US-based Traveler Study cohort Alena J Markmann[1], Huy Tu[2], Stephen D. Graham[3], Matthew H. Collins[4], Sean Diehl[2], Aravinda de Silva[3] [1]Department of Medicine, Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA [2]Cellular, Molecular, and Biomedical Sciences Program, University of Vermont, Burlington, Vermont, USA [3]Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA [4]Department of Medicine, Emory University, Atlanta, Georgia, USA, and Hope Clinic of the Emory Vaccine Center, Division of Infectious Diseases, Department of Medicine, School of Medicine, Emory University, Decatur, Georgia, USA Zika virus is an enveloped, positive-sense RNA arbovirus in the family *Flaviviridae.* The recent Zika virus epidemic in Latin America in 2015-2016 has been associated with significant neurologic sequelae as well as severe congenital abnormalities that were not seen in past Zika virus outbreaks. A fundamental question in the field is whether or not prior flaviviral exposure predisposed these populations to more severe disease manifestations. Prior work from multiple groups on B cell responses acutely after Zika virus infection or in early convalescence have identified differences in memory B cell (MBC) populations between flaviviral naïve and immune individuals. In order to better understand long-term adaptive immunity after Zika virus infection, we are examining the antibody and MBC responses in both primary and secondary Zika virus cases at late-convalescence. Our studies use late-convalescent samples from travelers and we have successfully developed a flow-based assay to screen MBCs using *in vitro* infected cells. Here we will show the results of MBC profiling and monoclonal antibody isolation from these travelers. This work is fundamental to understanding Zika-specific flaviviral memory B cell responses in late convalescence and whether or not prior flaviviral infections can influence this.

1503

DETERMINING THE BINDING SITES OF NEUTRALIZING ANTIBODIES ISOLATED FROM A ZIKA VIRUS INFECTED INDIVIDUAL

Stephen Graham[1], Alena Janda[2], Huy Tu[3], Sean Diehl[3], Aravinda de Silva[4]

[1]*Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC, United States,* [2]*Department of Medicine, Division of Infectious Disease, University of North Carolina School of Medicine, Chapel Hill, NC, United States,* [3]*Cellular, Molecular, and Biomedical Sciences Program, University of Vermont, Burlington, VT, United States,* [4]*Department of Microbiology and Immunology University of North Carolina School of Medicine, Chapel Hill, NC, United States*

Zika virus (ZIKV) is an emerging flavivirus that has rare but serious disease manifestations, including neurological complications and birth defects. The similarities between ZIKV and dengue virus (DENV), a closely-related flavivirus, can result in the formation of cross-reactive antibodies in infected individuals. Recent studies have shown that ZIKV disease manifestations can vary from individual to individual, depending on their DENV infection history. Through the Arbovirus Traveler Study at the University of North Carolina at Chapel Hill, two late-convalescent neutralizing monoclonal antibodies (nAbs) from individual DT 172 have been isolated through memory B-cell isolation and sorting in collaboration with Sean Diehl's lab at the University of Vermont. Both a type-specific and a cross-reactive monoclonal antibody from donor DT 172 have been isolated, and their binding sites on the ZIKV envelope protein, which is the major ZIKV antigenic determinant, have been identified. Mapping antigenic epitopes on the envelope protein of ZIKV using highly neutralizing monoclonal antibodies from late convalescence is critical to informing ZIKV vaccine and diagnostic testing development.

1504

ZIKV ANTIBODY DEPENDENT ENHANCEMENT OF INFECTION MEDIATED BY WNV AND DENV SEROPOSITIVE CORD-BLOOD SAMPLES FROM MOTHERS IN EL PASO-TEXAS

Jeanette Orbegozo[1], Pedro M. Palermo[1], Anjali Joshi[2], Himanshu Garg[2], Douglas M. Watts[1]

[1]*University of Texas at El Paso, El Paso, TX, United States,* [2]*Texas Tech University Health Sciences Center, El Paso, TX, United States*

The recent Zika virus (ZIKV) outbreak and association with increased microcephaly cases are important public health concerns. ZIKV infection during the first trimester increased the likelihood of microcephaly and fetal demise because of infection of the fetal neuronal progenitor cells. A phenomenon known as antibody dependent enhancement (ADE) of dengue virus (DENV) infection has been demonstrated in epidemiological studies in DENV endemic areas. The mechanism involves preexisting flavivirus antibodies that serve to enhance the severity of a second infection with a different flavivirus. ADE has been observed in children born to DENV seropositive mothers instead of protecting by mutual cross reactivity to new infection with other DENV serotype resulting in a severe infection. Since ZIKV is closely related to flaviviruses like West

Nile virus (WNV) and DENV, prior exposure to these viruses could induce cross reactive antibodies that may enhance a subsequent ZIKV infection. Therefore, the aim of this study was to determine if WNV and DENV antibodies in cord blood enhanced ZIKV infection *in vitro*. A sero-surveillance study for WNV and DENV antibodies was conducted among mothers at the time of delivery in El Paso, Texas. From this study, a subset of 45 cord blood seropositive samples for DENV (n= 8) and WNV (n=37) were selected based on their reactivity in specific ELISAs and neutralization assays. The samples were used to determine neutralizing and enhancing titers against ZIKV, WNV and DENV using respective reporter virus particles (RVPs). ADE assay in K562 cells demonstrated that ZIKV infection was enhanced by WNV and DENV antibody positive samples. ZIKV infection was enhanced by 1:40-1:160 dilutions of the WNV and 1:40- 1:320 dilutions of DENV antibody positive samples respectively. This suggests that low titers of preexisting WNV and DENV antibodies could enhance ZIKV infection *in vitro*. Further studies are warranted to determine if low titers of pre-existing WNV and DENV antibodies in cord blood samples could enhance ZIKV infection *in-vivo* using an appropriate ZIKV disease murine model and to determine the clinical outcome, if any, in this model

1505

EXPERIMENTAL WEST NILE VIRUS TRANSMISSION CYCLES USING WILD BIRDS AND MOSQUITOES

Alex D. Byas[1], Angela M. Bosco-Lauth[1], Claudia Rückert[1], Alexis Robison[1], Michael C. Young[1], Dalit Talmi-Frank[1], Todd A. Felix[2], Aaron Brault[3], Richard Bowen[1], Gregory D. Ebel[1]

[1]*Colorado State University, Fort Collins, CO, United States,* [2]*Wildlife Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Lakewood, CO, United States,* [3]*Division of Vector-borne Diseases, National Center for Emerging Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States*

West Nile virus (WNV) emerged in North America in 1999 and continues to be the leading cause of mosquito-borne disease in the United States. The rapid adaptation of WNV to local transmission ecologies and its continued diversification highlight the ability of RNA viruses to evolve in response to changing conditions. Different WNV hosts impose unique evolutionary pressures on virus populations, with virus undergoing genetic diversification in mosquitoes and restriction in vertebrates. In addition, species-specific impacts of birds (American robins, house sparrows and American crows) and mosquitoes (*Culex (Cx.) pipiens*, *Cx. quinquefasciatus* and *Cx. tarsalis*) have been documented. However, the impact of distinct vector-vertebrate pairs on virus transmission and evolution remain poorly understood. Accordingly, we developed laboratory transmission systems involving pairings of ecologically-relevant mosquitoes and birds to evaluate whether the effects of evolutionary pressures including avian purifying selection and mosquito bottlenecks are maintained when WNV is forced into a combined vector-vertebrate system. Specifically, we predict that mutations that arise during infection of highly competent *Cx. tarsalis* will be removed by purifying selection following transmission to robins, ultimately resulting in fitness gains. Conversely, we expect that strong population bottlenecks imposed by the moderately competent vector *Cx. pipiens*, coupled with relatively weak purifying selection in crows will result in diminished virus fitness and possible extinction. A field isolate of WNV was used to infect wild birds and three consecutive cycles of bird-to-mosquito transmission were performed. Bird serum and mosquito saliva were sequenced to assess viral populations. Future work includes cell culture competition assays to assess the effects of specific bird-mosquito pairings on viral fitness and evolution. Ultimately this work will provide knowledge on how new viral genotypes emerge and help understand the evolutionary forces that shape their populations in nature.

1506

ARTHROPOD-BORNE VIRAL ENCEPHALITIDES IN THE DOMINICAN REPUBLIC: THE VIRAL-HUMAN INTERFACE AND UNDERESTIMATION IN A REGION WITH VIRAL CIRCULATION

Leandro Tapia[1], Miguel Delgadillo[2], Wenceslao Hernandez[2], Zayda Menier[2], Ricardo Domingo[1], Robert Paulino-Ramirez[1]

[1]*Institute for Tropical Medicine & Global Health - Universidad Iberoamericana, Santo Domingo, Dominican Republic,* [2]*School of Medicine, Universidad Iberoamericana, Santo Domingo, Dominican Republic*

West Nile Virus (WNV) and Eastern Equine Encephalitis Virus (EEEV) are well-established viral pathogens found in many tropical and sub-tropical countries. Viral dynamics have evolved over time due to climatologic factors, vector-host interactions, and of particular importance the influx of birds through new migration patterns.The aim of this study was to review data exploring the distribution of reported human and zoonotic infections for arthropod-borne related viruses capable of inducing encephalitis in the Dominican Republic within the indexed scientific literature. Following the Cochrane review methodology, we searched keywords: "West Nile Virus" AND "Eastern Equine Encephalitis" AND "St. Louis Encephalitis" AND "Venezuelan Equine Encephalitis" each linked with "Dominican Republic" in the English and Spanish languages in PubMed and Google Scholar databases. A total of 17 articles were identified, among them eight (8) met the inclusion criteria, five (5) for WNV, three (3) for EEEV, zero (0) for VEEV and zero (0) for SLEV. After eliminating duplicates, only six (6) articles were included in the analysis. Articles reporting positive cases were published between 1950-1978 identifying EEEV and between 2003-2006 identifying WNV, in avian, mosquito, and equine populations. The first report describing EEEV in La Hispaniola occurred as a result of an outbreak in 1948. The first report for WNV in the Dominican Republic was published in 2002, where the presence of virus and antibodies were identified in migratory and residential birds in two locations within the national territory. Some discrepancies were observed between articles regarding horse positive cases. Subsequent studies were performed investigating vectors and avian populations yielding no evidence of human infections. Supplemental studies should examine the current circulation of encephalitides and their impact on humans in the Dominican Republic to enhance surveillance and disease control regarding future outbreaks in a country with one of the largest touristic industries in the Caribbean.

1507

EXPANDING FORECASTS OF HUMAN ARBOVIRAL DISEASE: PREDICTING WEST NILE VIRUS IN LOUISIANA

Justin K. Davis[1], Raoult Ratard[2], Mike Wimberly[1]

[1]*University of Oklahoma, Norman, OK, United States,* [2]*Louisiana Department of Health, New Orleans, LA, United States*

West Nile virus threatens public health in many parts of the U.S., but annual incidence varies widely and there is need for forecasting risk to help target public health responses. We developed and implemented the Arbovirus Monitoring and Prediction (ArboMAP) system in South Dakota, which typically has the highest annual incidence out of all US states, and successfully predicted human risk in SD in 2016-2018 using weather and mosquito infection data. In 2019 we began predicting human risk in Louisiana, another area of high transmission, with distinct meteorological and other characteristics requiring modifications to ArboMAP. In particular, we discuss the extraordinary, disruptive impacts of hurricanes, which are more than just heavy rain and wind events, and perform model selection with new covariates summarizing hydrological conditions, which are necessary to characterize risk in a state where water balance is a constant concern. We investigate the possibility of using SD and LA as sentinels for risk in the whole country, and identify states in which risk cannot yet be accurately predicted with these data. This sets out a path for expansion towards a more comprehensive national arboviral prediction system.

1508

SEROLOGICAL EVIDENCE OF WEST NILE VIRUS INFECTION IN WHITE-TAILED DEER FROM 2014 TO 2018 IN TEXAS

Pedro M. Palermo[1], John C. Morrill[2], Douglas M. Watts[1]

[1]*University of Texas at El Paso, El Paso, TX, United States,* [2]*Orion Research & Management Services Inc., Gatesville, TX, United States*

White-tailed deers (WTD) are abundant mammals widely distributed across the United States. Due to this feature, WTD are considered to be excellent sentinels for detecting arboviral activity in certain geographic areas. Evidence of West Nile virus (WNV) antibody in WTD has been reported previously in several states. However, there is a lack of reports of West Nile infection in WTD from Texas even though is among the states with the highest clinically reported cases of West Nile neuroinvasive disease. Therefore, the aim of this study was to determine the prevalence of West Nile antibody in deers from Texas. Deer sera samples (n=526) were collected during the hunting season (fall and winter) in Travis County, Texas from 2014 to 2018 and tested for IgG antibody to Flavivirus by an indirect Enzyme-linked immunosorbent assay (ELISA). ELISA antibody-positive samples were further tested for West Nile and St. Louis encephalitis virus (SLEV) antibody by an 80% plaque-reduction neutralization tests (PRNT80). Overall, 9.51% (n=50) and 0.19% (n=1) of the deer samples had serological evidence of West Nile and St. Louis encephalitis infections respectively. WNV seroprevalence increased by age ($p<0.05$), and there was no significant difference between gender. Interestingly, 3% (n=16) of the samples were positive for Flavivirus antibody but negative for SLEV and WNV antibodies, suggesting that other Flaviviruses may be circulating in WTD from Texas. Finally, these results support the circulation of WNV among WTD and highlight the potential role of WTD as sentinels for WNV in Texas.

1509

SYSTEMATIC REVIEW OF MARBURG VIRUS VACCINE CLINICAL TRIALS

Melinda J. Hamer

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Background: Recent deadly outbreaks of Marburg virus underscore the need for an effective vaccine. Marburg virus outbreaks can be equally or more disastrous than Ebola. This systematic review aimed to determine progress towards a vaccine for Marburg virus. Methods: Article search criteria were developed to query PubMed for peer-reviewed articles from 2010 through 2018 on Marburg virus vaccine clinical trials in humans and non-human primates (NHP). Abstracts were reviewed by two authors. Relevant or possibly relevant articles were reviewed in full. Discrepancies were resolved by a third author. Data abstracted included year, author, title, mechanism of action, number of subjects, effectiveness or efficacy, and demographics. Assessment for risk of bias was done using the Syrcle tool for animal studies, and the Cochrane Collaboration risk of bias tool for human studies. Results: 83 articles were identified; 29 were related to Marburg vaccines. After full text review, 13 articles were selected. 215 human subjects were in three phase 1 trials, and 109 NHP in 10 trials. Vaccine mechanisms were DNA plasmids, recombinant vesicular stomatitis virus, adenovirus vectors, virus-like particles (VLP) and siRNA. A Phase 1 human study of MARV glycoprotein (GP) with adenovirus vector vaccine had 0 vaccine-related severe adverse events and 100% with detectable IgG response. Two human phase 1 studies of DNA plasmid vaccines had 4 adverse effects requiring vaccine discontinuation out of 128 participants and 31-80% immunogenicity. In NHP, an adenovirus vector vaccine with MARV GP was 100% effective in two challenge studies with 18 subjects. A NHP VLP vaccine challenge trial was 100% effective in 13 subjects. 4 articles were rated low risk of bias; 5 were moderate, 3 were high, and one with insufficient evidence to rate. Conclusion: The MARV glycoprotein vaccine with adenovirus vector had the most favorable safety profile, 100% protection in NHP challenge, and the best immunogenicity among vaccines tested in humans in this review. Further research is needed to develop this and other vaccines to limit the spread of this highly lethal virus.

1510

SOCIAL RESISTANCE DRIVES PERSISTENT TRANSMISSION OF EBOLA IN THE EASTERN DEMOCRATIC REPUBLIC OF CONGO, 2018: A MIXED-METHODS STUDY

Jack Underschultz[1], Claude Kasereka Masumbuko[2], Michael Hawkes[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Université Catholique de Graben, Butembo, Democratic Republic of the Congo*

The second largest Ebola epidemic in history is currently raging in Eastern Democratic Republic of Congo (DRC). Stubbornly persistent Ebola transmission has been associated with social resistance, ranging from passive non-compliance to overt acts of aggression toward Ebola reponse teams. We explored community resistance using focus group discussions and assessed the prevalence of resistant views using standardized questionnaires. Despite being generally cooperative and appreciative of the foreign-led Ebola response, focus group participants provided eyewitness accounts of aggressive resistance to control efforts, consistent with recent media reports. Mistrust of Ebola response teams was fueled by perceived inadequacies of the response effort ("herd medicine"), suspicion of mercenary motives, and violation of cultural burial mores ("makeshift plastic morgue"). Survey questionnaires found that the majority of respondents had compliant attitudes with respect to Ebola control. Nonetheless, 78/630 (12%) respondents believed that Ebola was fabricated and did not exist in the area, 482/630 (72%) were dissatisfied with or mistrustful of the Ebola response, 60/630 (9%) sympathized with perpetrators of overt hostility, and 102/630 (15%) expressed non-compliant intentions in the case of Ebola illness or death in a family member, including hiding from the health authorities, touching the body, or refusing an official burial team. Denial of the biomedical discourse and dissatisfaction/mistrust of the Ebola response were statistically significantly associated with indicators of social resistance. We concluded that social resistance to Ebola control efforts was prevalent among focus group and survey participants. Mistrust, with deep political and historical roots in this area besieged by chronic violence and neglected by the outside world, may fuel social resistance. Resistant attitudes may be refractory to short-lived community engagement efforts targeting the epidemic but not the broader humanitarian crisis in Eastern DRC.

1511

TRACKING EBOLA VIRUS GENOMIC DRIFT WITH A RESEQUENCING MICROARRAY

Carolyn Fisher[1], Bryan Lanning[1], Irina Tiper[1], Moussa Kourout[1], Krishnamurthy Konduru[1], Anjan Purkayastha[2], Gerardo Kaplan[1], Robert Duncan[1]

[1]*FDA Center for Biologics Evaluation and Research, Silver Spring, MD, United States,* [2]*OpenBox Bio, LLC, Vienna, VA, United States*

Currently, Ebola Virus Disease in the African Congo is the world's second-worst outbreak of the deadly disease. Diagnosis depends on Ebola-specific PCR assays for detection, but genomic sequencing information is needed for outbreak management. The ability to monitor genomic drift, to identify the Ebola strain and discover any mutations occurring are important for efficacy of diagnostics and therapeutics and disease surveillance. We have designed and tested an Ebola Virus Resequencing Microarray Assay (EBOV-RMA) to determine the sequence of the critical genes of the Ebola virus genome in a 12-hr protocol adaptable for use in remote areas. The EBOV-RMA is a high-density GeneChip manufactured by Affymetrix. We optimized multiplex PCR conditions to amplify and hybridization to provide the sequence of four Ebola species with <400,000 copies of viral genome, well below the amount typically isolated from patients. The Ebola sequences were obtained with a >98% base call rate. An in-house developed bioinformatic pipeline uses the raw base calls to search the

GenBank database and identify the closest sequence match. Sequence obtained for a repository sample of Zaire Ebola RNA was 99.97% identical to the Illumina Next Generation Sequencing (NGS) result for the same sample. To evaluate use of the chip to study genetic drift, we developed a recombinant vesicular stomatitis virus expressing green fluorescent protein (GFP) and EBOV glycoprotein (GP) genes of the Zaire strain (rVSV-ZEBOVgp-GFP). The recombinant virus was cultured in Vero E6 cells with the presence of the monoclonal antibody, KZ52, that binds the Ebola GP and neutralizes infection. Over two or three rounds of virus selection, EBOV-RMA sequence data showed GP coding changes, including the mutation N(506)D, that permitted escape from KZ52 binding in other studies; thus demonstrating the effectiveness of the RMA to detect genomic drift. The EBOV-RMA gives targeted sequence as accurately as NGS, identifies, and types samples on a phylogenetic tree. Genetic drift studies with the Ebola pseudovirus showed effectiveness at detecting changes in the genome of a replicating virus.

1512

ESTIMATING SUBNATIONAL FIRST-DOSE MEASLES-CONTAINING VACCINE (MCV1) COVERAGE USING MODEL-BASED GEOSTATISTICS IN LOW AND MIDDLE INCOME COUNTRIES FROM 2000 TO 2018

Alyssa N. Sbarra, Jason Q. Nguyen, Sam Rolfe, Lucas Earl, Ashley Marks, Natalie C. Galles, Di Zheng, Simon I. Hay, Jonathan F. Mosser, Stephen S. Lim

Institute of Health Metrics and Evaluation, Seattle, WA, United States

Estimates that capture local patterns of vaccine coverage are critical for understanding progress in childhood survival, strategic planning to improve health delivery systems, and preparing for associated-disease risk and burden. Recognizing the importance of subnational coverage, the Global Vaccine Action Plan (GVAP) and the Sustainable Development Goals (SDGs) on immunization coverage recognize the have set coverage targets to obtain 80% district-level coverage for all routine childhood immunizations, including the first doses of the measles-containing vaccine (MCV1). Data was collected from over 325 surveys conducted between 2000 and 2018, which included over 1,700,000 children aged 12-23 months old in 107 low and middle income countries. Using a Bayesian geostatistical model, annual high geographic resolution (5x5 km) estimates of routine MCV1 coverage, calibrated to the Global Burden of Diseases, Injuries, and Risk Factors Study 2019, were used to produce district-level coverage estimates for 113 low and middle income countries. Subnational MCV1 coverage reveals within-country inequities that would be overlooked if only focusing on national-level estimates, such as in Nigeria, where districts range from 3 to 96% MCV1 coverage. MCV1 coverage is generally increasing over time, however there are areas of consistently low coverage that suggest that there many persistent drivers of coverage inequities. Current trends in coverage are inadequate for reaching 80% target goal; less than 60% of districts have a 95% probability of reaching 80% target goals in 2018. Additional doses from supplementary immunization activity programs were considered to determine how coverage might have been impacted, by assuming a series of different geographic distributions of campaign doses, and additional locations for targeted SIA activity were identified.

1513

IDENTIFICATION OF A ROTAVIRUS OUTBREAK THROUGH PHYSICIAN CALL CENTER IN A RURAL COMMUNITY OF BANGLADESH

Afruna Rahman[1], Kyu Han Lee[2], Sanwarul Bari[1], Farzana Islam[1], Mustafizur Rahman[1], Muntasir Alam[1], Sabbir Ahmed[1], Shams El Arifeen[1], Emily Gurley[2]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States*

Delayed recognition of danger signs and seeking care for illness contribute to maternal and child mortality in Bangladesh. Mobile phones are widely available in Bangladesh; over 93% of households own cell phones in the study area. The objective of this study was to monitor trends in illness notification and investigate any unusual findings. Since September 2017, a private company has been providing toll-free access to physicians for all residents of Baliakandi, a rural sub-district with a population of 220,461. Physicians refer patients with danger signs to six designated facilities following referral guideline. From September 2017 to March 2019, total 6642 calls have been made; 2461 (37%) for illnesses among children under-5; diarrhoea, fever and cough being the most common causes. During November 2018 - January 2019, we noticed a peak in number of calls related to diarrhoea among under-5 children in the bi-weekly reporting graph. We received 128 calls in these 3 months, a number clearly greater compared to previous 5 months data when 77 calls were made between June-October 2018. In response, we visited the Baliakandi sub-district hospital to test stool samples for rotavirus among under-5 children coming to the facility with diarrhoea. From 16 January - 9 February 2019, we collected samples from 22 children (7 - 30 months) to be tested using commercial ELISA kit. Out of 22, 18 samples had detectable group A rotavirus antigen. We notified Baliakandi sub-district hospital and Institute of Epidemiology, Disease Control and Research (IEDCR) about the outbreak on 24 January 2019. IEDCR promptly contacted the hospital authority but as the situation improved, intervention was not required. We have developed a guideline to minimize the response time in the future. For a known disease, the hospital outbreak management team will start initial management; IEDCR team will provide support if required. For any unknown disease, IEDCR will send a team immediately upon notification. Our finding suggests that routine monitoring of illness notification through the call centre has potential to identify health crisis in a rural context.

1514

COMMUNITY BASED RESPIRATORY SYNCYTIAL VIRUS (RSV) MORTALITY STUDY IN KARACHI PAKISTAN: FORMATIVE PHASE

Fauzia A. Malik[1], Lauren B. Guterman[1], Saima Jamal[2], Asad Ali[2], Saad B. Omer[1], Abdul Momin Kazi[2]

[1]*Emory University, Atlanta, GA, United States,* [2]*Aga Khan University, Karachi, Pakistan*

Respiratory syncytial virus (RSV) is a respiratory pathogen with potentially high disease burden in developing countries. Global estimates suggest that RSV may contribute to morbidity and mortality in infants. In developing countries post-mortems on young infants are not often performed to identify the cause of infant death due to religious and cultural barriers, while taking a nasopharyngeal (NP) swab from a deceased infant would present challenges in any setting. We have less direct data on the incidence and severity of RSV and the associated early deaths in specific populations. The objectives of this study were to understand views of the community for taking nasopharyngeal swab (NP) sample of a deceased infant and understand how timely notification of infant deaths can be achieved. This is a qualitative exploratory study conducted from November 2017 to March 2018. Purposive sampling was used to conduct sixty in-depth interviews with the caregivers, elders, religious & local leaders, and funeral workers. Five focus group discussions were conducted with healthcare providers providing services within study catchment area. From the formative phase, we explored the acceptance of study procedures within the communities and assessed the benefits to families of deceased infants for participating in the study. Data from the in-depth interviews and focus group discussions with our key informants show that taking a nasopharyngeal swab at the point in the ritual bath when the nose is cleaned would be religiously and contextually acceptable. We obtained a Fatwa document from different mosques, stating that the procedures of our study are in accordance with Islam and other religious affiliations. Based on formative phase findings RSV mortality-based surveillance will give us the estimates of the burden of severe RSV infection and associated deaths in young infants. Surveillance phase activities will include collecting nasopharyngeal swabs from recently deceased infants and we will conduct ongoing community engagement.

1515

COMPARATIVE PATHOGENESIS OF BANGLADESH AND MALAYSIAN ISOLATES OF NIPAH VIRUS IN THE AFRICAN GREEN MONKEY

Mike Holbrook[1], Yu Cong[1], Dima Hammoud[2], Ji Hyun Lee[1], Elena Postnikova[1], Jonathan Kurtz[1], Louis Huzella[1], Vincent Munster[3]

[1]*NIAID Integrated Research Facility, Frederick, MD, United States,* [2]*Center for Infectious Disease Imaging, Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, MD, United States,* [3]*Virus Ecology Unit, Laboratory of Virology, Rocky Mountain Laboratories, Hamilton, MT, United States*

The development of animal models that accurately recapitulate human disease is an ongoing challenge in infectious disease research. In this project we focused on developing a nonhuman primate model for Nipah virus (NiV) infection by modifying the exposure dose and route to more accurately mimic potential human exposure. In the studies discussed here we used a relatively low dose (~500 pfu) and large particle (~12 um) aerosol exposure to replicate fomite or droplet exposure. Using this approach, we found that animals infected with the Malaysian isolate of NiV (NiV-M) developed distinct neurological lesions, with both restricted and facilitated diffusion, suggestive of microinfarctions and encephalitis, respectively. Animals that succumbed following infection with NiV-M survived until 16-22 days post-exposure. Animals exposed to the Bangladeshi isolate of NiV (NiV-B) that succumbed survived until 8-12 days post-infection when they developed a rapidly progressing hemorrhagic pulmonary disease with no evidence of neurological disease. Analysis of clinical data found few clear differences between animals exposed to either NiV-M or NiV-B. In general, animals that succumbed had increased glucose, slightly increased creatinine and decreased platelet levels. Animals that succumbed to NiV-B infection had decreased albumin levels possibly associated with the hemorrhage seen in the lungs of these animals. Of the two animals that survived NiV-M exposure, one seroconverted and had a NiV-specific IgM and IgG response and neutralizing antibody titer. The second survivor did not seroconvert. Two animals that succumbed to NiV-M exposure had IgM and IgG responses with one also demonstrating neutralizing antibody titers. Serological testing of animals exposed to NiV-B is pending. Through the use of a low exposure dose and large particle aerosol, an animal model that closely recapitulates human disease has been developed. Further work with this model and on-going analysis of immune response data could be useful for the development of immune modulators and antivirals for the treatment of NiV infection.

1516

GLOBAL EL NIÑO-SOUTHERN OSCILLATION TELECONNECTIONS AND PATTERNS OF DISEASE OUTBREAKS

Assaf Anyamba[1], Radina P. Soebiyanto[1], Jennifer L. Small[1], Sarah Hutchinson[1], Richard Damoah[1], Brett M. Forshey[2], Christine Toolin[2], Seth C. Britch[3], Compton J. Tucker[1], William Karesh[4], Wassila Thiaw[5], Jean-Paul Chretien[6], Jose L. Sanchez[2], Kenneth J. Linthicum[3]

[1]*NASA Goddard Space Flight Center, Greenbelt, MD, United States,* [2]*Department of Defense, Armed Forces Health Surveillance Branch, Silver Spring, MD, United States,* [3]*US Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, United States,* [4]*EcoHealth Alliance, New York, NY, United States,* [5]*National Oceanic and Atmospheric Administration, National Centers for Environmental Predictions, Climate Prediction Center, International Desks, College Park, MD, United States,* [6]*National Center for Medical Intelligence, Fort Detrick, MD, United States*

Climate variability associated with the El Niño-Southern Oscillation (ENSO) has marked effects on environmental conditions, favoring several infectious diseases outbreaks including Rift Valley fever, dengue, and cholera. Although studies have linked ENSO events with a particular disease transmission, the impacts of ENSO on climate conditions and disease outbreaks at global scale remains elusive. We analyzed patterns of some disease outbreaks during the strong 2015/2016 El Niño event in relation to climate anomalies derived from satellite measurements. Our results, published in February 2019, indicated disease outbreaks in El Niño-connected regions (southeast Asia, Tanzania, western US and Brazil) followed shifts in rainfall, temperature and vegetation, where both drought and flooding occurred in excess (14-81% rainfall departures from normal). Our results showed 2.5-28% increase in disease intensity during years with El Niño events than those without. Plague in Colorado and New Mexico, and cholera in Tanzania were significantly associated with above normal rainfall ($p<0.05$); while dengue in Brazil and southeast Asia were significantly associated with above normal land surface temperature ($p<0.05$). During this strong 2015/2016 El Niño event and under the umbrella of US interagency collaborative efforts, we issued alerts when elevated conditions for certain climate extremes were projected in regions with possible outbreaks of climate-sensitive diseases. The alerts led to Rift Valley fever vaccination in livestock in Kenya, averting a potentially damaging outbreaks and economic impacts. We continue to monitor reports of several climate-sensitive diseases worldwide from ProMED. After the 2015/2016 El Niño event, we observed a decreasing trend in intensity of several disease outbreaks. This work not only highlights the global impacts of ENSO in triggering or amplifying disease outbreaks but also demonstrates the prolonged impact of such climate perturbations. Given the changing and variable climate, climate observations and forecasts are going to be a critical tool in disease outbreak preparedness, prevention and control.

1517

SAFETY AND IMMUNOGENICITY OF A COMPRESSED SCHEDULE 2-DOSE HETEROLOGOUS EBOLA VACCINE REGIMEN IN HIV INFECTED AND UNINFECTED ADULTS

Julie A. Ake[1], Kristopher Paolino[2], Kristin Mills[2], Jack Hutter[2], Susan Biggs Cicatelli[3], Leigh Anne Eller[1], Michael Eller[1], Chi L. Tran[1], Lalaine Anova[1], Linda Jagodzinski[1], Lucy Ward[4], Nicole Kilgore[4], Janice Rusnak[4], Callie Bounds[4], Christopher Badorrek[4], Ine Ilsbroux[5], Dickson Anumendem Nkafu[6], Auguste Gaddah[6], Georgi Shukarev[5], Viki Bockstal[5], Kerstin Luhn[5], Macaya Douoguih[5], Cynthia Robinson[5]

[1]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Clinical Trials Center, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [3]*ICON Government and Public Health Solutions, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [4]*Medical Countermeasure Systems (MCS), Joint Vaccine Acquisition Program (JVAP), Fort Detrick, MD, United States,* [5]*Janssen Vaccines and Prevention, Leiden, Netherlands,* [6]*Janssen Research and Development, Beerse, Belgium*

Earlier studies of different regimens with Ad26.ZEBOV (Ad26) and MVA-BN-Filo (MVA) vaccines showed the strongest immune responses are induced with Ad26 as dose 1 followed by MVA 2 months later. Shorter schedules for heterologous vectored vaccines are desirable for accelerated induction of protection against Ebola virus disease (EVD). This pilot study (RV456/EBL2003) evaluated safety and immunogenicity of a 2-dose regimen of $1x10^8$ Inf U MVA-BN-Filo dose 1 followed by $5x10^{10}$ vp Ad26.ZEBOV administered intramuscularly 14 days apart in HIV infected & non-HIV infected adults in the US. Safety and binding antibody (ab) concentrations to the Zaire glycoprotein were co-primary objectives. Fifty non-HIVinfected and 25 antiretroviral therapy (ART) treated HIV infected adults received vaccine or placebo (4:1 ratio). Solicited adverse events (AEs) were collected for 7 days post vaccination. Unsolicited AEs were analyzed for 14 days post-dose 1 and 28 days post-dose 2 and serious AEs until the study's end. CD4 T cell counts and HIV viral loads were monitored for HIV infected adults. Binding ab responses were assessed via EBOV GP FANG ELISA at baseline, pre-dose 2, 21 days, 42 days, 6 months and 1 year post dose 2. Study participants were 63% male, with median age 47 years (range: 19-70). The regimen was well-tolerated in both the non-HIV infected and ART treated HIV infected groups. Local and systemic solicited AEs were generally mild or moderate with no vaccine related serious AEs. In HIV infected adults, vaccination had no effect on CD4 T cell counts and

no durable impact on HIV viral load suppression. 21 days post dose 2, 100% uninfected and 95% HIV infected adults responded to vaccination. In non-HIV infected adults, geometric mean ab concentration (GMC) of EBOV specific binding abs was 6286 EU/mL (n=36) and 2005 EU/mL (n=19) for HIV infected adults at day 21 post dose 2. The vaccine-induced immune response in non-HIV infected adults was similar to other studies in HIV infected adults. While ab response tended to be lower in HIV infected than in non-HIV infected adults, this accelerated vaccination regimen is well-tolerated and immunogenic in both populations.

1518

ENSURING COMPLETE INACTIVATION OF ARBOVIRUSES BY HEAT WITH STRINGENT SAFETY TESTING

Michael Parker, Jessica Shifflett, Sujatha Rashid

ATCC, Manassas, VA, United States

Inactivated viruses are useful for assay development at reduced biosafety levels and can serve as process controls in research and detection assays. Heat inactivation may be preferential to other inactivation methods due to cost-effectiveness and avoidance of chemical manipulation. However, standardized parameters for heat inactivation of arboviruses are not established for treatment temperature and duration, nor are they verified for safety by detection of residual viable virus, posing material biosafety concerns. For example, treatment of dengue virus at 56°C, under commonly used, published conditions, resulted in virus infectivity upon extended incubation period and serial passaging. In this study, yellow fever, zika, dengue type 2, chikungunya and mayaro viruses were incubated at 65°C completely submerged in a water bath, and inactivation was assessed at three time points. Complete inactivation was demonstrated by inoculating permissive host cells and incubating for 14 days under optimal growth conditions for two passages. Samples collected throughout incubation were tested for infectivity by monitoring cytopathic effect (CPE), immunofluorescence-staining (IFA) using virus-specific antibodies and qRT-PCR. Our results indicate that treated samples were not infectious by CPE/IFA. Significant reductions in Cq values over the 28-day incubation period suggest the presence of non-replicating virus in the samples. The heat-inactivated viruses were sufficiently stable for immunological (IFA) and molecular (qRT-PCR) assays, demonstrating their utility as controls for detection assay development. Our results demonstrate that specific, reproducible conditions for heat inactivation can be established that are applicable to multiple arboviruses and ensure material safety compliance. Heat-inactivated viruses with quantified viral genome copy number are available through BEI Resources.

1519

URBAN ARBOVIRAL EPIDEMICS AND HEALTH SYSTEM RESPONSE IN EL SALVADOR

Mirna P. Amaya Amaya

University of Florida, Gainesville, FL, United States

Major historical, demographic and societal changes, including the growth of population centers in tropical countries, have impacted the emergence of arboviral diseases in Central America. The increase in vector borne diseases, has resulted in an overwhelmed vector control system and public health sector. At the same time, there has been a growing global concern on the shortage of health care workers. A recent joint World Health Organization and World Bank estimate calculates the shortage of Human Resources for Health (HRH) at 18 million by 2030, more than doubling the 2013 estimate of 7 million. Therefore, a greater focus has been placed on the importance of an adequate density of HRH within low and low-middle income countries, in order to sustain the country public health efforts. Through an extensive review of scientific literature, country reports, and in depth semi-structured interviews with key stakeholders; this paper explores the health system response to recent arboviral epidemics in El Salvador from a HRH perspective. The study aims to identify barriers healthcare workers face during health emergencies, using the recent Zika epidemic as a point of comparison. Low and Low-Middle income countries are disproportionately affected by arboviral diseases, due to an unequal distribution of wealth and resources. Strengthening health system infrastructures and processes for early identification of outbreaks, then becomes imperative. This would help overcome health disparities and adequately prepare for the necessary public health emergency response to epidemics. The study findings suggest that some of the main barriers that the health workforce encounter during health emergencies are the product of poverty, social inequality, security issues, a fragmented health system, as well as internal politics, among others. However, there is a lot of hope for change and an unrelenting optimism on the part of El Salvador's healthcare workers; evident through their recommendations to the ministry of health, and also through personal accounts of ingenuity in overcoming the barriers they face before, during, and after health emergencies.

1520

THE COMPOSITION AND CLINICAL RELEVANCE OF THE BLOOD VIROME IN FEBRILE PEDIATRIC OUTPATIENTS IN TANZANIA BY UNBIASED NEXT GENERATION SEQUENCING

Mary-Anne Hartley[1], Samuel Cordey[2], Florian Laubscher[2], Kristina Keitel[3], Thomas Junier[4], Francisco J. Pérez-Rodriguez[5], Gael Vieille[2], Josephine Samaka[6], Tarsis Mlaganile[6], Frank Kagoro[6], Zainab Mbarack[7], Mylène Docquier[8], Laurent Kaiser[2], Valérie D'Acremont[3]

[1]*University of Lausanne, Lausanne, Switzerland,* [2]*Division of Infectious Diseases and Laboratory of Virology, University of Geneva Hospitals, Geneva, Switzerland,* [3]*Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [4]*Swiss Institute of Bioinformatics, Geneva, Switzerland,* [5]*University of Geneva Medical School, Geneva, Switzerland,* [6]*Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania,* [7]*Mwananyamala Hospital, Dar es Salaam, United Republic of Tanzania,* [8]*UiGE*[3] *Genomics Platform, University of Geneva, Geneva, Switzerland*

Viruses are present in every human tissue with associations spanning from integral parts of the heritable human genome, to tolerated guests or unwelcome infections. Together, this diverse and changeable group of viruses comprise the human virome. The composition and role of the virome in human blood is largely unknown. We previously benchmarked a technique of unbiased next generation sequencing able to detect genetic traces of all vertebrate viruses. This retrospective cohort study investigates the potential clinical relevance of its extreme sensitivity by describing the composition and clinical associations of the blood virome in 803 febrile paediatric (2-59 months) Tanzanian outpatients. We found 62 viruses (25 RNA, 37 DNA) across 20 families, including several viruses novel to human blood from the Astroviridae, Circoviridae, Genomoviridae, Parvoviridae and Papilloviridae families. At least 1 virus/sample was detected (median=3; IQR=1-8; range=1-9). Most DNA viruses comprised ubiquitous commensal viruses (UCVs) of the Anelloviridae family; and 37% of samples (n=298/803) had only UCVs detectable. Patients with >1 non-UCVs were 3.6 months younger than those with none (CI95=1.7-2.4; p<0.001). Amongst numerous clinical associations, many aligned with known presentations. For example, children carrying classic gastroenteritis viruses were more likely to present with vomiting (Rotavirus: RR=2.0, p=0.001. Norovirus: RR=5.4, p=0.001); rhinoviruses were associated with pneumonia (RR=2.8, p=0.050); roseola viruses tended to present in younger children with fever only (RR=1.4, p=0.050); and HIV-1 was associated with higher severity (RR=6.2, p=0.019). Thus, we present a highly diverse virome with a wealth of clinical associations, many of which aligned with expected presentations. However, the complexity of these findings blurs human comprehension, and we conclude that the use of such high-resolution data would require objective pattern detection algorithms for unbiased validation. Further the results still need to be complemented by conventional techniques before they could be used at a clinical level.

1521

A METAPOPULATION MODEL FOR THE 2018 EBOLA VIRUS DISEASE OUTBREAK IN EQUATEUR PROVINCE IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Sophie Meakin, Mike Tildesley, Emma Davis, Matt Keeling

University of Warwick, Coventry, United Kingdom

Ebola virus disease is a viral haemorrhagic fever with high mortality that has caused a number of severe outbreaks in Central and West Africa, placing huge strains on already limited healthcare resources. Mathematical models matched to early case reporting data can be used to identify outbreaks that are at high risk of spreading and thus allocate resources more effectively. Here we consider the Ebola virus disease outbreak in Equateur Province in the Democratic Republic of the Congo, which was declared on 8 May 2018. We use a simple stochastic metapopulation model to capture the dynamics in the three affected health zones: Bikoro, Iboko and Wangata. We use approximate Bayesian computation methods to determine parameters by matching between reported and simulated cases. We use this framework to calculate the force of infection in each health zone and so indicate which areas are most at risk. by allowing the transmission parameter to change over time, we can also determine how the basic reproductive ratio changes over time as various intervention measures are implemented.

1522

CLINICAL DEVELOPMENT OF LHF-535 AS AN ORAL THERAPEUTIC FOR LASSA FEVER

Sean M. Amberg[1], Portia A. Vliett-Gregg[1], Alison E. Heald[2], Eric J. Tarcha[1], Jeff Posakony[1], Kristin M. Bedard[1], Clinical Network Services (CNS) Pty Ltd[3], Nucleus Network[4]

[1]*Kineta, Seattle, WA, United States,* [2]*Alison Heald Consulting, LLC, Seattle, WA, United States,* [3]*Hamilton QLD, Australia,* [4]*Melbourne VIC, Australia*

Lassa fever is a viral hemorrhagic disease endemic in West Africa. LHF-535 is a small molecule antiviral currently under development as a therapeutic option to treat Lassa fever and other viral hemorrhagic fevers of arenavirus origin. The human safety and pharmacokinetics of LHF-535 was evaluated in a single ascending dose double-blind phase 1a trial in healthy volunteers. Six cohorts of eight subjects each were randomized to LHF-535 (six per cohort) or placebo (two per cohort) and administered oral LHF-535 with weight-based dosing. Doses ranged from 0.3 mg/kg in the first cohort to 40 mg/kg in the last cohort. Plasma concentrations generally increased dose proportionally with respect to total exposure (mean AUC 1430 to 118,000 h·ng/mL). The frequency of treatment-emergent adverse events (TEAE) were similar between placebo (50%) and LHF-535 recipients (42%). TEAE frequency did not correlate with LHF-535 dose. Human exposure from oral LHF-535 reached exposures comparable to exposure provided by daily intraperitoneal dosing in guinea pigs at a dose that was fully protective against a lethal challenge with Lassa virus. These results support the continued clinical development of LHF-535.

1523

ZAIRE EBOLA VIRUS GLYCOPROTEIN - INSIGHTS FROM EPITOPE MAPPING AND INFECTIVITY ANALYSES

Edgar Davidson[1], Tabb Sullivan[1], Aubrey L. Bryan[1], Andrew Flyak[2], James E. Crowe[2], Benjamin J. Doranz[1]

[1]*Integral Molecular, Inc., Philadelphia, PA, United States,* [2]*Departments of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN, United States*

Recent disease outbreaks due to Ebola virus (EBOV) highlight the need to characterize the immune response to ebolaviruses to develop vaccines and therapies. We have used extensive GP mutation libraries to map the epitopes for >200 monoclonal antibodies (MAbs) targeting the EBOV surface glycoprotein, GP. A broad variety of MAbs has been mapped, as described in publications, including the ZMapp therapeutic MAb cocktail; MAbs from survivors of EBOV and Bundibugyo ebolavirus infection; cross-neutralizing MAbs targeting the GP membrane-proximal external region (MPER); a broadly cross-reactive MAb that blocks GP interaction with its endosomal receptor Niemann-Pick C1; GP binding activity in sera of mice injected with DNA encoding MAbs (DMAbs), and MAbs whose synergistic combination transforms a non-neutralizing MAb into a potent neutralizer. With the Viral Hemorrhagic Consortium, we characterized how MAb *in vitro* neutralization correlates with *in vivo* protection. The epitope maps expand our understanding of how the immune system recognizes EBOV GP, and allow correlation of epitopes with MAb neutralizing capabilities, to develop anti-EBOV therapeutics and vaccines. Mapping also identified mutations that increase the exposure of neutralizing epitopes and that affect EBOV function, impacting future anti-ebolavirus vaccine strategies. Such insights are being used design new immunogens to serve as improved vaccines. To identify GP residues important for EBOV infectivity, we performed infectivity assays with the full GP mutation library, using a lentivirus pseudotype system. We identified critical residues whose mutation abrogated infectivity without affecting GP conformational integrity. The locations of the residues suggest that they are crucial for GP conformational changes that cause virus-host membrane fusion. Additionally, to identify uncharacterized EBOV cellular receptors, we assayed wild-type GP infectivity in non-permissive cells individually expressing 5,300 unique human membrane proteins of our membrane proteome array (MPA). This has identified candidate membrane proteins that enable EBOV infectivity.

1524

SEROPREVALENCE OF THE *ARARAQUARA VIRUS* ANTIBODIES IN A POPULATION FROM A PROSPECTIVE COHORT IN SÃO JOSÉ DO RIO PRETO, SP, BRAZIL

Gislaine Celestino da Silva[1], Marcílio Jorge Fumagalli[2], Victor Miranda Hernandes[1], Bruno Henrique Milhim[1], Caroline Rodrigues da Silva[1], Lucas Celestino Araujo[1], Nathalia Zini[1], Eliane Aparecida Favaro[1], Luiz Tadeu Figueiredo[2], Ana Carolina Terzian[1], Mauricio Lacerda Nogueira[1]

[1]*São José do Rio Preto School of Medicine, São José do Rio Preto, Brazil,* [2]*Ribeirão Preto School of Medicine - University of São Paulo (FMRP-USP), Ribeirão Preto, Brazil*

Hantaviruses (family *Peribunyaviridae*, genus *Orthohantavirus*) are spherical and enveloped presenting glycoproteins in the surface with approximately 120 nm in size. The genome consists of a negative and tri-segmented ssRNA. The three RNA segments are defined as small (S), medium (M) and large (L) which encode N proteins, enveloped G1 and G2 glycoprotein, and viral RNA polymerase, respectively. Hantaviruses have as natural reservoirs wild rodents that can eliminate the virus through urine, saliva, and feces. Rodents can carry the virus for life without becoming ill. In Brazil, epidemiological surveys detected the genotype Araraquara virus (ARAV) in *Necromys lasiurus* rodents are predominantly found in the cerrado, a predominant biome in the São Paulo State. The aim of this study is to perform a serological survey to detect the presence of anti-ARAV IgM and/or IgG antibodies in patients from a prospective cohort study in a neighborhood of the municipality of São José do Rio Preto, SP, Brazil. A total of 831 patients were randomly selected, and submitted to an interview for investigation of patient›s health history, living conditions and workplace. So far 253 sera samples were collected and tested by an IgG/IgM indirect-ELISA using the N recombinant protein of ARAV as the antigen, as described previously. The cut-off was established as the mean value + 2 standard deviations control samples and showed a cut-off Optical Density (OD) equal to or greater than 0.300. Our results showed 0.8% (2/253) of positivity to ARAV-IgG, 0.4% (1/253) of undetermined and 98.8 % (250/253) of negative samples. None of the samples were positive to ARAV-IgM while 0.4% (1/253) of the samples were undetermined and (252/253) 99.6% were negative. São José do Rio Preto is endemic to Dengue and other arboviruses; however, several cases of the acute febrile disease are negative for these viruses. In this way, we will be

able to estimate the prevalence of the hantavirus antibodies, contributing to surveillance in the municipality and with a better diagnosis and patient care.

1525

THE SECRET 'LIVES' OF MOSQUITO-ASSOCIATED VIRUSES: METAGENOMIC RNA SHOTGUN SEQUENCING HELPS DECIPHER VIRAL ECOLOGY BUT SHOWS THAT HOST SPECIES IS THE MOST IMPORTANT DRIVER OF VIROME COMPOSITION

Panpim Thongsripong[1], James Angus Chandler[2], Amy B. Green[3], Pattamaporn Kittayapong[4], Bruce A. Wilcox[5], Durrell D. Kapan[6], Shannon N. Bennett[1]

[1]*Department of Microbiology, California Academy of Sciences, San Francisco, CA, United States,* [2]*Department of Molecular and Cell Biology, Berkeley, CA, United States,* [3]*Department of Microbiology, University of Hawai'i at Manoa, Honolulu, HI, United States,* [4]*Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Bangkok, Thailand,* [5]*Integrative Research and Education Program, Faculty of Public Health, Mahidol University, Bangkok, Thailand,* [6]*Department of Entomology and Center of Comparative Genomic, California Academy of Sciences, San Francisco, CA, United States*

The discovery of novel viruses in the environment has accelerated greatly in recent years with the accessibility of high-throughput *de novo* sequencing, yet challenges remain to interpret their biology and the major drivers of their diversity and evolution. Mosquitoes, because of their public health importance, are among organisms whose viromes are being intensely characterized. Even as the number of new mosquito-associated viruses exponentially increases, our understanding of the viral ecology and association with other mosquitoes' symbionts remains limited. Using RNA-seq, and a laboratory method to enrich microbial RNA, we characterized viruses with RNA-based genomes and other symbionts in three mosquito species: *Armigeres subalbatus*, *Culex fuscocephala*, and *Mansonia uniformis*, collected from three previously-characterized habitats along an urbanization gradient in Thailand. We found multiple novel and known viruses belong to at least 12 viral families. In addition, we investigated how the mosquito's virome changed across the host species and habitat, and found that the pattern of virus presence was defined primarily by the host species rather than by the geographical locations or habitats. This result suggests that the insect-associated viruses displayed relatively narrow host ranges but were capable of spreading through a mosquito population efficiently, at least within the geographical scale of this study. Other single-celled and multicellular microorganisms such as bacteria, alveolates, fungi, nematodes, and lophotrochozoan were also found. The biological role of the mosquito-associated viruses should be further validated. These results emphasize the importance of including ecological information in viromic studies, and characterizing other microorganisms associated with hosts, in order to gain further insights into viral ecology in systems where host specificity is driving both viral ecology and evolution.

1526

HEPATITIS E IN BANGLADESH: INSIGHTS FROM A NATIONAL SEROSURVEY

Andrew Azman[1], Kishor K. Paul[2], Taufiq Rahman Bhuiyan[2], Firdausi Qadri[2], Henrik Salje[3], Emily Gurley[1]

[1]*Johns Hopkins School of Public Health, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Institut Pasteur, Paris, France*

Hepatitis E is thought to be responsible for more than 70,000 deaths and another 3,000 stillbirths. Large outbreaks of HEV have been documented in South Asia and studies from Bangladesh suggest that up to 10% of maternal mortality may be caused by this vaccine-preventable disease. However, little is know about the geographic distribution and transmission intensity of hepatitis E in countries like Bangladesh. With an efficacious vaccine licensed for use in China and improved prospects for future WHO prequalification, endemic countries like Bangladesh need more detailed data on where HEV transmission occurs, thus providing important geographic targets for interventions. We conducted a nationally representative serosurvey across Bangladesh of 2,778 individuals from 70 randomly selected communities and tested serum samples for anti-IgG antibodies with the Wantai ELISA kit. We found that 20.9% of the population had evidence of historical HEV infection (IgG+) with large variation between communities (range 0-78%). Using Bayesian model-based geostatistical models, we explored the relationship between key individual- household- and community-level covariates and developed 5km by 5km maps HEV IgG positivity throughout the country. We found that males had a 2.3 fold (95% CrI 1.8-3.0) increased odds of being seropositive with increasing risk by age. A number of factors related to travel history, household wealth, community poverty and remoteness were associated with HEV seropositivity in univariate analyses, however, they were not significant in multivariable models. Our maps reveal Dhaka as a significant *foci* of HEV transmission with seroprevalence over 60% in some areas. In total, we estimate that 40.4 (35.6-45.6) million people in Bangladesh have been infected with HEV. These data provide insights into HEV epidemiology and can be used to guide future interventions and research in Bangladesh.

1527

EVALUATION OF A TWO TARGET RT-PCR ASSAY FOR DETECTION OF LASSA VIRUS

Ketan Patel[1], Bobbie Rae Erickson[1], Timothy Flietstra[1], Leonie-Sophie Hecht[2], Hussein El Halas[2], Stuart Nichol[1], John Klena[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Altona Diagnostics, Hamburg, Germany*

Lassa virus (LASV), a member of the family Arenaviridae, is an enveloped single stranded RNA virus, causing endemic disease in West Africa. Natural host of LASV is *Mastomys natalensis*, a small rodent. Human-to-human transmission is possible within the community as well as in the health care setting, via aerosols, contact with contaminated body fluids or re-use of contaminated medical equipment. LASV can cause Lassa Hemorrhagic Fever (LHF) with high fatality rates, reaching 15-20% among hospitalized patients. The considerable sequence diversity between LASV strains is challenging for the development of molecular diagnostic assays. The likelihood of false-negative results can be decreased significantly by using multiple genomic sequences as target regions, e.g. the L gene and the GPC gene. Here we present the performance evaluation of a real-time RT-PCR based molecular diagnostic test, comprised of two independent assays targeting specific sequences within the L and GPC genes, respectively. The reactivity of the RealStar® Lassa Virus RT-PCR Kit 2.0 toward the wide spectrum of virus variability was evaluated using infected Vero cell culture isolates of 39 different Lassa virus strains from different endemic areas: Nigeria, Guinea, Sierra Leone, Liberia, and Togo. All 39 tested samples were detected positive for Lassa virus RNA using the RealStar® Lassa virus RT-PCR Kit 2.0. The sensitivity and accuracy of the kit is 100% using the panel of Lassa Virus available at CDC. The RealStar® Lassa Virus RT-PCR Kit 2.0 detected all 39 strains representing 5 out of 6 linages from the Lassa fever endemic areas. Lineage V was not available for testing. The L and GPC assays showed strain and lineage dependent differences in reaction efficacy. The combined testing of GPC and L assay in one kit reduces the risk of non-reactivity towards certain strains or lineages.

1528

DRUG REPURPOSING FOR SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS

Jasper Chan, Shuofeng Yuan, Lei Wen, Zi-Wei Ye, Dong-Yan Jin, Kwok-Yung Yuen

The University of Hong Kong, Hong Kong, Hong Kong

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne bunyavirus that causes severe disease in human with a case-fatality rate of up to 30%. It causes an acute febrile illness,

known as SFTS, which is characterized by high fever, thrombocytopenia, and hemorrhagic complications in infected humans. Patients with SFTS may also develop other clinical manifestations such as systemic upset, coma, slurred speech, gastrointestinal upset, hepatosplenomegaly, and lymphadenopathy, and abnormal laboratory findings including leukopenia, prolonged activated partial thromboplastin time, deranged liver function tests, proteinuria, hematuria, and elevated creatinine kinase and lactate dehydrogenate levels. It is prevalently found in ticks and mammals in the Northeastern part of China, and parts of Japan and South Korea. There are currently very limited treatment options for SFTSV infection. In this study, we conducted a drug repurposing programme using an *in silico* structure-based screening approach to screen a large chemical library to identify potential inhibitors of the nucleocapsid protein (NP) of SFTSV. The *in vitro* antiviral activity of the drugs which potentially bind to SFTSV NP were assessed in Huh-7 cells using cytopathic effect inhibition, viral load reduction, and plaque reduction assays. Among the drugs predicted to bind with SFTSV NP, clofazimine, an antimicrobial for treating leprosy, was found to have the most potent antiviral activity *in vitro*. The IC_{50} and CC_{50} of clofazimine were 0.8 μM and 10.1 μM, respectively. Molecular docking predicted that clofazimine bound to the NP with high stability. The inhibition of SFTSV NP by clofazimine was validated by a mini-replicon assay which reflected the NP polymerase activity. Clofazimine-treated type I interferon receptor-deficient A129 mice had significantly improved clinical and virological parameters. Clinical trials should be considered to assess the treatment effects of clofazimine in patients infected with SFTSV.

1529

FIRST REPORT OF THE OROPOUCHE VIRUS IN COLOMBIA

Doris Esther Gomez[1], Jorge A. Egurrola[1], Cristopher Cruz[2], Margarita Ochoa[1], Carolina Guevara[2], Maria Silva[2], Julia S. Ampuero[2]

[1]*Universidad de Cartagena, Cartagena, Colombia,* [2]*Naval Medical Research Unit-6, Lima, Peru*

Oropouche fever is an emerging zoonotic disease caused by the Oropouche virus (OROV) belonging to the family Bunyaviridae, genus *Orthobunyavirus*. OROV was initially reported in Trinidad and Tobago in 1955 and since then human cases have been reported in Brazil, Peru, Panama and Ecuador. Here, we report a case of a 28-year-old female patient from Turbaco (Bolivar, Colombia) with 1-day history of fever, malaise, chills, myalgias, headache, retroocular pain, photophobia, dizziness, sore throat, anorexia, dysgeusia and nausea. A blood sample was taken after informed consent was obtained as part of a passive febrile surveillance study in the area with the approval of the NAMRU-6 and Cartagena University IRBs. A real time RT-PCR was negative for dengue, Zika and chikungunya virus. Subsequently, the sample was inoculated in Vero 76 cells, and cytopathic effect was observed four days after inoculation. Indirect immunofluorescence (IFI) was performed with pools of polyclonal antisera for flavivirus (yellow fever and dengue-3), alphavirus (Venezuelan equine encephalitis, Eastern equine encephalitis, and Mayaro), HAC (encephalomyocardiovirus, Allpahuayo and Tacaribe) and bunyavirus (Guaroa, caraparu and OROV). A positive signal was observed with the mix of bunyavirus antisera. Guaroa, caraparu and OROV-specific antisera was then individually used to perform IFI and a positive signal was observed with OROV antisera. The sample was also analyzed for OROV, Guaroa, Mayaro and Tacaribe by ELISA IgM using antigens produced in-house and no positive signal was detected. Cell culture supernatant from grown virus was used to sequence the complete genome by unbiased amplifications and sequencing on the Illumina MiSeq platform. The isolated OROV is similar to Ecuadorian and Peruvian strains in its three genome segments (GenBank: MK643115, MK643116, MK643117). This would be the first human case confirmed with infection by OROV in Colombia.

1530

THE ROLE OF RON4 IN *PLASMODIUM* SPOROZOITE INFECTION OF THE LIVER

Minami Baba, Mamoru Nozaki, Mayumi Tachibana, Motomi Torii, Takafumi Tsuboi, Tomoko Ishino

Ehime University, Toon, Japan

Rhoptry neck protein 4 (RON4) is known as one of the *Plasmodium* merozoite rhoptry proteins and discharged to the moving junction during invasion. Since RON4 is refractory to gene disruption, it is thought to be essential for merozoite invasion of erythrocytes. RON4 is also expressed and localized to rhoptries in sporozoites, other invasion form of *Plasmodium*. It was reported that RON4-repressed sporozoites show lower infectivity to cultured hepatoma cells *in vitro*. This finding indicates that RON4 in sporozoites has an important role for infection of hepatocytes similar to that in merozoites. However, it remains unclear when RON4 works in the liver infection. To elucidate the molecular mechanism of RON4 during sporozoite infection of the liver *in vivo*, we applied a stage-specific knockdown system by promoter swapping in a rodent malaria parasite strain, *Plasmodium berghei*. The *ron4* promoter region was replaced by an erythrocytic-stage specific promoter, *msp9* promoter, by homologous recombination. The parasite amount in the mouse liver at 1h after intravenously inoculation of 30,000 RON4-knockdown sporozoite was approximately 3-fold less than that of control, indicating that RON4 has a role in sporozoite migration from hepatic sinusoid towards liver parenchyma. The parasite amount in the mouse liver at 42h after RON4-knockdown sporozoite inoculation was also reduced to 10-fold less than the control sporozoite infection. Taken together, these results suggest that RON4 has a role(s) also in hepatocyte invasion and/or development in hepatocytes. In vitro gliding assay demonstrates that RON4-knockdown sporozoites has lower adhesion ability and circular movement than that of control sporozoites. This study indicates that RON4 is required for sporozoite targeting to the liver via involving in its migration ability.

1531

VISIBLE AND BIOCHEMICAL EVIDENCE OF ENDOTHELIAL GLYCOCALYX DEGRADATION IN TANZANIAN CHILDREN WITH FALCIPARUM MALARIA

Salvatore M. Florence[1], Ayam Kalingonji[1], Margaret A. Bush[2], Youwei Chen[2], Tsin W. Yeo[3], Nicholas M. Anstey[4], Matthew P. Rubach[5], Donald L. Granger[6], Esther D. MwaiKambo[1], J. Brice Weinberg[2]

[1]*Hubert Kairuki Medical Center, Dar es Salaam, United Republic of Tanzania,* [2]*Duke University and Veterans Affairs Medical Centers, Durham, NC, United States,* [3]*Lee Kong Chian School of Medicine, Singapore, Singapore,* [4]*Menzies School for Health Research, Darwin, Australia,* [5]*Duke University Medical Center, Durham, NC, United States,* [6]*University of Utah and Veterans Affairs Medical Centers, Salt Lake City, UT, United States*

Endothelial dysfunction in patients with *Plasmodium falciparum* malaria plays a major role in pathogenesis. The endothelial glycocalyx (eGC) composed of membrane-bound proteoglycans and glycoproteins is a gel-like layer covering the vessel luminal surface. The eGC functions to maintain vascular homeostasis by regulating permeability, modulating blood flow-induced shear stress signals including formation of nitric oxide, and inhibiting endothelial adherence of blood cells. We hypothesized that breakdown of the eGC occurs in malaria leading to endothelial activation and microvascular dysfunction. At the Hubert Kairuki Medical Center in Dar es Salaam, Tanzania, we measured eGC breakdown products glycosaminoglycans (GAG) and syndecan-1. We used dark-field SDF microscopy to visually quantify eGC damage at the axilla and pinna, including analysis of the perfused boundary region (PBR) that reflects eGC integrity. *P. falciparum* diagnosis was established by microscopy and RDT. Total urinary GAG levels were measured by dimethylmethylene blue assay, and plasma angiopoietin-2 & syndecan-1 by ELISA. We studied 59 healthy (HC), 49 moderately severe malaria (MSM), and 36 severe malaria (SM), age 1 to 10 years. Imaging revealed significantly higher

PBR in malaria vs HC (1.63±0.04 microns vs 1.51±0.04; p = 0.047). Total urinary GAG was higher in SM [13.4±1.0 mol/g creatinine (mean±SEM)] and MSM (12.4±1.0) than in HC subjects (4.3±0.4); p<0.0001. Plasma syndecan-1 was higher in malaria patients than HC (334±33 vs 79±7 ng/mL; p<0.0001). Both urine GAG and plasma syndecan-1 correlated significantly with plasma angiopoietin-2 (R=0.8 & R=0.7, respectively; p<0.0001), indicating an important relationship of GCX degradation and disease severity. Thus, Tanzanian children with falciparum malaria have evidence of glycocalyx degradation based on increased levels of urinary GAG and plasma syndecan-1, as well as increased eGC damage detected by visual imaging. It is likely that this glycocalyx damage contributes to the pathogenesis of vascular dysfunction. Agents that protect against glycocalyx damage may be useful as adjunctive treatments for malaria.

1532

PLACENTAL PATHOLOGY AND THE RISK OF PREECLAMPSIA IN WOMEN EXPOSED TO *PLASMODIUM FALCIPARUM* INFECTIONS IN THE PLACENTA

Dorotheah Obiri[1], Isaac Erskine[2], Kwame Adu-Bonsaffoh[3], Daniel Oduro[4], Kwadwo A. Kusi[5], Michael F. Ofori[5], Ben Gyan[5]

[1]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana,* [2]*Department of Pathology, Korle-Bu Teaching Hospital, Accra, Ghana,* [3]*Department of Obstetrics and Gynecology, Korle-Bu Teaching Hospital, Accra, Ghana,* [4]*Department of Animal Biology and Conservation Science, University of Ghana, Accra, Ghana,* [5]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana*

Preeclampsia (PE) is a placental disorder with multifactorial etiologies that present with different phenotypes. In malaria-endemic regions, high incidence of PE is reported, with debilitating maternal and fetal effects particularly among first time pregnant women. However, the relationship between placental pathology, *Plasmodium falciparum* infection in the placenta and PE is underexplored. A total of 134 placentas were sampled at delivery from 69 women without PE (non-PE group) and 65 women diagnosed with PE (PE group) at a tertiary hospital in Ghana. Demographic details were documented and placentas examined histologically for pathological lesions and placental malaria (PM). Placental pathology was increased in the PE group compared to the non-PE group, with syncytial knots being the specific pathology associated with PE ($P = 0.003$). Pathology was also found to be dependent on first-time pregnancy and blood pressure to induce PE using a regression model (AOR 2.8, 95% CI = 1.2 – 6.4). Of 133 placentas scored for placental parasite exposure, 64 (48.1%) and 21 (15.8%) represented active and past infections respectively. Placental parasite exposure was significantly higher in the PE group [39 (29.3%) and 15 (11.3%) active and past infections respectively] compared to the non-PE group [25 (18.8%) active infections and 6 (4.5%) past infections, $P < 0.0001$]. The absence of placental exposure was 10 (7.5%) in the PE and 38 (28.6%) in the non-PE group. Multivariate analysis showed placental pathology (AOR 3.0, 95% CI = 1.2 – 7.5), exposure to PM [(active infection: AOR 6.7, 95% CI = 2.3 – 19.1); (past infection: AOR 12.4, 95% CI = 3.0 – 51.0)], first pregnancy (AOR 6.6, 95% CI = 2.4 – 18.2) but not blood pressure to be significantly associated with PE. Placental histological changes and PM are risk factors for PE particularly in primigravids. Mechanisms associated with this finding should further be investigated.

1533

CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* HECT E3 UBIQUITIN LIGASE

Brajesh Kumar Singh, Xin-zhuan Su

National Institutes of Health, Rockville, MD, United States

The gene encoding *Plasmodium yoelii* HECT ubiquitin ligase (*Pyheul*) is a large essential gene (22.5 Kb) that regulates parasite growth and virulence. We have shown that *Pyheul* influences parasitemia and host mortality in mice. This finding inspires us to study this gene in the human malaria parasite *P. falciparum*. Protein ubiquitylation is an important post-translational regulation that has been shown to be necessary for life cycle progression and survival of *P. falciparum*. The *P. falciparum* E3 ubiquitin ligase is substantially variable and divergent compared to the homologs from other eukaryotes, which make the E3 ligase a potential parasite-specific target. To better understand the function of the ligase, we created a transgenic parasite line using pSli-HA-glmS plasmid that enables us to knock down the expression of E3 ubiquitin ligase. The pSli-HA-glmS plasmid is designed for C-terminal tagging of proteins with 3X-HA tag and addition of glmS sequence. The glmS ribozyme allows a candidate gene to be down regulated with the addition of glucosamine. Our study will help toward a better understanding of ubiquitylation and new ubiquitination targets. The ubiquitin targets may therefore represent excellent molecules for future development of anti-malarial drugs.

1534

A POTENTIAL ROLE FOR ANTIBODY-MEDIATED *THROMBOCYTOPENIA* IN PEDIATRIC CEREBRAL MALARIA

Iset Medina Vera[1], Anne Kessler[2], Visopo Harawa[3], Wilson Mandala[4], Stephen J. Rogerson[5], Terrie Taylor[6], Karl Seydel[6], Morayma Reyes[7], Kami Kim[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*New York University, New York, NY, United States,* [3]*Blantyre Malaria Project, Blantyre, Malawi,* [4]*Academy of Medical Sciences, Malawi University of Science and Technology, Malawi University of Science and Technology, Thyolo, Malawi,* [5]*The University of Melbourne, Melbourne, Australia,* [6]*Michigan State University, East Lansing, MI, United States,* [7]*Albert Einstein College of Medicine, Bronx, NY, United States*

Cerebral malaria (CM) is characterized by coma with peripheral *P. falciparum* parasitemia, parasite sequestration, and associated endothelial dysfunction, pathologic immune activation, and thrombocytopenia with intravascular coagulation. CM pathology is reminiscent of heparin-induced thrombocytopenia (HIT), a pro-thrombotic adverse response to exogenous heparin treatment where pathologic antibodies (Ab) recognize a neo-antigen within platelet factor 4 (PF4) complexed to heparin. PF4/heparin/Ab complexes cross-link platelets and other immune cells via Fcγ receptor leading to a positive feedback loop. Autoimmune HIT can occur in patients without prior heparin exposure. In CM, autoimmune, non-malaria antibodies have been described, but the contribution of antibody-mediated thrombocytopenia has not been addressed. We hypothesized that HIT-like antibodies may contribute to malaria pathogenesis. Children with uncomplicated malaria (UM, n=84) or CM (n=123) were recruited at Queen Elizabeth Central Hospital (Blantyre Malaria Project, Malawi; 2015-2017 seasons) and plasma was analyzed. In CM, median levels of HIT-like IgG in plasma were elevated relative to UM (OD: 0.27 *vs* 0.18, p=<0.0001). Notably, the proportion of CM (33 of 123) with IgG levels above the established clinical threshold (OD=0.4) defining HIT positivity was 22-fold higher than in UM (1 of 84). Furthermore, heparin-dependent and independent binding of IgG (confirmed by inhibition with high dose heparin) were observed. Levels of HIT-like IgG in acute infection decreased with convalescence (30 days; p=<0.0001). Analysis of non-malaria, auto-immune antibodies/complexes (anti-dsDNA antibodies and circulating immune complexes; CIC) showed no differences between UM and CM. Levels of soluble PF4 were elevated in UM relative to CM, indicating an early platelet activation that may result in consumptive coagulopathy as malaria progresses. Further studies will focus on analysis of platelet activation markers and functional studies on HIT. Overall, this preliminary work points to a role for auto-antibody mediated thrombocytopenia in severe malaria pathology.

1535

PLASMODIUM YOELII ERYTHROCYTE BINDING-LIKE PROTEIN MODULATES HOST CELL MEMBRANE, IMMUNITY AND VIRULENCE

Yu Chih Peng[1], Yanwei Qi[2], Cui Zhang[1], Xiangyu Yao[3], Jian Wu[1], Xia Lu[1], Keyla Tumas[1], Xiao He[1], Chen-Feng Qi[1], Anthony Holder[4], Osamu Kaneko[5], Timothy Myers[1], Carole Long[1], Jian Li[6], Xinzhuan Su[1]

[1]*National Institutes of Health, Rockville, MD, United States,* [2]*Guangzhou Medical University, Guangzhou, China,* [3]*Roche, Shanghai, China,* [4]*MRC National Institute, London, United Kingdom,* [5]*Nagasaki University, Nagasaki, Japan,* [6]*Xiamen University, Xiamen, China*

Malaria erythrocyte binding-like (EBL) proteins play a critical role in parasite invasion. Previously we identified a C741Y substitution at the trafficking domain VI of PyEBL and performed allelic exchanges between two isogenic parasites, leading to altered disease phenotypes and host immune response. In addition to expression in merozoites, PyEBL is also expressed in iRBCs, resulting in changed surface protein expression, PS exposure, and osmotic fragility. The parasites with C741 allele triggered stronger inflammatory cytokines and chemokines. The parasites with Y741 allele stimulated CD36 mediated phagocytosis, type I interferon signaling, T cell differentiation, isotype switching and improved host survival. This study reveals a previously unknown mechanism of PyEBL in regulating innate immune response and host pathogen interaction.

1536

THE IMPACT OF ALPHA-GLOBIN GENE VARIANTS ON ENDOTHELIAL FUNCTION IN ADULTS WITH SEVERE MALARIA

Jessica Nino de Rivera[1], Matthew Grigg[2], Dongying Ma[1], Yu Yang[1], Bridget Barber[2], Timothy William[2], Kim Piera[2], J. Brice Weinberg[3], Tsin W. Teo[2], Nicholas M. Anstey[2], Hans C. Ackerman[1]

[1]*National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [2]*Menzies School of Health Research, Darwin, Australia,* [3]*Duke University, Durham, NC, United States*

The human alpha globin genes HBA1 and HBA2 are highly polymorphic, with deletions and sequence variants that decrease the transcription or synthesis of functional alpha globin protein causing a spectrum of alpha thalassemia syndromes. While some variants have been associated with decreased susceptibility to malaria, precise mechanisms of protection remain to be elucidated. Recently, alpha globin was discovered to be expressed in vascular endothelial cells where it regulates nitric oxide signaling. Genetic or pharmacologic inhibition of alpha globin expression increases nitric oxide signaling in intact vessels; therefore, we hypothesized that human polymorphisms that inactivate alpha globin will increase endothelial nitric oxide signaling. This is relevant to the pathogenesis of malaria where the loss endothelial nitric oxide signaling may contribute to adhesion of infected blood cells, occlusion of small vessels, and impairment of vasoregulation. Using droplet digital PCR, we precisely quantified the known structural *HBA* variants -3.7, -4.2, --SEA, and --FIL as well as the Constant Spring and Adana sequence variants in two case-control studies of severe malaria conducted in Timika, Indonesia (n=324) and Sabah, Malaysia (n=194). We are now analyzing the association between alpha globin genotype and physiologic measures of endothelium-dependent vasodilation as well as biomarkers of endothelial activation such as angiopoietin-2 and osteoprotegerin. These studies will extend our understanding of the role of endothelial alpha globin in nitric oxide signaling and determine whether alpha globin gene variants alter endothelial function in severe malaria.

1537

ASSOCIATION OF EPCR POLYMORPHISMS RS867186-GG WITH PROTECTION AGAINST HUMAN CEREBRAL MALARIA (HCM)

Mingli Liu[1], Juan Cespedes[1], Bharti Praveen[2], Sri Krishna[2], Jonathan Stiles[1]

[1]*Morehouse School of Medicine, Atlanta, GA, United States,* [2]*National Institute for Research in Tribal Health (NIRTH), Jabalpur, India*

Cerebral malaria (CM) is characterized by the sequestration of *Plasmodium*-infected erythrocytes (pRBC) to host brain microvasculature beds via *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). Under normal situation, activated protein C (APC) bound to endothelial protein C receptor (EPCR) has cytoprotective properties by activating protease-activated receptor 1 (PAR1) while pRBC transports PfEMP1 to their membranes, which can bind EPCR in the same region as APC. As a result, APC is less capable of inducing cytoprotective effects via PAR1. Two studies involving adult malaria patients revealed that EPCR rs867186-G allele could mediate protection against severe malaria, while three other studies involving child malaria patients showed that EPCR gene variants are not associated with severe malaria or increased mortality among children with CM. We examined the association of the EPCR rs867186-G allele with protection against severe malaria. *1)* 47 malaria patient and 4 healthy control individual samples were collected from 2004 to 2007 at NSCB Medical College Hospital, India. CM and malaria associated complications were defined using WHO criteria. *2)* Genomic DNA was isolated from the peripheral blood mononuclear cells of subjects. Primer sequences were designed to contain rs867186 of the *PROCR* gene (NM 006404) and were used to amplify a 660 bp product. PCR products were purified, and DNA sequences were determined by Sanger Sequencing (www.Genewiz.com. Genewiz, NJ). *3)* Statistical analysis: Nonparametric tests compared groups, and the chi-square or Fisher's exact test for 2x2 table was used for categorical variable, if the expected value was less than 5, to analyze differences in allele frequencies. P-value <0.05 was considered statistically significant. Fisher's exact test showed that there is significantly higher rates of A/G plus G/G in CM patients compared to mild malaria ($p = 0.0034$); similarly, there is significantly higher rates of A/G in CM patients compared to mild malaria patients ($p = 0.0017$). Our results indicate that rs867186-GG or rs867186-GA genotypes do not mediate protection against HCM.

1538

USING SEASONAL MALARIA CHEMOPREVENTION (SMC) TO SCREEN FOR ACUTE MALNUTRITION

Moumouni Bonkoungou[1], Youssouf Sawadogo[1], Stanislas Nebie[1], Thierry Ouedraogo[1], Yacouba Savadogo[2], William Brieger[3], Gladys Tetteh[4], Blami Dao[4]

[1]*PMI Improving Malaria Care Project, Ouagadougou, Burkina Faso,* [2]*Ministry of Health, National Malaria Control Program, Ouagadougou, Burkina Faso,* [3]*Johns Hopkins University, Baltimore, MD, United States,* [4]*Jhpiego Baltimore, Baltimore, MD, United States*

Malaria and malnutrition remain a major public health burden in Burkina Faso for children under five years of age. In 2017 case fatality rate of malaria was 1.5 percent among children under five years of age and malaria was responsible for 35.9 percent of deaths in primary health facilities. In the same year, malnutrition was responsible for 4.6 percent of deaths in primary health facilities and 3.3 percent of deaths in hospitals. Malaria is aggravated by the presence of malnutrition. In 2018, the Burkina Faso Seasonal Malaria Chemoprevention (SMC) campaign integrated malnutrition screening in 65 out of 70 health districts. During the SMC campaign, community health workers administer sulfadoxine-pyrimethamine + amodiaquine (SP+AQ); they also screened for malnutrition using the Shakir sling to measure mid-upper arm circumference to detect for acute malnutrition. Children who are not severely malnourished receive the standard malaria preventative treatment by SP+AQ. Children diagnosed with severe malnutrition do not receive SP+AQ and are referred to health facilities for appropriate

case management. In October 2018, during the last monthly round of SMC, 419,705 children aged 6 to 59 months were screened by community distributors in 12 President's Malaria Initiative (PMI) supported health districts. Among these children, 2,009 cases of moderate acute malnutrition, and 525 cases of severe acute malnutrition (0.6 percent of children) were detected. In November, after the last round (October), 427 children with severe acute malnutrition have been reported by health facilities. This represents 81.3 percent of severe acute malnutrition detected during SMC. In 2017 malnutrition screening was not combined with SMC and children screened for the whole year were 988,529 compared to 1,177,316 in 2018 (with SMC), an increase of 19 percent of children benefited from screening. In the context of a limited resource country, SMC is a good strategy for the reduction of malaria cases as well as a great opportunity for the detection and management of malnutrition in children under five years of age.

1539

PROBING THE REGULATION OF TARGETABLE METABOLIC PATHWAYS IN MALARIA PARASITES

Philip M. Frasse, Audrey R. Odom John

Washington University School of Medicine, St. Louis, MO, United States

Malaria continues to be an enormous economic and global health threat. We study unique and targetable metabolic pathways in the malaria parasite, *Plasmodium falciparum*, to guide future antimalarial drug development. Using a drug resistance screen, we have identified mutations in multiple haloacid dehalogenase (HAD) enzymes that confer resistance to fosmidomycin, an inhibitor of the first committed step of the methylerythritol phosphate (MEP) pathway of isoprenoid biosynthesis in the parasites. We now use genetic, biochemical, and metabolomic approaches to determine the enzymatic functions of these enzymes and illuminate the mechanism(s) of resistance in our mutant parasites. We have found that parasites with mutations in HAD1 or HAD2 have increased availability of the isoprenoid precursor, deoxyxylulose-5-phosphate (DOXP), which outcompetes the inhibitor. We find that HAD enzymes are phosphatases that dephosphorylate intermediates of central carbon metabolism, in turn modulating DOXP levels. In addition, we have identified genetic changes in a related HAD protein, phosphomannomutase (PMM), in parasites evolved for resistance to the Malaria Box compound MMV665917. We hypothesize that MMV665917 targets mannose biology or central carbon metabolism, and ongoing studies address this hypothesis. Altogether, these HAD proteins and their homologs are involved in many branches of metabolism, and may be key to understanding the finely-tuned metabolic regulation in malaria parasites. We therefore are assessing the sensitivity of these resistant strains to other antimalarials to determine whether drug resistance by these mechanisms may sensitize parasites to other drugs. By expanding our understanding of parasite metabolism, we are informing future endeavors to target these unique pathways for therapeutics.

1540

ELEVATED LEVELS OF HEMOZOIN AND ERYTHROPHAGOCYTOSIS PREDICT LONGITUDINAL EPISODES OF SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN

Samuel B. Anyona[1], Evans Raballah[1], Elly Munde[1], Caroline Ndege[1], Qiuying Cheng[2], Paul Fenimore[3], Benjamin H. McMahon[3], Nick Hengartner[3], Collins Ouma[4], Christophe G. Lambert[2], Douglas J. Perkins[2]

[1]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya,* [2]*University of New Mexico Center for Global Health, Albuquerque, NM, United States,* [3]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [4]*Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Kisumu, Kenya*

In western Kenya, severe malaria manifests as severe malarial anemia [SMA; hemoglobin (Hb)<5.0 g/dL] in children <48 months. Our previous studies showed that enhanced uptake of *Plasmodium falciparum* hemozoin (*Pf*Hz, malarial pigment) in cultured peripheral blood causes dysregulation in inflammatory mediators known to impact on the development of SMA. We have also shown that elevated levels of pigment-containing monocytes (PCM) in Kenyan children increases the risk of developing SMA in cross-sectional analyses. However, the impact of naturally-acquired *Pf*Hz and erythrophagocytosis on the development of longitudinal episodes of SMA are unexplored. As such, we determined levels of intraleukocytic *Pf*Hz and erythrophagocytosis in circulating mononuclear cells in children (n=1,610) over a 36 month follow-up period during the phase of naturally-acquired malarial immunity (>19,000 visits). Logistic regression modeling (controlling for anemia promoting covariates) over 36 months of follow-up revealed that presence of PCM increased the odds of SMA up to 3.54-fold (P=8.63x10^{-17}), while erythrophagocytosis enhanced the odds of SMA up to 67.36-fold (P=3.90x10^{-3}). Taken together, these results suggest that phagocytosis of *Pf*Hz and erythrophagocytosis by mononuclear cells increase the longitudinal risk of SMA.

1541

TEMPERATURE DRIVES MALARIA TRANSMISSION: IMPLICATIONS FOR DISEASE CONTROL

Courtney Murdock[1], Kerri Miazgowicz[1], Erin Mordecai[2], Sadie Ryan[3], Richard Hall[1]

[1]*University of Georgia, Athens, GA, United States,* [2]*Stanford University, Stanford, CA, United States,* [3]*University of Florida, Gainesville, FL, United States*

Dramatic reductions in disease burdens of human malaria in the last 20 years has led to ambitious calls for eradicating the disease by 2030. However, eradication hinges on our ability to eliminate transmission 1) from both symptomatic and asymptomatic hosts, 2) across highly heterogeneous landscapes created by biotic and abiotic factors, and 3) with the emergence of multi-drug resistance to the last remaining anti-malarials, artemisinin-combination therapies. Despite growing research efforts to develop new therapeutics and vaccines, mitigating malaria transmission still largely depends on conventional mosquito control methods (e.g. bed nets, indoor residual spraying). Developing tools that will allow us to successfully predict outbreaks and efficiently target current and future interventions to specific times and locations will aid effective mosquito and disease control. Current work revolves around experimentally testing common assumptions made by mathematical models that predict transmission, identifying additional sources of ecological variation these models should incorporate, and building new models to improve prediction. This includes assessing how transmission models perform when key mosquito life history traits (e.g. daily per capita mortality rate, egg production, and biting rate) change across the lifespan of the mosquito vector, in thermally variable environments, across different species of *Anopheles* mosquitoes, and with parasite infection. Results from these studies combine both empirical and mathematical models to resolve uncertainty in current malaria models.

1542

SINGLE STEP MEROZOITE RELEASE STRATEGY FOR RED BLOOD CELL INVASION ASSAYS

Jurgen Bosch, Quentin D. Watson, Rajeev K. Mehlotra, Howard J. Meyerson, Peter A. Zimmerman

Case Western Reserve University, Cleveland, OH, United States

Investigating fundamental features of malaria blood stage infection has relied on synchronization of parasites propagating in culture.

Traditionally, parasite growth has been synchronized by exposing cultures to refrigeration, treating 5% D-sorbitol or mannitol, sedimentation in Plasmagel or gelatin. More recently, parasite synchronization has been achieved by isolating a brood of merozoites and adding them to target red blood cells (RBC). In this method, late-developmental stages are purified from cultures using magnetic columns followed by a mechanical rupture through a syringe filter. This method requires at least 6-8h and four steps (E64 inhibition of parasite growth/propagation, magnetic column enrichment, schizont maturation, mechanical rupture). Here we demonstrate a novel, single-step chemical treatment to rapidly and efficiently release merozoites from infected RBC. 40 μL of resuspended parasite culture was mixed with 1 mL of the treatment solution. The cells are then incubated on ice. We varied treatment exposure (2, 5 and 10 minutes) and resting in complete media (0, 10, 30 minutes) times to optimize parasite invasion. Extent of RBC lysis/merozoite liberation and parasite invasion was monitored by imaging flow cytometry (IFC), counting 200000 cells. Invasion assays occurred over 72 hr. Results showed that 2-5 minute treatment times were optimal, while 10 minutes diminish parasite invasiveness completely. At these optimal treatment times, highest levels of parasite RBC invasion was observed at 10 minutes of resting, although all of the resting times were compatible with onward parasite growth. Optimal starting parasitemia and invasion culture seed volume was 0.2 μL of the RBC lysate is added to 7.2 μL of packed RBC (final hematocrit of 4%).

1543

MODELING RESISTANCE-CONFERRING MUTATIONS AND ANTIMALARIAL DRUG INTERACTIONS IN THE PFCRT 3.2 Å CRYO-EM STRUCTURE

Kathryn J. Wicht, Jonathan Kim, Yong Zi Tan, Filippo Mancia, David A. Fidock

Columbia University Medical Centre, New York, NY, United States

Resistance to weak-base 4-aminoquinoline antimalarials has primarily been attributed to mutations in the 49 kDa drug *P. falciparum* chloroquine resistance transporter (PfCRT), resident on the parasite's digestive vacuole (DV) membrane. These mutations first arose in the field from chloroquine (CQ) drug pressure. More recently, PfCRT mutations associated with resistance to piperaquine (PPQ), a partner drug of artemisinin-based combination therapies, have been reported. In this study, we performed computational protein modeling based on our structure of the CQ-resistant (CQ-R) 7G8 PfCRT isoform from South America. This structure was solved by single-particle cryo-electron microscopy to 3.2 Å resolution in the open-to-DV conformation. Here, we mutated CQ and PPQ resistance-conferring mutations *in silico* and docked antimalarials into the structures. Residue changes on the transmembrane (TM) helices contributing to CQ resistance (CQ-R) or PPQ resistance (PPQ-R) map to a central cavity, implicating this as the major site of drug interaction. Leveraging the 2-fold pseudo-symmetry of PfCRT's ten TM helices, a homology model was built by performing sequence alignment of the symmetrically related TM pairs in order to generate the closed conformation of the transporter. Subsequent mapping of mutations, drug docking and molecular dynamic simulations suggest differences in the way that PfCRT interacts with PPQ vs CQ to regulate their transport away from the acidic DV (pH ~5). Simulation analyses revealed favorable H-bonding between positively charged drugs and negatively charged or polar residues in the binding cavity, except in the case of CQ with the CQ-sensitive isoform, where CQ cannot access the binding site. These findings support the hypothesis that transport of protonated CQ^{2+} out of the DV is controlled by drug access to PfCRT's binding cavity, whereas protonated PPQ^{4+} transport may be influenced by deprotonation and release from PfCRT in the closed-to-DV conformation. These structural insights provide new opportunities to define approaches for blocking PfCRT's ability to function as a pleiotropic drug resistance transporter.

1544

MOLECULAR STUDIES OF *PFDHPS* AND *PFDHFR* DURING SEASONAL MALARIA CHEMOPREVENTION AT THREE STUDY SITES IN MALI

Youssouf Diarra[1], Lassina Doumbia[1], Oumar Kone[1], Ibrahim Keita[1], Lansana Sangare[1], Haidara D. Bouye[1], Vincent Sanogo[1], Bassi Coulibaly[1], Amadou Bouare[1], Abdoul K. Diallo[1], Zakaria Haidara[1], Modibo Telly[1], Jules Mihigo[2], Erin Eckert[3], Moustapha Coulibaly[1], Etienne Coulibaly[1], Moucart Diallo[1], Ababacar Maiga[1], Donald J. Krogstad[4], Ousmane A. Koita[1]

[1]*University of the Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*President's Malaria Initiative, United States Agency for International Development, Bamako, Mali,* [3]*President's Malaria Initiative, United States Agency for International Development, Washington, DC, United States,* [4]*Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

Sulfadoxine-pyrimethamine (SP) in combination with amodiaquine is now recommended by the World Health Organization for seasonal malaria chemoprevention (SMC) in children from 3 to 59 months of age in the Sahel region of Africa. Since 2012, SP has also been used to prevent plasmodial infection and malarial disease in pregnant women (Intermittent Preventive Treatment of Malaria during pregnancy, IPTp-SP). For this reason, there is concern about the spread of parasites resistant to sulfadoxine-pyrimethamine from selective pressure to the intensive use of SP in SMC campaigns and for IPTp. The objective of this study was to examine the frequency of quintuple mutants in the *Pfdhfr* and *Pfdhps* genes and to identify potential new mutations using Sanger sequencing during and after the 2018 SMC campaign in children and in pregnant women at the time of their first visit to the Ante-Natal Clinic. Of the 428 children with confirmed malaria who were enrolled in this study, 254 received SP/AQ during the 2018 SMC campaign. The preliminary results show the presence of the mutation at position 540 in the *Pfdhps* gene with an amino acid change from K to E (Lysine to Glutamate). This mutation may be essential for the resistance of *Pfdhps* to sulfadoxine. In addition, new mutations have been found with other codon changes in the same gene. Processing of the remaining isolates should clarify the impact of SMC on the polymorphisms in the *Pfdhps* and *Pfdhfr* genes in Mali.

1545

USE OF ARTEMISININ COMBINATION THERAPIES HAS NOT CHANGED THE GENETIC DIVERSITY OF THE K13 PROPELLER DOMAIN IN UGANDAN *PLASMODIUM FALCIPARUM* POPULATIONS

Melissa D. Conrad[1], Victor Asua[2], Stephanie A. Rasmussen[3], Patrick Tumwebaze[2], Adoke Yeka[4], Roland A. Cooper[3], Samuel L. Nsobya[2], Moses Kamya[4], Grant Dorsey[1], Philip J. Rosenthal[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Infectious Disease Research Collaboration, Kampala, Uganda,* [3]*Dominican University, San Rafael, CA, United States,* [4]*Makerere University, Kampala, Uganda*

Artemisinin combination therapies (ACTs) are used to treat uncomplicated malaria in most endemic regions and have been extremely important in decreasing morbidity and mortality. However, resistance to artemisinins, defined as delayed clearance after therapy, has emerged in Southeast Asia, and the potential spread of resistance to high transmission areas, such as much of sub-Saharan Africa, could have devastating consequences. Artemisinin resistance has been associated with multiple non-synonymous SNPs (NS-SNPS) in the propeller domain of the *K13* gene (*K13PD*) in Southeast Asia, yet the relevance of such mutations in sub-Saharan Africa is unclear. Over 200 NS-SNPs, including some that have been associated with resistance in Southeast Asia, have been reported at low prevalence in clinical isolates from Africa, but the delayed clearance phenotype is uncommon and ACTs continue to be highly efficacious. Hypothesizing that changes in the diversity of *K13PD* following ACT exposure would serve as

an early indication of the evolution of artemisinin resistance in Uganda, we compared the genetic diversity of the *K13PD* locus in clinical isolates collected before and after the implementation of ACT use from seven sites across the country. We detected NS-SNPs in the *K13PD* locus in 17 of 682 (2.5%) clinical isolates collected between 1999 and 2004 and in 21 of 574 (3.7%) isolates collected between 2012 and 2016 (p = 0.25), representing a total of 26 different polymorphisms at 24 codons. Individual NS-SNPs generally were detected only once and were never found in more than 0.7% of the total isolates. Two SNPs (P574L and A675V) associated with delayed clearance in Southeast Asia were seen in samples collected between 2012 and 2016, but each in a single isolate. No differences in diversity following implementation of ACT use were found at any of seven sites across Uganda. In conclusion, we found no evidence that selection by ACTs is impacting on *K13PD* diversity in Uganda.

1546

TREATMENT PILOT IN BURKINA FASO, 2017-2018

Ousmane Badolo, Mathurin Dodo, Thierry Ouedraogo, Moumouni Bonkoungou, Youssouf Sawadogo, Stanislas Nebie, Blami Dao, Gladys Tetteh, William Brieger

Jhpiego, Ouagadougou, Burkina Faso

Malaria is the main cause of morbidity, hospitalization and death in health facilities in Burkina Faso. The Improving Malaria Care (IMC) project funded by the US President's Malaria Initiative (PMI) and implemented by Jhpiego provides technical and logistical support to the National Malaria Control Program (NMCP) to implement pre-referral treatment of severe malaria with rectal artesunate in children under 5 by 1244 Community-based Health Workers (CHWs) covering 117 health centers in four health districts in the Sahel region. This study aims to describe the process and compare malaria-attributable deaths before and after the pilot. In August 2018, a stakeholder orientation workshop discussed the implementation of the pre-referral treatment policy. This was followed by an advocacy workshop at regional level, a training-of-trainers session and training of CHWs on the pre-referral treatment. A total of 564 children under 5 with RDT-confirmed severe malaria received pre-referral treatment using rectal artesunate during the period October to December 2018. In 2017, from October to December, before the IMC-supported intervention, 4,159 cases of severe malaria and 154 deaths from malaria were recorded in the Sahel region, with a case fatality proportion of 3.7%. From October to December 2018, 5383 cases of severe malaria and 128 deaths from malaria among children under 5 were recorded with a case fatality proportion of 2.4%. Despite the increase in cases of severe malaria (29.4%) between 2017 and 2018, the number of malaria-attributable deaths decreased by 16.8%, and the case fatality proportion decreased by 35.8%. While a reduction in deaths from malaria in the Sahel region is seen, we saw an increase in other regions. For example, the number of malaria related deaths increased from 28 to 103 in North region, from 48 to 125 in Center East region and from 85 to 143 in East region. The pre-referral treatment of severe malaria cases may have contributed to reducing the number of malaria related deaths in the Sahel region. Based on this result, PMI is planning support for extension of this intervention to cover the 6 health districts of the Center-North region.

1547

ANTI-PARASITIC PROPERTIES FOR NEEM DERIVATIVES: POTENTIAL NOVEL THERAPEUTIC OPTIONS FOR CONTROLLING RESISTANT *PLASMODIUM FALCIPARUM* MALARIA

Angela O. Achieng[1], Caroline Ndege[1], Bernard Guyah[2], Collins Ouma[2], Cristian Bologa[3], Tudor Oprea[3], Douglas J. Perkins[4]

[1]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya,* [2]*Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University, Kisumu, Kenya,* [3]*Division of Translational Informatics, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, United States,* [4]*University of New Mexico Center for Global Health, Albuquerque, NM, United States*

Malaria control largely depends on the use of chemotherapy. However, the emergence and spread of multi-drug resistant *Plasmodium Spp*. provides significant challenges to malaria control. Conventional antimalarials were primarily discovered and adopted for malaria treatment based on their *in vitro* anti-plasmodial activity; with little knowledge of their molecular mechanism of action. *Azadirachta indica* (neem) is traditionally used as herbal therapy to treat malaria in Asia and Africa. Neem antimalarial activity is primarily attributed to its terpenoid derivatives. The potential use of neem derivatives in combinatorial therapeutics, and the molecular mechanism(s) underlying their anti-plasmodial activity remain largely unexplored. This study investigated *in vitro* anti-plasmodial efficacy of neem leaf extract and 17 neem derivatives individually, and in combination with dihydroartemisinin (DHA) against *Plasmodium falciparum* D6 and W2 strains using the SYBR® GREEN I assay. *In silico* molecular docking studies were also conducted for identification of plausible putative parasite targets for characterization of anti-plasmodial mechanisms of action for neem. *In vitro* screening experiments identified 10 compounds with potent anti-parasitic activity (IC_{50} <2μM) against *Pf*D6 and *Pf*W2, and with low or no cytoxicity on human cells. In addition, significant synergistic interactions were observed between five neem derivatives in combination with DHA. Molecular docking identified *P. falciparum* histone deacetylase 1 protein *(Pf*HDAC1) as a target for a methyl 2, 5-dihydroxycinnamate neem derivative. Additional *in vitro* target bioassay confirmed that methyl 2, 5-dihydroxycinnamate possess antimalarial potential through binding to *Pf*HDAC1. Taken together, these results suggest that neem derivatives are promising novel candidates for use as antimalarial drugs.

1548

ASSESSMENT OF PARASITE CLEARANCE AFTER TREATMENT WITH PYRONARIDINE-ARTESUNATE, ARTESUNATE-AMODIAQUINE, ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININ-PIPERAQUINE IN *PLASMODIUM FALCIPARUM* MALARIA: RESULTS FROM THE WEST AFRICAN NETWORK (WANECAM) TRIAL

Issiaka Soulama[1], Issaka Sagara[2], Habib Beavogui[3], Jean Bosco Ouedraogo[4], Sodiomon B. Sirima[5], Abdoulaye Djimde[2]

[1]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [2]*Malaria Research and Training Center, Bamako, Mali,* [3]*Centre National de Formation et de Recherche en Sante Rurale (CNFRSR), Conakry, Guinea,* [4]*Institut de Recherche en Sciences de la Santé, Institut des Sciences et techniques (INSTech), Bobo, Burkina Faso,* [5]*Centre National de Recherche et de Formation sur le Paludisme, Groupe de Recherche Action en Santé, Ouagadougou, Burkina Faso*

Reports from the Greater Mekong Subregion (GMS) showed the emergence of delayed parasite clearance with known artemisinin based combination therapies. We conducted the WANECAM to comparatively assess the antimalarial safety/efficacy and monitor the parasite clearance time in patients treated for uncomplicated *P. falciparum* with three-day regimens of pyronaridine-artesunate (PA), artesunate-amodiaquine (ASAQ), artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP). A randomized phase IIIb/IV comparative, multicentre, open-label parallel 3 arms trial was conducted in Burkina Faso, Guinea and Mali in patients aged more than six months with 42 days of follow-up. We monitored patients over a 2-year period to capture the incidence of repeated episodes of malaria and to assess PA and DP safety and efficacy, compared with AL or ASAQ (no direct comparison between PA and DP). Treatment was directly observed, and blood smear samples were collected twice daily (12 h ±2 h) until parasite clearance. The endpoint of interest for this present sub-study was parasite clearance slope half-life for the first episode of malaria. The estimates of parasite clearance time (PCE) was derived using the WorldWide Antimalarial Resistance Network (WWARN) PCE tool. PCE estimates were summarized from well-fitting profiles (with R-squared >0.8). A total of 4,710 patients were recruited in the study

with the PCR-adjusted efficacy on day 28 in the per protocol population estimated at 99.8%, 99.7%, 99.3%, and 99.9% in PA, ASAQ, AL, and DP arms respectively. Parasite clearance slope half-life was available from 4,205 (89.3%) participants for the first episode with the profile adjudged as good fitting for 2,136 (50.1%) participants. The day 2 parasite positive proportion was 1.3% (58/ 4632) and day 3 positive proportion was 0.06% (3/ 4627). The overall median parasite clearance slope-half-life was 2.8 h (inter-quartile range (IQR): 2.4 to 3.2h) and this was similar between the study drugs. The four ACTs included in this trial demonstrated excellent efficacy and had comparable parasite clearance slope half-life.

1549

COMBINATION THERAPIES FACILITATING THE SPREAD OF ARTEMISININ-RESISTANCE IN THE GREATER MEKONG SUBREGION

Kristan A. Schneider[1], Ananias A. Escalante[2]

[1]*University of Applied Sciences Mittweida, Mittweida, Germany,* [2]*Temple University, Philadelphia, PA, United States*

The spread of artemisinin resistance due to several mutations in the Kelch13 gene in the the Greater Mekong Subregion is a source of concern, and its containment a public health priority. Susceptibility of *Plasmodium falciparum* to antimalarial drugs is typically evaluated by therapeutic efficacy studies. While many confounding factors contribute to the clinical outcome it is difficult to identify mutations associated with drug resistance if these are at low frequency in the initial phase of their spread. From a clinical perspective the focus lies on therapeutic efficacy of chemotherapy. However, the spread of drug resistance is an evolutionary process, determined by the relative fitnesses of resistance-conferring haplotypes. Although therapeutic efficacy is related to evolutionary fitness, the former is just one of many so-called fitness components contributing to determine the latter. When considering the complex compounds of fitness components, it becomes clear that certain drug combinations can facilitate the spread of artemisinin resistance despite improved therapeutic efficacy. This fact, that sounds like a paradox at the first sight, is the consequence of the parasite's life history. By using a formal qualitative model, this argument can be put forward in a simple way without digging into mathematical details. Formalizing this argument give rise to new study designs to be conducted in the future to get better estimates on the spread of drug resistance and new strategies for containment.

1550

CHARACTERIZATION OF DORMANT *PLASMODIUM FALCIPARUM* PARASITES IN HUMAN PARTICIPANTS FOLLOWING ARTESUNATE THERAPY

Chris Peatey[1], Nanhua Chen[1], Karryn Gresty[1], Karen Anderson[1], Paul Pickering[1], Rebecca Watts[2], James McCarthy[2], **Qin Cheng**[1]

[1]*Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia,* [2]*QIMR Berghofer Medical Research Institute, Brisbane, Australia*

Artemisinin (ART) monotherapy has been associated with frequent recrudescence. ART-induced dormancy, shown *in vitro* and in animal models, provides a plausible explanation. However, there has been no documentation and characterisation of ART-induced dormancy in human infection. In an *Plasmodium falciparum* induced blood stage malaria (IBSM) infection study, participants were infected with either 3D7 (ART-sensitive) or K13M (ART-resistant) and blood samples were collected from participants prior to, and 48-72 hrs post artesunate treatment. We characterised ART-induced dormant parasites using microscopy, FACs, *in vitro* culture and molecular methods. Dormant parasites were observed in peripheral blood samples from all participants 48-72 hrs post artesunate treatment. Both pre- and post-treatment blood samples seeded parasite growth *in vitro*. Compared to pre-treatment samples, post-treatment samples showed a 10-12 day delay to reach 2% parasitemia *in vitro*. Recovered parasites had the same K13 genotype as pre-treatment parasites. Upregulation > 80 fold, of an apicoplast enzyme encoding gene, the molecular signature of ART-induced dormancy, was detected 48 hrs post-artesunate treatment. Combined with the observed dynamics of parasitemia *in vivo*, this study provides strong evidence for the presence of ART-induced dormant parasites in patients infected with ART-sensitive *P. falciparum* which go on to cause recrudescence. Combination treatment regimens with partner drugs targeting dormant parasites likely improve efficacy of ART antimalarials.

1551

IN-VITRO AND *EX-VIVO* SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO ANTIMALARIAL DRUGS IN BINH PHUOC PROVINCE OF VIETNAM FROM 2018 TO 2019

Thu Huong Pham, Tong Thanh Nguyen, Thanh Viet Ngo, Thuy Thu Do, Guy Thwaites, Hien Tinh Tran

Oxford University Clinical Research Unit - Vietnam, Ho Chi Minh, Vietnam

The reduced susceptibility of anti-malarial drugs to *Plasmodium falciparum* highlights the urgent need to monitor the efficacy of the current regimens and identify the alternative potent compounds for the treatment. To assess the susceptibility of *Plasmodium falciparum* to antimalarial drugs at the study sites, *in-vitro* and *ex-vivo* drug sensitivity assays in *Plasmodium falciparum* malaria patients were undertaken. A total of 69 *Plasmodium falciparum* isolates from patients with uncomplicated malaria in Binh Phuoc Province of Vietnam, where multidrug-resistant strains of *P. falciparum* have been documented, were collected from 2018 to 2019. Three *in-vitro* tests were used: The World Health Organization schizont inhibition assay (WHO microtest) in which parasites are exposed to different concentrations of antimalarial drugs; the ring-stage survival assay (RSA) parasites are exposed to 700 nM dihydroartemisinin for 6 hours whereas in the piperaquine survival assay (PSA) parasites are expressed to 200 nM piperaquine for 48 hours. The development of parasites from trophozoits to schizonts (microtest); from ring stages to trophozoits (RSA, PSA) were assessed. The microtest assay showed 33% (18/55) of parasites resistant to mefloquine (MQ), 82% (45/55) resistant to chloroquine (CQ), and 31% (17/55) resistant to piperaquine (PIP). Only 2% (1/55) of the isolates were resistant to quinine (QN). The RSA and PSA assay showed the resistance rates to DHA and PIP were 91% (63/69) and 83% (57/69), respectively. Lumefantrine (LM) and amodiaquine (AQ) with the low inhibitory concentrations - IC_{50} (mean IC_{50}: LM=33 nM and AQ=20.21 nM), were highly active against *P.falciparum*. This study reconfirmed that RSA and PSA; are useful surveillance tools to monitor the artemisinin and piperaquine resistance in Vietnam and SEA countries where the artemisinin and artemisinin-based combination (ACTs) therapy resistance are worsening, and help to adjust the management of malaria patients.

1552

GAMETOCYTE CLEARANCE IN KENYAN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AFTER ARTEMETHER LUMEFANTRINE OR DIHYDROARTEMISININ PIPERAQUINE TREATMENT

Francis T. Kimani, Protus O. Omondi, Kelvin K. Thiong'o, Eva A. Nambati, Edwin K. Too, William K. Chege, Maureen A. Otinga

Kenya Medical Research Institute, Nairobi, Kenya

The efficacy and safety of Artemether-lumefantrine(AL) and Dihydroartemisinin-piperaquine phosphate (DP) against asexual parasite population has been well documented. However, the effect of these antimalarials on sexual parasites is still unclear. Children from western Kenya with uncomplicated *P. falciparum* malaria were assigned randomly to AL or DP treatment. A total of 334 dried blood spot samples were collected for up to 5 weeks after treatment. *P. falciparum* gametocytes were detected by *Pfs25* qRT-PCR. Gametocytes prevalence, density and duration of gametocyte carriage were determined. At baseline all the 334 children had positive asexual parasite by microscopy, 12% (40/334) had detectable gametocyte by microscopy and 83.7% (253/302) had gametocytes by RT-qPCR. Overall, 12% of the study population at baseline was gametocyte positive by microscopy vs 83.7% by RT-qPCR a 10-fold

higher than microscopy. The prevalence of gametocytes decreased from 84.6% (125/148) to 7.04% (5/71) at day 42 in AL group and from 82.4 % (127/154) to 14.5% (11/74) in DP group. The duration of gametocyte carriage as estimated by qRT-PCR was slightly shorter in AL group(4.5days) than in DP group (5.1days) but not significant (p=0.301). Submicroscopic gametocytes persisted in some patients up to day 28 despite a significant number of patients being negative for asexual parasites by microscopy on day 7. The study indicates that AL may clear *Plasmodium falciparum* gemetocytes slightly faster than DP. However, presence of submicroscopic gametocyte densities after highlights the limitation of interventions that aim to reduce malaria transmission by use of antimalarial drugs therefore a gametocidal drug in combination to ACTs will be useful in blocking malaria transmission more efficiently.

1553

EXAMINING THE EPISTATIC INTERACTION BETWEEN PLASMEPSIN II AND PFCRT IN *PLASMODIUM. FALCIPARUM* PIPERAQUINE RESISTANCE

Sachel Mok[1], Leila Ross[1], Satish Dhingra[1], Tomas Yeo[1], Melanie Shears[2], Abhai K. Tripathi[2], Lise Musset[3], Photini Sinnis[2], David A. Fidock[1]

[1]*Columbia University Medical Center, New York, NY, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Institut Pasteur de la Guyane, Cayenne, French Guiana*

Clinical failure to the artemisinin combination partner drug, piperaquine (PPQ), has recently emerged in the Greater Mekong Subregion countries of Cambodia, Vietnam and Thailand. A genome-wide association study identified *Plasmodium falciparum* plasmepsin II (*pfpm2*) gene amplification as a molecular marker of resistance. However, genetic modifications by conditional knockdowns or overexpression did not phenocopy resistance, defined *in vitro* as >10% survival of parasites exposed to 200nM PPQ for 48h. Recent evidence indicates that resistance is also associated with novel variants of PfCRT on the Dd2 haplotype background, including mutations F145I and G353V that have recently emerged in Cambodia. To elucidate molecular determinants of PPQ resistance, we implemented a genetic cross between the PPQ-resistant Cambodian isolate PH1008-C and the sensitive NF54 line, using four huHep FRG-NOD mice transfused with human erythrocytes. Asexual blood stage progeny were cloned and whole-genome sequenced to generate a high resolution genetic map. To date, we have 13 unique genetic recombinants each represented by 1 to 22 clones. Interestingly, progeny with distinct recombinant patterns segregated by mouse, suggesting that host factors can exert selective pressures to favor certain parasite genotypes independent of virulent antigens. Genetic association analyses reveal that PPQ IC_{50s} were positively associated with *pfpm2* copy number in progeny expressing wild-type PfCRT. The combination of *pfpm2* with Dd2+M343L PfCRT gave the highest resistance, with a 5-fold increase in IC_{50} relative to NF54. In parallel, we derived clones from the PPQ-resistant S. American isolate S170 that harbors the 7G8+C350R variant, and observed significant association of multi-copy *pfpm2* with percent survival at lethal PPQ concentrations, yet only a 1.3-fold shift in IC_{50}. This suggests that PPQ response is modulated by an epistatic interaction between *pfpm2* and *pfcrt*. Using transfection, we are investigating the contribution of *pfpm2* to PPQ-R on a panel of contemporary *pfcrt* alleles, which will help us to predict the PPQ response of clinical isolates.

1554

MOLECULAR MECHANISMS AND SELECTIVE REVERSAL OF PIPERAQUINE, LUMEFANTRINE AND AMODIAQUINE RESISTANCE IN *PLASMODIUM BERGHEI* ANKA.

Fagdéba David Bara[1], Loise Ndung'u[1], Noah Machuki Onchieku[1], Beatrice Irungu[2], Peter Mwitari[2], Francis Kimani[3], Damaris Matoke-Muhia[3], Gabriel Magoma[1], Alexis Nzila[4], Daniel Kiboi[5]

[1]*Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI), Nairobi, Kenya,* [2]*Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya,* [3]*Centre for Biotechnology Research and Development, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya,* [4]*King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia,* [5]*Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya*

Antimalarial drugs, lumefantrine (LM), amodiaquine (AQ) and piperaquine (PQ) are essential components in artemisinin-based combination therapies. Using a model malaria parasite *Plasmodium berghei* we studied the mechanisms of resistance and reversal to LM, AQ, and PQ. Here, we used *P. berghei* parasites resistant to LM, PQ or AQ selected by *in vivo* drug pressure. We tested the ability of known resistance reversing agents (RA): probenecid, verapamil, or cyproheptadine to restore susceptibility of the resistant parasite to LM, AQ or PQ in the standard 4-day suppressive test. We first tested RA alone to identify the highest dose that does not inhibit parasite growth. Oral treatment with LM, AQ, and PQ alone or in combination with RA was administered for a total of four daily doses. Parasite density was estimated microscopically (×100) 96 hours post parasite inoculation. Parent strain was sensitive to LM, AQ and PQ with an ED_{90} of 3.52, 4.29 and 3.93mg/kg respectively. LM resistant (LM^R), AQ resistant (AQ^R) and PQ resistant (PQ^R) parasites obtained after 1-2 years of drug pressure had ED_{90} of 52.06, 20.32 and 63.39mg/kg respectively. At 5mg/kg, cyproheptadine restored LM activity by 65% against LM^R but failed to restore PQ activity against PQ^R. Probenecid (400mg/kg) and verapamil (50mg/kg) did not chemo-sensitize either LM^R to LM or PQ^R to PQ. We have previously shown that PQ^R is also resistant to LM (ED_{90} 97.25mg/kg). Surprisingly, these three chemosensitizers restored LM potency against PQ^R. Our data revealed that cyproheptadine restores LM activity in LM^R and also indicate that the selection of PQ^R is associated with LM decreased efficacy; however, this efficacy can be restored by chemosensitizers. We have currently employed a reverse genetics approach (using PlasmoGEM vectors) to delete or overexpress selected genes associated with mediating transport and metabolism of drugs. We aim to measure the impact of the deletion or elevated expression on parasite susceptibility to antimalarial drug alone and in combination with RA. We hope to provide clues on the possible molecular mechanisms of LM, AQ and PQ resistance and the interaction with the selected RA.

1555

IDENTIFICATION OF THE *PLASMODIUM FALCIPARUM* ACETYL-COA SYNTHETASE AS AN EMERGING ANTIPLASMODIAL DRUG TARGET

Robert L. Summers[1], Manu Vanaerschot[2], James M. Murithi[2], Charisse F. Pasaje[3], Madeline R. Luth[4], Pamela Magistrado-Coxen[1], Emma F. Carpenter[5], Jade Bath[2], João P. Pisco[6], Avinash S. Punekar[6], Beatriz Baragaña[6], Ian H. Gilbert[6], Justin T. Munro[7], Manuel Llinás[7], Jacquin C. Niles[3], Sabine Ottilie[4], Elizabeth A. Winzeler[4], Marcus C. Lee[5], David A. Fidock[2], Amanda K. Lukens[8], Dyann F. Wirth[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Columbia University, New York, NY, United States,* [3]*Massachusetts Institute of Technology, Cambridge, MA, United States,* [4]*University of California San Diego, La Jolla, CA, United States,* [5]*Wellcome Sanger Institute, Hinxton, United Kingdom,* [6]*University of Dundee, Dundee, United Kingdom,* [7]*Pennsylvania State University, University Park, PA, United States,* [8]*Broad Institute, Cambridge, MA, United States*

The evolution of drug resistance by the malaria parasite *Plasmodium falciparum* highlights the need for new generations of antimalarial compounds with novel modes of action. In recent years high-throughput phenotypic screening has identified thousands of new drug-like antiplasmodial compounds, however the mode of action of many remains unknown. The Malaria Drug Accelerator (MalDA) has applied a range of chemogenomic approaches to identify the targets of promising new antimalarial compounds and thereby aid the development of novel drug targets for malaria. By conducting resistance selection experiments and using whole genome sequencing, we identified mutations in the parasite's

Acetyl-CoA Synthetase (PF3D7_0627800; PfAcAS) which confer resistance to two structurally distinct antimalarial compounds, MMV084978 and MMV019721. Allelic exchange using the CRISPR/Cas9 system confirmed that the A597V mutation was sufficient to confer resistance to both compounds. Orthologues of PfAcAS in eukaryotes catalyze the formation of the central metabolite acetyl-CoA from acetate, coenzyme A and ATP, and participate in a range of essential processes including fatty acid biosynthesis, nutrient sensing and acquisition, and epigenetic regulation. Homology modelling of PfAcAS revealed that the A597V and T648M mutations cluster around the predicted active site of the protein, suggesting that MMV019721 and MMV084978 may act by competing with the substrates of PfAcAS. Such active-site mutations may be expected to impair the function of the protein and reduce parasite fitness, however *in vitro* competitive growth experiments between wildtype and drug-resistant parasite lines demonstrated that parasites bearing the T648M or A597V mutations do not incur a fitness cost relative to wildtype parasites, whereas preliminary data indicate a small fitness-cost associated with the A597V mutation. These findings identify the malaria parasite's Acetyl-CoA Synthetase as the target of structurally distinct antiplasmodial compounds and support the development of inhibitors of PfAcAS as antimalarial compounds with a novel mode of action.

1556

INVESTIGATION OF MOLECULAR MARKERS OF ANTIMALARIAL RESISTANCE DURING A THERAPEUTIC EFFICACY STUDY CONDUCTED IN THE DEMOCRATIC REPUBLIC OF THE CONGO, 2017

P. Mandoko[1], J. Matangila[2], E. Mukomena[3], P. Mitashi[2], J. Likwela[4], D. Mbongi[2], Samaly Souza[5], Gireesh Subramaniam[5], Naomi Lucchi[5], **Eric Halsey**[6], Leah Moriarty[6], Venkatachalam Udhayakumar[5], D. Mumba[1], G. Mesia[7]

[1]*National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo,* [2]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [3]*National Malaria Control Programme and University of Lubumbashi, Lubumbashi, Democratic Republic of the Congo,* [4]*National Malaria Control Programme and University of Kisangani, Kisangani, Democratic Republic of the Congo,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*Centers for Disease Control and Prevention, President's Malaria Initiative, Atlanta, GA, United States,* [7]*University of Kinshasa and Central Africa Clinical Research Network, Kinshasa, Democratic Republic of the Congo*

Due to the threat of emerging antimalarial resistance, the World Health Organization recommends incorporating surveillance for molecular markers of antimalarial resistance into routine therapeutic efficacy studies (TESs). In 2017, a TES of artemether-lumefantrine, artesunate-amodiaquine (ASAQ), and dihydroartemisinin-piperaquine was conducted in six sites in the Democratic Republic of Congo (DRC). Mutations in *K13, Pfcrt,* and *Pfmdr1* were identified by Sanger sequencing samples from 260 children experiencing treatment failure. For the *K13* gene, no artemisinin resistance associated mutations were found in the 245 and 229 successfully sequenced pre-treatment and post-treatment samples, respectively. The *Pfcrt* gene was investigated only for the 47 subjects in the ASAQ arm. All 47 pre-treatment samples were successfully sequenced and 38 showed a K76T mutation, associated with chloroquine resistance. Of the 258 pre-treatment samples successfully sequenced for the *Pfmdr1* gene, 133 (70 single and 63 mixed infections) carried the NYD haplotype (corresponding to codons 86, 184, and 1246), 82 (31 single and 51 mixed infection) carried the NFD haplotype, and 40 (20 single and 20 mixed infection) carried the YYY haplotype. The implications of these haplotypes remain unclear, with some reports linking NFD and YYY haplotypes to lumefantrine and amodiaquine resistance, respectively. While the *K13* findings are reassuring for DRC, we are conducting further *Pfmdr1* analyses, including comparing frequencies of haplotypes between subjects who did or did not experience a recrudescent infection.

1557

DISTRIBUTION OF PFCRT MUTATIONS ASSOCIATED WITH PIPERAQUINE RESISTANCE IN CAMBODIA

Biraj Shrestha[1], Zalak Shah[1], Andrew P. Morgan[2], Matthew Adams[1], Piyaporn Saingam[3], Chaiyaporn Chaisatit[3], Paphavee L. Ketwalha[3], Christian Parobek[2], Huy Rekol[4], Soklyda Chann[3], Michele D. Spring[3], Mariusz Wojnarski[3], Mark M. Fukuda[3], Brian A. Vesely[3], David L. Saunders[1], Philip L. Smith[3], Chanthap Lon[3], Jessica T. Lin[2], Norman C. Waters[1], Shannon T. Harrison[1]

[1]*University of Maryland Baltimore, Baltimore, MD, United States,* [2]*University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [3]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [4]*National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*

Resistance to artemisinins and key partner drugs such as piperaquine (PPQ) have become well-established in regions of Cambodia and neighboring countries. In 2016, Cambodia replaced dihydroartemisinin-piperaquine with artesunate-mefloquine as the first-line therapy. We and others have previously shown that mutations within the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) are associated with reduced susceptibility to PPQ. Subsequent gene-editing studies have shown that these mutations can independently confer PPQ resistance in parasites with single copy plasmepsin II. To better understand the emergence and distribution of PfCRT mutations associated with PPQ resistance, we are examining parasites from 478 clinical infections collected from eight provinces in Cambodia from 2009-2017. We are using PacBio amplicon sequencing or whole genome sequencing data to identify PfCRT mutations and quantitative PCR (or read coverage for samples with whole genome sequences available) to estimate plasmepsin II (pfpm2) copy number. In this dataset, amplified pfpm2 is observed in <1% of samples in 2009, does not reach high frequency until 2013 (62%) and 2014 (79%), then decreases in frequency in 2016 (48%) and 2017 (55%). Our preliminary data from a site in northern Cambodia identify multiple PfCRT mutations, including mutations H97Y, F145I, A195V, I218F, and G353Y. F145I is first observed in 2013 (24%), peaks in 2014 (34%), then decreases in prevalence in 2016 (24%) and 2017 (13%). However, H97Y, I218F, and G353V each increase in prevalence to 24% in 2017 from 10%, 13%, and 20% in 2016, respectively. Whole-genome sequencing will be performed on parasites with PfCRT mutations of interest to determine parasite origins. This work provides insight into the emergence, dynamics, and origins of PfCRT mutations contributing to PPQ resistance.

1558

SELECTION OF DRUG-RESISTANCE MARKERS FOLLOWING TREATMENT WITH ARTEMETHER-LUMEFANTRINE IN HIV-INFECTED AND HIV-UNINFECTED CHILDREN AND ASSOCIATION WITH LUMEFANTRINE PK EXPOSURE

Richard Kajubi[1], Ou Joyce[2], Hanna Ehrlich[3], Justin Goodwin[4], Tracey L. Freeman[3], Liusheng Huang[5], Norah Mwebaza[1], Francesca Aweeka[5], **Sunil Parikh**[3]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*Yale University, New Haven, CT, United States,* [3]*Yale School of Public Health, New Haven, CT, United States,* [4]*Yale School of Medicine, New Haven, CT, United States,* [5]*University of California San Francisco, San Francsisco, CA, United States*

The use of artemisinin-based combination therapies has been associated with the directional selection of mutations in key transporters, with *pfmdr1* N86 and *pfcrt* K76 appearing to confer lower susceptibility to lumefantrine. The relationship between drug levels and resistance selection has not been extensively evaluated. We used samples from a previously conducted PK/PD study of artemether-lumefantrine (AL) for the treatment of malaria in HIV-uninfected and HIV-infected children followed for 42 days in Tororo, Uganda. Paired samples for those individuals who had recurrent malaria by 42 days (n=185 cases) are being genotyped 1) to assess complexity of infection (six microsatellites) and 3) to analyze SNPs

using luminex-based assays (*pfmdr* N86Y, Y184F, and *pfcrt* K76T). 96% if cases were new infections at 28 days (n=11 recrudescent infections) in our high transmission setting. Transporter genotypes were compared between day 0 and day of failure, and grouped as wild-type (WT), mixed, or mutant. Thus far, n= 133 paired samples have been analyzed for *pfmdr* N86Y. On day 0, 81% were wild-type, 2.% mutant, and 17% mixed. At the time of recurrent parasitemia, N86Y genotype had not changed in 77% (n=103), WT was selected for in 18% (n=24), and mutant in 5% (n=6). For Y184F, n= 154 paired samples have been analyzed with 15% wild-type, 18% mutant, and 66% mixed on day 0. At the time of recurrent parasitemia, Y184F genotype did not demonstrate directional selection. The K76T mutation on *pfcrt* was genotype on day 0 (n=140 paired samples), with WT in 26%, mutant in 52%, and mixed in 22%. At the time of recurrent parasitemia, K76T genotype had not changed in 36% (n=45), WT was selected for in 42% (n=53) and mutant in 22% (n=27). Remaining samples will be completed for transporter genotyping. Following completion, the relationship between resistance selection (N86Y and K76T) and drug exposure will be assessed using previously quantified intensive and population PK parameters for lumefantrine. Final analysis will be presented.

1559

DUAL PRONGED ATTACK ON MALARIA: DRUGS WITH ANTIPARASITIC AND IMMUNOMODULATORY PROPERTIES

Jessica Simpson[1], Yash Gupta[2], Steven Goicoechea[1], Whelton A. Miller III[2], Brijesh Rathi[3], Ravi Durvasula[2], Prakasha Kempaiah[2]

[1]*Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States,* [2]*Loyola University Chicago Stritch School of Medicine and Department of Medicine, Loyola University Medical Center, Maywood, IL, United States,* [3]*Department of Chemistry, Hansraj College University Enclave, University of Delhi, Delhi, India and Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States*

Despite recent advancements in reducing the disease burden of malaria caused by *Plasmodium falciparum*, malaria still remains a significant global health problem. Although artemisinin combination therapies are first-line therapy for malaria, there is an urgent need for new drugs due to the development of drug resistant parasites. Additionally, it is well established that the severity of the disease depends upon the host immune response. In malaria, the erythrocytic stages are the only symptomatic stages, but the parasite evades the host immune system at multiple stages. Thus, a molecule with anti-parasitic activity and beneficial immunomodulatory effect on the host response to infection, could be more beneficial as a therapeutic drug. In this study, as part of our larger drug repurposing effort, we investigated three of the identified FDA-approved drug compounds identified to have anti-malarial activity in PfD6 and Dd2 strains with a mean 50% growth inhibitory concentration (IC50) in the erythrocyte stage of the parasite (IC50<2μg/ml). Using the in-vitro malaria (Hemozoin (PfHz) treated PBMCs) model, we found that these drugs rescued the dysregulated inflammatory mediators known to be modulated during malaria by PfHz. Fexofenadine Hydrochloride (antihistamine), Simvastatin (statin: lipid-lowering agent), and Posaconazole (antifungal) are immunomodulatory and active against *P. falciparum* strains (drug sensitive and resistant). We employed a reverse docking and datamining in-silico strategy to identify the targets of these compounds. The predicted interactions were validated by target modelling followed by molecular docking and molecular dynamics simulations. Currently, we are performing target specific assays to validate in-silico data to discover novel molecules with dual properties.

1560

SPATIAL PATTERNS OF ANTI-MALARIAL DRUG QUALITY AND AVAILABILITY PROVIDED BY PRIVATE AND PUBLIC OUTLETS IN EQUATORIAL GUINEA

Jordan M. Smith[1], Jeremias Nzamio[1], Restituto Mba Nguema[1], Norberto Bosepa Cuba Cuba[1], Gninoussa Akadiri[1], Wonder P. Phiri[1], Carlos Cortes[1], Matilde Riloha Rivas[2], Harpakash Kaur[3], Guillermo A. Garcia[4]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Equatorial Guinea, Malabo, Equatorial Guinea,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Medical Care Development International, Silver Spring, MD, United States*

Falsified and substandard artemisinin-based combination therapies (ACTs) represent a serious concern for malaria endemic countries, as they can endanger patients, contribute to drug resistance, and undermine public trust in national health systems. In order for Ministries of Health to take action against this threat, evidence on the prevalence of poor-quality ACTs must be provided through systematic assessments of drug quality availability. The Bioko Island Malaria Control Project (BIMCP) has carried out two such assessments in Equatorial Guinea, with the latest occurring in 2018 (drug content analysis currently underway). The latest assessment was undertaken throughout Bioko Island and in each of the five provincial seats within the continental region of Equatorial Guinea, during which 660 antimalarials were purchased from all 548 drug outlets using a mystery client sampling approach. This study aims to describe the spatial patterns of drug outlets selling ACTs in a socio-demographic and geo-political context. Prevalence of falsified and/or substandard ACTs will be associated with the outlet type and neighborhood characteristics within which they are sold, with special attention being given to outlet proximity to ports of entry. Prevalence of high-quality ACTs distributed through the public sector at no cost but were ultimately purchased from private outlets will also be quantified to describe patterns of commercial redistribution of freely available ACTs. The findings of this study will highlight the importance of national authorities to track the sale of ineffective ACTs and the commercial redistribution of high-quality freely available ACTs, which together may drive therapeutic failure and strain public health systems.

1561

DUAL SITE AND MECHANISM OF ACTION OF ARTEMISININ ANTIMALARIALS

Wenchuan Ma[1], **Victoria A. Balta**[2], Katy N. Olafson[1], Ognjen S. Miljanić[1], David J. Sullivan[2], Peter G. Vekilov[1], Jeffrey D. Rimer[1]

[1]*University of Houston, Houston, TX, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

The delayed clearance of *Plasmodium falciparum* parasites following artemisinin combination therapy is characterized by a ring stage-resistance to artemisinins measured by elevated drug pulse concentrations to inhibit ring stages. The trophozoite stages remain sensitive to low nanomolar drug. The phenotype is closely associated with the Kelch13 genotypic changes at numerous amino acids. Here we investigated for parasiticidal activity and mechanism of action on heme crystallization the heme-artemisinin adduct metabolite, previously thought to be inactive after heme or iron cleavage of the endoperoxide bridge. Heme-artemisinin adduct was synthesized in reducing conditions, column purified and validated by mass spectrometry with sharp peaks at *m/z* 838 and 898, with the absence of *m/z* 282 of free artemisinin. Observations of beta-hematin crystal growth inhibition in the presence of ART and H-ART by time-resolved *in situ* atomic force microscopy (AFM) revealed that the addition of non-activated ART had no effect on the surface features and the kinetics of layer nucleation and growth. In contrast heme artemisinin adduct demonstrated near irreversible heme crystal growth inhibition by absorbing at growth sites to prevent subsequent addition. Biologic validation of exogenous heme-artemisinin adduct effect on parasites showed 50% inhibition in the 10 to 70 nmolar range varying by

artemisinin or artesunate as the heme adduct in both resistant Kelch13 mutant and sensitive parasites. After a 6 hour exogenous 700 nM H-ART pulse of trophozoites, followed by extensively SDS/ bicarbonate/water washes to purify hemozoin, H-ART is detected with hemozoin heme by a mass spectrometry at *m/z*s 838. The previously thought inactive heme-artemisinin adduct kills parasites when exogenous in culture medium. Artemisinins work in the parasite cytosol at ring stages and in the cytosol and digestive vacuole at the trophozoite stage via suicide activation by iron or heme to generate radicals which damage bystander proteins. In the digestive vacuole there is an additional quinoline-like mechanism of heme crystal inhibition by the heme-artemisinin adduct.

1562

MOLECULAR SURVEILLANCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE IN NIGERIA

Ifeyinwa C. Aniebo[1], Wellington A. Oyibo[2], Oladipo Oladosu[2], Ndukwe Kalu Ukoha[3], Lilian Anomnachi[3], Gordon A. Awandare[4], Kelechi Ohiri[3]

[1]*Harvard T.H Chan School of Public Health, Boston, MA, United States,* [2]*ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine, University of Lagos, Lagos, Nigeria,* [3]*Health Strategy and Delivery Foundation, Abuja, Nigeria,* [4]*West Africa Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana*

Monitoring drug resistance is essential for early detection and subsequent prevention of the spread of drug resistance. We investigate the presence of mutations associated with malaria drug resistance in Lagos, Nigeria.A cross-sectional study was carried out in health facilities and households across 12 local government areas. Finger-prick blood samples from 200 consenting individuals of all ages provided blood films for microscopic examination and blood spots on filter paper. All samples were screened for malaria parasites using nested PCR. Direct sequencing was used to determine the frequency distribution of genetic variants in the anti-malarial drug-resistant *P. falciparum* genes in malaria-positive isolates. Complexity of infection of all samples were investigated using both the COIL and McCOIL.One hundred and nine (109) samples were positive for *P.falciparum*. The frequency of chloroquine resistant haplotype (CVIET) was 32% and NFD haplotype in *Pfmdr1* was 39%. There were 8 nonsynonymous mutations identified in *Pfkelch13* gene, two have been described while the others have not been described or validated in any known study. Mutations in *Pfdhfr* were present with the triple mutants having the highest frequency at 95% (IRNI). The quadruple mutation at position 164L was identified at very low frequency (0.9%). Mutations in *Pfdhps* were identified with haplotype frequency 35% (SGKAA), 17% (AGKGS) and 8.3% (AGKAA) being the majority. All samples were WT at positions V127M and D128Y/H in the *Pfarps10* protein, D193Y in ferredoxin, N326S in *Pfcrt* and T484I in *Pfmdr2*, however, 18% of the samples had the I356T mutation (VDDNTT). All samples were wildtype for *P.falciparum* exonuclease and *Plasmepsin*. Our results show that chloroquine appears to be in use even though Nigeria had a first-line treatment policy change to ACT's in 2005. The results show the presence of drug resistance to commonly used antimalarials and suggests piperaquine is likely to remain highly efficacious. Consequently, it is imperative to implement a national surveillance system for monitoring malaria drug resistance to inform resilient response to malaria control in Nigeria.

1563

MALARIA PARASITEMIA INCIDENCE AMONG DIFFERENT AGE GROUPS IN A STABLE TRANSMISSION AREA OF MALI RECEIVING SEASONAL MALARIA CHEMOPREVENTION

Abdoulaye Katile[1], Bourama Kamate[1], Cheick O. Guindo[1], Mamady Kone[1], Bakary Traore[1], Jacob Dara[1], Ousmane A. Poudiougo[1], Bayaya Haidara[1], Amatigue Zeguime[1], Allaye Tolo[1], M'Bouye Doucoure[1], Boucary Ouologuem[1], Souleymane Traore[1], Sidiki Perou[1], Baba Djiguiba[1], Mahamadou S. Sissoko[1], Issaka Sagara[1], Jen C.C. Hume[2], Jennifer Kwan[3], Patrick E. Duffy[2]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

The intensity and seasonality of malaria transmission influence the age distribution of parasitemia and disease, and age-specific incidence information is used by policymakers to plan the allocation of diagnostic and therapeutic tools. Doneguebougou is in a holoendemic area where malaria transmission is highly seasonal, typically from June to December. Under-five year old children have been receiving seasonal malaria chemoprevention (monthly sulfadoxine-pyrimethamine + amodiaquine) from August through November since 2014. We aimed to determine the current malaria parasitemia incidence rate among different age groups. These data were collected during a community study of the dynamics of malaria transmission. We screened and enrolled 597 volunteers aged 6 months to 65 years and followed for 5 months (August to December 2018). Blood samples were collected at routine monthly visits and the time of any clinical illness. Malaria infection diagnosis and the density of parasitemia were determined by RDT and blood smear respectively. The population was relatively young with a mean age of 19 years with 54% of the study group female. Among our study population, under-fives represented 18%, 5-12 year olds 28%, 13-18 year olds 20%, and >18 year olds 34%. *Plasmodium falciparum* was the most frequently encountered species at 94.4% of positive blood smears, while 3.9% were recorded as *Plasmodium malariae.* A total of 22% of the subjects carried *Plasmodium falciparum* gametocytes: 7% in under-fives years, 23% in 5-12 year olds, 31% in 13-18 year olds and 22% in >18 years olds. This higher incidence of malaria parasitemia in older versus younger children may reflect a shift in an area receiving seasonal malaria chemoprevention and should continue to be monitored. This data and additional clinical malaria cases will be presented with additional follow up of the cohort.

1564

DECREASING *IN VITRO* ARTEMISININ SENSITIVITY OF *PLASMODIUM FALCIPARUM* ACROSS INDIA

Rimi Chakrabarti[1], John White[1], Prasad H. Babar[1], Shiva Kumar[1], Devaraja Mudeppa Gouda[1], Anjali Mascarenhas[1], Ligia Pereira[1], Rashmi Dash[1], Jennifer N. Maki[1], Ambika Sharma[1], Kabita Gogoi[2], Devojit K. Sarma[2], Ipsita P. Bhowmick[2], Suresh Kumar[1], Edwin Gomes[3], Jagadish Mahanta[2], Pradyumna K. Mohapatra[2], Laura Chery[1], Pradipsinh Rathod[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Regional Medical Research Center - Northeast Region, Dibrugarh, India,* [3]*Goa Medical College and Hospital, Bambolim, India*

The geographic proximity and ecological similarities of northeastern India to the Greater Mekong subregion may affect the long-term management and sustainability of Artemisinin Combination Therapy (ACT) in India. ACT has been used to treat uncomplicated *Plasmodium falciparum* infections in India since 2004. However, since 2008, decreasing artemisinin effectiveness is seen throughout the Greater Mekong region. The resistant SE Asian parasites could propagate further to the 1.2 billion people of India who live in malaria transmission areas, and possibly even to Africa. To layout baseline data to track changing ACT resistance in

Indian parasites, 11 *P. falciparum* isolates from northeast India and 10 isolated from southwest India were studied *in vitro.* Ring Stage Survival Assay (RSA) showed significant parasite resistance to dihydroartemisinin from a remarkable 50% of the 2014-2015 northeast Indian samples. Even more surprising, 2/10 samples from the southwest region of India, from as far back as 2012, also showed decreased sensitivity to artemisinin. This establishes that RSAs will be useful for tracking changes in artemisinin effectiveness against *P. falciparum* in India with respect to geography and time, and for estimating the magnitude of parasite resistance. Such cell-based assays will also guide placement of clinical studies to assess patient responses to ACT in different parts of India. In the present work, Kelch gene sequences in Indian isolates were found to be unique but could not predict ACT resistance as measured by RSA. Unique Indian Kelch genotypes may still help track movements and evolution of artemisinin resistant parasites in India.

1565

CHARACTERIZATION AND ANALYSIS OF FALSE-NEGATIVE RAPID DIAGNOSTIC TESTS DUE TO *PFHRP2* AND *PFHRP3* DELETIONS IN WESTERN KENYA *PLASMODIUM FALCIPARUM* POPULATION

Nathaniel Idquival Dizon[1], Samuel Elberts[1], Karina Rivas[1], Janet Oyieko[2], Carolyne Kifude[2], Shirley Luckhart[3], V. Ann Stewart[1]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*US Army Medical Research Directorate-Kenya, Kisumu, Kenya,* [3]*University of Idaho, Moscow, ID, United States*

To combat rising drug costs and increasing drug resistance for *P. falciparum* infections, the WHO has recommended that only patients whose infections are confirmed via rapid diagnostic test (RDT) or microscopy be treated with anti-malarial drugs. Many of the common malarial RDTs rely on the detection of histidine-rich protein 2 and 3 (*PfHRP2/PfHRP3*) antigens. Recent published reports indicate that genetic polymorphisms in these genes include deletions, and that such polymorphisms may lead to false-negative RDT results. An increased prevalence of false negative RDT results due to *PfHRP2/3* gene mutations have become a concern, as the use of RDTs as a diagnostic tool has increased over microscopy. Using PCR we did a pilot study to determine the presence of *PfHRP2/3* deletions in blood samples from a population of asymptomatic adults who presented for voluntary HIV testing in Kisumu, Kenya. All human samples used were collected as part of a larger cross-sectional molecular epidemiology study of HIV and malaria status in pre-treatment asymptomatic adult individuals. Two single copy *P. falciparum* housekeeping genes were then compared and used to confirm parasitemia of *PfHRP2/3* negative blood samples, and determine a lower limit of DNA-based gene detection. We failed to amplify a significant number of *PfHRP2*, *PfHRP3,* and dual *PfHRP2/3* gene targets in our asymptomatic, *Plasmodium* 18S-positive sample population. We will obtain deep sequence information to confirm and further characterize any mutations in this sample set and compare with RDT results above a reasonable threshold of sensitivity. Our study will inform implications for the utility of current diagnostic assays for malaria control and eradication efforts.

1566

DETECTION OF DRUG RESISTANCE SNPS IN *PLASMODIUM FALCIPARUM* WITH THE CRISPR-BASED DIAGNOSTIC SHERLOCK

Clark H. Cunningham, Jonathan J. Juliano, Jonathan B. Parr

The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

CRISPR-based diagnostics are an exciting and rapidly-growing technology with a wide range of applications for detecting tropical diseases. These technologies combine the base-pairing abilities of CRISPR RNAs (crRNAs) and nuclease activity of Cas proteins to detect specific nucleic acid sequences with extreme sensitivity, achieving single-copy limits of detection. One promising CRISPR-based diagnostic assay is SHERLOCK, or Specific High-Sensitivity Enzymatic Reporter UnLOCKing. As previously published, SHERLOCK pairs isothermal recombinase-polymerase amplification (RPA), *in vitro* transcription, crRNA base-pairing, and collateral cleavage of fluorescent RNA reporters by Cas13a to detect nucleic acid targets. We previously developed SHERLOCK assays capable of detecting malaria, with pan-*Plasmodium*, *P. falciparum* and *P. vivax* species-specific SHERLOCK assays capable of detecting parasite DNA at levels at or below the sensitivity of current PCR assays. We are now harnessing SHERLOCK's ability to discriminate single nucleotide polymorphisms (SNPs) to develop assays capable of detecting drug resistance mutations in *P. falciparum*. We will present results from our ongoing efforts to adapt these assays for use at the point-of-care, involving both lateral flow and handheld fluorometry. We will also present optimized methods for streamlined sample collection and preparation. Taken together, these findings will demonstrate SHERLOCK's potential to improve patient care and molecular surveillance in diverse settings.

1567

QUALITY OF MALARIA CASE MANAGEMENT IN OUTPATIENT AT HEALTH FACILITIES IN RWANDA

Jean Louis Ndikumana Mangala[1], Aline Uwimana[1], Noella Umulisa[2], Michee S. Kabera[1], Aimable Mbituyumuremyi[1], Jean Damascene Niyonzima[1], Didier Uyizeye[2], Jeanine U. Condo[1]

[1]*Rwanda Biomedical Center, Kigali, Rwanda,* [2]*Maternal and Child Survival Program/JHPIEGO, Kigali, Rwanda*

Malaria remains the world's most important tropical parasitic disease, and one of the major public health challenges in the poorest countries of the world, particularly in sub-Saharan Africa. Although significant progress has been made, malaria remains one of the most important diseases in Rwanda, causing significant morbidity, mortality, and economic loss for the country. The objective of the Rwanda Malaria Health Facility Survey (RMHFS) was to assess health facility readiness for malaria diagnosis and treatment and to evaluate the supply chain and the quality of malaria case management. This survey was conducted in 100 health centers between July and September 2018. Facility Inventory Sheet, Health Worker Interview, Observation of Outpatient Consultations Tool and Exit Interviews were used for data collection after obtaining a signed consent form from respondents with respect to their confidentiality and anonymity. Eight teams of five interviewer-observers conducted the RMHFS. For 961 patients present at time of the survey, 152 patients were diagnosed of malaria using microscopy, with a positivity rate of 15.8%. Artemether and Lumefantrine for the treatment of simple malaria cases were available at 100% compared to pre-transfer treatment (Quinine and Artesunate) that were available at 88% in health facilities. Health providers in public health facilities trained in malaria case management and Integrated Management of Childhood Illnesses in the past 3 years represent 44.3% and 62.6% respectively compared to 37% and 61.1% in faith-based health facilities. Overall, 98.7% confirmed simple malaria cases were diagnosed using blood smear or rapid diagnostic test and received first line antimalarial treatment according to National policy at health centers. Findings of RMHFS show that although Rwanda is striving to improve access to early diagnosis and appropriate case management to reduce malaria burden through 24/7 continuity of care in all surveyed health facilities; but there is still need for training of staff in malaria case management and increasing availability of pre-transfer drugs.

1568

EVALUATION OF A NEW POINT OF CARE MALARIA DIAGNOSTIC DEVICE GAZELLE™: A PILOT STUDY

Praveen K. Bharti[1], Rajat Kumar[1], Priyaleela Thota[2], Anil K. Verma[1], Sweta Srivas[1], Tyler Witte[2], Anne Rocheleau[2], Mrigendra Singh[3], S. Rajasubramaniam[1], Aparup Das[1]

[1]*National Institute of Research in Tribal Health, Jabalpur, India,* [2]*Hemex Health, Portland, OR, United States,* [3]*National Institute of Malaria Research-Field station (NIMR-FS), Jabalpur, India*

Despite a considerable progress in control and management of malaria in India, malaria remains a major public health problem especially in rural and tribal parts of the country. Prompt and accurate diagnosis is very critical for management of malaria and also to achieve malaria elimination goals. However, diagnosis is a challenge in resource poor settings because of lack of infrastructure and unavailability of skilled healthcare workers. Currently, microscopy and RDT are the standard tools for malaria diagnosis in Primary/Community health centres as well as in the private sector. Although inexpensive, microscopy is labor intensive, time consuming, requires skilled manpower and has poor sensitivity and specificity, especially for low parasitemia. On the other hand, RDTs provide faster turnarounds, easy handling and storage but cannot reliably detect low density parasitemia and sensitivity is affected by malaria species and variants. In this pilot study, we evaluated the performance of Gazelle™, a point of care malaria diagnostic device which employs the principle of Magneto-Optical Detection (MOD) to detect hemozoin (a paramagnetic by-product of all malaria parasite species) in blood sample for diagnosis of malaria. In the pilot study conducted with a total of 300 samples, the accuracy of Gazelle™ was found to be 94.7%, 94.3% and 95.4% in comparison to microscopy, RDTs and PCR respectively. When compared with PCR, Gazelle™ had higher accuracy than microscopy and RDTs. Gazelle is faster than microscopy, RDT, and PCR and it is a field-oriented device. Notably, the performance of Gazelle™ was comparable with microscopy; specificity of Gazelle™ was higher than RDTs. A validation study with a larger sample size (~1000 samples) is currently ongoing and the final results will be presented at the conference. Gazelle™ may be an alternative potential diagnostic solution for settings where there is a need for speed, accuracy and ease of use.

1569

ASSESSMENT OF MALARIA USING NEW HIGHLY SENSITIVE RAPID DIAGNOSTIC TEST IN TWO TOWNSHIPS SELECTED FOR SUBNATIONAL MALARIA ELIMINATION IN MYANMAR

Myaing Myaing Nyunt[1], Nay Yi Yi Lin[2], Zaw Lin[2], Aung Thi[3], Tint Wai[2], Poe Poe Aung[2], Zaw Win Thein[2], Thura Htay[2], Zin Min Tun[2], Aye Kyawt Paing[2], Drzayar Han[2], Kaythwe Han[2], Christopher Plowe[1], Norbert Odero[1], Alyssa `. Platt[1], Elizabeth Turner[1], Manfred Meng[1]

[1]*Duke University, Durham, NC, United States,* [2]*Myanmar Ministry of Health and Sports, Yangon, Myanmar,* [3]*Ministry of Health and Sports, Yangon, Myanmar*

Malaria-associated morbidity and mortality have been progressively declining in Myanmar in the last five years. Myanmar is committed to eliminate malaria by 2030, and five regions with the lowest malaria burden have been slated for subnational elimination. An extensive cross-sectional survey of malaria was conducted in January-December, 2018 in two townships in Mandalay, one of the five regions, in preparation for the assessment of different approaches such as targeted mass drug administration or focal screen and treatment using appropriately-sensitive diagnostics for accelerated elimination. The major objectives were to evaluate the baseline prevalence of asymptomatic malaria reservoir and to assess the performance characteristics of a new highly sensitive rapid diagnostic test (hsRDT), at a population level. Villages with potential malaria transmission were selected using a multistage cluster sampling with lot quality assurance method. Extensive data on demographic information, malaria history, occupational, travel, migration, mobility information were collected from 11,128 consented participants. Finger prick blood was collected for testing of malaria, using routine conventional rapid diagnostic test (cRDT), and new highly sensitive RDT (hsRDT). Blood was also collected as dried blood spots for ultrasensitive PCR testing, as gold standard, conducted at the malaria laboratory at the Department of Medical Research in Yangon, Myanmar. External quality control was provided by an independent analytical team at the malaria laboratory at Duke Global Health Institute, Duke University. Preliminary analyses showed that hsRDT identified 2–3-folds more *P. falciparum* infections than cRDT. Individual and village-based performance characteristics of hsRDT, using usPCR as gold standard, heterogeneity of malaria in the study sites, and risk factors associated with asymptomatic malaria will be presented. The potential role of the new diagnostics hsRDT in identifying populations with low-density malaria in pre-elimination setting will be discussed.

1570

A REPORT ON THE INTEGRATION OF A MALARIA RAPID DIAGNOSTIC TEST IN A POINT OF CARE CLINICAL DECISION SUPPORT PLATFORM, MEDSCINC, FOR USE IN PRIMARY HEALTHCARE SETTINGS IN KANO STATE, NIGERIA

Karell G. Pellé[1], Meg McLaughlin[2], Aisha Giwa[3], Ezra Mount-Finette[2], Sam Scarpino[4], Nada Haidar[3], Fatima Adamu[3], Temitope Adeyoju[3], Nirmal Ravi[3], Adam Thompson[3], Barry Finette[5], Sabine Dittrich[1]

[1]*Foundation for Innovative New Diagnostics, Geneva, Switzerland,* [2]*THINKMD, Burlington, VT, United States,* [3]*eHealth Africa, Kano, Nigeria,* [4]*Northeastern University, Boston, MA, United States,* [5]*THINKMD and University of Vermont College of Medicine, Burlington, VT, United States*

In primary health care (PHC) settings in developing countries, clinical algorithms such as WHO's Integrated Management of Childhood Illness (IMCI) are widely used by healthcare workers (HCWs) to provide treatment and care. However, adherence to these paper guidelines is often poor. One solution to this issue is to provide computed clinical care algorithms or clinical decision support systems (CDSS). In these algorithms, tools such as thermometers or malaria RDTs (mRDTs) are recommended to perform accurate assessments and provide disease diagnoses. MEDSCINC is a point of care (POC) CDS platform that determines disease risks for several childhood illnesses based on Bayesian cluster pattern logic independent of diagnostic tests such as malaria RDTs. As malaria is a common childhood febrile illness in sub-Saharan Africa, we hypothesized that integrating mRDT results within the algorithm is feasible and would improve malaria assessment compared to standard care, IMCI. In a pilot in Kano State Nigeria, 7 HCWs in 5 PHCs were trained to use a modified MEDSCINC algorithm that integrates an mRDT. In total, 555 children ages 2-59 months were assessed with the platform during a 4-week period. With the platform, mRDTs were performed on all 480 children who presented with fever or a history of fever, per IMCI protocol. Of those, 66.7% were mRDT positive and 33.3% mRDT negative. In terms of performance accuracy, MEDSCINC had a sensitivity of 42.8% (95% CI: 37.3-48.4) and a specificity of 63.8% (95% CI: 55.7-71.1). In addition, the MEDSCINC malaria assessments positive predictive value (71%) was better than historical (50%) and current (67%) Nigeria IMCI screening. This study shows the feasibility of integrating mRDTs into CDSS and provides insight into the accuracy of MEDSCINC to predict malaria risk, enabling algorithm improvements tailored to high burden settings during and after use. It also shows the importance of validating new or predictive algorithms alone and with POC diagnostics whenever available and highlights the need for publicly available datasets generated from relevant patient cohorts to develop and improve clinical algorithms.

1571

FIELD PERFORMANCE OF CONVENTIONAL AND HIGH-SENSITIVITY MALARIA RAPID DIAGNOSTIC TESTS IN TWO TRANSMISSION SETTINGS IN HAITI

Eric Rogier[1], Karen E. Hamre[1], Vena Joseph[2], Mateusz Plucinski[1], Jacquelin Presume[3], Ithamare Romilus[3], Gina Mondelus[3], Tamara Elisme[3], Lotus L. van den Hoogen[4], Jean F. Lemoine[5], Chris Drakeley[4], Ruth Ashton[2], Michelle A. Chang[1], Alexandre Existe[3], Jacques Boncy[3], Gillian Stresman[4], Thomas Druetz[6], Thomas Eisele[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [3]*Laboratorie National de Santé Publique, Port au Prince, Haiti,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Ministère de la Santé Publique et de la Population, Port au Prince, Haiti,* [6]*University of Montreal School of Public Health, Montreal, QC, Canada*

Accurate diagnosis of malaria serves as the foundation for multiple elimination and control strategies. Finding active infections becomes increasingly difficult as *Plasmodium falciparum* transmission is reduced in an area, and new diagnostic tools could serve to more accurately detect asymptomatic infection and estimate prevalence rates in these low-transmission and elimination settings. Malaria elimination in Haiti relies on histidine-rich protein 2 (HRP2) field-deployable rapid diagnostic tests (RDTs) for routine identification of *P. falciparum* infections in both clinical and community settings. In 2017, two easy-access group (EAG) and one household-based survey took place in Haiti for which enrollees (N=32,506 total) were tested by a conventional RDT (cRDT) and high-sensitivity RDT (hsRDT). Subsets of participants were selected from each survey and filter paper blood samples assayed for HRP2 and malaria DNA in a laboratory (n=1,154 total) for direct comparison with RDT results. Both cRDT and hsRDTs were able to detect low concentrations of HRP2 antigen in participant's blood with sensitivity estimates between 2.6 and 14.6 ng/mL. In comparison with the laboratory HRP2 assay, the sensitivity estimates in the two EAGs were 86.3% and 95.0% for the cRDT and 88.2% and 96.0% for the hsRDT. Specificity in the two EAGs was 99.6% and 92.9% for the cRDT and 98.0% and 90.0% for the hsRDT. In the EAG surveys, we observed no significant differences between the estimated detection levels for HRP2 and parasite DNA in host blood. In the household-based survey, the hsRDT returned a significantly higher number of positive tests than DNA detection in all age categories, but this represented a very small proportion (less than 0.2%) of all participants in the survey. In this low-transmission setting, both types of RDTs performed well. The hsRDT identified more antigen positive persons than the cRDT in all three surveys, but this was only significant in the household survey, and was a small proportion of additional positives from all three surveys.

1572

COMPARISON AND EVALUATION OF TWO PCR ASSAYS FOR THE QUALITATIVE DETECTION OF *PLASMODIUM* SPECIES IN CLINICAL SPECIMENS

Lynne Sloan, Emily Fernholz, Heather Arguello, Susan Schneider, Bobbi Pritt

Mayo Clinic, Rochester, MN, United States

Malaria is a major tropical disease caused primarily by four members of the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale.* A fifth species, *P. knowlesi,* is responsible for a significant number of malaria cases in parts of Southeast Asia. While microscopic examination of Giemsa-stained thick and thin blood films remains the gold standard method for laboratory diagnosis, PCR-based tests are increasingly available and offer increased sensitivity and specificity over conventional microscopy. We compared the performance of a commercially-available real-time TaqMan PCR assay to a previously-published, laboratory developed real-time FRET probe-based PCR test (LCMAL) for the detection and differentiation of *Plasmodium* species in clinical specimens. Forty-six archived microscopy positive whole blood specimens were concurrently tested by the LCMAL and the RealStar® Malaria PCR by Altona Diagnostics (RSMAL) using Roche LightCyclers following DNA extraction using the Roche MagNA Pure LC 2.0. Both PCR assays detect and differentiate the five *Plasmodium* spp., but the LCMAL cannot reliably differentiate *P. vivax* from *P. ovale,* which is a known limitation of this assay. The LCMAL detected *Plasmodium* DNA from all 46 specimens as follows: 19 *P. falciparum*, 2 *P. vivax*, 3 *P. malariae*, 6 *P. ovale* and 16 *P. vivax/P. ovale.* In comparison, the RSMAL PCR assay detected 45 of 46 specimens: 18 *P. falciparum*, 11 *P. vivax*, 3 *P. malariae*, and 13 *P. ovale*; the single discordant result (LCMAL+/RSMAL-) contained a very low level of *P. falciparum* (cycle threshold >35 cycles). Original microscopy results showed: 19 *P. falciparum*, 9 *P. vivax*, 3 *P. malariae*, 4 *P. ovale,* 10 *P. vivax/P. ovale* and 1 *Plasmodium* sp., NOS. The RSMAL PCR showed an excellent (97.8%) agreement with the LCMAL. While both PCR assays compared well to the original pre-archival microscopy results, the RSMAL had the added advantage over the LCMAL and microscopy of reliably identifying *P. vivax/P. ovale* results to the species level. As a commercially-available kit, the RSMAL may be easier to implement in a clinical laboratory than the LCMAL which requires separately-sourced custom reagents.

1573

EVALUATION OF A PAN *PLASMODIUM* LACTATE DEHYDROGENASE DETECTION ASSAY USING MICROCAPILLARY CYTOMETRY

Xuemei Wan, Julie Clor, **Kamala Tyagarajan**

Luminex Corporation, Hayward, CA, United States

The rise of HRP2 deletions worldwide has increased the importance of *Plasmodium* Lactate Dehydrogenase (LDH) markers for malarial *Plasmodium* species detection. While several RDTs have been developed for detection of Pan LDH, sensitivity of RDT-based detection for this antigen remains poor. We have recently demonstrated that the microcapillary-based, low-cost Muse® Cell Analyzer and bead-based immunoassays have the capability to provide high sensitivity, yes/no confirmation on the presence of multiple *Plasmodium* antigens. Using this approach, the performance of the research use only Muse® Malaria *P.f.-P.v.* Detection Assay for the detection of *P. falciparum* HRP2, *P. falciparum* LDH, and *P. vivax* LDH was shown. Here we describe a new assay on the Muse System that allows for detection of the Pan LDH antigen region in different *Plasmodium* species. The assay was developed to allow for high sensitivity detection of LDH from *P. falciparum*, *P. vivax,* and *P. malariae,* allowing for better characterization of samples. Our data demonstrate the Pan LDH assay is capable of LDH antigen detection at low ng/mL and is superior to RDTs. Further, linear response is observed over a wide dynamic range of LDH antigen concentrations. Analysis of frozen blood samples with the Pan LDH Assay demonstrates that the assay can universally detect *P. falciparum* LDH, *P. vivax.* LDH, and *P. malariae* LDH, and LDH from mixed infections. Clear responses can be observed in a wide range of parasitemia. Testing of *P. malariae* samples with the Muse Malaria *P.f.-P.v.* Detection Assay demonstrates no cross-reactivity of the assay. Dilution studies demonstrate the capability to detect Pan LDH from *P. malariae* samples down to 50-100 parasites/µL, and more sensitive detection capability with *P. vivax* and *P. falciparum* samples down to <20 parasites/µL. The availability of a simplified, yes/no, sensitive Pan LDH-based assay on the Muse Cell Analyzer provides a powerful tool for malarial researchers to optimally characterize *Plasmodium* antigens.

1574

PERFORMANCE EVALUATION OF A NOVEL MULTIPLEXED LATERAL FLOW ASSAY TO IDENTIFY COMMON CAUSES OF FEVER IN ASIA AND INFORM TREATMENT DECISIONS

Sonia Arafah[1], Stuart Blacksell[2], Mark Mayo[3], Bart Currie[3], Aurelien Macé[1], Stefano Ongarello[1], Angelo Gunasekera[4], Javan Esfandiari[4], Sabine Dittrich[1]

[1]*FIND, Geneva, Switzerland,* [2]*Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University and Oxford*

University, Bangkok, Thailand, [3]Menzies School of Health Research, Darwin, Australia, [4]Chembio Diagnostic Systems, Inc., Medford, NY, United States

Fever is the most frequent symptom in patients seeking care globally and particularly in low and middle income countries (LMICs); several infectious causes of fever have been identified and are due to parasitic, viral or bacterial agents. In South East Asia, malaria, dengue, *Rickettsial pathogens* (like *Orientia tsutsugamushi* and *R. typhi*), *Leptospira* and *Burkholderia pseudomallei* have been described as causes of febrile illness with a high prevalence, depending on the population and setting. Patient management is however often driven by detection of malaria with RDTs: malaria negative cases are usually treated with broad spectrum antibiotics, contributing to the raise of global antimicrobial resistance. To circumvent those common practices, more guidance is required to inform treatment decision and allow more targeted treatment of Acute Febrile Illness (AFI). Conventional laboratory methods require a lot of resources and are not always performed in LMICs. Several single-plex RDTs are currently on the market and could help clinicians but due to cost, procurement challenges, and limited data on their performance, they are rarely used. Chembio has developed a multiplex lateral flow immunoassay (DPP® Fever Panel II Assay) that is able to detect serum IgM and specific antigens of common treatable causes of AFI in Asia (malaria, dengue, chikungunya, scrub and murine typhus, *Leptospirosis* and *Melioidosis*). This multiplex RDT aims to support treatment decisions at level 2 or 1 facilities with all bacterial pathogens requiring a change to specific antibiotics (eg. Doxycycline). A retrospective clinical study is being conducted in Thailand and Australia to assess the performance (sensitivity, specificity, positive and negative predictive values) of the test. A total of 510 samples are being tested from 3 countries (Bangladesh, Sri Lanka, Australia). All test lines are assessed in comparison to the appropriate gold standard diagnostic (e.g. IFA, MAT, blood culture). The presented results represent the first data from this novel tool and are instrumental to inform its utility and impact on prescribing practices and health outcomes in the region.

1575

DISTINGUISHING BETWEEN *PLASMODIUM FALCIPARUM* AND *P. VIVAX* BY CONSIDERING BROWNIAN RELAXATION TIMES USING MAGENTO-OPTICAL DETECTION (MOD)

Robert J. Deissler[1], D'Arbra Blankenship[2], Emma McCann[2], Brian T. Grimberg[2]

[1]*Department of Physics, Case Western Reserve University, Cleveland, OH, United States,* [2]*Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States*

As pointed out by the CDC, once a determination of malaria infection has been made, one of the factors that should guide treatment is the infecting *Plasmodium* species. Here we propose a method to distinguish between *P. falciparum* and *P. vivax* using our magneto-optical detection (MOD) device, which relies on the magnetic and optical properties of the hemozoin produced by the infecting parasites. A beam of polarized light is projected through a blood sample. As a magnetic field is applied to the blood sample, the hemozoin crystals align, causing a change in the transmitted light intensity. When the field is removed, the hemozoin relax back to their random orientations due to the thermal motion of the blood molecules, causing the light intensity to return to its previous value. The time for this relaxation to take place, referred to as the Brownian relaxation time, depends on the size of the hemozoin crystals, being proportional to the cube of the length of the crystals. Since the length of *P. vixax* crystals are roughly an order of magnitude less than the full length of *P. falciparum* crystals, the Brownian relaxation time of the *P. vixax* crystals will be roughly three orders of magnitude less than the relaxation time of the *P. falciparum* crystals. In order to distinguish between these two crystal sizes based on the relaxation times, we found that it was necessary to increase the viscosity of the blood samples. In this way we found that it was possible to distinguish between *P. falciparum* and *P. vivax* infections.

1576

VALIDATION OF BREATH BIOMARKERS FOR NONINVASIVE DIAGNOSIS OF MALARIA

Amalia Berna[1], Lucy Bollinger[2], Josephine Banda[3], Patricia Mawindo[3], Tasha Evanoff[1], Diana Culbertson[4], Karl Seydel[5], **Audrey R. Odom John**[1]

[1]*Washington University School of Medicine, St. Louis, MO, United States,* [2]*University of Washington, Seattle, WA, United States,* [3]*University of Malawi College of Medicine, Blantyre, Malawi,* [4]*Lao Friends Hospital for Children, Luang Prabang, Lao People's Democratic Republic,* [5]*Michigan State College of Osteopathic Medicine, East Lansing, MI, United States*

Despite substantial worldwide investment in malaria control, *Plasmodium falciparum* infection still remains a serious global health problem. The most widely available and sensitive RDTs rely on detection of a *P. falciparum*-specific protein, HRP2. Unfortunately, HRP2-based RDTs possess critical weaknesses, including rising incidence of *hrp2*- parasite strains. In clinical settings, there is a pressing need for new highly sensitive and specific tests that are simple, affordable. We previously used state-of-the-art mass spectrometry techniques to identify volatile organic compounds (VOCs) produced by cultured *P. falciparum*. Recently, we have found that uncomplicated falciparum malaria leads to characteristic changes in breath VOC composition. Using just six breath biomarkers, we diagnosed falciparum malaria noninvasively with over 82% accuracy in children from Lilongwe, Malawi. Here we present work from our ongoing studies to determine the reproducible changes in breath composition in response to malaria infection, in an independent cohort of children in a malaria endemic area. In addition, we evaluate breath composition before and after antimalarial use, to establish biomarkers that change in response to treatment. We are currently enrolling children aged 4-12 with and without uncomplicated *P. falciparum* malaria in Blantyre, Malawi for breath analysis. Blood smear test or RDT is used to diagnose malaria. Furthermore, blood samples will also be used for PCR quantification of asexual parasites and gametocytes. We expect that our data will provide robust evidence that infections such as malaria cause specific, reproducible changes in breath VOCs. Critically, our studies will determine the limit of parasite detection by breath VOCs. In addition, our work will inform the fundamental biological principles that drive breath volatile production during clinical pediatric malaria.

1577

EVALUATION OF A NOVEL HEMATOLOGY ANALYZER FOR MALARIA DIAGNOSIS USING FINGER-PRICK BLOOD IN AN ENDEMIC AREA OF COLOMBIA

Tatiana Maria Lopera-Mesa[1], Lina Zuluaga-Idarraga[1], Alexandra Rios[1], Veronica Sierra[1], Edwar Garzón[1], Ikki Takehara[2], Yuji Toya[2], Chiaki Takeuchi[2], Kinya Uchihashi[2], Alberto Tobón-Castaño[2]

[1]*Universidad de Antioquia, Medellin, Colombia,* [2]*Sysmex Corporation, Kobe, Japan*

As defined by WHO guidelines, finger-prick blood collection is a standard method of malaria diagnosis, and is preferable for new diagnosis methods in endemic areas. The XN-30 is an analyzer that requires only small amounts of fresh blood (20uL min.), and is able to perform simultaneously complete blood-cell count (CBC) with 8 parameters as well as quantitative analysis of malaria-infected cells, for research purposes. This study describes the XN-30 performance for malaria parameters compared to microscopy and real-time PCR (RT-PCR), when measured from finger-prick blood, and the performance of CBC parameters between venous and finger-prick blood in an endemic area of Colombia where *P. falciparum* and *P. vivax* are present. A cohort of 60 subjects with acute febrile syndrome were enrolled (30 malaria negative and 30 malaria positive). Malaria diagnosis was done by microscopy and RDT in the field, and it was later confirmed by RT-PCR. Venous and finger-prick blood in EDTA collected from single patients were processed on the XN-30. The sensitivity, specificity, positive/negative predictive values (PPV, NPV), and likelihood ratio of positive and negative tests (LRP and LRN) were

calculated. The intra-class correlation coefficient (ICC) and Bland-Altman plot were used to evaluate concordance of parasitemia. Compared with microscopy, *Plasmodium* detection in the XN-30 showed a sensitivity of 93.6% (95% CI 83.3 - 100), specificity of 100% (95% CI 98.2 - 100), PPV of 100% (95% CI 98.3 - 100) and NPV of 93.3% (95% CI 82.7 - 100). Concordance analysis of parasitemia between microscopy thick smear and the XN-30 showed ICC 0.89 (95% CI 0.78 - 0.95) and compared with thin smear ICC 0.99 (95% CI 0.97 - 0.99). In concordance analysis of the XN-30 CBC parameters between venous and finger-prick blood, ICC of WBC/RBC/HGB/HCT/MCV/PLT were 0.82 (95% CI 0.71 - 0.89) / 0.86 (95% CI 0.78 - 0.92)/ 0.93 (95% CI 0.88 - 0.96)/ 0.90 (95% CI 0.84 - 0.94)/ 0.88 (95% CI 0.80 - 0.92) / 0.94 (95% CI 0.90 - 0.96) respectively. These results suggest that the XN-30 is effective for malaria detection and CBC parameters, even when analyzing finger-prick collected blood.

1578

INFORMATION ON MALARIA DIAGNOSIS AND TREATMENT INCLUDED IN HEALTH MANAGEMENT INFORMATION SYSTEMS IN 23 COUNTRIES

Emily Stammer, Kate Gilroy, Michel Pacqué

John Snow Inc/MCSP, Washington, DC, United States

Most low and middle-income countries use data from health management information systems (HMIS) to monitor malaria programs on a routine basis. WHO provides guidance for malaria control program indicators, however the data elements needed for reporting these indicators are often not included in national HMIS. The USAID Maternal and Child Survival Program conducted a review of child health data elements, including malaria, in 23 low and middle-income countries' HMIS to identify commonalities and gaps. We collected national HMIS registers, summary forms and patient forms used at the facility and community level and extracted the data elements on each form or register into a standardized template. A number of malaria data elements are more commonly collected at the community level than the facility level. At the community level, 10 out of 23 countries consistently capture (in both registers and summary forms) information about malaria diagnosis using rapid diagnostic tests (RDT) among children under five years of age (< 5) compared with 7 out of 23 countries capturing the same information at the facility level. We found that 9 out of 23 countries consistently capture the treatment of RDT+ children < 5 at the community level compared with 3 out of the 23 at the facility level. At the facility level, the majority of data related to malaria diagnosis and treatment is collected only in summary forms, indicating the summary forms rely on data aggregated from open "diagnosis" fields in registers, which has implications for quality of the data. Finally, about half of the countries do not report on malaria treatment at all in their community (10 of 23 countries) or facility forms (13 of 23 countries). Not all countries are routinely collecting and reporting WHO-recommended malaria diagnosis and treatment data elements, which are necessary to monitor malaria management and epidemiology across geographic areas, time or service locations. The findings can help country and global stakeholders understand what data elements are available in each country's routine HMIS to calculate key indicators and advocate for inclusion of priority indicators during HMIS reviews.

1579

MALARIA MICROSCOPY PARAMETERS AFFECTING ATTAINMENT OF COMPETENCY LEVELS IN NATIONAL COMPETENCY ASSESSMENTS IN GHANA, 2019

Alexander Asamoah, Mildred Komey, Akosua Gyasi Darkwa, Keziah Lawrencia Malm

National Malaria Control Program, Accra, Ghana

Malaria tests are now the bedrock of malaria diagnosis. As microscopy remain the gold standard for testing, the competency of malaria microscopists to retain prescribers' confidence and improve case management cannot be overemphasized. As such the National Malaria Control Programme set out to determine the competency levels of regional malaria microscopists, certify them and determine parameters that affect attainment of competency levels in a national competency assessment in malaria microscopy for onward training of laboratory scientists. A 5-day national competency assessment in malaria microscopy was conducted in 3 sessions across the country from November 2018 to April 2019. Malaria microscopists were purposively selected to participate in the assessment. Each microscopist examined 74 blood slides obtained from Slide Bank. The microscropists' assessments were scored and graded using a standardized computer-based template. The assessment scores for malaria microscopy parameters (parasite detection, species identification and density determination) was used to determine competency levels. Analysis of Odds Ratio was done using Epi-Info version 7 stat cal to determine associations between parameters. Of 80 microscopists assessed, 61% (49) attained either Level A or B competence. All regions obtained at least one certified microscopist with Level A (expert) competence. The proportion of microscopists who could not attain competency levels in all three areas assessed was 26% (8/31), two was 16% (5/31) and in one area was 55% (17/31). Species identification scores accounted for 39% (12/31) of microscopists not attaining competency levels. The odds of attaining competency levels with species identification was about 3 times OR=2.85(CI:1.29-6.30) whilst parasite density was about 2 times OR=1.69(CI:0.74-3.89) than compared with parasite detection. Microscopists that attained Levels A and B were certified as National malaria microscopists. Species identification was significantly associated with attainment of competency levels. We recommend regular training in species identification for effective case management.

1580

OPENMM - A LOW-COST, MODULAR, AND AUTONOMOUS MICROSCOPE FOR MALARIA DIAGNOSIS AND BEYOND

Hongquan Li, Hazel Soto-Montoya, Lucas F. Valenzuela, Maxime Voisin, Manu Prakash

Stanford University, Stanford, CA, United States

Microscopy has been the gold standard for the diagnosis of malaria since its first adoption more than a century ago. Today the practice of manually examining stained blood smear with an oil immersion objective remains largely the same. While more than 200 million patients were tested by microscopic examination in the year of 2016, implementation of microscopy-based diagnosis is limited by the requirement of trained technicians, very low throughput and presence of errors due to human factors. With the advent of artificial intelligence and ever-increasing compute power at the edge, automated robotic microscopes are poised to enable a new era in the diagnosis of malaria and many other diseases but current platforms remain cost prohibitive. Here we present openMM, a low-cost, open-source, modular and autonomous microscopy platform capable of brightfield/darkfield and fluorescent imaging. Modularity of the platform allows rapid reconfiguration of the microscope and makes it easy to tailor the platform to specific applications. Motorized slide scanning, autofocus as well as software processing pipelines make the platform autonomous and give rise to high throughput. The cost of 300 USD makes wide deployment of the tool in resource-limited settings possible. At low magnification, initial tests in the lab suggest achievable detection limit of better than 150 parasites/ul of blood for *Plasmodium* Falciparum, with imaging speed of more than 1 million blood cells per minute. We have also developed a high magnification module, using which morphology of ring stage parasites are clearly resolved. With about two orders of magnitude in cost reduction, openMM will open up access to networks of robotic microscopes and bring together communities with diverse expertise and accesses to complementary resources for collectively advancing microscopy-based disease diagnostics.

1581

MALARIA DIAGNOSIS IN THE MOBILE GOLD MINERS' POPULATION OF SURINAME IN 2018: AN EVALUATION OF THE MALARIA SERVICE DELIVERS' SYSTEM USING RAPID DIAGNOSTIC TEST

Hedley Cairo

Ministry of Health Malaria Program, Paramaribo, Suriname

Currently malaria infections in Suriname occur mainly among persons engaged in small-scale gold mining and related activities. Since 2006, lay persons living in mining areas have been trained to perform malaria diagnosis with Rapid Diagnostic Test (RDT) and to treat malaria. Blood films are also prepared for each subject tested. Blood smear prepared by the Malaria Service Deliverers (MSDs) in the miners' communities are sent to TropClinic malaria laboratory in the Capital Paramaribo. The TropClinic is staffed with trained malaria microscopy technicians. In Suriname and many other countries, microscopy is considered the gold standard for malaria diagnosis. A study was conducted to evaluate the accuracy of MSD system using RDTs in the field compared to microscopy done at the central clinic for malaria diagnosis in Suriname. The EpiTools and MedCalc websites were used for the calculation of the RDT test performance characteristics. In 2018, 2265 subjects were tested by the MSDs with RDT. For 2022 (89.3%) RDT result a microscopy result was available for comparison. The majority of malaria cases, 88 (37.3%) of the 236 cases diagnosed in Suriname in 2018, were diagnosed by the MSD network. Considering microscopy as the reference test, the sensitivity and specificity of the MSD system were respectively 88.1% [95%CI: 77.8 - 94.7] and 98.4% [95%CI: 97.8 - 98.9] . The PPV was 65.5% [95%CI: 57.0 - 73.2]. The NPV system was 99.6% [95%CI: 99.2 - 99.8]. The overall accuracy of the MSD system was 98.1% [95% CI: 97.37 - 98.6]. Early and accurate malaria diagnosis is essential to initiate prompt and adequate treatment. The sensitivity of the MSD system calculated was below the 95% recommended by the World Health Organization. Continuous training of the workers and ensuring optimal conditions for the storage of RDTs in the field could improve the performance of the MSDs. These result should be interpreted with some degree of reserve, because there are studies in which RDT and microscopy are compared to PCR as reference test revealing a higher sensitivity for RDT. This study emphasizes the need of a QA & QC system for diagnostics to achieve the malaria elimination goal.

1582

ADVANTAGES, DISADVANTAGES AND PITFALLS IN THE DETECTION OF MIXED-SPECIES MALARIA CASES IN A NON-ENDEMIC SETTING

Alexander Oberli, Trang Ha Thu Nguyen, Konrad Mühlethaler

Institute for Infectious Diseases, Bern, Switzerland

A precise laboratory diagnosis of the *Plasmodium* spp. causing malaria is of critical importance with regard of a species targeted treatment. So far, microscopic detection and identification of *Plasmodium* spp. in Giemsa-stained blood smears is the gold standard for the laboratory diagnosis of malaria. Nevertheless, in clinical practice mixed-species malaria infections are often not detected by light microscopy (LM) or rapid diagnostic test (RDT), as a notable decrease in the number of parasites of one species may occur. Mixed species infections in humans with *P. vivax* and *P. ovale* have only been described in a triple or quadriple species infection together with *P. falciparum* and/or *P. malariae*. The case of an 8-year-old girl migrating with her family from Afghanistan to Central Europe is to our knowledge the first report of a mixed *P. vivax* / *P.ovale* infection confirmed by LM and molecular diagnostic tools. Subsequent molecular testing of other family members revealed a submicroscopic *P.vivax* / *P. falciparum* mixed infection of one family member, indicating that microscopic examination of blood smears does not reliably distinguish *Plasmodium* spp., especially when one species dominates the other numerically. To our knowledge this is the first documentation of an uncommon two-species mixed infection comprising both *P. vivax* and *P. ovale*, confirmed by light microscopy and different molecular diagnostic tools. Here, we discuss and compare advantages, disadvantages and pitfalls of LM, RDTs, commercially available multiplex rtPCR and species-specific singleplex rtPCR for the detection of mixed-species infections in the case of a migrating refugee family but also in a general non-endemic setting.

1583

HIGHLY SENSITIVE MALARIA DIAGNOSITCS USING A NOVEL SET OF ANTI-PFHRP2 ANTIBODIES

Rolf Fendel[1], Andrea Kreidenweiss[1], Johanna Griesbaum[1], Sofia Dembski[2], Torsten Klockenbring[3]

[1]*Institute of Tropical Medicine, Tübingen, Germany,* [2]*Fraunhofer Institute for Silicate Research ISC, Würzburg, Germany,* [3]*Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany*

Despite global efforts to stop malaria , *Plasmodium falciparum* transmission is ongoing, and mortality remains high. WHO recommends diagnostic confirmation before starting antimalarial treatment. Most rapid diagnostic tests currently in use are detecting *Plasmodium falciparum* histidine rich protein (PfHRP2). Overall, current PfHRP2-RDTs have an acceptable performance but fail to detect malaria parasites a lower parasitemia. In the present work, we generated a novel set of anti-PfHRP2 antibodies with high affinity against PfHRP2 antigen. Several protein assays (ELISA, TR-FRET, etc.) were conducted to compare the performance and detection limit of our antibodies to commercially available anti-HRP2 antibody sets The novel anti-HRP2 antibodies specifically detected various HRP2 antigen products at considerably lower quantities. The limit of detection using the novel antibodies was below 10 pg/ml which was significantly lower than the commercially available antibodies under the same experimental setup. In conclusion, the present work describes a novel set of anti-HRP2 antibodies with high potential for use in malaria diagnostic tests. In combination with new technological approaches (TR-FRET), sensitive and simple assays for malaria diagnosis are within reach.

1584

SYSTEMATIC REVIEW OF STATISTICAL METHODS FOR ANALYSIS OF SAFETY DATA IN MALARIA CHEMOPREVENTION AND TREATMENT IN PREGNANCY CLINICAL TRIALS

Noel P. Patson[1], Miriam K. Laufer[2], Mavuto Mukaka[3], Alinune Kabaghe[4], Don Mathanga[4], Victor Mwapasa[4], Lawrence Kazembe[5], Kennedy N. Otwombe[1], Marinus J. Eijkemans[6], Tobias Chirwa[1]

[1]*University of the Witwatersrand, Johannesburg, Johannesburg, South Africa,* [2]*University of Maryland, School of Medicine, USA, Baltimore, MA, United States,* [3]*Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [4]*University of Malawi, College of Medicine, Blantyre, Malawi,* [5]*University of Namibia, Department of Biostatistics, Windhoek, Namibia,* [6]*University Medical Center Utrecht, Utrecht, Netherlands*

Improved statistical analysis of safety data in premarketing randomized controlled trials (RCTs) aids in generation of high quality drug safety evidence through efficient use of data. Currently, lack of high quality antimalarial drug safety evidence during pregnancy may have limited introduction of antimalarial drug treatment and prevention options. Pregnant women are systematically excluded from RCTs due to fear of potential drug harms. Although some RCTs are done in pregnant women, the collected safety data can be complex; the data may have multiple outcomes, repeatedly measured over time, and non-adherence to the treatment induced by adverse events (AEs) may be present. This complex data need powerful statistical analysis methods beyond the traditional descriptive approaches to account for the event-dependence, missing data and time-dependencies. We conducted a systematic review to establish the current practice in statistical analysis methods in antimalarial drug safety data in pregnancy trials. We systematically searched five databases (PubMed, Embase, Scopus, Malaria in Pregnancy Library (MiPL) and Cochrane Central Register of Controlled Trials (CENTRAL)) for original

English articles reporting Phase III RCTs on antimalarial drugs in pregnancy published from 2010 to 2018. A total of 26 RCTs, included in this review, collected multiple safety outcomes including AEs. Statistical analysis of the RCTs used descriptive statistics, mainly proportions (94.4%, 24/26). The commonly used inferential statistical method was Fisher`s exact test (61.5%, 16/26). Negative binomial, Poisson and logistic regression were used in three trials only. Five trials addressed the subject of missing data but did not explore a potential link with safety outcomes. Only two trials used graphs next to tables to aid in summarizing patterns of safety outcomes. Current statistical analysis of antimalarial drug safety data in pregnancy trials is predominantly descriptive and univariate. Further attention is required to consider methods that include time-varying dependence of AEs, informative censoring, recurrence of AEs and correlation.

1585

EFFICACY AND TOLERANCE OF "SAYE", AN HERBAL REMEDY IN THE TREATMENT OF MALARIA

Maminata Coulibaly Traore[1], Ibrahima Fayama[2], Toussaint Rouamba[1], Sibidou Yougbare[1], Daniel Valia[1], Innocent Valea[1], Ollo Da[3], Jean Claude W Ouedraogo[2], Serge R. Yerbanga[4], Halidou Tinto[1]

[1]*URCN/IRSS, Nanoro, Burkina Faso,* [2]*Université Ouaga 1, Ouagadougou, Burkina Faso,* [3]*IRSS/DRO, Bobo-Dioulasso, Burkina Faso,* [4]*IRSS-DRO, Bobo-Dioulasso, Burkina Faso*

Saye is an antimalarial phytomedicine composed of a mixture of three plants: *Cassia alata* Linn, *Cochlospermum planchonii* Hook, *Phyllanthus amarus* Schum and Thonn) widely used for the treatment of malaria in Burkina Faso. Saye has shown interesting *in vitro* and *vivo* antiplasmodial activity without toxicity. A clinical trial was then conducted to demonstrate its efficacy and tolerance. This was an open-label Phase II/III clinical trial in patients with uncomplicated *P. falciparum* malaria in Nanoro, Burkina Faso. A total of 57 patients meeting the inclusion criteria were included and actively followed up for 42 days with a daily appointments the first 7 days during which, they received 3 capsules of Saye dosed at 130.5mg, 3 times per day and weekly follow up visits. The PCR -unadjusted cure rates were 54%, 45%, 33% and 25% at days 7, 14, 28 and 42, respectively. Day-42 PCR-corrected adequate clinical and parasitological response (ACPR) with Saye was 45.1 [95% CI (31.4 - 59.5)]. A total of 168 mild adverse events (AE) were reported during the 42 days follow up and mainly represented by vomiting (7%), diarrhoea (13%), abdominal pain (13.9%), headache (11.3%), cough (6.1%), asthenia (5.2%), hyperthermia (7.8%), anemia (41.2%), thrombopenia (5.9%), hyper-transaminases (17.6), hyperglycemia (13.7%) and leucopenia (5.9%). Only, few of them (10%) were found related to the study medication. Saye was able to cure a little more than half of the patients with malaria, with no toxic effect. Saye was well tolerated by patients. However, further studies should be conducted comparing Saye to an ACT with probably new treatment regimens or new Saye compositions.

1586

ASSESSMENT OF POTENTIAL PHARMACOKINETIC DRUG INTERACTION BETWEEN ARTEMETHER-LUMEFANTRINE (AL) AND PRIMAQUINE

Jay Prakash Jain[1], Helen Gu[2], Katalin Csermak Renner[3], Pramod J Math[4]

[1]*Novartis Institutes for BioMedical Research, Inc., Emeryville, CA, United States,* [2]*Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States,* [3]*Novartis Pharma AG, Basel, Switzerland,* [4]*Novartis Healthcare Pvt. Ltd., Hyderabad, India*

Until the approval of tafenoquine, primaquine (PQ) has been the only available radical treatment for *Plasmodium vivax* and *P. ovale* malaria due to its hypnozoitocide activity, PQ has high *P. falciparum* gametocytocidal activity as well. WHO recommends PQ with other antimalarials including artemisinin based combination therapy (ACT) as cure for *P. vivax, P. ovale* and mixed infections and as low single dose with ACT for reducing transmission. It has been reported that PQ's activity is primarily dependent on its metabolism mediated by cytochrome P450 (CYP)2D6 to form active metabolites. Artemether-Lumefantrine (AL) is one of the most used ACT and because lumefantrine (LUM) is a potent CYP2D6 inhibitor it can potentially impact the PQ metabolism thereby alter its efficacy and safety. The objective was to assess the PK-related drug interactions for PQ as a CYP2D6 victim drug with LUM. To achieve this, integrated *in vitro* and in silico studies were conducted. The inhibitory effect of LUM on PQ metabolism was investigated in human liver microsomes and hepatocytes. The results showed that *in vitro* hepatic clearance of PQ was affected by changed in intrinsic clearance (CLint) in the presence of LUM. Additionally, physiologically-based pharmacokinetic (PBPK) models for PQ and LUM were developed. The predicted PQ exposure would be increased by ~1.4 fold when co-administration with 3-QD of LUM. Although the impact on the metabolites could not be studied inhibition of CYP2D6 in exposure dependent manner was seen in silico. Other effects (e.g., polymorphism of CYP2D6) can influence the PQ activity, which were not studied. Overall, there seems to be a theoretical potential reduction of PQ efficacy due to the CYP2D6 inhibition by LUM, especially in patients who are not extensive CYP2D6 metabolizers. However, Novartis safety database did not reveal a significant risk of loss of efficacy due to the interaction. Recent evidence suggests that the risk of recurrence after *P. vivax* malaria with AL-PQ is low. Due to the lack of clear clinical data, complex PQ metabolism and pharmacodynamic relationship, guidance on the need for dose adjustment, if any, cannot be provided.

1587

A POTENTIALLY SAFER RADICAL CURE REGIMEN OF PRIMAQUINE - EARLY RESULTS FROM A PRIMAQUINE CHALLENGE STUDY IN HEALTHY GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT MALES

Podjanee Jittamala[1], James Watson[2], Sasithon Pukrittayakamee[1], Borimas Hanboonkunupakarn[1], Cindy Chu[3], Germana Bancone[3], Joel Tarning[2], François Nosten[3], Nicholas Day[2], Nicholas White[2], **Walter Taylor**[2]

[1]*Mahidol University, Bangkok, Thailand,* [2]*MORU, Bangkok, Thailand,* [3]*SMRU, Mae Sot, Thailand*

Primaquine is the only widely used drug for the radical cure of *Plasmodium vivax* malaria. In areas with high relapse rates, the WHO recommended primaquine dose is 0.5 mg base/kg/day x 14 days. This is highly efficacious but contraindicated in patients with G6PD deficiency (less than 30% of normal enzyme activity). A once weekly regimen of 0.75 mg/kg for 8 weeks is recommended currently in all G6PD deficient variants but may cause clinically significant haemolysis in some patients. These concerns and the difficulty of testing for G6PDd in low resource settings mean primaquine is little used. We aim to develop a safe and efficacious primaquine regimen for G6PD deficient patients that obviates the need for G6PD testing. Mechanistic within-host modelling of primaquine induced haemolysis in G6PD deficiency showed that it is theoretically possible to deliver a safe radical curative primaquine regimen over 20 days by inducing controlled haemolysis with gradually escalating doses (7.5, 15, 22.5 & 30 mg). A Phase 1 study in G6PD deficient healthy volunteers has commenced to test and refine this predicted "optimal" primaquine regimen. Optimal is defined both in terms of the absolute and relative reductions in haemoglobin concentrations and the daily rate of haemolysis. In total 20 healthy volunteers will be recruited in two sites in Thailand. The trial design is adaptive, with rigorous pre-specified alterations in the administered regimen, depending on the observed outcomes. Recruitment in Bangkok started in December 2018 and should finish in June 2019. The safety of the initial regimen has been demonstrated in 5 volunteers (baseline haemoglobins ca. 14 to 16 g/dL) with one of three common G6PDd mutations: Mahidol, Viangchan, and Canton. Fractional haemoglobin falls ranged from 15 to 25%. These early preliminary results provide proof-of-concept of the safety of ascending doses primaquine. The final analysis, using our prior developed mechanistic model, will identify an

optimal ascending dose primaquine regimen that will be subsequently field tested for safety and efficacy in G6PD normal and deficient patients with *P. vivax* infection.

1588

EFFECT OF DIHYDROARTEMISININ-PIPERAQUINE AND ARTEMETHER-LUMEFANTRINE WITH AND WITHOUT PRIMAQUINE ON *PLASMODIUM VIVAX* RECURRENCE: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS

Robert J. Commons[1], on behalf of the WWARN Vivax ACT Recurrence Study Group[2]

[1]*Menzies School of Health Research, Red Hill, Australia,* [2]*WorldWide Antimalarial Resistance Network, Oxford, United Kingdom*

Artemisinin-based combination therapy (ACT) is recommended for uncomplicated *Plasmodium vivax* malaria in regions with emerging chloroquine resistance. This study aimed to determine the risk of recurrent *P. vivax* following dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL) with or without primaquine. Clinical efficacy studies of uncomplicated *P. vivax* treated with DP or AL and published between January 2000 and January 2018 were identified. Individual patient data from available eligible studies were pooled using standardised methodology. The effect of mg/kg dose of piperaquine/lumefantrine, ACT administered, and primaquine on the rate of *P. vivax* recurrence between days 7 and 42 after starting treatment were investigated by Cox regression analyses. Secondary outcomes were the risk of recurrence assessed on days 28, and 63. 2,017 patients from 19 studies were included. The risk of recurrent *P. vivax* at day 42 was significantly higher in 384 patients treated with AL alone (44.0% [95%CI 38.7-49.8]) compared to 812 patients treated with DP alone (9.3% [7.1-12.2]); adjusted hazard ratio (AHR) 12.63 [95%CI 6.40-24.92], p<0.0001. A higher dose of piperaquine was associated with decreased rates of recurrence at days 42 and 63; AHRs [95%CI] for every 5 mg/kg increase 0.63 [0.48-0.84], p=0.0013 and 0.83 [0.73-0.94], p=0.0033 respectively. In patients with symptomatic recurrence after AL the mean haemoglobin increased 0.13 g/dL [95%CI 0.01-0.26] for every five days that recurrence was delayed; p=0.0407. Co-administration of primaquine reduced substantially the rate of recurrence at day 42 after AL (AHR=0.20 [0.10-0.41], p<0.0001), and at day 63 after DP (AHR=0.08 [0.01-0.70], p=0.0233). The risks of *P. vivax* recurrence at day 42 are lower following treatment with DP compared with AL, reflecting a longer duration of post-treatment prophylaxis. These risks are reduced substantially by co-administration with primaquine. Delaying *P. vivax* recurrence is associated with a small improvement in haemoglobin. This highlights benefits of radical cure and prolonged post-treatment prophylaxis.

1589

PHARMAKOCINETICS, EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE DISPERSIBLE TABLET FORMULATION (1:12) IN THE TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN NEONATES AND INFANTS <5 KG BODY WEIGHT

Cornelis Winnips[1], Jay Prakash Jain[2], Guoquin Su[3], Celine Risterucci[1], Marc Cousin[1], W. Lin[3], Katalin Csermak Renner[1]

[1]*Novartis Pharma AG, Basel, Switzerland,* [2]*Novartis Institutes for Biomedical Research Inc., Emeryville, CA, United States,* [3]*Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States*

In neonates/infants <5 kg of body weight (BW) with uncomplicated *P. falciparum* malaria, WHO recommends artemisinin-based combination therapy (ACT) at the same mg/kg BW as in those ≥5 kg, but no ACT is currently approved for this population. In a recent trial in infants <5 kg BW and >28 days, Coartem dispersible (AL), at 20mg artemether:120mg lumefantrine BID for 3 days yielded artemether exposures whose safety was not previously established. This open-label, single-arm, multicenter study in Sub-Saharan Africa will assess a new formulation of AL with a 1:12 ratio, in infants <5 kg, at a starting dose of 5mg:60mg (bid for 3 days) based on Physiologically based Pharmacokinetic modeling. It will use a staggered approach by enrolling inpatients in two sequential cohorts of 20 infants each: age >28 days (Cohort 1) and age ≤28 days (Cohort 2). Exposure will be checked in a few patients prior to completing each cohort with or without dose adjustment. An independent data monitoring committee will review efficacy and safety data on an ongoing basis, and at completion of Cohort 1, recommend whether to proceed to Cohort 2. Exclusion criteria include severe malaria, signs and symptoms of a critical condition, hepatic or renal abnormality, and major neurological malformation. The main objectives are to evaluate PK (e.g. artemether C_{max}), efficacy (e.g. PCR-corrected parasitological cure up to day 43; time to parasite clearance), safety and tolerability of the new AL tablet. Neurodevelopment status will be checked at Day 4 and at 12 months of age. Use of antipyretics and rescue medication per local guidelines is allowed. Protocol approval is sought from ethics committees in Switzerland and in participating countries. Written informed consent will be obtained from all parents/guardians. Results are expected in 2022.

1590

COHORT EVENT MONITORING STUDY OF 8572 MALARIA CASES TO EVALUATE, IN REAL-LIFE SETTING, THE SAFETY AND TOLERABILITY OF THE FIXED-DOSE COMBINATION THERAPY PYRONARIDINE-ARTESUNATE FOR THE TREATMENT OF UNCOMPLICATED MALARIA

Michael Ramharter[1], Gaston T. Lutete[2], Ghyslain Mombo-Ngoma[3], Serge-Brice Assi[4], Jude D. Bigoga[5], Felix Koukouikila-Koussounda[6], Pierre-Michel Ntamabyaliro Nsengi[2], Francine Ntoumi[6], Mirjam Groger[1], Diane Egger-Adam[7], Matthias Karnahl[7], Jorge Liz[8], Robert M. Miller[9], Sarah Arbe-Barnes[9], Jangsik Shin[10], Peter G. Kremsner[7], Isabelle Borghini-Fuhrer[8], Stephan Duparc[8]

[1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany,* [2]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [3]*CERMEL, Lamabrene, Gabon,* [4]*Institut Pierre Richet / Institut National de Santé Publique, Bouaké, Côte D'Ivoire,* [5]*University of Yaounde, Yaounde, Cameroon,* [6]*FCRM, Brazzaville, Republic of the Congo ,* [7]*Institut für Tropenmedizin, Tübingen, Tübingen, Germany,* [8]*Medicines for Malaria Venture, Geneva, Switzerland,* [9]*Artemida Pharma Limited, Stevenage, United Kingdom,* [10]*Shin Poong Pharmaceutical Co., Ltd., Seoul, Republic of Korea*

Pyronaridine-artesunate (Pyramax™, PA) has received a positive opinion under article 58 by EMA and is approved in African and Asian countries for the treatment of uncomplicated vivax and falciparum malaria. PA is highly efficacious and is well tolerated apart from, in a small number of patients, asymptomatic, transient rises in liver transaminases. This large clinical Phase IIIb/IV study has evaluated the safety of PA in a real-life setting including hitherto underrepresented patient populations such as children <1 year. The study was conducted in Cameroon, Democratic Republic of Congo, Gabon, Ivory Coast and the Republic of Congo. Patients above 5kg bodyweight presenting with uncomplicated malaria at local health facilities were included. The primary objective was to evaluate any hepatic safety events in a subgroup of patients enrolled with asymptomatic transaminases >2xULN from blood taken immediately prior to treatment. PA was given according to the label information as a once daily dose for 3 days; the first dose under supervision by the health care provider and patients were seen again on D7 and D28 at home by a community health worker. Further liver function testing was performed if there were any hepatic signs or symptoms. Between June 2017 and March 2019, 8,572 uncomplicated malaria episodes including 142 with elevated baseline liver enzymes and 147 in children <1 year of age, were treated with PA. No episode of clinically apparent drug induced liver injury was detected. 9 mostly mild (0.1%) hypersensitivity reactions occurred. 33 (0.4%) serious adverse events occurred in the course of this study of which only 3 (0.03%) events were judged as related to PA. D28 crude and PCR corrected cure rate will be presented. PA has confirmed its very good safety and efficacy profile for the treatment of uncomplicated malaria in

Africa in this unselected patient population. This includes patients with raised liver enzymes at baseline, children below one year of age and patients with comorbidities.

1591

A PHASE 1A, FIRST-IN-HUMAN, DOSE-ESCALATION STUDY OF M5717: A FIRST-IN-CLASS INHIBITOR OF *PLASMODIUM FALCIPARUM* EUKARYOTIC TRANSLATION ELONGATION FACTOR 2

James McCarthy[1], Oezkan Yalkinoglu[2], Arnand Odedra[1], Rebecca Webster[1], **Claude Oeuvray**[3], Aliona Tappert[2], Deon Bezuidenhout[4], Justin Wilkins[5], Akash Khandelwal[2], Wilhelmina Bagchus[6]

[1]*QIMR Berghofer Medical Research Institute, Heston, Australia,* [2]*Merck KGaA, Darmstadt, Germany,* [3]*The Global Health Institute of Merck, Eysin, Switzerland,* [4]*Merck PTY, Modderfontein, South Africa,* [5]*Occams, Werl, Germany,* [6]*Merck Institute for Pharmacometrics, Lausanne, Switzerland*

M5717 is a first-in-class inhibitor of the *Plasmodium falciparum* translation elongation factor 2. Preclinical data predicting good oral bioavailability, potent antimalarial activity, and favorable safety profile prompted the conduct of a phase 1a, first-in-human, safety and pharmacokinetic (PK) study in healthy adult volunteers (ClinicalTrials.gov Identifier: NCT03261401). The study is a randomized, placebo-controlled design, employing dose escalation with an adaptive component allowing for modifications to the dose increments based on PK and safety data. Currently (April 2019), seven doses have been tested in fasted volunteers (50 - 1250 mg); typical cohort size was six active and two placebo volunteers. M5717 was well tolerated, with no serious adverse events (SAEs), severe adverse events (AEs), or clinically significant electrocardiogram findings noted. All AEs were mild or moderate and transient, and mostly considered unrelated to M5717. Exposures following single oral doses of M5717 were non-linear across the dose range. The most parsimonious model describing M5717 PK was two-compartmental, with a recirculation component to describe secondary peaks; central volume of distribution (V2/F) and absorption rate constant (ka) were strongly inversely correlated with the administered dose. The median time to maximum concentration (t_{max}) was 2.9 hours (range 1-7), and the median terminal half-life ($t_{1/2}$) was over 6 days and appeared independent of dose. None of the volunteers receiving 1250 mg M5717 exceeded the exposure threshold area under the curve (AUC) of 318,200 hr*ng/mL defined from the no-observed-adverse-effect-level (NOAEL) in pre-clinical toxicology studies in rats. In summary, results to date show that M5717 is well tolerated and has favorable PK enabling single oral dose administration. These results have prompted the initiation of a phase 1b study in which healthy volunteers were inoculated with blood-stage *P. falciparum* parasites and dosed with M5717 to investigate its antimalarial activity.

1592

SAFETY OF *SIPHONOCHILUS AETHIOPICUS* (AFRICAN GINGER) FOR THE TREATMENT OF TANZANIAN ADULTS AGED 18 TO 45 YEARS BY USING CONTROLLED HUMAN MALARIA INFECTION (CHMI)

Florence Milando

Ifakara Health Institute, Bagamoyo, United Republic of Tanzania

Malaria is a key public health challenge occurring mostly in the Africa region and causes significant morbidity and mortality. According to the WHO 2018 report, an estimated 219 million cases of malaria occurred worldwide in the year 2017. The scientific investigation of antimalarial herbal remedy to prove its efficacy and safety may be of benefit to the worldwide agenda of malaria control and elimination.This will be the first time such an approach is used in a controlled clinical trial setting for evaluation of herbal remedies for malaria and hence open up the possibility of testing many more malaria products in Africa. The object of this study is to evaluate the safety and tolerability of *Siphonochilus aethiopicus* (African Ginger) given as preventive or curative medicine among Tanzanian healthy adult aged 18 to 45 years by using the Control Human Malaria Infection model. The study design of the trial will be single center phase IIb and open label clinical trial with a population of 18 healthy participants aged 18 to 45 years residing in Bagamoyo District or nearby districts. The participants will be divided into two subgroups of 9 participants each. The first subgroup (G1) will receive a malaria herbal remedy 2 day before the CHMI to evaluate its preventive efficacy (liver stage activities of antimalarial). The second subgroup (G2) will be treated with an antimalarial herbal remedy as soon as diagnosed positive with blood slide after CHMI to evaluate its curative efficacy (blood stage activities of the herbal remedy). The safety and tolerability will be evaluated through assessment of the following;(i) Clinical significance abnormal values of laboratory tests during the follow up period of 42 days (ii) Significant changes in physical examination results comparing to baseline during the follow up period of 42 days (iii) AEs during the follow up period of 28 days or serious adverse events (SAE) during the follow up period of 42 days. The study will be commenced in June 2019 and the complete monitored data will be available for presentation in October 2019.

1593

EFFICACY OF *SIPHONOCHILUS AETHIOPICUS* (AFRICAN GINGER) FOR THE TREATMENT OF TANZANIAN HEALTHY ADULTS AGED 18 TO 45 YEARS BY USING CONTROLLED HUMAN MALARIA INFECTION (CHMI)

Kamaka Ramadhani Kassimu

Ifakara Health Institute, Bagamoyo, United Republic of Tanzania

Malaria is a disease of poverty and still a major global health problem especially in Africa. Almost all African countries are located in a malaria endemic area. It was estimated that 219 million cases of malaria occurred worldwide in 2017. The scientific investigation of antimalarial herbal remedy to prove its efficacy and safety may be of benefit to the worldwide agenda of malaria control and elimination. The objective of this study is to evaluate the parasitic suppression activities and protective efficacy of *Siphonochilus aethiopicus* (African Ginger) given as preventive or curative medicine among healthy Tanzanian adults aged 18 to 45 years by using the Control Human Malaria Infection (CHMI) model. The study design of the trial will be single center phase IIb and open label clinical trial with a population of 18 healthy participants aged 18 to 45 years residing in Bagamoyo District or nearby districts. The participants will be divided into two subgroups of 9 participants each. The first subgroup (G1) will receive a malaria herbal remedy 2 day before the CHMI to evaluate its preventive efficacy (liver stage activities of antimalarial). The second subgroup (G2) will be treated with an antimalarial herbal remedy as soon as diagnosed positive with blood slide after CHMI to evaluate its curative efficacy (blood stage activities of the herbal remedy). The efficacy will be evaluated through ;(i) Early Treatment Failure (ETF) (ii) Asexual parasite clearance time (PCT). (iii) Adequate Clinical and Parasitological Response (ACPR) for treatment at 28 days and 42days after PCR correction of reinfection (ACPR 28 and 42 after PCR correction of reinfection (iii) Proportion of participants from group 1 with development of *Pf* parasitemia by TBS following CHMI. The study will be commenced in June 2019 and the complete monitored data will be available for presentation in October 2019.

1594

PHARMACOLOGICAL PROPERTIES OF PIPERAQUINE IN HEALTHY VOLUNTEERS WITH INDUCED BLOOD-STAGE *P. FALCIPARUM* MALARIA INFECTION: A MECHANISTIC MODELLING APPROACH

Thanaporn Wattanakul[1], Richard Hoglund[1], Joerg Möhrle[2], James McCarthy[3], **Joel Tarning**[1]

[1]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*Medicines for Malaria Venture, Geneva, Switzerland,* [3]*QIMR Berghofer Medical Research Institute, Brisbane, Australia*

Dihydroartemisinin-piperaquine is a recommended first-line therapy for malaria. Piperaquine is also under consideration for other drug combinations. The aim of this study was to develop a semi-mechanistic pharmacokinetic-pharmacodynamic (PK/PD) model describing the parasite dynamics in healthy volunteers in the induced blood stage *P. falciparum* model. The PK/PD model developed was used to predict treatment failures in the presence of multidrug resistant infections, as well as optimal dosing regimens for these infections. 24 volunteers participated in the study. Each participant was inoculated with a total of 1,800 viable *falciparum* infected erythrocytes (ACTRN12613000565741). Parasite densities and piperaquine levels were measured by qPCR and LC-MS, respectively. Nonlinear mixed-effect modelling was used to characterise the PK properties of piperaquine and the *in vivo* parasite dynamics in response to piperaquine. Piperaquine PK was well described by a three-compartment disposition model. A semi-mechanistic parasite dynamics model was developed, including maturation of parasites, sequestration of mature parasites, synchronicity of infections and multiplication of parasites, as described in natural infections with *falciparum* malaria. Piperaquine-associated parasite killing was estimated using an E_{MAX}-function. The final PK/PD model described parasite dynamics adequately, with simulations predicting a higher probability of treatment failures when parasites were resistant to piperaquine alone compared to dihydroartemisinin alone (3.38% vs 1.69%). Modelling and simulation predicted that the ideal additional drug candidate in novel triple-combination therapies should have a 48h parasite reduction ratio of $\geq 10^3$, with ≥3 week duration of action, to combat multidrug resistant infections. In conclusion, a semi-mechanistic model describing *falciparum* parasite dynamics was successfully developed and implemented using nonlinear mixed-effects modelling. This will be a highly useful tool to assess novel antimalarial drug combinations including piperaquine, and its effect on multidrug resistant infections.

1595

EXTENDED DURATION ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF MALARIA IN HIV-UNINFECTED CHILDREN IN UGANDA: THE EXALT RANDOMIZED CONTROLLED PK/PD STUDY

Mwebaza Norah[1], Whalen Meghan[2], Francis Orukan[1], Kacey Richards[3], Martina Wade[3], Were Moses[1], Liusheng Huang[2], Richard Kajubi[1], Francesca Aweeka[2], Sunil Parikh[3]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*University of California San Francisco, San Francsisco, CA, United States,* [3]*Yale School of Public Health, New Haven, CT, United States*

Artemether-lumefantrine (AL) is the most widely used artemisinin-based combination therapy. Recent data, including a comprehensive meta-analysis, suggest that lumefantrine (LR) exposure is lower in young children and that absorption is dose-limited. We are conducting a prospective PK/PD study of extended duration AL in children ages 6 months-17 years in a high transmission area in Uganda. Children with uncomplicated *P. falciparum* infection are randomized to 3-day (standard 6-dose) or 5-day (10 dose) AL with intensive or population PK sampling for artemether, DHA, and LR quantification by LC MS/MS out to day 21 and clinical follow-up to 42 days. Thus far, n=349 children have been screened, n=167 enrolled (median age 5 years, range 1-14), and n=7 withdrawn. The targets for enrolment are n=100 and n=120 episodes for intensive and population PK sampling, respectively. Outcome data for n=84 participants (3-day (n=47) and 5-day (n=37)) are presented. Thus far, 28-day treatment outcomes in the 3-day (n=47) versus 5-day (n=37) are as follows: ACPR (38% vs 70%), LPF (51% vs 19%), LCF (11% vs 11%), respectively. At 42-days, 3-day vs 5-day treatment outcomes in the 3-day (n=47) versus 5-day (n=37) are as follows: ACPR (28% vs 38%), LPF (51% vs 32%), LCF (21% vs 30%). PK parameters for n=18 children have been completed thus far (n=9 each arm). Terminal LR concentrations were significantly increased in the 5-day versus 3-day regimen on days 7,14, and 21 (1090, 186, 91.5 ng/mL vs 321, 118, 62.2 ng/mL; all p-values <0.005). LR area under the concentration-time curve (AUC) measured from the time of the last dose over 21 days indicates a geometric mean $AUC_{0\text{-infinity}}$ of 322 ug*h/ml and 255 ug*h/ml (p =0.20) for the 5-day and 3-day regimens, respectively. Preliminary results demonstrate that twice daily AL over 5 days significantly enhances terminal LR concentrations out to day 21 with modest increases in $AUC_{0\text{-infinity}}$. Changes in PK exposure coincide with a reduced risk of malaria at 28 days, with risk reduction appearing to diminish by 42 days in our high transmission setting. Enrollment began in February 2018 and completed PK/PD and safety data are expected in Fall 2019.

1596

MOLECULAR EPIDEMIOLOGICAL STUDY OF MULTIDRUG-RESISTANT FALCIPARUM MALARIA IN THE CENTRAL HIGHLANDS OF VIETNAM IN 2018-2019

Huynh H. Quang[1], Marina Chavchich[2], Nguyen T. Trinh[1], Nguyen D. Manh[3], Michael D. Edstein[2], Kimberly A. Edgel[4], Nicholas J. Martin[4]

[1]*Institute of Malariology, Parasitology and Entomology, Quy Nhon, Vietnam,* [2]*Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia,* [3]*Military Institute of Preventive Medicine, Hanoi, Vietnam,* [4]*U.S. Naval Medical Research Unit TWO, Singapore, Singapore*

Dihydroartemisinin-piperaquine (DHA-PPQ) used to treat *Plasmodium falciparum* in Vietnam is now failing at a rate of >50% in Binh Phuoc province. This is due to the spread of resistant parasites, particularly those containing mutation C580Y in the Kelch 13 propeller gene responsible for artemisinin resistance combined with amplification of plasmepsins 2 and 3, which is implicated in piperaquine resistance (i.e. KEL1/PLA1 clade). Furthermore, mutations in the *Pfcrt* gene responsible for resistance to chloroquine may also be contributing to the successful spread of these "super-resistant" parasites. It is of utmost importance to evaluate if KEL1/PLA1 parasites are spreading north of Binh Phuoc province and to consider alternative treatment options to avoid selection of highly resistant parasites and future treatment failures. To characterize the markers of drug resistance, we conducted a survey of symptomatic malaria in several provinces of the central highlands of Vietnam from June 2018 to March 2019. Of the 102 *P. falciparum* patients recruited so far, 33 were from Dak Nong, 46 from Dak Lak, 7 from Gia Lai, and 16 from Kon Tum province. *P. falciparum* malaria was confirmed in all samples by blood film microscopy and HRP2-3 based rapid diagnostic testing, indicating absence of *hrp2-3* deletions in these samples. Sequencing of the Kelch 13 gene revealed that the C580Y mutation was present in the majority of the samples (73.5%, 75/102) and only two participants had parasites containing the I539T mutation. Twenty three (22.5%) of the participants had parasites with amplification of plasmepsins 2 and 3. KEL1/PLA1 parasites were present in 27.3% (9/33) of participants' samples from Dak Nong, 6.5% (3/46) from Dak Lak, 42.9% (3/7) from Gia Lai, and 6.3% (1/16) from Kon Tum province. Characterization of other markers including Exo415, *Pfcrt* and *Pfmdr1* genes is ongoing. These preliminary findings suggest that the KEL1/PLA1 parasites are now present in Dak Nong province and in the central highlands of Vietnam. These data, collected jointly with Vietnamese public health officials, will inform future malaria treatment guidelines.

1597

ASSESSMENT THE QUALITY OF ROUTINE MALARIA DATA IN MADAGASCAR

Solo Harimalala Rajaobary

National Malaria Control Program, Antananarivo, Madagascar

The availability of quality data is critical for informed decision-making. By 2018, more than 7% of inconsistencies were found in centrally transmitted reports. This study's objective is to evaluate the quality of the data transmitted between different levels of the health system. This study was a routine data analysis of monthly activity reports (RMAs) from January to June 2018 in 20 health facilities (HF), 8 health districts, 2 health regional directorates and the central level. The Routine Data Quality Assessment (RDQA) tool was used for the review. The reported results audit focused on the accuracy of the data transmitted, the promptness of reporting, the completeness and availability of reports. A recount was performed to assess the quality of the positive rapid diagnostic test (RDT +) indicator. Quality data is determined by an accuracy between 95% and 105%, and discrepancy by the difference between the cases reported and the cases recounted by the recounted cases. Overestimation occurs when the ratio of recounted data to reported data is less than 95%. At the health facility level, data verification showed accuracy between 95 to 105% and overestimation to less than 70%. The source documents were 80% complete and 75% available. An overestimate of 91% was observed at the district level, and 84% at the regional level. The promptness of reporting was 90% with availability of 92% at this level. At the regional level, these rates were respectively 78% and 89% and an overestimate at 84% was observed. At the central level, promptness was at 38% and availability at 96%. A 7% discrepancy was reported at the HF level, 8% at the health district level and 41% at the regional level. Between the HF level and the central level, an 8% discrepancy was observed for the 20 HF supervised. The data quality anomalies identified at HF level were reflected at different levels of the health system. The availability of quality data remains a challenge for the Ministry of Health.

1598

HOUSEHOLD MEMBERS OF PERSONS WITH MALARIA IN HIGHLAND KENYA AREAS OF UNSTABLE TRANSMISSION ARE AT INCREASED RISK OF DEVELOPING CLINICAL MALARIA WITHIN 30 DAYS

George Ayodo[1], Lindsey B. Turnbull[2], Veronicah Knight Adhiambo[1], Chandy C. John[2]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States*

The national malaria strategy in Kenya is that only microscopy- or RDT-confirmed cases of malaria are treated with anti-malarial drugs. Screen and treat studies suggest that individuals in the household of a person with malaria may be at higher risk of subsequent malaria than individuals in malaria negative households. In the present prospective case-control study, we assessed whether clinical malaria was more frequent in individuals living in households with compared to without a person with microscopy confirmed malaria within the past 30 days. From 2007-2017, all persons from Kipsamoite and Kapsisiywa, two highland Kenya areas of unstable malaria transmission who came to site health centers with symptoms of fever or headache were tested for malaria by microscopy. Individuals who visited the health center and had microscopy-confirmed malaria were designated cases. Individuals who visited the clinic in the same week as a case for symptoms of malaria, but were microscopy negative were designated controls. Visits by household members of cases or controls within 30 days of the case or control visit were assessed for the presence of microscopy-confirmed malaria. The analysis was thus limited to encounters whereby multiple household members visited the clinic within a 30-day period. Of 7098 total visits, 70 cases and 516 controls were identified. 87 health center visits within 30 days of the case/control visit were from case households and 599 were from control households, of which 40 (46.0%) and 39 (6.5%) were microscopy positive respectively. Persons in the household of a case had 12-fold increase in the odds of developing microscopy-confirmed malaria within 30 days of the index (case/control) health center visit compared to persons in a control household (OR 12.22, 95% CI 7.18, 20.81, p <0.0001). In this area of unstable malaria transmission, household members of individuals with confirmed malaria have a substantially increased risk of malaria within 30 days of the case illness. Future studies should assess whether anti-malarial treatment of household members of persons with confirmed malaria can decrease subsequent malaria incidence.

1599

POOR AGREEMENT BETWEEN FACILITY RECORD DATA, ROUTINE HEALTH INFORMATION SYSTEM DATA, AND EXIT INTERVIEW DATA DURING A HEALTH FACILITY SURVEY IN MOZAMBIQUE: CAUSE FOR CONCERN WITH REGARD TO ROUTINE DATA QUALITY?

Baltazar Candrinho[1], Mariana Da Silva[2], Guidion Mathe[2], Mercia Dimene[2], Ana Rita Chico[3], Ana Cristina Castel-Branco[3], Frederico Brito[4], Marcel Andela[3], Gabriel Ponce de Leon[5], Abu Saifodine[6], Rose Zulliger[7], Mathew Plucinski[5], James Colborn[3]

[1]*Baltazar Candrinho, Maputo, Mozambique,* [2]*National Malaria Control Program, Maputo, Mozambique,* [3]*Clinton Health Access Initiative, Maputo, Mozambique,* [4]*UNICEF, Maputo, Mozambique,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*United States Agency for International Development, Maputo, Mozambique,* [7]*Centers for Disease Control and Prevention, Maputo, Mozambique*

Malaria data reported through Mozambique's routine health management information system (HMIS) is the primary source of data for measuring transmission trends and evaluating program impacts. Previous studies showed major discrepancies between HMIS data and health facility record data; few studies exist that compare HMIS to a gold standard measure of case data. This study used data collected through a health facility survey conducted April-May 2018 in Maputo Province (low malaria burden), Cabo Delgado (moderate) and Zambézia (high). Facilities were stratified by type and randomly sampled. Data collection included exit interviews and clinical re-examinations of 20 randomly selected patients, comparison of exit interview data with health facility records, and routine data abstraction to compare with HMIS data. A total of 1,840 patients from 117 health facilities were included; 72% had fever / history of fever. Retrospective data abstracted from health facility registers aligned poorly with HMIS data from the same period. The absolute number of all-cause outpatients, suspect malaria cases, and confirmed malaria cases recorded in facility registries and reported through the HMIS were significantly different in all provinces; in all cases numbers were higher for HMIS than for facility registries. Specifically, 42,431 all-cause outpatient visits were abstracted from facility registers in Maputo Province, compared with 87,992 in the HMIS (difference=45,561). Factors associated with agreement between registry and exit interview data and RDT results were patients being under the age of 5 and clinicians testing and treating according to national treatment guidelines (p<0.01 for all provinces). Similarly, the presence / absence of fever was more likely to match when clinicians correctly tested and treated in Zambézia (p=0.04), and in smaller facilities in Maputo and Cabo Delgado (p<0.05). These results suggest care should be taken in assuming the accuracy of data reported through Mozambique's routine HMIS, and improving case management practices could potentially lead to concurrent improvements in the quality of malaria indicators.

1600

THE ASSOCIATION BETWEEN *PLASMODIUM FALCIPARUM* INFECTION IN THE FIRST SIX MONTHS OF LIFE AND SUBSEQUENT INFECTION AMONG CHILDREN UNDER 24 MONTHS IN MALAWI, 2016-2018

Liana R. Andronescu[1], Andrea G. Buchwald[2], Andy Bauleni[3], Patricia Mawindo[3], Don P. Mathanga[3], Miriam K. Laufer[1]

[1]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Colorado School of Public Health, University of Colorado, Denver, CO, United States,* [3]*Malaria Alert Center, University of Malawi College of Medicine, Blantyre, Malawi*

In high transmission settings, infants under six months comprise up to 10% of malaria-related hospitalizations in children under five years old. Despite the prevalence of malaria infection and disease in this age group, the epidemiology is not well understood and incidence is rarely assessed. In the first six months of life, infants may either have increased immunity to infection due to presence of maternal antibodies or increased susceptibility due to exposure *in utero*. The impact of early malaria infection on subsequent disease is unclear. We aimed to determine if *P. falciparum* infection in the first six months of life alters the risk of subsequent clinical malaria up to 24 months of age. To address this question, infants in Malawi were followed at two sites from birth until 24 months of age. Study participants were scheduled for clinic visits at three-month intervals and asked to visit the clinic between appointments in the event of illness. RDTs were performed at all scheduled appointments and if malaria signs and symptoms were present at sick visits. Positive RDTs were confirmed by microscopy. Preliminary data from one study site included 94 participants with a mean follow-up time of 21.48 (SD: 6.54) months and a mean of 2.37 (SD: 4.30) infections. In the first six months of follow-up, 27 infections were reported for an incidence rate of 5.1 *P. falciparum* infections per 100 person-months of observation. In the following 18 months, there were 207 infections for an incidence rate of 14.6 infections per 100 person-months. A Poisson model, adjusted for season of birth, indicates that for every infection occurring before six months, an additional 1.73 (95% CI 0.97, 3.07) infections may be observed between six and 24 months, suggesting early infection is associated with increased risk of subsequent infection. These results might be due to increased exposure to *P. falciparum* or an impact on immunity among infants who are infected in the first six months of life. Analysis of our final analysis will include data from both health centers and covariates for bed net use and village to assess exposure.

1601

SPATIAL MODELING OF CATCHMENT AREAS FOR ESTIMATING MALARIA INCIDENCE USING HEALTH FACILITY SURVEILLANCE DATA IN UGANDA

Adrienne Epstein[1], Victor Kamya[2], Sarah Staedke[3], Arthur Mpimbaza[2], Asadu Sserwanga[2], Jane Namuganga[2], James Kapisi[2], Isabel Rodriguez-Barraquer[1], Moses Kamya[2], Grant Dorsey[1], Bryan Greenhouse[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

High quality measures of malaria are essential to target populations at risk, measure changes in disease burden, and evaluate the impact of interventions. A recommended indicator in high transmission settings is incidence, or the number of cases divided by the population at risk. Quantifying this denominator is challenging using health facility data because catchment areas are not well defined. The aim of this analysis is to model catchment areas around Malaria References Centers supported by the Uganda Malaria Surveillance Project. Using outpatient data from 34 Malaria References Centers from January 2017 to March 2019 (n = 950,674 outpatient visits of which 247,487 were malaria cases), we estimated travel times from each patient's village of residence to the health facility. We specified Poisson generalized additive models to evaluate the relationship between travel time and attendance. The outcome was the count of outpatient visits for each unique village/facility pair. We included covariates accounting for travel times to competing facilities and random effects for sentinel facilities. We used the modeled relationships to estimate catchment area population sizes by determining the threshold beyond which attendance appreciably declined. Villages with travel times within this threshold were considered to be part of the health facility's catchment area. To calculate catchment area populations, we applied a correction factor to the village populations to account for the probability of attendance. Preliminary results suggest catchment sizes ranged from 1,464 individuals to 7,822 individuals. In addition to travel time, attendance was driven by the proximity of alternative nearby facilities; fewer facilities within a 1 hour travel time of a patient's village was positively associated with attendance. We used these population estimates to estimate malaria incidence, which ranged from 31/1,000 to 505/1,000 across the sites. We will next validate these estimates by comparing them to known incidences from cohort studies in 3 districts where Malaria References Centers are located.

1602

EPIDEMIOLOGY OF *PLASMODIUM VIVAX* IN DUFFY NEGATIVE INDIVIDUALS

Lauren Bradley, Guiyun Yan

University of California - Irvine, Irvine, CA, United States

Plasmodium vivax is the most common malarial species worldwide however, it is relatively rare in sub-Saharan Africa. In order to establish blood-stage infection *P. vivax* utilizes the Duffy antigen as the receptor for invasion. It has long been understood that Duffy negativity, or the lack of expression of the Duffy gene, yields resistance to blood-stage infection of *P. vivax*. Recently, however, studies have found cases of *P. vivax* infection in Duffy negative individuals in a number of countries in Africa and South America. Little is currently known about the extent of *P. vivax* infections and parasitemia in Duffy-negative people in the African community. This study aims to investigate the effect of Duffy genotypes on *P. vivax* prevalence and parasitemia, and whether the copy number of the Duffy binding protein gene has any impact on *P. vivax* parasitemia. It is possible that certain Duffy genotypes and phenotypes within the human population allows for increased infectivity by *P. vivax*. We are examining these questions using *P. vivax* samples from the cross-sectional survey and hospital-based passive case detection in a malaria epidemic and an endemic areas in Ethiopia.

1603

SPATIOTEMPORAL EPIDEMIOLOGY OF MALARIA IN SOUTHERN VENEZUELA: A CRITICAL *PLASMODIUM* HOT-SPOT IN LATIN AMERICA

Maria E. Grillet[1], Jorge Moreno[2], Jan E. Conn[3], Juan V. Hernandez[4], Maria F, Vicenti[5], Adriana Tami[6], Alberto Paniz-Mondolfi[7], Martin Llewellyn[8], Ananias A. Escalante[9]

[1]*Instituto de Zoología y Ecología Tropical, Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela,* [2]*Centro de Investigaciones de Campo "Dr. Francesco Vitanza," Servicio Autónomo Instituto de Altos Estudios, MPPS, Tumeremo, Edo Bolivar, Bolivarian Republic of Venezuela,* [3]*Wadsworth Center, New York State Department of Health, Albany, NY, United States,* [4]*University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen, Netherlands,* [5]*University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen, Groningen, Netherlands,* [6]*University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen, Netherlands,* [7]*Infectious Diseases Research Incubator. Instituto de Investigaciones Biomédicas, Clinica IDB, Barquisimeto, Bolivarian Republic*

of Venezuela, [8]Institute of Biodiversity, Animal Health, and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom, [9]Temple University, Philadelphia, PA, United States

Despite advances in malaria control worldwide, Latin America has undergone a setback mainly due to Venezuela. The Venezuelan cases represented 57% of the regional malaria burden in 2017, and the country was among those with the largest increases worldwide. Historically, the southeastern part of the country (near the Brazilian border), has accounted for ~70% of malaria. Thus, this study aims to describe the spatial variation of cases in the last decade, for *P. vivax* and *P. falciparum*, to detect the occurrence of hot spots, describe the spatial progression of the epidemic, and predict spread across the country. Venezuela reported around 1,207,348 malaria cases in the whole period, with overall incidence rates (per 1,000 person-years) ranging from 5.2 (2007) to 28 (2017). Overall, ~61% of the cases were in in the south. From 2012-2017, *P. vivax* malaria in that region increased from 25,843 to 146,885 (468%) with 496,847 cases and a mean ratio of *P. vivax*/*P. falciparum* of 3.04. Annual malaria incidence during the last 10 years mostly occurred in the eastern area of Bolivar state where two significant and persistent local clustering of *P. vivax* and *P. falciparum* were detected. Average malaria incidence within the cluster area was 5.2 times greater than outside the cluster. Since 2014, local malaria transmission has reemerged in new areas producing a significant change in the epidemiological landscape. The disease is now propagating across the country, increasing local transmission in the traditional endemic *foci* and re-emerging in northern urban and peri-urban landscapes. Massive increases in transmission in southern Venezuela are positively correlated with an increase in uncontrolled illegal gold mining and deforestation, exacerbated by the deterioration of public health care. Changes in migration, as a result of the Venezuelan economic collapse, have driven the malaria spillover into the rest of the country and Latin America, a global threat for the WHO Regional Technical Strategy for Malaria.

1604

ADAPTING MALARIA INDICATOR SURVEYS TO IMPROVE UPON TRAVEL DATA RELEVANT FOR MALARIA EPIDEMIOLOGY

Carlos A. Guerra[1], Daniel T. Citron[2], Olivier Tresor Donfack[3], David L. Smith[2], Guillermo A. Garcia[1]

[1]*Medical Care Development International, Silver Spring, MD, United States,* [2]*Institute for Health Metrics and Evaluation, Seattle, WA, United States,* [3]*Medical Care Development International, Malabo, Equatorial Guinea*

Malaria indicator surveys (MIS) are increasingly used for monitoring and evaluating the impact of malaria interventions and to provide input data for modeling malaria prevalence and indicators. Some MIS include questions about history of travel but only a few have been used to explore the relationships between human mobility, malaria prevalence and importation. A recent study on Bioko Island that used MIS data to characterize human mobility unveiled clear relationships between human travel to mainland Equatorial Guinea and odds of malaria infection, strongly suggesting that many who return to the island could be acquiring their infections during their travels. The study also revealed limitations of the MIS travel data and prompted modifications to the questionnaires. Here, we present preliminary analyses of the expanded travel information captured by the 2018 MIS on Bioko Island. Questions related to travel were modified to include more detail about travel, including pinpointing travel destination as well as frequency and duration of travel. The questionnaire was also expanded to record professional sector of adult interviewees in an attempt to identify high-risk groups of travelers. The data showed that 72.7% of travellers to mainland were bound to three out of thirteen districts and 47% to a single district, Bata. The level of detail of within-island travel improved significantly and provided new insights into human movement and its possible role in mixing parasites brought in to Bioko from mainland Equatorial Guinea. On average, travellers made 1.4 trips to mainland every 8 weeks and spent 18 nights away per trip. It was also possible to identify that 80.5% of travelers belonged to three job sectors. The new travel data have important implications for the design and implementation of strategies designed to reduce malaria importation rates to Bioko. We show that by implementing simple modifications and extensions to MIS travel questionnaires useful travel information can be improved substantially.

1605

REVEALING THE MALARIA MAP: USING SOFTWARE IN A NOVEL APPROACH TO GEOSPATIAL TARGETING OF MALARIA INTERVENTIONS AND SBC MESSAGING

Christina M. Riley[1], Frazer Bwalya[1], Todd Jennings[2], Derek Pollard[1], Anne C. Martin[1], Javan Chanda[2], Reuben Zulu[3], Emmanuel Kooma[3], John Miller[2], Anna M. Winters[1]

[1]*Akros, Lusaka, Zambia,* [2]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [3]*National Malaria Elimination Centre, Ministry of Health, Lusaka, Zambia*

The Zambia Ministry of Health is aiming to eliminate malaria through an ambitious strategy emphasizing a scalable intervention package (SIP) that includes indoor residual spraying (IRS) and targeted mass drug administration (MDA). To optimize delivery of these interventions, accurate data on performance of service delivery teams, intervention uptake among community members, and focused Social Behavior Change (SBC) messaging is necessary. Operational service delivery information for census-type intervention campaigns was collected from 2017 to 2019 in Southern Province, Zambia using Reveal, an in-field spatial intelligence tool that plans and tracks delivery of field intervention activities down to household level. Information was combined with satellite-based household enumerations to develop dashboards of service delivery statistics and location information to understand coverage and to support targeted SBC messaging. In addition to improved SIP coverage at district and village level from 2017 to 2019, SBC messaging data among individuals present at the time of the MDA R2 campaign in 2019 (N=36,786) shows that approximately 90% of all individuals self-reported that they had heard malaria messaging in the previous year. More than 50% specifically reported having heard that malaria was dangerous, 33% recalled hearing messages on the importance of IRS and MDA in combatting malaria and 10% cited testing and treatment-seeking messaging in cases of fever. Individuals highly preferred health facilities (63%) and community health workers (55%) to village meetings (18%), radio (8%), or TV (3%) as their source for malaria information and messaging, supporting intervention service delivery for malaria messaging delivery method. Geospatial differences in messaging awareness and preferred source of messaging were observed. The Reveal platform continuously improved field team operational performance through regular review and data feedback. Using Reveal, geo-spatial differences uncovered in newly acquired malaria messaging data will be optimized in similar fashion to further improve impact of malaria SIPs.

1606

RISK FACTORS OF MALARIA INFECTION IN A LOW ENDEMIC DISTRICT WITH INTENSIFIED VECTOR CONTROL IN MAPUTO PROVINCE, SOUTH OF MOZAMBIQUE

Julia Montana Lopez[1], Wilson Simone[2], Beatriz Galatas[1], Caterina Guinovart[3], Fernando Laice[2], Arlindo Chidimatembue[2], Regina Rabinovich[4], Baltazar Candrinho[5], Francisco Saute[1], Pedro Aide[6]

[1]*Barcelona Institute for Global Health - Centro de Investigaçao em Saude de Manhiça, Manhiça, Mozambique,* [2]*Centro de Investigaçao em Saude de Manhiça, Manhiça, Mozambique,* [3]*Barcelona Institute for Global Health, Barcelona, Spain,* [4]*Barcelona Institute for Global Health - Harvard TH Chan School of Public Health, Boston, MA, Barcelona, Spain,* [5]*National Malaria*

Control Program, Ministry of Health, Maputo, Mozambique, [6]Centro de Investigaçao em Saude de Manhiça - National Institute of Health, Ministry of Health, Manhiça, Mozambique

Southern Mozambique is targeted by a regional malaria elimination initiative. In Moamba district, the NMCP led a universal coverage of vector control tools (VC) with distribution of long-lasting insecticidal nets (LLINs) in late 2017 and Indoor Residual Spraying (IRS) campaigns with Actellic in the dry seasons of 2015-2018. Incidence reported by Ministry of Health declined from about 256 malaria cases per 1000 in 2015 to 84 per 1000 in 2018. An age-stratified cross-sectional survey was carried out in Moamba district (Maputo province) in May-June 2018, to estimate malaria infection prevalence after the deployment of VC. Using a structured questionnaire, data on demographics, use of malaria control tools and travel history were collected from 2,919 individuals selected from 30 clusters using a two-stage cluster sampling method with probability proportional to size. A rapid diagnostic test (RDT) was done in all participants. Multivariable logistic regression models adjusting for clustering at the neighbourhood and household levels via random effects were used to identify the main risk factors associated with RDT positive malaria infections. Data presented are preliminary. Overall weighted malaria prevalence was 2.5% (95% CI 1.6%-3.8%), and most cases (41 of 68) clustered near Maputo. Under 5 prevalence was 1.5% (0.8%-2.7%), 3.9% (2.3%-6.4%) in 5-15 year olds and 2.0% (1.1%-3.4%) in >=15 year olds. 76% of participants reported sleeping under a bednet the previous night and 45% of households reported receiving IRS in the previous 12 months. The main risk factors for infection were travel to malaria endemic areas in Mozambique (OR=17.9 (1.3-249.6), p=0.03) and living near Maputo (OR=4.2 (1.4-12.8), p=0.01). No association was found between RDT positivity and VC variables (sleeping under a net, living in a sprayed household, both, or neither), age or gender. These findings suggest that importation from higher malaria transmission areas may hinder elimination in the south. The lack of impact of VC may be explained by insufficient coverage estimates or changes in the mosquito population and its behaviour as a result of intensified VC for years.

1607

COMPARATIVE EFFECTIVENESS TRIAL OF TWO COMMUNITY CASE MANAGEMENT TECHNIQUES FOLLOWING WITHDRAWAL OF INDOOR RESIDUAL SPRAYING IN NE UGANDA

Dorothy Echodu[1], Kathryn Colborn[2], Ronald Mulebeke[3], Thomas Eganyu[4], Humphrey Wanzira[3], Fred Bukenya[4], Richard Elliott[5], Joaniter Nankabirwa[6], Jimmy Opigo[7], Adoke Yeka[8]

[1]*Pilgrim Africa, Seattle, WA, United States,* [2]*University of Colorado Denver, Denver, CO, United States,* [3]*Pilgrim Africa, Kampala, Uganda,* [4]*Pilgrim Africa, Toroma, Uganda,* [5]*Boise State University, Boise, ID, United States,* [6]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [7]*National Malaria Control Program, Kampala, Uganda,* [8]*University of Makerere, Kampala, Uganda*

Vector control should be pursued and maintained as long as malaria transmission is ongoing. Budget constraints faced by high burden countries make this a challenge in the case of indoor residual spraying (IRS). We present the protocol of a prospective two year trial (2019-2021) evaluating alternative cost effective intervention combinations to maintain malaria reduction gains post IRS in a high burden setting. IRS with pirimiphos-methyl was implemented every 6-8 months in 55 villages in Katakwi District in Uganda from 2016-2018 to achieve malaria prevalence reduction below 10% after the last round of IRS. We will conduct a cluster-randomized effectiveness trial of the combination of PermaNet 3.0 deltamethrin- piperonyl butoxide (PBO) nets and either integrated community case management (iCCM) for malaria, pneumonia and diarrhea in children under 5, or proactive community case management (ProCCM). ProCCM includes active all ages malaria case detection and treatment, and active detection and treatment of pneumonia and diarrhea in children under 5. A total of 27 villages have been randomized to iCCM + PBO nets and 28 villages to ProCCM + PBO nets using covariate-constrained randomization. Malaria incidence data will be recorded weekly by community health workers in both arms using mobile devices, and also recorded on paper as per national guidelines. Community level cross-sectional Pf prevalence and serology surveys at baseline, midline and endline, and continuous HMIS monitoring from all public health facilities in the study area will be conducted. Entomological surveillance using CDC light-traps and pyrethrum spray catches is also planned. Primary study outcomes are all ages Pf prevalence measured in cross-sectional surveys, and annual parasite incidence in children under 5 at public health facilities. Secondary outcomes include changes in transmission indicators measured by entomology and serology, as well as incidence data from the intervention arms. Baseline prevalence data together with preliminary incidence and entomology data will be presented. This study is expected to provide evidence to guide sustainable IRS exit.

1608

MALARIA AND OTHER PARASITIC INFECTIONS IN PREGNANCY IN GHANA: BURDEN AND EFFECT

Gifty D. Ampofo, Matilda Aberese-Ako, Harry Tagbor

University of Health and Allied Sciences, Ho, Ghana

Uptake of antenatal clinic (ANC) interventions to control malaria and anemia in pregnancy has improved in Ghana yet maternal anemia prevalence and low birth weight (LBW) incidence have remained the same over the past decade. It is known that intestinal helminths and schistosomiasis co-exist with malaria and may be contributing to the persisting maternal anemia and low birth weight but their specific contributions are not known. This study therefore sets out to determine the relative contributions of malaria, intestinal helminthiasis and schistosomiasis and/or co-infections to maternal anemia and low birth weight. A health facilities-based cohort study began in June 2018 and is on-going, in the Ashanti and Volta Regions of Ghana, to follow pregnant women from booking until delivery of 2500 live births. At recruitment, demographic, socio-economic, obstetric and medical histories are recorded. Blood, urine and stool samples are taken for a full blood count (FBC) and detection of parasitic infections. FBC and malaria parasitemia are measured again at subsequent visits. Stool and urine samples are taken again prior to delivery. All other morbidities that these women experienced are also recorded as are interventions delivered during ANC visits. At delivery, the birth weight and any adverse birth outcomes are recorded. Data is being captured electronically and STATA 15 is being used for data management and subsequent analysis. Currently, a total of 2461 women of all gravidity have been enrolled. Their mean age is 27.18 years (sd=6.59). At baseline their mean hemoglobin concentration, anemia and malaria parasitemia prevalence are 10.84g/dl (sd=1.53), 51.64% and 6.52% respectively. Only 61.48% have bednets and 47.18% slept under one the night prior to enrolment. Out of 454 live births, 419 have recorded birth weights. The mean birth weight is 3.0kg (sd=0.49) and 9.30% are low birth weight. Malaria parasitemia (at baseline) seems low while maternal anemia and LBW incidence appear high. Factors accounting for these findings will be understood at the end of the study.

1609

PREVALENCE OF MALARIA IN EARLY PREGNANCY AMONG NULLIPAROUS WOMEN IN THE DEMOCRATIC REPUBLIC OF THE CONGO, KENYA, ZAMBIA AND PAKISTAN

Sequoia I. Leuba[1], Melissa Bauserman[1], Carl L. Bose[1], Antoinette K. Tshefu[2], Waldemar A. Carlo[3], Musaku Mwenechanya[4], Edward A. Liechty[5], Fabian Esamai[6], Robert L. Goldenberg[7], Saleem Jessani[8], Elizabeth M. McClure[9], Jennifer J. Hemingway-Foday[9], Steven Meshnick[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo,* [3]*University of Alabama at Birmingham, Birmingham, AL, United States,* [4]*University Teaching Hospital, Lusaka, Zambia,* [5]*School of Medicine, Indiana University, Indianapolis, IN, United States,* [6]*Department of Child*

Health and Paediatrics, Moi University School of Medicine, Eldoret, Kenya, [7]Columbia University, New York, NY, United States, [8]Aga Khan University, Karachi, Pakistan, [9]RTI International, Research Triangle Park, NC, United States

In malaria-endemic countries, an estimated 1 in 4 women are infected with malaria during their pregnancy, and this infection can cause maternal anemia, preterm birth, stillbirth, and low birth weight. However, likely due to the late enrollment of antenatal care in most malaria-endemic areas, few studies have examined malaria infection in early pregnancy. This is the first large multisite study of malaria in early pregnancy. This is an ancillary study, added to the Global Network's trial of low-dose aspirin in early pregnancy (ASPIRIN). At early pregnancy (i.e., 6-13 weeks), dried blood spots were obtained from 3261 pregnant women in four sites of the Global Network (Democratic Republic of the Congo (DRC), Kenya, Zambia, and Pakistan). These dried blood spots were subsequently analyzed for *Plasmodium falciparum* infection by qPCR. The prevalence of *P. falciparum* among nulliparous pregnant women ranged from 1.0% to 59.8%. The prevalence in the DRC was 59.8% (666/1113; 95% CI: 57.0%, 62.7%), in Kenya was 33.2% (220/663; 95% CI: 29.6%, 36.8%), in Zambia was 5.4% (31/572; 95% CI: 3.6%, 7.3%), and in Pakistan was 1.0% (4/411; 95% CI: 0.3%, 2.5%). Risk factors of *Plasmodium falciparum* infection in early pregnancy and associations with birth outcomes will be presented. These results show differing levels of malaria in early pregnancy depending on the location. Future steps include determining risk factors of malaria in early pregnancy and its effects on birth outcomes.

1610

MULTIPLICITY OF INFECTION AND PARASITE DENSITY BY AGE IN SYMPTOMATIC MALARIA EPISODES IN SOUTHERN MALAWI

Alaina Halbach[1], Andrea Buchwald[1], Dominique Earland[1], Alick Sixpence[2], Mabvuto Chimenya[2], Milius Damson[2], Karl Seydel[3], Don Mathanga[2], Terrie Taylor[3], Miriam Laufer[1]

[1]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States, [2]Malaria Alert Center, University of Malawi College of Medicine, Blantyre, Malawi, [3]College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States*

Malaria remains an important cause of morbidity and mortality worldwide despite improvements in public health interventions. Individuals repeatedly exposed to malaria over time can develop a state of semi-immunity with asymptomatic infections. While overall parasite density has been found to decrease with age, we hypothesize that in order for symptomatic disease to occur in adults who have developed this semi-immunity, their parasite burden, defined by parasite density (PD) and multiplicity of infection (MOI), must be higher than in children. This assessment is part of a 2 year longitudinal malaria cohort study in Mfera, Malawi with age stratified enrollment of 120 subject ages 1-50 presenting with uncomplicated *Plasmodium falciparum* malaria. Subjects presented for monthly follow-up visits as well as for sick visits. Blood smears and dried blood spots on filter paper were collected at all visits. PD (parasites/μL) was determined via smear and MOI was determined through MSP and GLURP genotyping from the dried blood spots. The PD and MOI for symptomatic episodes were compared across age groups. There were 459 symptomatic episodes from 110 subjects. The mean PD decreased significantly across age groups (7,581 parasites/μL in the adults compared to 65,499 and 48,322 in children under 5 years and children aged 5 to 15 years, respectively, p<.001). The mean MOI was significantly less in adults compared to the two younger age groups (1.88 clones detected per episode in adults compared to 2.51 and 2.58 clones per episode in children under 5 and children 5 to 15, respectively, p<.005). The decreased parasite burden among adults may be explained by their semi-immune responses that limit parasite density, even in symptomatic infections, thus reducing the overall parasite burden in adults. Alternatively, symptomatic infections may occur when novel parasite proteins are expressed.

1611

PREVALENCE OF MICROSCOPIC AND SUBMICROSCOPIC *PLASMODIUM SPP.* INFECTIONS AND ASSOCIATED FACTORS IN INDIGENOUS AND NON-INDIGENOUS COMMUNITIES IN COLOMBIA

Jehidys Estella Montiel Ramos, Luisa F. Carbal Reyes, Veronica Sierra Cifuentes, Juan C. Perez, Gabriel J. Velez, Daniel C. Aguirre Acevedo, Lina M. Zuluaga Idarraga, Cesar H. Segura Latorre, Alberto Tobon Castaño, Ana M. Vasquez Cardona

Universidad de Antioquia, Medellín, Colombia

Prevalence of asymptomatic and submicroscopic infections (SI) by *Plasmodium* in Colombia has not widely explored. There is a great diversity of indigenous communities in Colombia living in endemic areas; however, the burden of infection in these populations has not extensively studied. The prevalence of *Plasmodium* infection and its associated factors in indigenous and non-indigenous population was explored in two endemic villages from Antioquia. A Community-based cross-sectional survey was conducted between Nov 2016-Nov 2017 in 7 villages of Turbo and El Bagre municipalities from Antioquia. All inhabitants of all ages that were willing to participate were included. Sociodemographic and clinical data from each subject were recorded as well as household information. Parasitological diagnosis was performed by microscopy and nested PCR. The prevalence of microscopy and SI was estimated and a adjusted GEE model was used to explore risk factors associated with parasitemia, taking into account the correlation between subjects at a household level. Among 713 participants enrolled, 60,1% were from indigenous communities. *Plasmodium* spp. was detected in 30 subjects (4,2%, CI 95% 2,9-5,9), from those 29 were in indigenous population. 47% of infections were afebrile and most of them submicroscopic (10/14). Microscopic and submicroscopic prevalence was 2,5% (CI 95% 1,6-3,9) and 1,7% (CI 95% 0,9-2,9) respectively. In El Bagre all infections occurred in indigenous participants (3,9%, CI 95% 2,2-7,1) and 81% were submicroscopic. By contrast, in Turbo the highest prevalence occurred in indigenous people (11,5%; CI 95%: 7,3-17,5) but 88,8% were microscopic. Living in an indigenous population increase the incidence rate of infection compare with non-indigenous population (IRR 19,4; CI 95% 2,3-166,7). Most of infections were detected in indigenous communities. Not only microscopic but also SI can contribute to malaria transmission. Thus, prevention and control strategies targeting to a particular reservoir such as indigenous population, rather than relying on passive detection of clinically malaria, might be essential to achieve elimination

1612

SUBCLINICAL *PLASMODIUM FALCIPARUM* INFECTION AMONG CHILDREN AND ADULTS RESIDING IN A HIGH MALARIA TRANSMISSION COMMUNITY

Tamaki Kobayashi[1], Matthew M. Ippolito[2], Jay Sikalima[3], James S. Lupiya[3], Mike Chaponda[3], William J. Moss[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]Tropical Diseases Research Centre, Ndola, Zambia*

Chronic, asymptomatic, and subpatent malaria are overlapping subclinical syndromes classified according to the extent or duration of parasitemia and the presence or absence of parasite-attributable pathology. Subclinical malaria is incompletely characterized in terms of its natural history, clinical consequences, and epidemiological contribution. We investigated demographic and clinical features of subclinical malaria to assess potential risk factors and elucidate its relevance to malaria control. Longitudinal and cross-sectional surveys were conducted between 2013 and 2017 in adults and children residing in a high malaria transmission area of northern Zambia (n=2,249). *Plasmodium falciparum* parasitemia was measured by polymerase chain reaction (PCR), microscopy, and rapid diagnostic test (RDT). Demographic, clinical data (temperature, hemoglobin, self-reported symptoms), bed net use, indoor residual spraying, and recent antimalarial treatment were recorded. Parasite prevalence was 31% by microscopy,

53% by RDT, and 55% by PCR. Participants were stratified according to PCR, microscopy, and RDT results: patent (+/+/+), subpatent (+/-/-), recent (-/-/+), and no (-/-/-) parasitemia. Among PCR-positive individuals, 66% (n=816) had subclinical malaria defined as temperature <38°C and no self-reported fever, chills, headache, vomiting, diarrhea, or cough within the prior 48 hours. Participants with patent (n=592) or recent (n=183) parasitemia tended to be younger (median age = 10 y, IQR 5-20) than those with subpatent (n=253) or no parasitemia (n=762) (25 y, IQR 10-41). Participants with patent or recent parasitemia were more likely to have anemia compared to no parasitemia (OR=1.8, 95%CI 1.5, 2.2). Those with no parasitemia were more likely to report sleeping under a bed net compared to those with patent, subpatent or recent parasitemia (OR=2.5, 95%CI 1.7, 2.5). This study identified a high prevalence of subclinical malaria in a holoendemic area. Characterization of this diverse group will inform public health and clinical approaches to malaria control in similar transmission settings.

1613

SEVERE MALARIA SURVEILLANCE IN A RURAL DISTRICT HOSPITAL IN NORTHERN ZAMBIA

Matthew M. Ippolito[1], Jean-Bertin Kabuya[2], James S. Lupiya[2], Luc Kambale Kamavu[3], Jay Sikalima[2], Mike Chaponda[2], Proscovia Miiye Banda[3], William J. Moss[4]

[1]*Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*Tropical Diseases Research Centre, Ndola, Zambia,* [3]*St. Paul's General Hospital, Nchelenge, Zambia,* [4]*Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States*

Severe malaria surveillance is routinely performed in Zambia by hospital-based Health Management Information System (HMIS) personnel. HMIS surveillance relies on information aggregated from ward registers. Supplementation of HMIS data with hospital- and patient-level data from additional sources may provide greater resolution of severe malaria clinical epidemiology. To assess hospital-based methods of severe malaria surveillance, we conducted a single center study that evaluated four data collection approaches over the period June 2017 to March 2019. Aggregate data were collected from HMIS records and central pharmacy artesunate inventories. Individual-level data were collected from artesunate administration records and laboratory blood transfusion logbooks. Means and standard deviations of cases per month were calculated and compared using Student's *t* test and one-way analysis of variance. The HMIS monthly case estimate (79 ± 40) was systematically greater than estimates based on artesunate inventory (56 ± 38), artesunate treatment courses (54 ± 32), and pediatric blood transfusions (45 ± 18) (P=0.02). Age was similar between artesunate-treated patients and transfused patients (median 24 months, interquartile range 14-43) and similar across years of surveillance. Partial or complete blood product stockouts were recorded for 95 days over the 667-day surveillance period (14%). Accurate accounting of severe malaria case burden can help focus resources in a timely and effective manner. Shifts over time in case volume, age distribution, and allocation of blood transfusion may reveal underlying changes in local malaria epidemiology. Alternative data sources can supplement existing register-based HMIS surveillance.

1614

PRELIMINARY FINDINGS AND LOGISTICAL CHALLENGES FROM AN INTENSIVE LONGITUDINAL COHORT STUDY OF MALARIA TRANSMISSION IN A PRE-ELIMINATION SETTING IN SOUTHERN ZAMBIA

Jessica Schue[1], Japhet Matoba[2], Jennifer C. Stevenson[2], Harry Hamapumbu[2], Ben Katowa[2], Michael Musonda[2], Tamaki Kobayashi[1], Timothy Shields[1], Andre Hackman[1], Philip E. Thuma[2], William J. Moss[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Macha Resarch Trust, Choma, Zambia*

The Southern Province has the lowest *P. falciparum* prevalence in children under 5 years in Zambia and is targeted for malaria elimination. An intensive cohort was designed to understand how malaria transmission is sustained in a near-elimination setting. The study is a longitudinal cohort of geographically clustered households in Choma District, Zambia. Households are visited monthly for up to two years and all persons over 3 months of age are eligible. Blood is collected at each visit and qPCR targeting *Pfcyt-b* is performed. Passive case detection occurs at the rural health center and all persons who test positive by RDT are eligible. Data collection began in October 2018. As of March 2019, 171 households were enrolled and 9 refused to participate. In the enrolled households, there were 845 eligible persons of which 795 (94%) consented. The median household size was 5 (range:1-17) and 83% of households own at least one bed net. Each person has a median number of 4 visits, with 264 completing all visits in the first 6 months. Bed net usage the previous night ranged from 38% in October 2018 to 49% in March 2019. On average, 27.7% (range: 12.6-42.6) participants traveled in the previous 4 weeks. Bed net use declined during travel, 75.9% of all travelers reported not using a bed net. Laboratory results through mid-February identified 29 qPCR positive samples, for a parasite prevalence of 2.2% within the cohort. Eight confirmed cases were enrolled at the health center. Public events in the area impacted monthly sampling targets. Sampling members has been a challenge, especially for school age children and farmers. Community mobilization and monthly dissemination meetings of aggregated results help to maintain high levels of acceptance. Data collection continues through September 2020. *Pfcyt-b* positive samples will be genotyped to determine the proportion resulting from local transmission vs. importations. The longitudinal genetic data and the travel information will be used to construct a malaria transmission network. Risk factors for malaria at the household and individual level will be identified.

1615

WHEN FEVERS REIGNITE: AN ASSESSMENT OF *PLASMODIUM VIVAX* RECURRENCES IN PANAMA

Carmela M. Jackman[1], Bernardo Garcia Espinosa[2], Madeline E. Baird[2], Nicholas Presley[2], Darlene Bhavnani[2], Lizbeth Cerezo[1]

[1]*Ministerio de Salud de la República de Panamá, Panama City, Panama,* [2]*Clinton Health Access Initiative, Boston, MA, United States*

Panama is committed to eliminate malaria by 2020. Over the last five years, malaria cases have become increasingly concentrated in indigenous *Comarca* regions yet reduction of national cases has stalled in this period. A successful elimination campaign will tailor activities to the species makeup of the local context but while *Plasmodium vivax* accounts for all local cases in Panama, little is known about the burden of relapses. To address this gap, this study sought to quantify *P. vivax* recurrences and describe the population in which they occur. Currently, Panama's malaria case data is stored in free-text files without validation or unique ID numbers. This retrospective cohort study compiled annual data and applied Jaro-Winkler distances on patient names to identify individuals with multiple malaria incidents. Per national guidelines, cases are confirmed by microscopy and followed up with a control blood slide for nine months. Recurrences were defined as successive episodes of *P. vivax* malaria in this timeframe. To assess risk factors related to recurrence, odds ratios (OR) were calculated from patient demographics. Of the 2,168 *P.*

vivax cases reported from January 1, 2016 to December 31, 2018, 11.0% were identified as recurrences. This proportion increased over time, yielding 5.8% in 2016 to 16.2% in 2018. Median time to recurrence was 118 days (IQR: 71-173 days). Among cases detected in the Guna Yala *Comarca*, 13.8% were recurrences, the highest proportion versus other endemic regions where this figure ranged from 3.5% to 12.3%. Highest odds of recurring were found in males (OR: 1.36; 95% CI: 1.04 - 1.77), and in those aged 0 - 4 years (OR: 1.65; 95% CI: 1.09 - 2.51) when compared to individuals over 40 years old. Trends observed in this study coincide with efforts toward strengthening reporting and case detection through improved community case management in the 2016-2018 period. This work highlights the importance of quantifying recurrence and assessing its contribution to ongoing transmission. As Panama approaches elimination, this study provides evidence on *P. vivax* recurrences to describe progress toward this goal and inform case management strategies.

1616

PLASMODIUM SPECIES FREQUENCY AT BANCOUMANA, A MALARIA VACCINE TESTING CENTER IN MALI

M'Bouye Doucoure[1], Amatigue Zeguime[1], Sintry Sanogo[1], Moussa B. Kanoute[1], Bourama Samake[1], Aissata Doumbia[1], Drissa Dembele[1], Aly Togora[1], Mahamadoun H. Assadou[1], Boubacar Traore[1], Jordyn Manucci[2], Agnes Mwakingwe-Omari[3], Jen C.C. Hume[3], Patrick E. Duffy[3], Issaka Sagara[1], Ogobara Doumbo[1]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Division of Intramural Research, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Malaria transmission blocking vaccines (TBVs) that target the sexual stages of the parasite in the mosquito host have emerged as a potential tool for malaria elimination. Recent phase 1 TBV clinical trials have shown promising activity, prompting further evaluation in phase 2 trials. In support of such trials, we initiated a study in Bancoumana, Mali, a rural and malaria-endemic site 60 km south of Bamako, to characterize community-specific malaria epidemiology. Here, we present first year data (Feb 2018 to Jan 2019) on *Plasmodium* species frequencies and parasite infection prevalence. A total 873 volunteers stratified into three age groups (0-4 years, 5-14 years and 15-65 years) were recruited and followed monthly for clinical and laboratory assessment (i.e. clinical exam, blood smears and hemoglobin level). As expected, *P. falciparum* (*Pf*) was most prevalent at 11.3% (1092/9634) with 3.5% (335/9634) gametocyte carriers; for *P. malariae* and *P. ovale,* the annual prevalence was 0.64% (62/9634) and 0.15% (14/9634) respectively. *Pf* prevalence was significantly higher in Feb [19.4% (162/836)] compared to transmission season in Oct [13.2% (105/794), p=0.001] and Nov [13.3% (106/796), p=0.001]. The lowest prevalence was observed in Jan [6.3% (49/775)]. *P. falciparum* gametocyte carriage rates (GCR) peaked in Feb [7.7% (64/836), while *P. malariae* and *P. ovale* GCRs varied between 0% and 1.8% throughout the year. Overall, malaria prevalence was lower in 2018-2019 compared to previous assessments in Oct 2011 [21.5% (50/233)] and Oct 2012 [38.2% (26/68)] (Assadou et al). *Pf* was most prevalent in 5-14 year olds [7.7% (744/9634) with a GCR of 2.29% (221/9634)], followed by participants 15-65 years [3.0% (291/9634) with a GCR of 0.94% (91/9634)] and 0-4 years [0.59% (57/9634) with a GCR of 0.24% (23/9634)]. *Pf* density ranged between 40 to 409,320 parasites/µl. *Pf* coinfected with *P. malariae* in 0.29% (28/9634) of cases, and with *P. ovale* in 0.03% (3/9634) of cases. Prevalence and parasite density were highest among children aged 5-14, and *P. falciparum* gametocyte carriage throughout the year enables continual transmission.

1617

CHARACTERIZATION OF ENDEMIC YEAR-ROUND MALARIA TRANSMISSION IN THE CHITTAGONG HILL TRACTS OF BANGLADESH

Forrest K. Jones[1], Amy Wesolowski[1], Ching S. Phru[2], Mohammad S. Hossain[2], David J. Sullivan[1], Wasif A. Khan[2], Emily S. Gurley[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh*

In Bangladesh, 18 million people live in malaria endemic regions. Most malaria cases occur in the remote Chittagong Hill Tracts on the eastern border. Surveillance in this area has relied on both facility-based and field-based detection which is likely incomplete from limited access to hard to reach areas. Accurate incidence estimates could improve elimination plans. Using data from community-based malaria surveillance, we estimated incidence and described demographic, temporal, and geographic heterogeneity. During Sep 2010 - Oct 2013, United Nations Development Programme and Hill District Council staff regularly visited remote communities in 70 unions and tested people with febrile illness for malaria using either slide microscopy or rapid diagnostic test. We collected health register data from mobile clinics and community health workers and calculated incidence rates using the expected annual number of cases and population estimates from the 2011 Bangladesh Census. Among 43,783 patients tested, 10,334 were diagnosed with malaria. Overall, the incidence rate was 4.3 cases per 1000 person-years [PY]) while the median case age was 14 (IQR: 6 - 28.5). Individuals <5 years had significantly higher incidence rates (6.4 cases per 1000 PY) than those 5 - 14 years and ≥15 years (5.5 & 3.6 cases per 1000 PY). For those ≥15 years, men had higher incidence rates than women (4.0 vs 3.0 cases per 1000 PY), but not significantly so. Monthly incidence remained ≥1 case per 1000 PY throughout the entire study period. Among 70 unions, the median incidence was 2.8 cases per 1000 PY (IQR: 1.2 - 5.5); the three unions with the highest incidence (48, 28 & 28 cases per 1000 PY) also had the most cases. In the Hill Tracts, malaria maintains a robust, year-round transmission cycle afflicting both sexes and all age groups, with a higher burden borne by children. Most unions sustain high transmission as defined by the WHO (≥1 case per 1000 PY) with some having rates comparable to parts of sub-Saharan Africa. Elimination in Bangladesh will require sustained control strategies for all ages while targeting geographic areas of highest incidence.

1618

QUANTIFYING THE ROLE OF AGE AND *PLASMODIUM FALCIPARUM* INFECTION TO ANEMIA PREVALENCE AMONG CHILDREN IN UGANDA

John M. Henry[1], David L. Smith[1], Moses Kamya[2], John Rek[3], Bryan Greenhouse[4], Isabel Rodriguez-Barraquer[4], Grant Dorsey[4]

[1]*University of Washington, Seattle, WA, United States,* [2]*Makerere University, Kampala, Uganda,* [3]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [4]*University of California San Francisco, San Francisco, CA, United States*

The link between malaria infection and anemia is well established, but the mechanisms of interaction between the human host and protozoan parasite which lead to anemia are complex and noisy. In particular, those mechanisms may depend on a wide variety of factors including the age and exposure history of the host. This makes determining the relative contribution of malaria infection to the burden of anemia difficult. Using hemoglobin concentration measurements and *P. falciparum* parasite counts from children under 10 years old obtained from the Program for Resistance, Immunology, Surveillance, and Modeling (PRISM) study currently underway in Uganda, we have found strong evidence for the existence of an age- and infection status-dependent pattern in hemoglobin concentration. Applying the known statistical properties of the data-fitted Ornstein-Uhlenbeck process to determine the probability an individual is below the hemoglobin levels which categorize anemia

conditioned on infection status, we estimated the impact of malaria infection on anemia. After correcting for age, we show that *P. falciparum* infection accounts for an average drop of approximately .835 g/dl in girls and .651 g/dl in boys, which accounted for age-specific differences in the prevalence of mild, moderate, and severe anemia. In this population, the model suggests *P. falciparum* increased odds of anemia by a factor of about 1.54 for girls and 1.42 for boys. Further study is needed for individuals during and after adolescence and in locations that may have different baseline levels of nutrition and elevation, as these are also strong drivers of hemoglobin concentration. Improving these estimates may have programmatic implications in evaluating the relative impact of different public health interventions focused on reducing the burden of malaria and anemia.

1619

EVALUATION OF A LINK BETWEEN MALARIA AND HYPERTENSION IN THE UNITED STATES: A CROSS-SECTIONAL POPULATION-BASED COHORT ANALYSIS

Morgan Birabaharan, Andrew Strunk, Amit Garg, Stefan Hagmann

Donald and Barbara Zucker School of Medicine, Hempstead, NY, United States

Malaria has growing recognition for its association with hypertension (HTN). Low birth weight, malnutrition and chronic inflammation in the setting of malaria in malaria-holoendemic areas have been discussed as possible patho-mechanisms. We aimed to assess if predominant singular or sporadic malaria infection in the context of travel exposure in a population residing in a malaria non-endemic country also shows an association with HTN. A cross-sectional analysis was performed using Explorys, a large database with US patients seen in the inpatient and outpatient settings (63 million unique lives, 27 healthcare organizations, all census regions and insurance types represented). The analysis included adult patients with an active status in the database during April 2014-April 2019 and had demographic data on age, gender, race, and body mass index. The Systematized Nomenclature of Medicine - Clinical Terms (SNOMED-CT) of 'malaria' and 'essential hypertension' were used to identify patients with malaria and HTN. We assessed overall HTN prevalence among patients with and without past malaria infection, and within demographic subgroups. A total of 4,670 malaria patients were compared with the control cohort of approximately ~18 million non-malaria patients. The prevalence of HTN in the malaria-cohort was 43.6% (2,080/4,670) compared to 38.2% (6,897,190/18,062,810) in the general population. Patients with malaria were more likely to be male (57.0% vs 43.4%), >65 years of age (41.3% vs 28.9%), African American (25.9% vs 12.6%), and diabetic (18.4% vs 14.9%), but were less likely to be a smoker (22.1% vs 29.3%), obese (41.2% vs 45.9%), or an alcoholic (3.2% vs 3.9%). In subgroup analysis, the prevalence of HTN was highest among patients who were male, >65 years of age, and white. Patients with history of malaria and one modifiable risk factor had higher prevalence of HTN than the general population with the same modifiable risk factor; obese (58.8% vs 50.1%), tobacco smoker (61.1% vs 46.0%), diabetic (87.2% vs 82.3%), and alcoholism (73.3% vs 54.2%). In this observational study we observed a higher prevalence of HTN among those with a history of malaria infection. As most individuals in the U.S. diagnosed with malaria suffer from travel-associated malaria, our findings suggest that even singular or rare exposure to malaria may be associated with higher odds of also having HTN. Further investigation of the malaria-HTN link should also include travel-associated malaria cases.

1620

EPIDEMIOLOGIC AND CLINICAL PROFILE OF SEVERE MALARIA CASES TREATED AT THE BEFELATANANA UNIVERSITY HOSPITAL, MADAGASCAR FROM JANUARY 2018 TO FEBRUARY 2019

Hitsy A. Razafindrazaka[1], Jocelyn Razafindrakoto[2], Volatiana Andriananja[1], Mihaja Raberahona[1], Rajaonarison Mahan[1], Laurent Kapesa[2], Mamy Randria:[1]

[1]*Department of Infectious Diseases, Befelatanana University Hospital, Antananarivo, Madagascar,* [2]*USAID/PMI, Antananarivo, Madagascar*

Madagascar has five malaria transmission zones, based on transmission length and the intensity. In general, the central highlands (CHL) has low malaria transmission and the coastal areas have high malaria transmission. Malaria is the 4th leading cause of hospital mortality in 2017. We described the epidemiologic and clinical profile of severe malaria cases admitted to the Befelatanana University Hospital, situated in the CHL. We conducted chart abstractions of all severe malaria cases from January 1, 2018 to February 28, 2019. Cases were defined as a person admitted at with positive malaria Rapid Diagnostic Test (RDT) and at least one WHO-defined severe malaria symptom. Data were captured and analyzed using EPI-INFO 7.0, and the P-value and OR were calculated by Fisher›s exact test. A total of 73 patients were admitted to the analysis: 19 were female (26%) and 54 (74%) male. The mean age was 35 years (range 10-50). Sixty-four (88%) cases were residents of the CHL, and reported having travelled to high transmission zones. None of them took malaria chemoprophylaxis. Two cases were autochthonous and seven cases were not specified. During their travel, 13 cases worked as masons (18%), 13 as seasonal farmers (18%), 11 as street vendors (15%), 10 drivers (13 %), and 17 unspecified. All 64 cases developed severe malaria after returning to the CHL. The symptoms were fever in all of them, shaking in 37 cases (51%), sweat in 32 cases (45%) and headache in 30 cases (41%). The main symptoms of severity were prostration (71%), confusion (67%), kidney failure (16%), and coma (12 %). The treatment was injectable Artesunate in 63 cases (86.3%) and injectable quinine in 10 cases (21.92%), which was followed by ACT treatment. Median time to discharge was 4.33 days. The parasitemia was negative at Day 3 in 29 out of 31 cases (94%) who tested by microscopy. Eight cases died: 2 women and 6 men. The majority of cases were CHL residents travelling to high transmission zones. This suggests that they should be sensitized on prevention such as consistent use of LLIN. Clinicians in the low transmission also CHL should be regularly targeted to refresher training on malaria case management.

1621

EVALUATING GUIDELINES FOR COMMUNITY HEALTH WORKER PROTOCOLS IN ZAMBIA BASED ON DATA FROM TRIALS AND ROUTINE REPORTING

Caitlin A. Bever[1], Reine Rutagwera[2], Hannah Slater[3], John Miller[2], Kammerle Schneider[3], Thom Eisele[4], Edward Wenger[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*PATH, Lusaka, Zambia,* [3]*PATH, Seattle, WA, United States,* [4]*Tulane University, New Orleans, LA, United States*

The Zambian National Malaria Elimination Centre has rolled out a vast community health worker (CHW) program to improve access to timely malaria diagnosis and treatment in rural areas. In addition to addressing primary cases, CHW duties include conducting reactive follow-ups, in which those living in the vicinity of a primary case are tested, and treated if positive. The original guidelines for CHWs mandated that reactive follow-ups include all neighbors within 140m of the index household but the routine reporting data has shown that this is an unachievable standard in regions where the transmission intensity, and therefore primary caseload, is high. A revised set of recommendations dictated that in these high transmission intensity regions, CHWs should still attempt to perform at least five follow-ups per month for maintenance of skills and

continued awareness within the community. In this work, we set out to further improve the regional CHW guidelines by balancing the operational difficulties faced by each CHW with the epidemiological benefits expected from a given number of reactive follow-ups. Using data from a large-scale mass drug administration trial conducted in Southern Province from 2011-2016 as well as routine reporting data collected by CHWs from 2014 until now, we determine the expected number of infections treated per follow-up as a function of local transmission intensity and population distribution. These results are weighed against previously observed CHW performance to recommend achievable protocols that can be adjusted in response to major changes in transmission. Finally, we identify those health facility catchment areas where it is expected that the current allotment is insufficient for the local population and suggest specific placement for additional CHWs.

1622

MALARIA TRANSMISSION MONITORING IN LAGOS STATE, NIGERIA

Ndukwe Ukoha[1], Ify Aniebo[1], Kelechi Ohiri[1], Wellington A. Oyibo[2]

[1]*Health Services Delivery Foundation, Abuja, Nigeria,* [2]*ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine, University of Lagos, Lagos, Nigeria*

Malaria prevalence in Nigeria in the last 15 years has seen successive decline in prevalence from the Malaria Indicator Survey conducted in 2010 and 2015 from 42% in 2010 to 27% in 2015. Indeed in 2015, the prevalence of malaria was 0% in Lagos State. This prompted the use a surrogate measurement in elucidating the transmission of malaria in Lagos. The objective of this transmission intensity monitoring was to provide a snapshot on malaria transmission using antigens. Samples for this study was obtained from asymptomatic individuals in communities in Lagos. A sub-set of the Lagos MIS samples were tested by indirect ELISA for total immunoglobulin (Ig)-G Ab against a chimeric *Pf* antigen. In a sub-set of 142 asymptomatic samples from which ELISA was conducted to measure *Plasmodium falciparum* antibodies, 7 of the individuals were positive (4.9%) by microscopy but the Pf ELISA detected antibodies in 80 persons with antibody prevalence of 56.3%. High antibody levels were expressed in children less than 15 years compared with the older age groups and this indicated that malaria transmission occurred more at the younger population. Our antibody results provided several significant indicators of transmission in Lagos and this was also evident in the high levels of Pf antibody in Ikorodu, an area identified as a high malaria transmission area when compared to the urban areas where transmission is low. These results demonstrated that malaria transmission is ongoing in Lagos.

1623

EPIDEMIOLOGY OF MALARIA IN KT ZONE, SOUTHERN ETHIOPIA: A FIVE YEAR DATA ANALYSIS, 2011-2015

Abraham Lere Keshabo, **Adamu Addissie Nuramo**

Addis Ababa University, Addis Ababa, Ethiopia

Between 2000 and 2015, the number of malaria cases declined by 42% while the malaria death rate declined by 66% in the African Region. However, Malaria is a major public health challenge in Ethiopia, contributing 4% of all cases in Africa. It makes approximately 68% of the population the country at risk. Therefore, this surveillance data analysis needed to analyze magnitude, trends, and geographical distribution of the disease in Kembata-Tembaro Zone from 2011 to 2015. A descriptive study was employed for analysis of data on malaria indicators from the Integrated Disease Surveillance and Response System database for the years 2011-2015. The surveillance data were analyzed to show incidence, trends and variation in risk by reporting woredas by using charts, graphs and tables. In the Zone, the average estimated annual incidence of reported total malaria in the overall population was 69 per 1000 persons and confirmed malaria were 54 per 1,000 per year over the five years (2011 to 2015). As of the calendar years 2011-2015, the annual incidence of total malaria report dropped from 119/1000 to 9/1000 and reported malaria in-patient admissions and deaths dropped from 1.7/1000 to 0.9 per 1,000 per year and 1.3/100,000 to 0.4/100,000 respectively. In addition, laboratory test increases from 60% in 2011 to 90.2% in 2015. We conclude that the magnitude of Malaria in Kembata Tembaro Zone declined (dropped significantly from 119/1000 to 9/1000) from 2011-2015. From all woredas, kedida woreda is most frequently affected woreda in the Zone. Even though different malaria control strategies were designed to roll back to its minimum level in Kembata Tembaro Zone, still malaria cases were not decreased as expected. Therefore, the zonal health department should maintain such reduction in both morbidity and mortality due to malaria.

1624

IDENTIFICATION OF EXPRESSED *VAR*S IN WHOLE BLOOD CLINICAL SAMPLES WITH A CUSTOM CAPTURE ARRAY VERSUS RNA ENRICHMENT METHODS

Emily M. Stucke[1], Antoine Dara[2], Ankit Dwivedi[1], Theresa Hodges[1], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Karim Troaore[2], Boureima Guindo[2], Bourama Tangara[2], Amadou Niangaly[2], Modibo Daou[2], Issa Diarra[2], Youssouf Tolo[2], Mody Sissoko[2], Albert E. Zhou[1], Matthew B. Laurens[1], Amed Ouattara[1], Bourema Kouriba[2], Ogobar K. Duombo[2], Shannon Takala-Harrison[1], David Serre[1], Mahamadou A. Thera[2], Christopher V. Plowe[3], Mark A. Travassos[1], Joana C. Silva[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Sciences, Techniques and Technologies, Bamako, Mali,* [3]*Duke University, Durham, NC, United States*

The *var* gene family encodes *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1) antigens. These highly diverse antigens are displayed on the surface of infected erythrocytes and play a critical role in immune evasion and sequestration. Studies of *var* expression using non-leukocyte-depleted blood are challenging due to the predominance of host genetic material and lack of conserved *var* segments to allow primer anchoring and sequence amplification. To address these barriers, we compared two enrichment methods for parasite RNA extracted from whole blood clinical samples—globin and rRNA depletion followed by polyA selection *vs.* a custom capture array based on Roche's SeqCap EZ Enrichment System. The capture array was designed with probes covering the 3D7 reference genome and an additional >4,000 full-length *var* gene sequences. We tested each method on the same samples from Malian children with severe or uncomplicated malaria infections, and sequenced using Illumina. *Var*-like transcripts were identified from the *de novo* assembly of non-human reads and annotated. For each sample, we compared transcript length and number of unique transcripts generated from each enrichment method. To determine if each method yielded identical *var* sequences, we compared sequences generated by each method. We then quantified *var* expression to determine if expression correlated between methods. Depletion of the most abundant human RNAs followed by polyA selection produced transcripts with greater median length in samples with the highest parasitemias compared to the capture method. The capture array produced the longest maximum length and largest numbers of transcripts for each sample, particularly for samples with low parasitemia (<2,000 parasites/μL). The capture method produced more unique fragments, including up to 20 distinct acidic terminal sequence domains per sample. The two methods produced different quantitative *var* profiles. Further evaluation will include expression analyses of samples with known *var* repertoires to determine the method best suited to analyze *var* expression in studies with both low and high parasitemia samples.

1625

GLOBAL GENETIC DIVERSITY AND POPULATION STRUCTURE OF *PLASMODIUM FALCIPARUM* TRANSMISSION VACCINE TARGETS PFS47, PFS48/45 AND PFS230

Ankit Dwivedi[1], Alvaro Molina-Cruz[2], Giovanna Carpi[3], Kara Moser[1], Carolina Barillas-Mury[2], Joana C. Silva[1]

[1]*Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [3]*Department of Biological Sciences, Purdue University, West Lafayette, IN, United States*

The *Plasmodium falciparum* (*Pf*) protein Pfs47 has been shown to mediate evasion of the mosquito complement-like immune system, enabling parasite survival and transmission. Pfs47 is a potential target of interventions aimed at preventing malaria transmission, as are Pfs48/45 and Pfs230, other 6-cystine family members expressed during the sexual stage of the parasite life cycle. Population genetics and experimental transmission studies, using multiple *Pf* strains and mosquito species, suggest that *P. falciparum* adaptation to local mosquito populations has led to regional selection at the *Pfs47* locus. Less is known about genetic diversity and selection on *Pfs48/45* and *Pfs230* locus. In this study we analyze whole genome sequence data of ~710 parasite samples originating from 8 geographical locations in South America, Africa, SE Asia and Oceania, which differ in intensity of malaria transmission and *Anopheles* mosquito vector species. We investigate genetic differentiation at all sexual stage 6-cys protein family *loci* between global populations based on nucleotide diversity (ϖ), haplotype diversity (*Hd*) and fixation index (F_{ST}). Genetic differentiation at *Pfs48/45* and *Pfs230* is higher than expected by chance, as observed in the case of *Pfs47*. Furthermore, haplotype diversity networks of these 6-cys *loci* show strong geographic structure with varying diversity in local populations. We also investigated potential functional association of other *loci* with *Pfs47* based on linkage disequilibrium (LD) statistics (*r2*). We generated a list of *Pf loci* in LD with *Pfs47*, which includes other sexual stage 6-cys protein family *loci*. Further investigation of functional processes and metabolic pathways associated with the *loci* in LD with *Pfs47* is underway. These genes could be functionally associated with *Pfs47* and/or potentially involved in survival of the parasite in the mosquito vector, representing potential targets for transmission-blocking strategies. The genetic diversity and population structure observed in Pfs47, Pf48/45 and Pfs230 *loci* could be important for the successful design of transmission blocking vaccines based on these targets.

1626

THE APOLIPOPROTEIN E3/E3 GENOTYPE IS ASSOCIATED WITH PROTECTION FROM SEVERE MALARIA IN UGANDAN CHILDREN

Giselle Lima-Cooper[1], Benson J. Ouma[2], Andrea L. Conroy[1], Katrina Co[1], Dibyadyuti Datta[1], Robert O. Opoka[3], Chandy C. John[1]

[1]*Ryan White Center for Pediatric Infectious Diseases and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Department of Microbiology, Makerere University, Kampala, Uganda,* [3]*Department of Paediatrics and Child Health, Makerere University, Kampala, Uganda*

Apolipoprotein E (APOE), a protein involved in the transport and metabolism of lipids and brain injury repair, has been implicated in the pathogenesis of experimental cerebral malaria, but there is conflicting data on whether the APOE alleles (E2, E3, E4) or genotypes are associated with increased risk of severe malaria in humans. We investigated associations between APOE polymorphisms and disease severity in two cohorts of Ugandan children enrolled in prospective studies of severe malaria pathogenesis. The first cohort consisted of children ages 5-12 years with cerebral malaria (CM, n=92) or uncomplicated malaria (UM, n=94), and asymptomatic community children (CC, n=108). Children with UM or CC had not had severe malaria, so were combined as a "protected" comparator group to CM. Children with the APOE E4 allele or the APOE E3/E4 genotype had an increased risk of CM (odds ratio (OR) [95% confidence interval (CI)], 1.71 [1.03, 2.84] and 1.85 [1.09, 3.14], respectively), while children with the APOE E3/E3 genotype had decreased risk of CM (OR [95% CI], 0.57 [0.33, 0.98]). The second cohort consisted of children ages 18 months - 12 years with CM (n=269) or severe malaria anemia (SMA, n=233), and asymptomatic community children (CC, n=216). In this cohort, children with CM and SMA had similar frequencies of APOE alleles and were combined into a single severe malaria group. Children with the APOE4 allele or APOE4/E4 genotype had a higher risk of severe malaria (OR [95% CI], 1.68 [1.16, 2.44] and 11.25 [1.51, 83.60], respectively), while children with the APOE E3 allele or APOE E3/E3 genotype had a decreased risk of severe malaria (OR [95% CI], 0.43 [0.22, 0.84], and 0.54 [0.37, 0.78], respectively). Children with the APOE E4/E4 or E3/E3 genotypes also had significantly increased or decreased risks, respectively, for CM alone. In two independent cohorts of Ugandan children, APOE E3/E3 was associated with protection from severe malaria and APOE4 with increased risk for severe malaria. Future studies should confirm these associations in other populations, and if confirmed, determine the mechanisms by which APOE E3/E3 may protect from severe malaria.

1627

MALARIA IN VENEZUELA: CHANGES IN THE COMPLEXITY OF INFECTION REFLECTS THE INCREMENT IN TRANSMISSION INTENSITY

M. Andreina Pacheco[1], David A. Forero-Peña[2], Melynar Chavero[3], Angel Gamardo[4], Luisamy Figuera[3], Leopoldo Villegas[5], María E. Grillet[6], Kristan Schneider[7], Ananias A. Escalante[1]

[1]*Temple University, Philadelphia, PA, United States,* [2]*Escuela de Ciencias de la Salud, Universidad de Oriente, Núcleo Bolívar, Ciudad Bolívar, Bolivarian Republic of Venezuela,* [3]*Departamento de Medicina Interna, Complejo Hospitalario Universitario "Ruíz y Páez", Ciudad Bolívar, Bolivarian Republic of Venezuela,* [4]*Biomedical Research Institute and Therapeutic Vaccines, Ciudad Bolívar, Bolivarian Republic of Venezuela,* [5]*Asociación Civil Impacto Social (Tumeremo, Venezuela) and Global Development One, Silver Spring, MD, United States,* [6]*Instituto de Zoología y Ecología Tropical, Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela,* [7]*University of Applied Sciences Mittweida, Mittweida, Germany*

Malaria incidence has reached staggering numbers in Venezuela in a matter of years. Historically, Bolivar State has accounted for approximately 70% of the Venezuelan cases with many clustered in the Sifontes municipality, a region characterized by an extractive economy including gold mining. It has been proposed that changes in migration to this area, as a result of the Venezuelan economic crisis, drove a malaria spillover to the rest of the country and the region. Here, we studied *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) populations from the municipality of Sifontes. Samples collected in 2018 were compared with samples from 2003 (106 *Pf* and 104 *Pv*). A total of 77 *P. falciparum* and 94 *P. vivax* isolates from 2018 were genotyped by using eight standardized microsatellite *loci*. In addition, mutations linked to drug resistance (*Pfdhfr*, *Pfdhps*, and *Pfcrt*) and the *Pfk13* gene associated with artemisinin delayed parasite clearance in *P. falciparum* were analyzed. Consistent with the increase in transmission, polyclonal infections raised from 1.9% in 2003 to 39% in 2018 in *P. falciparum* and from 15.4% to 68% in *P. vivax*. Although the circulating *P. falciparum* parasites still harboring drug-resistant mutations in *Pfdhfr*, *Pfdhps*, and *Pfcrt*; mutations associated with artemisinin delayed parasite clearance was not found in the *Pfk13* gene. Interesting, for both parasites, the genetic diversity (He: 0.98 for *P. falciparum* and He: 1.0 for *P. vivax*) increased in 2018. The samples from 2003 and 2018 have several alleles per locus in common but do not share multi-locus genotypes. Bayesian clustering using the Structure v2.3.4 software yield two populations linked to the time of sampling showing that the parasites populations temporarily changed. Whereas the situation on mutations linked with drug resistance in *P. falciparum* seems to remain stable, all other observations are consistent with an increase in

transmission. These parasite genetic data will support studies directed to test the hypothesis that the outburst of malaria in Venezuela originated in Sifontes as a malaria hotspot.

1628

DEMOGRAPHIC AND EVOLUTIONARY INSIGHTS FROM RECENT LARGE-SCALE WHOLE-GENOME SEQUENCING EFFORTS OF *PLASMODIUM FALCIPARUM* IN SOUTH AMERICA

Angela M. Early[1], Pablo Cardenas Ramirez[2], Manuela Carrasquilla[3], Horace Cox[4], Luana C. Mathieu[5], Aimee R. Taylor[3], Caroline O. Buckee[3], Angelica Knudson[6], Lise Musset[5], Socrates Herrera[7], Julian C. Rayner[8], Daniel E. Neafsey[3], Vladimir Corredor[6]

[1]*Broad Institute, Cambridge, MA, United States,* [2]*Massachusetts Institute of Technology, Cambridge, MA, United States,* [3]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [4]*Guyana Ministry of Public Health, Georgetown, Guyana,* [5]*Institut Pasteur de la Guyane, Cayenne, French Guiana,* [6]*National University of Colombia, Bogota, Colombia,* [7]*Institute of Immunology, Cali, Colombia,* [8]*Wellcome Sanger Institute, Hinxton, United Kingdom*

Genomic diversity in *Plasmodium falciparum* is undercharacterized in many parts of South America, considering the significance of local disease burden, challenges to disease elimination, and the propensity for the *de novo* emergence of drug resistance mutations in this region. Here, we report the first whole-genome sequencing-based survey of *P. falciparum* from the Pacific coast of Colombia, a regional hotspot for malaria transmission that is estimated to account for 25% of the *P. falciparum* malaria cases in the Americas. Of the 151 clinical samples sequenced, only a small proportion of infections (0.1) are multiclonal. There is also a high degree of relatedness among parasites. Parasite pairs have a median identity-by-descent (IBD) rate across their genomes of 0.20, with 14% showing strong evidence of clonality (IBD > 0.75). This high clonality and low number of multiclonal infections suggest that recombination between unrelated lineages is low, allowing a small number of distinct clonal lineages to persist. Despite the low genetic diversity in the population, we observed multiple segregating haplotypes at known drug resistance genes including *pfcrt*, *pfdhps*, *pfdhfr*, and *pfmdr1*. We compared these Colombian results with those from the only other South American region with extensive population-level genomic data: the Guiana Shield. There is sufficient population structure to largely differentiate parasites from the two regions allowing infections to be flagged as potentially imported and placing directionality on parasite migration. The joint analysis of these data sets demonstrates how genome-wide sequencing and targeted molecular surveillance across the Americas is increasing our ability to (1) detect the early emergence of drug resistance, (2) track regional parasite movement, and (3) measure declines in diversity that could mark successful intervention strategies. These data, however, are only a first step. Continued dense sampling across the continent is needed to detect the potential spread of the *pfkelch13* C580Y mutation from Guyana and to monitor the expected increase of exported parasites from Venezuela.

1629

COMPARATIVE TRANSCRIPTOMICS OF *PLASMODIUM FALCIPARUM* IN NORMAL AND SICKLE-TRAIT ERYTHROCYTES USING RNA SEQUENCING

Joseph W. Saelens, Jens E. Petersen, Betsy Freedman, Steve B. Haase, Steve M. Taylor

Duke University, Durham, NC, United States

Sickle-trait hemoglobin (HbAS) confers near-complete protection from severe, life-threatening falciparum malaria in African children, but the molecular mechanisms by which HbAS confers these protective phenotypes remain incompletely understood; our preliminary data suggest that HbAS allows normal erythrocyte invasion and maturation, but disrupts the structured transcriptional program of the parasite. To further investigate this, we performed time-series comparative transcriptomic analyses of the asexual stage of *Plasmodium falciparum* in normal (HbAA) and HbAS erythrocytes using RNA sequencing. In separate experiments, late schizonts of *P. falciparum* reference strains 3D7 and FUP were isolated using Percoll gradients and inoculated into parallel cultures in HbAA and HbAS erythrocytes. Newly-invaded rings were purified using sorbitol, and over 48 hours parasites from each independent culture were sampled for light microscopy and RNA isolation every 3 hours. RNA from each timepoint was sequenced on the Illumina NovaSeq6000, generating ~120 million 150bp paired-end reads for each of 16 timepoints over the asexual cycle. We quantified transcripts using STAR and modeled differential expression of parasite transcripts between HbAA and HbAS erythrocytes with DESeq2. We identified periodic gene expression using persistent homology, Lomb-Scargle, JTK-CYCLE, and de Lichtenberg algorithms, and measured the impact of HbAS on *P. falciparum's* periodic gene expression using the Characterizing Loss of Cell Cycle Synchrony (CLOCCS) software. We then analyzed our time series data using the Local Edge Machine (LEM) software to infer *P. falciparum's* periodic regulatory networks that are disrupted in sickle-trait. These analyses reveal HbAS broadly disrupts the highly-structured gene expression of *P. falciparum,* suggesting that sickle-trait confers its protective effect by neutralizing the transcriptional programs that underlie the parasite's pathogenic mechanisms. Understanding these aberrant effects on parasites of HbAS will help to identify new targets for therapeutic and preventive malaria measures.

1630

EVOLUTION AND EXPANSION OF MULTI-DRUG RESISTANT MALARIA IN SOUTHEAST ASIA

William L. Hamilton[1], Roberto Amato[1], Rob W. van der Pluijm[2], Arjen M. Dondorp[2], Dominic P. Kwiatkowski[1], Olivo Miotto[2], MalariaGEN Community Project[3], GenRe-Mekong Project[3], TRACII collaborations[3]

[1]*Wellcome Trust Sanger Institute, Hinxton, United Kingdom,* [2]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand*

The evolution of drug resistant malaria poses a major challenge to elimination efforts. A multidrug resistant co-lineage of *Plasmodium falciparum* malaria, named KEL1/PLA1, spread across Cambodia 2008-2013, causing high treatment failure rates to the front-line therapy. Through the MalariaGEN Community Project, we have analysed whole genome sequencing data from >1,600 *P. falciparum* clinical samples collected 2008-2018 from across Southeast Asia to investigate the epidemiology of multi-drug resistance emergence, evolution and expansion. We found that KEL1/PLA1 parasites spread rapidly from 2015 into all of the regions surrounding Cambodia, maintaining a high level of genetic relatedness reflecting their common origin. However, several genetic subgroups have recently emerged within this lineage with diverse geographical distributions. Some of these KEL1/PLA1 subgroups carry recently emerging mutations in the chloroquine resistance transporter (*crt*) gene, which arise in association with a constellation of other mutations that have accumulated over decades in Southeast Asia. Our findings highlight both the utility of pathogen genomics for revealing large-scale processes in evolutionary biology, and the need for ongoing surveillance and rapid public health responsiveness in the face of continual parasite evolution.

1631

CHARACTERIZING *PLASMODIUM FALCIPARUM* GENETIC DIVERSITY IN TWO VILLAGES OF MALI BOUGOULA-HAMEAU AND FALADJE

Aoua Coulibaly[1], Aminatou Kone[1], Antoine Dara[1], Abdoulaye Djimde[1], Nicola Mulder[2], Olivo Miotto[3]

[1]*University of Science, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*University of Cape Town, Cape Town, South Africa,* [3]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand*

The genetic diversity of *P. falciparum* populations is a major factor in the parasites ability to adapt to changes in its environment, enabling the

development of drug resistance and the evasion of host immune system. Therefore, characterizing the genetic diversity and understanding the main drivers of the genetic diversity and differentiation would be critical in implementation of new strategies for malaria control programs. Studies have demonstrated markedly lower level of population structure in West African parasite populations, and given the difficulties in separating populations by geographical origin using current tools, it is necessary to develop new methods that can discriminate *P. falciparum* populations from West African countries. The aim of this study is to identify genetic variations that can discriminate between parasite populations in two villages of Mali, Bougoula-Hameau and Faladje. Overall 433 dried blood spots samples from both villages were subjected to DNA extraction. DNA was amplified using sWGA and sequenced on Illumina platform. In total, 1000000 SNPs were identified from the dataset. Series of filtering steps were conducted on the potential SNPs and samples, to eliminate artifacts and produce a list of high quality SNPs and samples. Population genetic analysis was performed including population structure and inter population differentiation. Principal component analysis (PCA) was done on all samples, and neighbor joining trees were constructed for all samples. The parasite populations showed a low genetic differentiation (Fst<0.02). When performing the same analyses after selecting a set of SNPs with higher Fst, we observed a remarkable separation between the groups. Analyses are ongoing to qualify how stable the differentiating SNPs are using a larger dataset, and higher resolution data.

1632

WHOLE TRANSCRIPTOME IDENTIFICATION OF MICRORNAS ASSOCIATED WITH SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN

Qiuying Cheng[1], Caroline Ndege[2], Samuel B. Anyona[2], Christophe G. Lambert[1], Douglas J. Perkins[1]

[1]*University of New Mexico, Albuquerque, NM, United States,* [2]*University of New Mexico-Kenya Global Health Programs, Kisumu, Kenya*

Molecular mechanisms that mediate the development of pediatric severe malarial anemia (SMA, Hb<5.0g/dL) are only partially defined. MicroRNAs (miRs), a group of non-coding RNAs ~22 nucleotides in length, have been shown to modulate host gene expression profiles. Thus, we utilized whole-transcriptome analysis of peripheral blood to compare miR levels in children (6-36 months of age) with non-SMA (Hb>5.0g/dL, n=43) and SMA (n=17) from a holoendemic *Plasmodium falciparum* transmission region of western Kenya, Siaya. Transcriptome measurements were performed using the HumanHT-12 v4 expression BeadChip kit (Illumina®) which covers 228 distinct human miRs. Here, we present the results for miRs which potentially target immune response genes known to be important in the pathogenesis of SMA. The preliminary findings presented highlight the potentially important role of miRs in the pathogenesis of SMA.

1633

PIPERAQUINE RESISTANCE IS ASSOCIATED WITH DIFFERENTIAL VACUOLAR ACCUMULATION AND PEPTIDOMIC PROFILES IN *PLASMODIUM FALCIPARUM*

John Okombo[1], Sachel Mok[1], Edward Owen[2], Zbynek Bozdech[3], Manuel Llinas[2], David Fidock[1]

[1]*Columbia University, New York City, NY, United States,* [2]*Pennsylvania State University, Pennsylvannia, PA, United States,* [3]*Nanyang Technological University, Jurong West, Singapore*

Recent reports document treatment failures with the first-line therapy combination of dihydroartemisinin plus piperaquine (PPQ) in Cambodia and emerging resistance in French Guiana. The absence of effective alternative malaria treatments underlines the need to define the molecular interactions linked to PPQ resistance (PPQ-R) to curb its spread. Analysis of genome-edited lines complemented by whole-genome sequence data and phenotypic studies recently revealed that novel mutations in the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) can mediate PPQ-R. Since PfCRT mutations conferring resistance to chloroquine (CQ), an antimalarial with a common putative mode of action, have previously been implicated in altered drug transport kinetics and changes in hemoglobin metabolism. The aim was to use peptidomics, gene expression studies, vacuolar drug accumulation assays and a heme fractionation protocol on isogenic *P. falciparum* lines expressing wildtype ($Dd2^{Dd2\ GCO3}$) and mutant pfcrt alleles ($Dd2^{Dd2}$, $Dd2^{Dd2\ F145I}$, $Dd2^{Dd2\ G353V)}$ to gain insight into phenotypes associated with mutations that modulate PPQ activity. Preliminary data suggest that lines harboring PfCRT mutations differ in peptidomic profiles compared to their isogenic parent. In addition, microarray-based transcriptomics reveal an upregulation of genes involved in the protein export pathway and hemoglobin digestion, possibly to offset defects in peptide transport. The PPQ-R $Dd2^{Dd2\ F145I}$ and $Dd2^{Dd2\ G353V}$ lines accrued [^{3}H] CQ to higher levels compared to $Dd2^{Dd2}$ but exhibited only marginal ablation in [^{3}H] PPQ accumulation relative to the PPQ-sensitive $Dd2^{Dd2\ GCO3}$, implying potential contribution of other physiological interactions – possibly binding to PfCRT at higher PPQ concentrations eliciting conformational changes that permit drug expulsion. Fractionation studies on the tested lines showed that PPQ inhibited heme detoxification in a manner not dissimilar to CQ. Our results provide evidence that changes in peptide levels, gene expression, drug accumulation and possible involvement of binding to PfCRT play a role in the PPQ-R phenotype.

1634

HIGH THROUGHPUT PHENOTYPIC SCREEN UNRAVELS *PLASMODIUM FALCIPARUM* GENES ESSENTIAL TO MALARIA TRANSMISSION

Jyotsna Chawla

University of South Florida, Tampa, FL, United States

Transmission to new hosts which is crucial to a parasite's life cycle is mediated by sexual stages, called gametocytes in the deadly *Plasmodium falciparum.* Circulating in the human host mature gametocytes are picked up during a blood meal and are primed for survival and development in the mosquito vector. Critical, yet neglected with no drugs targeting this stage, gametocytes propagate through the population unchecked, making it a prime target to block malaria transmission. Previous work in our laboratory achieved a saturation level mutagenesis of *P. falciparum* by creating random insertions of *piggyBac* transposon elements to generate >38,000 mutants. In this study, we characterized genes dispensable for asexual development for their likely importance in sexual stage development. These genes are characterized for their expression patterns, GO pathways, conservation with other *Plasmodium spp.* and other identifiable features. Our next goal is to validate mutant phenotype and define significance in gametocyte formation. Through this study, we anticipate closing an important gap in the *P. falciparum* life cycle and lay the foundation for new antimalarial intervention strategies.

1635

LONGITUDINAL GENOTYPING USING AMPLICON DEEP-SEQUENCING TO DESCRIBE RESIDUAL PARASITEMIA IN THE SETTING OF RAPIDLY DECLINING TRANSMISSION IN NAGONGERA, UGANDA

Jessica Briggs[1], Noam Teyssier[1], Joaniter Nankabirwa[2], John Rek[2], Emmanuel Arinaitwe[2], Moses Kamya[2], Grant Dorsey[1], Isabel Rodriguez-Barraquer[1], Bryan Greenhouse[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda*

Characterizing the reservoir of asymptomatic parasitemia that persists following effective malaria control interventions is critical for designing strategies to sustain gains and eventually eliminate malaria. In Nagongera, Uganda, 2 rounds of LLIN distribution and 6 rounds of IRS over 4 years have reduced the incidence of malaria from 2.29 cases per person-year (ppy) to 0.05 cases ppy. Despite this dramatic reduction in malaria incidence, in a cohort of participants (n=448) followed monthly over one

year from October 2017-October 2018, 26% of the participants were parasitemic by ultrasensitive *var*ATS quantitative PCR (qPCR) at one or more timepoints. To investigate the reservoir of parasitemia in this cohort, we performed qPCR monthly and genotyped all positive samples using an amplicon-based deep sequencing method targeting a highly variable region of *P. falciparum* apical membrane antigen-1. Of 782 available positive qPCR samples, we successfully genotyped 717 (92%). We then designated clones seen in the first three months as baseline infections and any clone seen afterwards a new infection. Three skips were allowed before calling the same clone in an individual new, to allow for imperfect detectability. Molecular force of infection (mFOI) was calculated as the number of new infection events divided by person time. At the cohort level, the proportion of participants with infections persistent from baseline declined from 15.4% to 4.7%. There were only 10 episodes of malaria; therefore, the majority of these infections cleared without antimalarials. All episodes of malaria occurred in the setting of acquiring a new infection within the last 28 days. Overall mFOI was 0.21 events ppy compared to an incidence of malaria of 0.05 cases ppy; thus mFOI was able to capture additional transmission events not reflected by malaria incidence. We show that amplicon deep sequencing of longitudinal samples can successfully be used to characterize asymptomatic parasitemia; in our cohort, declining prevalence of parasitemia is primarily driven through clearance of old infections.

1636

ACCURATE ASSEMBLY OF MULTIGENE FAMILIES AND OTHER REGIONS OF HIGH DIVERSITY IN *PLASMODIUM FALCIPARUM* FROM WHOLE GENOME SEQUENCING WITH NOVEL ASSEMBLER PATHWEAVER

Nicholas J. Hathaway[1], Jeffrey A. Bailey[2]

[1]*University of Massachusetts Medical School, Worcester, MA, United States,* [2]*Brown University, Providence, RI, United States*

Plasmodium multigene families play key roles in facilitating infection and host immune evasion but due to their interrelated and highly diverse nature have been a challenge to study and fully characterize using standard whole-genome sequencing and analysis methods. While other technologies such as long read sequencing can be used the greater cost and more cumbersome sample requirements have inhibited their adoption. Thus the vast majority of genomic studies of *Plasmodium* have relied on short read sequencing data. To leverage this wealth of data, we have developed a novel local assembler, PathWeaver, which after standard reference alignment takes shotgun whole genome sequencing data from specific genes or gene families and optimally de novo reconstructs the genes of interest. The algorithm can iteratively recruit mismapped and unmapped reads overcoming limitations of the reference genome. On control genomes, it was able to accurately assemble the VAR, RIFIN, STEVOR, and SURFIN gene families. In comparison to other metagenomic assemblers, metaSPAdes, MEGAHIT, savage and MetaVelvet), PathWeaver was showed improved performance on both multigene families as well as other highly variable genes including AMA1, CSP, and TRAP. PathWeaver was then used on all currently publicly available whole genome sequencing data which contained 4054 field samples that spanned across South America, Africa, and South East Asia and 33 lab isolates. This enabled the more fully characterization of the number and diversity of VAR, RIFIN, STEVOR, and SURFIN genes.

1637

HOST AND PARASITE TRANSCRIPTOMIC CHANGES UPON SUCCESSIVE *PLASMODIUM FALCIPARUM* INFECTIONS IN MALIAN CHILDREN

Katie R. Bradwell[1], Drissa Coulibaly[2], Matthew B. Laurens[3], Ahmadou Dembélé[2], Youssouf Tolo[2], Abdoulaye K. Koné[2], Karim Traoré[2], Amadou Niangaly[2], Andrea A. Berry[3], Bourema Kouriba[2], Kirsten E. Lyke[3], Shannon Takala-Harrison[3], Ogobara K. Doumbo[2], Christopher V. Plowe[4], Mahamadou A. Thera[2], Mark Travassos[3], David Serre[1]

[1]*Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Malaria Research and Training Center, University Science, Techniques and Technologies, Bamako, Mali,* [3]*Malaria Research Program, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [4]*Duke Global Health Institute, Duke University, Durham, NC, United States*

In malaria-endemic regions, children have to overcome successive encounters with *P. falciparum* parasites before developing immunity, first against severe disease and later against uncomplicated malaria. Understanding cellular and molecular interactions between host and parasites during infections can provide critical insights on processes underlying this stepwise acquisition of immunity, as well as to how parasites adapt to more "malaria-experienced" hosts. Here, we describe joint analyses of host and parasite gene expression from blood samples collected from five consecutive *P. falciparum* symptomatic infections in three Malian children. We generated and sequenced 15 polyA-selected RNA-seq libraries after globin reduction to simultaneously characterize host and parasite transcriptomes. Unsupervised clustering analysis revealed that host gene expression profiles primarily clustered by individual, while the parasite gene expression profiles differentiated early vs. late infections. This pattern was also supported by gene-by-gene analysis that revealed that a greater number of host genes were differentially expressed according to the individual rather than by the number of prior malaria episodes experienced (4,581 vs. 1,042, FDR=0.2), while more parasite genes were differentially expressed according to the number of prior infections (0 vs. 68). Gene co-expression analysis highlighted more than 1,000 human/parasite gene pairs whose expression co-vary across infections (R^2>0.8), revealing potential host/pathogen interactions throughout the course of the infections. We also showed that the dual RNA-seq data enable statistical assessment of the proportions of i) the different white blood cell subsets and ii) the parasite developmental stages. Finally, we leveraged the read sequences generated to analyze allelic variations in transcribed regions and rigorously assess the polyclonality of each infection. Overall, our findings suggest complex interactions between the host's acquisition of immunity and the parasite's escape mechanisms over successive infections.

1638

GLOBAL STRUCTURE OF THE *VAR* GENES ENCODING THE MAJOR VARIANT SURFACE ANTIGEN OF *PLASMODIUM FALCIPARUM*

Gerry Tonkin-Hill[1], Shazia Ruybal-Pesántez[1], **Kathryn E. Tiedje**[1], Virginie Rougeron[2], Sedigheh Zakeri[3], Tepanata Pumpaibool[4], Pingchai Harnyuttanakorn[4], OraLee H. Branch[5], Lastenia Ruiz-Mesia[6], Michael F. Duffy[1], Thomas S. Rask[1], Franck Prugnolle[7], Yao-Ban Yao-Ban Chan[8], Anthony T. Papenfuss[9], Karen P. Day[1]

[1]*University of Melbourne/Bio*[21] *Institute, Parkville, Australia,* [2]*MIVEGEC, University of Montpellier, Montpellier, France,* [3]*Malaria and Vector Research Group, Pasteur Institute of Iran, Tehran, Islamic Republic of Iran,* [4]*Chulalongkorn University, Bangkok, Thailand,* [5]*Concordia University, Portland, OR, United States,* [6]*Universidad Nacional de la Amazonía*

Peruana, Iquitos, Peru, [7]MIVEGEC, University of Montpellier, Montepellier, France, [8]University of Melbourne, Parkville, Australia, [9]Walter and Eliza Hall Institute, Parkville, Australia

Malaria remains a major public health problem in many countries. Unlike influenza and HIV, where diversity in immunodominant surface antigens is understood geographically, relatively little is known about the global population structure of PfEMP1, the major variant surface antigen of the malaria parasite *Plasmodium falciparum.* The extreme diversity of the *var* genes that encode PfEMP1 has so far hindered efforts to understand their population structure. A Jumping Hidden Markov Model (JHMM) that considers *var* gene recombination was used to reconstruct each sequence in the dataset as an imperfect mosaic of donor sequences. By leveraging this approach that we designed specifically for the analysis of *var* genes and applying it to a global dataset of *var* sequences from ten countries, we describe *var* gene population structure at a global scale for the first time. The sensitivity of the approach allowed for the comparison of the global dataset to ape samples of other *Plasmodium* species. Using the JHMM method, we were able to distinguish all countries within the global data set. We describe multiple sub-populations in South America consistent with the "Out of Africa" hypothesis where *P. falciparum* was introduced into South America from Africa, likely due to the transatlantic slave trade. We provide compelling evidence for *var* population structure within Africa with Uganda, Ghana and Gabon showing distinct proportion profiles using the JHMM method. Additionally, we identify a number of highly conserved *var* types that are present globally and have previously not been well characterized. These findings will inform efforts in potential vaccine design and suggest that the analysis of *var* genes is a promising tool in the surveillance of the rapidly evolving transmission dynamics of *P. falciparum*.

1639

IN SILICO CAPTURE AND ASSEMBLY OF HIGHLY VARIABLE LOCI

Theresa K. Hodges[1], James Matsumura[1], Ankit Dwivedi[1], Kara A. Moser[1], Andrea A. Berry[2], Shannon Takala-Harrison[3], Jonathan Crabtree[1], Joana Carneiro Da Silva[1]

[1]*Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Malaria Research Program, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States*

The reconstruction of highly variable protein-coding genes in *Plasmodium falciparum* remains challenging, despite recent advances in DNA sequencing technologies and whole genome assembly algorithms. In particular, genes with length polymorphisms and low complexity regions are difficult to assemble. Many of these genes contribute to antigenic variability in the parasite and are potential targets for vaccine design. We present a novel pipeline to reconstruct the sequence of target gene(s) from whole genome sequencing (WGS) data from new pathogen isolates. The first step involves the recruitment of WGS reads from targeted genes by using GSNAP to map read data to one or more reference allelic sequences from the *loci* of interest and retaining successfully map reads and their mate pairs. For each targeted gene, de novo assembly of all recruited reads is performed using SPAdes and the resulting assembly is assessed by aligning it back to the reference genome. Assembly is further improved by *de novo* assembling the recruited reads using Hierarchical Genome Assembly (HGA) and Scaffold Builder, based on user-defined thresholds. These steps are repeated for recruited reads that remain unassembled, using the SMALT aligner and/or iterative rounds with different assembly parameter values, until no further improvements can be made to the assembly, at which point the assembled fragment(s) is considered final. The pipeline was validated using short read WGS data from four *P. falciparum* strains from different continents, including 3D7 and NF166 from Africa, 7G8 from South America and NF135 from SE Asia. PacBio-based reference for each strain were used as positive control and confirmed the accurate reconstruction of >95% of all protein-coding *loci* over 90% of their length. Subsequently, we used this pipeline to reconstruct 228 malaria vaccine antigens from 459 *P. falciparum* isolates from seven different geographic areas, using short read WGS data for each isolate. Of these, 210 *loci* were fully reconstructed from >350 strains. Total amount of starting DNA material is a critical factor in the successful reconstruction of allelic sequences for target *loci*.

1640

MOLECULAR EPIDEMIOLOGY OF MALARIA UNDER SHIFTING AGRICULTURAL PRACTICES IN AFRICA

Elizabeth Hemming-Schroeder[1], Daibin Zhong[1], Amanda Chie[1], Harrysone Atieli[1], Andrew Githeko[2], Guiyun Yan[1]

[1]*University of California Irvine, Irvine, CA, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya*

Africa bears a disproportionate amount of the global malaria burden, accounting for roughly 90% of worldwide malaria cases and deaths. At the same time, millions of sub-Saharan African residents are also afflicted by chronic hunger, food insecurity, and famine. To help combat food insecurity, investments in dams and irrigation in sub-Saharan Africa have increased substantially over the past decade. However, it is not clear if water resource development will have unforeseen consequences for malaria control. Therefore, the objective of this study is to assess the impacts of shifting agricultural practices on malaria epidemiology by measuring differences in population genetic diversity and infection complexity across irrigation zones. To achieve this objective, we collected malaria parasites from 30 clusters in three irrigation zones in Homa Bay, Kenya. Malaria parasites were sequenced on the Illumina Miseq (2x300 bp read length) at four polymorphic genes (*pfmsp1*, *pfcsp*, *pfama1*, and *pfcpmp*) and four genes associated with drug resistance (*pfk13, pfmdr1, pfdhfr,* and *pfdhps*) to measure population level spatial genetic structure and within-host genetic diversity. A pilot test of 8 PCR reactions were successfully amplified, sequenced, and joined for the four polymorphic genes. An average of 22653 reads per sample passed quality filtering, 27931 reads for *pfcsp,* 11737 reads for *pfama1,* 24792 for *pfmsp1,* and 26151 for *pfcpmp.* These methods will be implemented, with the addition of drug resistance markers to compare population and within-host diversity across irrigation zones. This study will inform how the construction of irrigation schemes and shifting agricultural practices modulates malaria epidemiology and risk. As population growth is expected to continue in Africa, water resource development is also expected to help meet food security demands. Understanding the interactions between these development activities and malaria epidemiology is critical to maintaining effective malaria control strategies.

1641

EPHRIN B LIGANDS REGULATE HUMORAL IMMUNE RESPONSE TO *PLASMODIUM* PARASITE

Adesola C. Olatunde[1], Patrice N. Mimche[1], spencer O. Seely[1], Taryn P. Stewart[1], Franklin Maloba[2], Balotin Fogang[2], Lawrence Ayong[2], Tracey J. Lamb[1]

[1]*University of Utah, Salt Lake City, UT, United States,* [2]*Centre Pasteur du Cameroun, Yaounde, Cameroon*

Malaria remains one of the infectious diseases that cause high morbidity and mortality worldwide. Despite several attempts to induce protective immune responses against *Plasmodium* parasites that cause malaria, sterile immunity against this pathogen through vaccination is yet to be achieved. Generation of protective humoral immunity, characterized by a high affinity and long-lived antibody response, against any pathogen relies on robust germinal center (GC) reactions orchestrated by follicular helper T (Tfh) cells. The molecular and cellular mechanisms that prevent the development of a long-lived protective humoral response specifically in the case of *Plasmodium* are poorly understood. Emerging data have demonstrated that ephrin B1, a ligand for the Eph B receptor tyrosine kinase subfamily, is involved in Tfh cell recruitment, retention and contact-dependent interaction with GC B cells. Here we show an upregulation in the expression of Ephrin B ligands on both Tfh and GC B cells that peak

at day 17 post infection of wild type mice with non-lethal *P. yoelii* XNL. Selective deficiency of Ephrin B1/B2 on B cells ($CD19^{cre+}$Ephrin $B1/B2^{fl/fl}$ mice) led to lethality in some mice infected with an otherwise non-lethal *P. yoelii* XNL infection. We observed an increase in the expression of Ephrin B on peripheral Tfh cells from human with both severe and cerebral malaria. Therefore we investigated the role that Tfh-expressed ephrin B ligands may play in generating an efficacious antibody response to *Plasmodium* infection. Unlike $CD19^{cre+}$Ephrin $B1/B2^{fl/fl}$ mice, $CD4^{cre+}$Eprhin $B1/B2^{fl/fl}$ mice were able to control this infection. Our data show a requirement for EphrinB/EphB signaling for development of *Plasmodium* humoral responses with B cell expression of ephrin B1/B2 ligands a requirement in mice for generating a protective response against primary acute *Plasmodium* infection

1642

MALIAN CHILDREN WITH SEVERE MALARIA EXHIBIT DISTINCT PFEMP1 ANTIBODY PROFILES THAT DIFFER BY BLOOD TYPE

Albert E. Zhou[1], Paul Han[1], Andrea A. Berry[1], Drissa Coulibaly[2], Emily M. Stucke[1], Amed Ouattara[1], Biraj Shrestha[1], Matthew B. Laurens[1], Rie Nakajima[3], Aarti Jain[3], Omid Taghavian[3], Joshua M. Obiero[3], Li Liang[3], Algis Jasinskas[3], Amadou Niangaly[2], Bourema Kouriba[2], Abdoulaye Kone[2], Ogobara K. Doumbo[2], J. Alexandra Rowe[4], Shannon Takala-Harrison[1], Kirsten E. Lyke[1], Christopher V. Plowe[5], Philip L. Felgner[3], Mahamadou A. Thera[2], Mark A. Travassos[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali,* [3]*Vaccine Research and Development Center, Department of Physiology and Biophysics, School of Medicine, University of California Irvine, Irvine, CA, United States,* [4]*Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom,* [5]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Severe malaria caused by *Plasmodium falciparum* primarily affects children in sub-Saharan Africa. Severe malaria pathogenesis is poorly understood but likely involves deep tissue sequestration and rosetting of infected erythrocytes. ABO blood type and *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) variant surface antigen expression appear to play critical roles in rosetting. Depending on blood type, parasites infecting subjects may express certain subsets of PfEMP1s, such as DBLα1 domains, associated with severe disease pathogenesis. For each blood type, we predicted that Malian children with severe malaria would demonstrate a unique PfEMP1 serological profile that reflects the lack of antibody responses to PfEMP1s potentially involved in pathogenesis. We also predicted that severe malaria cases would have less recognition of and lowered seroreactivity to a subset of PfEMP1 protein fragments than controls with uncomplicated malaria. We probed a custom protein microarray populated with reference strain and field-derived PfEMP1s associated with severe malaria pathogenesis with sera from a 2000-2003 Malian severe malaria case-control study. Our findings suggest that sera from children with severe malaria differed in the number of PfEMP1s recognized depending on the blood type: sera from blood type A severe malaria cases (n=27) recognized 91.6% of PfEMP1 fragments, sera from blood type AB cases (n=7) recognized 21.8% of PfEMP1 protein fragments, and sera from blood type O cases (n=13) recognized 81.6% of PfEMP1 protein fragments. In addition, sera from blood types A, AB, and O severe malaria cases reacted less intensely to 67, 12, and 34 PfEMP1s fragments, respectively, than uncomplicated malaria sera. Analyses investigating the differential serological responses to DBLα1-PfEMP1s are currently ongoing. These findings may inform our understanding of severe malaria pathogenesis and the design of vaccines to protect individuals from severe malaria.

1643

IMMUNOMODULATION OF PREGNANCY-ASSOCIATED MALARIA AND ITS EFFECT ON INFANT IMMUNE RESPONSE AGAINST VACCINE ANTIGENS

Eliana M. Arango Florez[1], Catalina Alvarez Larrotta[1], Olga M. Agudelo Garcia[1], Amanda Maestre[1], Stephanie K. Yanow[2], Jaime Carmona Fonseca[1]

[1]*Universidad de Antioquia, Medellin, Colombia,* [2]*University of Alberta, Edmonton, AB, Canada*

Pregnant women have an increased risk of plasmodial infections. Submicroscopic plasmodial infections (SPI) are a very common finding in pregnant women living in low malaria transmission setting areas. SPI are considered chronic because they are usually asymptomatic and undiagnosed by routine tests (thick blood smear-TBS). In chronic infections, there is persistence of the antigenic stimulus that changes the expression of immune mediators. This promotes constant regulation, including increases in regulatory T cells, which can suppress unrelated immune responses in a non-antigen-specific manner, and cause T cell exhaustion, less robust effector functions and alteration in memory T cell differentiation. This study aimed to evaluate the immunomodulatory effect of plasmodial infection during pregnancy and its association with immune responses generated in infants against five vaccines included in the Colombian immunization plan (tetanus toxoid, hepatitis B, BCG, rotavirus, and pneumococci). Three groups of pregnant women and their infants (6 months old) were evaluated: 1) with SPI at delivery (n=15); 2) with a history of malaria during pregnancy (n=17); and 3) without malaria infection during pregnancy or at delivery (n=25). Malaria infection was diagnosed by quantitative real-time PCR (qPCR) and TBS. The relative expression of mediators associated with inflammation, anti-inflammation, regulation and co-stimulation of the immune response were quantified in peripheral blood of women and infants and placental tissue by qPCR (using the $\Delta\Delta$CT method). The infant serum levels of IgG anti-the five vaccines and of IgA anti-rotavirus were quantified by ELISA. Compared with the uninfected group, SPI at delivery increased the expression of FOXP3 in maternal peripheral blood, of IL10 and IL13 in placental tissue, and of IL10, IL13, FOXP3, TNFRII, and CD163 in infant peripheral blood. Malaria infections at delivery (submicroscopic) and during pregnancy (microscopic) were associated with decreased expression of CD40 in placenta and infant blood, as well as decreased levels of IgG anti-tetanus, rotavirus and pneumococci in 6 month old infants

1644

MATERNAL TRANSFER OF IMMUNOGLOBULIN G AND ITS IMMUNITY AGAINST *PLASMODIUM FALCIPARUM* INFECTION AMONG CHILDREN ENROLLED IN A UGANDAN BIRTH COHORT

Erick Okek

Makerere University, Kampala, Uganda

Pregnant mothers from malaria endemic areas transfer immune IgG to their babies. This paper estimates the efficiency of transfer of the IgG and associates the effects of maternal exposure to amount transferred. To estimate the efficiency of transfer, we ran a total of 583 maternal and cord blood pair using luminex. Each Magpix plate contained 40 pairs of cord and maternal samples. IgG response against a sixteen panel of common *Plasmodium falciparum* antigens were estimated; antigens were coupled with beads in the U.K.Each bead region was pooled in a buffer, mixed with samples to allow binding, several washings done, mixed and incubated with secondary IgG. Amount of antigen-antibody complex was determined by a Magpix machine. Positive control were obtained from adult individuals with history of malaria episodes, negative controls obtained from six Caucasians from the U.K .To determine the effects of maternal malaria incidence, maternal malaria prophylaxes, gravidity and child sex on amount of IgG transfer ,we did multivariate analyses in STATA using data from the mother study. To determine whether the amount of IgG transferred has any association to child malaria incidence,

we did spearman rank and found weak correlation. IgG against PfAMA1 is the most efficiently transferred at about 95%-100%. IgG transfer against most malaria antigens (GLURP, Etramp, MSP, RH5 and EBA) varies between 40% to 100% irrespective of maternal exposure. Children whose mothers had placental malaria had higher IgG response against Etramp, had very little malaria incidence during the first year of life. Maternal malaria prophylaxis, gravidity and sex had very little effects on the amount of IgG transferred. On average, it took a minimal of 3 months for a child to develop the first episode of malaria.PfAMA1 antigens are formed at earlier stages of *P. falciparum* infections thus most individuals will make robust antibodies against it thus facilitating its efficient transfer. This birth cohort was in malaria endemic area meaning almost all participants were exposed to malaria accounting for little effects of malaria incidence during pregnancy on IgG transfer.

1645

CHARACTERIZATION OF DIFFERENCES IN HOST IMMUNE GENE EXPRESSION PROFILE IN MALARIA-PROTECTED AND MALARIA-SUSCEPTIBLE CHILDREN

Gillian Mbambo, Ankit Dwivedi, Kirsten E. Lyke, Joana C. Silva

University of Maryland School of Medicine, Baltimore, MD, United States

Individuals in malaria endemic regions differ in the level of susceptibility to clinical *Plasmodium falciparum* infection, despite comparable exposure. We hypothesize that children with lower susceptibility to malaria exhibit a higher expression of genes involved in the activation of an immune response, or genes that play a role in immune effector function, in response to malaria infection, when compared with more susceptible children. To address our hypothesis, we used Peripheral Blood Mononuclear Cells (PBMCs) collected from Malian children (4-6 years of age) who participated in the control arm of an AMA1-based blood-stage vaccine efficacy study. PBMCs were collected over a transmission season, with samples from the beginning of the malaria season (Day 0), peak malaria season (Day 90) and the end of malaria season (Day 150). Children who experienced no clinical malaria episodes over the course of the malaria season were classified as "protected" whereas age-matched children that experienced two or more clinical malaria episodes were classified as "susceptible". PBMCs were stimulated with *P. falciparum* schizonts (Pfsz), *Staphylococcus aureus* enterotoxin B (SEB) as a positive control, and media as a negative control. We used mass cytometry to quantify phenotype and cytokine production post-antigen stimulation and identified a subset of children with T cell effector response including any combination of TNFα, IL-2, IFNγ transcriptional master cell regulators (Tbet and RORγt). This subset of subjects with cells with malaria antigen recognition is being used to identify differences in gene expression profiles that are associated with patterns of protective immune responses to malaria in malaria-protected vs. malaria-susceptible children. Characterizing differentially expressed genes between protected and susceptible individuals will help identify genes important for protection against malaria infections and further our understanding of the host's immunological response throughout a transmission season.

1645

CHARACTERIZATION OF DIFFERENCES IN HOST IMMUNE GENE EXPRESSION PROFILE IN MALARIA-PROTECTED AND MALARIA-SUSCEPTIBLE CHILDREN

Gillian Mbambo, Ankit Dwivedi, Kirsten E. Lyke, Joana C. Silva

University of Maryland School of Medicine, Baltimore, MD, United States

Individuals in malaria endemic regions differ in the level of susceptibility to clinical *Plasmodium falciparum* infection, despite comparable exposure. We hypothesize that children with lower susceptibility to malaria exhibit a higher expression of genes involved in the activation of an immune response, or genes that play a role in immune effector function, in response to malaria infection, when compared with more susceptible children. To address our hypothesis, we used Peripheral Blood Mononuclear Cells (PBMCs) collected from Malian children (4-6 years of age) who participated in the control arm of an AMA1-based blood-stage vaccine efficacy study. PBMCs were collected over a transmission season, with samples from the beginning of the malaria season (Day 0), peak malaria season (Day 90) and the end of malaria season (Day 150). Children who experienced no clinical malaria episodes over the course of the malaria season were classified as "protected" whereas age-matched children that experienced two or more clinical malaria episodes were classified as "susceptible". PBMCs were stimulated with *P. falciparum* schizonts (Pfsz), *Staphylococcus aureus* enterotoxin B (SEB) as a positive control, and media as a negative control. We used mass cytometry to quantify phenotype and cytokine production post-antigen stimulation and identified a subset of children with T cell effector response including any combination of TNFα, IL-2, IFNγ transcriptional master cell regulators (Tbet and RORγt). This subset of subjects with cells with malaria antigen recognition is being used to identify differences in gene expression profiles that are associated with patterns of protective immune responses to malaria in malaria-protected vs. malaria-susceptible children. Characterizing differentially expressed genes between protected and susceptible individuals will help identify genes important for protection against malaria infections and further our understanding of the host's immunological response throughout a transmission season.

1646

CHARACTERIZATION OF NATURALLY-ACQUIRED HUMAN ANTIBODIES TO *PLASMODIUM VIVAX* RETICULOCYTE BINDING PROTEIN 2B (PVRBP2)

Christopher L. King[1], Li-Jin Chan[2], Lenore Carias[1], Melanie Dietrich[2], Camila Franca[2], Sebastian Menant[2], Wai-Hong Tham[2]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Walter and Eliza Hall Institute, Melbourne, Australia*

The *Plasmodium vivax* reticulocyte binding protein 2b (PvRBP2b) has recently been identified to play a critical role in restricting parasite invasion into reticulocytes and is a potential vaccine candidate for *P. vivax* malaria. Naturally acquired immunity (NAI) protects against malaria in individuals residing in endemic areas and the passive transfer of antibodies from malaria immune individuals confers protection. A successful vaccine often models an effective immune response by natural infection. Previous studies have shown antibodies to PvRBP2b correlate with protection against clinical vivax malaria. Here we show that *P. vivax* exposed individuals acquire antibodies that block PvRBP2b binding to reticulocytes and monoclonal antibodies (mAbs) obtained from such individuals with NAI replicate this blocking activity. We also report the structure of PvRBP2b in complex with these inhibitory mAbs and identify the blocking epitopes by X-ray crystallography. These studies reveal that naturally acquired human mAbs target PvRBP2b at the binding interface with both human transferrin receptor and transferrin. Studies are currently underway to evaluate whether these human mAbs have evolved to engage polymorphic variants of PvRBP2b, whether these mAbs can block *P. vivax* invasion of reticulocytes, correlate with protection *in vivo*, and if the epitopes recognized by these mAbs are broadly conserved. A compendium of globally conserved epitopes of neutralizing human antibodies enhances the ability to develop strain-transcending RBP- and other Pv invasion ligand-based vaccines and therapeutics against *P. vivax*.

1647

CEREBRAL MALARIA, SICKLE CELL DISEASE AND BURKITT LYMPHOMA: TH1/TH2 CYTOKINE EXPRESSIONS AND CELL ADHESION MOLECULES

Olakunle O. Kassim[1], Muheez A. Durosinmi[2], Samuel K. Martin[3], Gail Hollowell[1], Kwashie A. AkoNai[2]

[1]*Howard University College of Medicine, Washington, DC, United States,* [2]*Obafemi Awolowo University, Ile-Ife, Nigeria,* [3]*Walter Reed Army Research Institute, Washington, DC, United States*

Plasmodium falciparum (Pf) plays a major role in the pathogenesis of cerebral malaria (CM), sickle cell disease (SCD) and Burkitt lymphoma (BL).

We sought to determine the prevalence of immunological factors that are common to all three diseases. Our three study groups consisted of children with CM, SCD and BL who were enrolled at Obafemi Awolowo University Teaching Hospital (OAU) in Ile-Ife, southwestern Nigeria. For these groups and the matched controls, we determined and compared the serum concentrations of endotoxin (LPS), Th1/Th2 cytokines (TNF-α, IL-1, IL-6, IL-10), cell adhesion molecules (ICAM-1 and VCAM-1), EB-VCA and EBNA-1 IgG antibodies and anti-malaria IgG, IgM, IgA and IgG subclasses to a Pf ring stage antigen. Similar determinations were done for serum iron, transferrin, α-1 antitrypsin and α-2 macroglobulin which are protease inhibitors. We analyzed the results for associations between the LPS and Th1/Th2 levels and between EBNA/EB-VCA/Pf antibodies and BL. Results showed that IgM antibodies to the malaria antigen were significantly elevated in BL, SCD and CM. But all the four IgG subclasses were decreased in SCD, BL and SCD. While Th1 and Th2 cytokine levels were 3-10X higher in CM, SCD and BL children than in controls, the highest values came from children with CM. Total serum iron, transferrin and iron binding capacity were significantly decreased in BL and SCD, but with highest decrease in SCD. While there were no differences in EBV-VCA levels for BL and control children, anti-EBNA-1 IgG antibodies were significantly elevated in BL. On the other hand, both ICAM-1 and VCAM-1 levels were twice as elevated in children with BL, SCD and CM. Odds ratio determinations showed an increased risk for BL with combined elevated anti-malaria and EBNA1 IgG antibodies. Dose response curves showed significant correlations between serum LPS and Th1/Th2 cytokines and between TNF-α and ICAM-1 and VCAM-1. The results elucidate the distinct immunological interactions in each of the malaria-associated diseases.

1648

THE EFFECT OF KILLER IMMUNOGLOBULIN-LIKE RECEPTOR GENOTYPE ON MALARIA INCIDENCE AND PARASITEMIA

Jean Digitale[1], Isabel Rodriguez-Barraquer[1], Perri Callaway[1], John Rek[2], Emmanuel Arinaitwe[2], Grant Dorsey[1], Moses Kamya[3], Margaret E. Feeney[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*Makerere University College of Health Sciences, Kampala, Uganda*

Killer cell immunoglobulin-like receptors (KIR) are expressed by malaria-specific effector T cells, and may influence the immune response to malaria. KIR genotypes are highly heterogeneous; little is understood about their relationship to antimalarial immunity. We evaluated the association of KIR genotypes with measures of immunity to malaria. Data were collected from Ugandan subjects enrolled from randomly selected households at 3 East Africa ICEMR sites with a range of malaria transmission. These analyses included 658 children aged 6 months to 10 years and 234 adults with immunogenetic and clinical data. KIR genotypes were determined by sequencing. Outcome data were obtained via routine quarterly follow-up of participants and care-seeking at study clinics when ill. Exposures of interest were KIR haplotype (A vs. B), number of activating KIR genes, and the presence of 9 KIR genes. Outcomes of interest were parasitemia (presence/absence and level, at both routine and ill visits), malaria incidence rate, and probability of symptoms if infected. We estimated multi-level models with random effects at the individual level, controlling for age group and sex. In models of malaria incidence and risk of parasitemia, we also controlled for household mosquito exposure levels. Future planned analyses will incorporate household random effects and HLA class I ligands for each KIR, and will control for multiple testing using the false discovery rate approach. 34% of respondents had Haplotype A; the mean number of activating KIR was 2.19 (SD=1.35). Each additional activating KIR was associated with an 8.5% decrease in parasite density at active malaria visits (95% CI: -16.2%, -0.11%), but not at routine quarterly visits, and did not appear to affect malaria incidence or the probability of symptoms if infected. *KIR2DS1* was protective against malaria incidence (incidence rate ratio: 0.81, 95% CI: 0.68, 0.96, p=0.02), with no effect on other outcomes. The presence of other KIR genes and haplotype (A vs. B) had no effect on any outcomes. The number of activating KIR and presence of *KIR2DS1* may impact parasitemia levels and malaria incidence rate, respectively.

1649

A SERINE PROTEASE HELPS CYTOTOXIC LYMPHOCYTE LYSE INFECTED RBCS AND MANAGE PARASITE DEATH

Gunjan Arora, Javier Manzella-Lapeira, Eric O. Long

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, Rockville, MD, United States

The morbidity and mortality caused by the malaria parasite *Plasmodium falciparum* occur during the blood-stage of infection. Antibodies acquired through years of exposure to *P. falciparum* can limit parasite replication and protect from malaria symptoms. A potential mechanism for inhibition of *P. falciparum* growth in RBCs is antibody-dependent cellular cytotoxicity (ADCC) by natural killer (NK) cells, which are activated by FcγRIIIa binding to IgG. We have shown that IgG isolated from malaria-exposed Malian adults induced lysis of infected RBCs by primary human NK cells, and inhibited parasite growth. Antibody-dependent NK cell-mediated lysis of RBCs was highly selective for infected RBCs in a mixed culture with uninfected RBCs. The *P. falciparum* antigen PfEMP1, which is expressed on the surface of infected RBCs, was a dominant target of antibodies that promoted NK cell-dependent lysis of infected RBCs and inhibition of *P. falciparum* growth. A human monoclonal antibody to the *P. falciparum* antigen RIFIN, also expressed on the RBC surface, stimulated lysis of RIFIN+ infected RBCs by NK cells, provided the RIFIN antibody carried an intact Fc receptor-binding segment. Cytotoxic granules released by NK cells contain Granzyme B, a potent protease that can effectively lyse infected RBCs and mediate death of intracellular parasite. Lysis of infected RBCs and parasite death can be prevented by inhibiting Granzyme activity. Importantly, ADCC was dependent on Granzyme but not Caspase activation. These results implicate acquired immunity through ADCC by NK cells towards *P. falciparum*-infected RBCs, antibody-based vaccine strategies that target blood-stage malaria parasites should consider this new mechanism of action.

1650

GENERATING LONG-LIVED EFFECTOR/MEMORY T CELLS WITH MOUSE CYTOMEGALOVIRUS VACCINATION TO PROLONG MALARIA IMMUNITY

Komi Gbedande[1], Samad A. Ibitokou[1], Florentin Aussenac[1], Mariapia degli Esposti[2], Michael G. Brown[3], Robin Stephens[1]

[1]*Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch (UTMB), Galveston, TX, United States,* [2]*Centre for Experimental Immunology, Lions Eye Institute, Nedlands, Western Australia, Australia,* [3]*Department of Medicine, University of Virginia, Charlottesville, VA, United States*

Malaria represents a challenge for vaccine development in part because long-lived T cell responses are required to maintain immunity. Immunity to infection with *Plasmodium spp.* is known to decay; and in mouse models, this decay correlates with loss of malaria-specific T cells. Various effective whole parasite vaccines have been proposed, but long-term protection has been elusive. Our recent work has identified effector T cells (Teff), such as those generated by persistent and recurring *Plasmodium* infections, to provide the best protection from malaria. However, Teff are short-lived; explaining the decay of natural immunity seen on emigration from an endemic area. The aim of the current study is to design a system allowing us to prolong generation of protective Teff. Cytomegalovirus (CMV) is the most successful T cell-inducing vaccine vector to date; and as a chronic virus, it can function as a vector for persistent expression of pathogen epitopes. Therefore, the goal of our novel contribution is to prolong protection by live-attenuated malaria vaccine strategies using chronic vaccination with a mouse cytomegalovirus (MCMV). Our preliminary data shows that MCMV infection protects against *P. chabaudi,* and also activates T cells driving them to an effector phenotype similar to that seen

in response to *P. chabaudi* infection. When we expressed the *P. chabaudi* MSP-1 epitope B5 as an MCMV immediate early gene, (MCMV-B5), we could see proliferation of B5 TCR Tg T cells, which is maintained by MCMV-B5 infection. We are currently testing the MCMV-B5 as a boosting strategy for vaccines that are effective, but shorter-lived in order to prolong immunity to malaria by promoting survival and cytokine production of protective effector T cells. Using MCMV as adjuvant to prolong generation of *Plasmodium*-specific effector T cells, we will also be able to study mechanisms of generation and protection.

1651

TH1 DIFFERENTIATION, BUT NOT ANTIBODY PRODUCTION, CORRELATES WITH PROTECTION FROM REINFECTION IN *PLASMODIUM* INFECTION, AND IS REGULATED BY STAT3 IN T CELLS

Victor H. Carpio[1], Florentin Aussenac[1], Kyle D. Wilson[1], Alexander Dent[2], **Robin Stephens**[1]

[1]*Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, TX, United States,* [2]*Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, United States*

In response to infection with *Plasmodium spp.*, IFN-γ^+ T helper-1 (Th1) cells drive control of parasite by phagocytosis, while IL-21$^+$ T follicular helper (Tfh) cells promote clearance by antibodies. Our data show that rather than separate subsets, many effector T cells maintain a hybrid Th1/Tfh phenotype (IFN-γ^+IL-21$^+$CXCR5$^+$) throughout infection. Although the germinal center appears with delayed kinetics, a small but stable fraction of GC-Tfh (CXCR5^{hi}PD-1^{hi}) is present, supporting reports that Tfh in malaria are less functional due to inflammatory cytokines. Persistence of parasite drives the hybrid phenotype, as early drug-cure reduced the hybrid Th1/Tfh phenotype, increasing Th1-like cells (IFN-γ^+IL-21$^-$CXCR5$^-$). We investigated the molecular regulation of Th1/Tfh T cells and found that CD4-intrinsic Bcl6, Blimp-1 and STAT3 coordinately regulate T-bet expression. Upon infection of mice deficient in the Th1 master regulator, T-bet, CD4 T cells made very little IFN-γ, and 40% of the animals died. Although Bcl6 can bind T-bet, bcl6 and Blimp-1 mainly regulate the level of CXCR5 on hybrid T cells. T cell specific deficiency of Tfh-promoting transcription factor, STAT3 (STAT3 TKO) had a more profound effect. Infected STAT3 TKO mice showed a decrease in the hybrid Tfh/Th1 phenotype, with a concomitant increase in Th1 cells. Strikingly, and despite reduced antibody production, STAT3 TKO mice were completely protected from reinfection. T-bet deficient mice, which had no Th1 memory differentiation, took longer to control parasite re-challenge; reinforcing the interpretation that the improved Th1 response was protective in re-challenge. In summary, the data demonstrate that T cell intrinsic STAT3 regulates the plastic differentiation state of mixed-lineage Th cells in persistent infection, and can determine the outcome of infection. While the host cytokine response to *Plasmodium* infection is surely highly evolved to optimize outcomes for both the parasite and the host, this mechanistic knowledge suggests the feasibility of manipulating immune responses towards better outcomes in malaria.

1652

CIRCULATING T FOLLICULAR HELPER CELL DYNAMICS DURING VACCINATIONS WITH TRANSMISSION BLOCKING CONJUGATED VACCINES PFS230-EPA AND PFS25-EPA ADJUVANTED WITH AS01

Kalifa Diarra[1], Kendrick Highsmith[2], Kadidia B. Cisse[1], Boubacar Dembele[1], Irfan Zaidi[2], Mahamadoun H. Assadou[1], Mamady Kone[1], Issaka Sagara[1], Sara A. Healy[2], Patrick E. Duffy[2]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Transmission blocking vaccines (TBV) for malaria provide an additional tool to limit the spread of malaria. Pfs25 and Pfs230 are among the two leading antigens that have been used to make conjugate vaccines and are the only TBVs to have been tested in malaria endemic countries using either Alhydrogel® or AS01 as the adjuvant. Both vaccines induce functional antibodies that can prevent development of Pf in the mosquitoes. Efficient antibody production by B cells requires interaction with a specialized subset of CD4 T cells called T follicular helper (Tfh) cells that produce IL-21 and can be identified by their expression of the homing marker CXCR5. The current study explored the dynamics of circulating Tfh cells during a small pilot study in healthy Malian adults that were vaccinated with either conjugated Pfs25-EPA/AS01, Pfs230-EPA/AS01, both Pfs25-EPA/AS01 and Pfs230-EPA/AS01 vaccines or a comparator vaccine at a 0, 1 and 6 month schedule. The levels of circulating Tfh cells were measured in real-time with a simple whole blood *ex vivo* assay using flow cytometry. Circulating Tfh cells were defined as CD4 T cells that co-expressed CXCR5 and PD1. The results showed that the levels of circulating Tfh cells were similar in subjects that received either the Pfs25, Pfs230 or both vaccines.

1653

HOSPITAL-DERIVED ANTIBODY PROFILES OF MALARIA PATIENTS IN SOUTHWEST INDIA

Apoorva Venkatesh[1], Aarti Jain[2], Huw Davies[2], Ligia Periera[3], Jennifer Maki[3], Edwin Gomes[4], Phillip L. Felgner[2], Sanjeeva Srivastava[1], Swati Patankar[1], Pradipsinh K Rathod[3]

[1]*Indian Insitute of Technology, Mumbai, India,* [2]*University of California Irvine, Irvine, CA, United States,* [3]*University of Washington, Seattle, WA, United States,* [4]*Goa Medical College and Hospital, Bambolim, India*

Naturally acquired immunity to malaria across the globe varies in intensity and protective powers. In Asia, particularly in India, there are unique opportunities for exploring and understanding malaria immunity relative to host age, co-occurrence of *Plasmodium falciparum* and *P. vivax* infections, varying travel history, and varying disease severity. A US NIH ICEMR (South Asia) team examined the level of immunity in an Indian malaria patient population at a tertiary care hospital in Goa, India. Sera from 200 patients of different ages, in different seasons, infected with *P. falciparum* or *P. vivax* or both species, and with different clinical severity were applied to an established protein array system with over 1,000 *P. falciparum* and *P. vivax* antigens. Differential binding of patient IgG to different antigens was measured. IgG reactivity towards *P. falciparum* antigens was very strong. Of 248 seropositive *P. falciparum* antigens, the strongest were VAR, MSP10, HSP70, PTP5, AP2, AMA1, and SYN6. In *P. vivax* patients, ETRAMPs, MSPs, and ApiAP2, sexual stage antigen s16, RON3 were the strongest IgG binders. Both *P. falciparum* and *P. vivax* patients also revealed strong binding to new antigens with unknown functions. Seropositives showed antigens unique to the young (HSP40, ACS6, GCVH) or to non-severe malaria (MSP3.8 and PHIST). Together, these studies confidently help define antigens for surveillance and possibly for disease protection, in many different parts of India and the world.

1654

PROTECTION-ASSOCIATED IMMUNE RESPONSES FOLLOWING VACCINATION WITH RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES

Nina Hertoghs[1], Katharine V. Schwedhelm[2], Ying Du[1], Fergal Duffy[1], Stefan H. Kappe[1], M. Juliana McElrath[2], Stephen C. De Rosa[2], Kenneth D. Stuart[1]

[1]*Seattle Children's Research Institute, Seattle, WA, United States,* [2]*Fred Hutchinson Cancer Research Center, Seattle, WA, United States*

Vaccines that prevent malaria infection would be valuable tools in the effort to eliminate and eradicate malaria, a disease that each year results in over 200 million cases and approximately 400,000 deaths. Radiation-attenuated sporozoites (RAS) have been established to confer sterilizing protection, although the mechanism behind this protection is incompletely understood. *In vivo* animal studies have provided some mechanistic insight into how anti-malarial immunity is achieved, although some of these mechanisms are challenging to study in humans. This project aims to understand protective anti-malarial immunity in humans and in this way, aid the development of a malaria vaccine. To this end, we performed immunological analyses of samples from clinical trials in which volunteers were vaccinated with *Plasmodium falciparum* RAS and whose protection from malaria infection was assayed by controlled human malaria infection (CHMI). Blood samples collected at multiple timepoints during the immunization schedule and after CHMI were analyzed to compare immune responses between protected and non-protected volunteers using whole blood RNAseq and flow cytometry on PBMCs. The RNAseq analysis includes responses of at least 13,000 genes at each time point and identified vaccine induced changes in the expression levels of numerous genes of which a proportion correlated with protection. Furthermore, three flow cytometric staining panels were developed, and an existing panel was optimized by using BD FACSymphony technology which can analyze up to 28 markers in each panel. Together, these four panels cover all major arms of the immune system and give a unique opportunity to broadly characterize vaccine-induced immune responses and how these develop over time. Additionally, these data help to assess whether the transcriptomic changes reflect changes in the immune cell composition of the blood due to cell trafficking or proliferation. The flow cytometric and RNAseq data are being integrated to provide insights into specific cell types and processes that are involved in the vaccine-induced protection and to guide further malaria vaccine development.

1655

PROTECTIVE ANTIGENS AND ANTIBODIES DURING THE PRE-ERYTHROCYTIC STAGE OF MALARIA

Rahwa A. Osman, Suzanne McDermott, Kenneth D. Stuart

The Centre for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA, United States

Malaria causes over 200 million cases and 445,000 deaths globally every year. A highly efficacious vaccine that prevents infection is an urgently needed tool to combat this situation. Immunization with liver-stage arresting, radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS) confers sterilizing protection. In addition, high anti-malaria antibody titers have been previously shown to be important for protection. These findings indicate that adaptive immune responses induced by malaria antigens during the pre-erythrocytic (PE) stage of the *P. falciparum* life cycle are suitable targets for better vaccine design. However, the protective antigens and antibodies associated to this protection are still unknown. To identify these elusive antibodies and cognate PE antigens, we are using samples from PfRAS vaccine trials in which protection was determined by Controlled Human Malaria Infection (CHMI) in combination with state-of-the-art molecular immunological approaches. We are identifying and characterizing B-cell receptor repertoires that are associated with protection using next generation sequencing of single chains as well as natively paired heavy and light chains. We expect these studies will identify protective antibodies, and clonal diversity and frequency associated with protection following malaria vaccination. To identify protective antigens, we are generating yeast antigen display libraries that comprehensively represent the *P. falciparum* proteome. We will screen these libraries with serum samples from protected individuals following PfRAS immunization and CHMI. Thus, our work will inform and improved vaccine design approaches and expand our knowledge of protective malaria antigens and host antibodies, as well as providing important tools to further malaria research.

1656

ENSEMBLE MODELING FOR PRECLINICAL ANTIMALARIAL DRUG DEVELOPMENT: PROVIDING MECHANISTIC INSIGHTS INTO PARASITE-HOST BEHAVIOR

Lydia Burgert[1], Matthias Rottmann[1], Sergio Wittilin[1], Andreas Krause[2], Nathalie Gobeau[3], Joerg Moehrle[3], Melissa Penny[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Idorsia Pharmaceuticals Ltd, Basel, Switzerland,* [3]*Medicines for Malaria Venture, Geneva, Switzerland*

Emerging drug resistance and late stage drug attrition fuel the need to search for new antimalarial compounds to be used in combination therapies. Host-parasite dynamics and their interactions with drugs are important to understand throughout the antimalarial drug development process to facilitate meaningful translation of results between and within clinical stages and thereby speed up the development process. Using an ensemble of mechanistic within-host parasite growth and antimalarial action models we assessed host-parasite interactions in the two main preclinical drug testing systems of murine parasite: *P. berghei* in normal mice and human parasite *P. falciparum* in immunodeficient NOD $^{scidIL\text{-}2R'c\text{-}/\text{-}}$ (SCID)-mice. Control and treatment data of four different antimalarials (ACT451840, Chloroquine, MMV390048 and OZ439) from three different laboratories were used to compare drug action between pre-clinical experimental systems. We identified changes in the ability of the parasite to infect erythrocytes as a driver of differences in parasite growth patterns observed between experiments and laboratories. We found that properties of the host-parasite system such as growth, clearance characteristics and resource availability are mainly driving *P. berghei* infection. In contrast, experimental set-up in SCID mice (e.g. human erythrocyte injections) replaces many host functions and therefore influences *P. falciparum* behavior and thus drug action. A comparison of drug efficacy parameters between host-parasite systems did not allow a direct translation of drug action between experimental systems. Additionally, we identified the importance of understanding parasite recrudescence behavior following non-curative treatment and elucidate potential pitfalls for human equivalent dose prediction. We conclude that additional parasite behavior such as parasite dormancy or changes in growth behavior might play an important role in explaining observed parasite recrudescence patterns. Taken together, our findings demonstrate the need of assessing the clinical relevance of gained mechanistic insights for human dose prediction.

1657

DEFINING MINIMAL TARGET PRODUCT PROFILES OF NEW MALARIA INTERVENTIONS: A MODELLING STUDY

Melissa A. Penny[1], Guojing Yang[1], Flavia Camponovo[1], Nakul Chitnis[1], Ewan Cameron[2], Monica Golumbeanu[1]

[1]*Swiss Tropical and Public Health Insitute, Basel, Switzerland,* [2]*Big Data Institute, Oxford, United Kingdom*

Delivery strategies and profiles of new malaria interventions for control and elimination will necessarily differ from those of existing interventions. Modelling and simulation provides understanding of how interventions interact to reduce malaria in different epidemiological settings without the need to test all combinations of interventions in the field. However, models to date have generally only been used at specific stages of development, rather than in generating evidence for decision-making along the whole development pathway. We present a new approach to use models and machine learning to identify key determinants of intervention impact

and thus aid decision making and more efficient resource allocation in the development of new interventions. Our framework 1) provides a new approach for using complex models to answer questions for product development; 2) guides thinking on operational, health system, or intervention factors determining impact and thus define the minimal profile of interventions for different settings; and 3) provides a way to iterate the precision of optimal profiles of new interventions as further detail becomes available. We applied our framework to many novel malaria interventions currently in development targeting different parasite and/or vector targets for a range of follow-up time points and delivery modalities. We found that at very low transmission, increasing levels of effective case management with existing or new interventions is likely to be core for facilitating further burden reduction. Conversely, for all other settings, coverage, rather than intervention efficacy, is the main driver of impact. While for some interventions high coverage deployment, or increasing efficacy, might be difficult or impossible to achieve, our analysis shows that this can be alleviated through deployment of interventions in combination, targeting different parts of the parasite or vector life-cycle. In addition, increasing the frequency of deployments can dramatically decrease the minimum coverage or minimum target product profile required in order to achieve different health goals.

1658

PLASMODIUM VIVAX LACTATE DEHYDROGENASE IN INDUCED BLOOD STAGE MALARIA INFECTION: UNDERSTANDING BIOMARKER DYNAMICS FOR THE PURPOSE OF MALARIA ELIMINATION

Sumudu Britton[1], Lachlan Webb[1], Deborah Akinlotan[1], Ihn K. Jang[2], Bridget Barber[1], Ellie Sherrard-Smith[3], Kim Piera[4], Anstey Nicholas[4], Gonzalo J. Domingo[2], McCarthy S. James[1]

[1]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [2]*PATH, Seattle, WA, United States,* [3]*Imperial College London, London, United Kingdom,* [4]*Menzies School of Health Research, Darwin, Australia*

Current rapid diagnostic tests (RDTs) based on lactate dehydrogenase (LDH) lack sufficient sensitivity to reliably diagnose *Plasmodium vivax* infection. A model of biomarker dynamics would inform optimisation of this assay as well as the development of new biomarkers for improved *P. vivax* RDTs for malaria elimination. We aimed to measure key assay parameters to develop a mathematical model of the dynamics of LDH during *P. vivax* infection. Eight malaria-naïve subjects from a *P. vivax* induced blood stage malaria study had blood samples collected at inoculation, and at sequential time-points up to and following treatment with chloroquine. Parasite density was measured by 18S qPCR; *P. vivax* LDH (PvLDH) was measured using the Quansys ELISA twice-daily to 3 days post treatment, and then daily until 5 days post treatment. We used data from 7 subjects who had sufficiently measurable levels of PvLDH to calculate a log-linear decay rate of LDH, and then converted this rate into an elimination half-life. The mean elimination half-life of PvLDH was 16 hours (range 12.6- 19.8). The correlation between parasitemia and PvLDH was 0.89 (95%CI 0.84-0.93). The observed level of PvLDH at any given time point was 0.37 pg/parasite (95% CI 0.346-0.398). Parasitemia and PvLDH appeared to follow out-of-phase 48-hour patterns. When a 24-hour lag was introduced to PvLDH in order to better model, the time pattern, the correlation between parasitemia and PvLDH was stronger ($r = 0.94$, 95%CI 0.91-0.96), which suggests a stage-specific or time-dependent relationship that will require explanation. Our study found a shorter PvLDH elimination half-life than previously described, which may contribute to the poor sensitivity of RDTs as a result of rapid clearance. We also found a strong correlation between parasitemia and PvLDH. We are now using key parameters identified in this study (half-life of PvLDH and the correlation between parasitemia and PvLDH) to develop a mathematical model to describe the dynamics of within-host parasite growth, and PvLDH production and decay over the course of a *P. vivax* infection.

1659

PREDICTING MALARIA ELIMINATION USING MATHEMATICAL MODELLING AND MACHINE LEARNING

Theresa Reiker, Monica Golumbeanu, Munir Winkel, Emilie Pothin, Nakul Chitnis, Thomas A. Smith, Melissa Penny

Swiss Tropical and Public Health Institute, Basel, Switzerland

Over the last decade, malaria transmission has decreased in many settings, and for some countries the strategic aim has shifted to elimination. An open question in planning is how to predict the probability of elimination in a given setting with a given set of interventions. Modelling and simulation provide crucial guidance in understanding potential intervention impact and determining optimal strategies. With recent computational advances, most applied modelling work is based on complex computer simulations. Nonetheless, predicting the probability of elimination for a specific strategy remains difficult, as this is inherently based on model and intervention assumptions as well as population sizes. Elimination is a binary, highly stochastic event and when many possible interventions are considered, elimination probabilities are difficult to derive. Here, we propose adding machine learning methods to current methods of simulation as an additional step in predicting disease progression and the impact of interventions. Specifically, we discuss definitions of elimination in the presence of importation and the implication for predictions/ interpretations, sampling approaches (full factorial; Latin hypercube; adaptive), and the use of, and performance of different machine learning emulators (especially Gaussian Processes) on top of simulation results to convert binary outcomes into probabilities. We provide a general framework to assist in setting up a modelling study and incorporating machine learning methods for predicting elimination of malaria. This framework is also applicable to other infectious diseases.

1660

COST ANALYSIS OF MALARIA CONTROL AND ELIMINATION ACTIVITIES IN HETEROGENEOUS MALARIA TRANSMISSION AREAS OF MYANMAR

Kyaw Myint Tun[1], Ann Levin[2], Zaw Tun Win[1], Bo Bo Thet Ko[1], Thant Zin Aung[1], Sway Minn Htet[1], May Aung Lin[1], Taylor Price[3], Hala Jassim AlMossawi[3], Sara Oliphant[3], Zaw Wutt Hmone[1], Neeraj Kak[3]

[1]*University Research Co., Myanmar, U.S. President's Malaria Initiative (PMI) Defeat Malaria Project, Yangon, Myanmar,* [2]*Levin & Morgan, LLC., Bethesda, MD, United States,* [3]*University Research Co., LLC, Chevy Chase, MD, United States*

PMI's Defeat Malaria project is being implemented in 31 townships of 3 States/Regions and has contributed to a significant decline of malaria incidence through the implementation of proven interventions. The resulting epidemiological picture is becoming increasingly heterogeneous, ranging from nearly malaria-free zones to *foci* of persistent transmission, thus requiring the deployment of both control as well as elimination activities. The cost analysis of malaria control activities, with considerations for potential elimination activity costs, is important for projecting resource requirements of interventions in heterogeneous malaria transmission settings. For this purpose, both financial and economic costs of Defeat Malaria were estimated, separated into recurrent and capital costs, and micro-costing and sensitivity analysis were conducted. The cost estimates were based on current project costs and case scenarios of initial malaria elimination activities conducted in low transmission areas. The estimated annual financial and economic direct costs in 2017 per Defeat Malaria output - cost per village malaria worker (VMW), per person tested, and per person treated - were $1,276 and $1,633 per VMW, $14 and $18 per person tested, and $710 and $909 for person treated, respectively. The annual cost for malaria control per VMW ranged from $1,146 to $2,282, considering the costs of training, supervision, monthly meetings, and incentives. The main cost drivers were monitoring/supervision and surveillance (i.e. monthly township meetings and data analysis), comprising over 40% of the total costs, followed by diagnosis, treatment, and

training. Sensitivity analysis revealed that the cost per VMW declines as more VMWs are added to the program, since there are several fixed costs associated with the program. Some approaches to lowering the cost of monthly meetings were examined. The findings from this study will serve as a baseline analysis for estimating the costs of malaria elimination activities, and developing an investment case showing the benefits of investing in malaria control and elimination compared to counterfactual scenarios

1661

MODELING *PLASMODIUM FALCIPARUM* INFECTION IN AN IMMUNOCOMPROMISED JUVENILE MOUSE MODEL

Jeanine A. Ursitti[1], Biraj Shrestha[2], Amed Ouattara[2], Matthew Adams[2], Christopher V. Plowe[3], Mark A. Travassos[2], Steven A. Fisher[1]

[1]*Departments of Medicine and Physiology, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Malaria Research Program, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Cerebral malaria, caused by infection with *Plasmodium falciparum*, leads to the deaths of hundreds of thousands of children under the age of 5 years, predominantly in Africa, each year. Binding of infected erythrocytes to the endothelial protein C receptor (EPCR) and other putative receptors, with subsequent sequestration of parasites in the brain vasculature, has been proposed as critical to pathogenesis. The limitations of current *in vivo* models, however, have hindered testing this hypothesis and developing new therapies. The goal of our experiments is to develop a novel model to specifically study the blood stage of *P. falciparum* malaria by injecting *P. falciparum* infected RBCs (iRBCs) into immune deficient juvenile mice (NBSGW, NOD.Cg-*KitW-41J Tyr* $^{+}$ *Prkdcscid Il2rgtm1Wjl*/ThomJ). Control (CON) and *P. falciparum* infected human RBCs (iRBCs; strain HB3var03) were cultured and labeled with the fluorophore CFDA-SE just prior to injection. Juvenile mice, at postnatal day 12-14, were injected intraperitoneally with a single aliquot of CON and iRBCs (100 µl, 7% parasitemia) and assayed at 4h and 24h post-injection. qPCR of blood samples showed that this approach yielded significant levels of HuRBCs in the blood (HBB gene; n=11 with n=6 positive, average= 0.09% human RBC hematocrit and range=0.001-0.296) and detectable parasitemia at 4h and 24h (Plasmepsin IV gene; n=11 with n=6 positive, average=0.031% and range=0.004-0.07). Histological assays showed aggregates of the fluorescently labeled iRBCs in brain and lung blood vessels of mice treated with iRBCs, but such aggregates are considerably lower in samples from CON injected mice. We conclude that IP injection of *P. falciparum* iRBCs into immunocompromised juvenile mice results in significant and persistent "blood humanization" and parasitemia. This technique may provide a novel model by which to test *P. falciparum*-genotype/disease-phenotype correlations using field samples as well as to study the pathogenesis of cerebral malaria and new therapies. We are continuing to test additional aspects of this model including humanization of the immune system.

1662

ANTIMALARIAL DRUG-RESISTANCE EVOLUTION DURING AND AFTER MASS DRUG ADMINISTRATION

Maciej F. Boni, Thu N-A Tran, Tran Dang Nguyen

Pennsylvania State University, University Park, PA, United States

Mass drug administration (MDA) of antimalarial drugs in malaria-endemic areas may be critical for helping near-elimination regions reach elimination. One concern in MDA implementation is insufficient participation of residents in a given transmission area, which may result in incomplete elimination and a subsequent rebound of malaria prevalence. A second potential concern (addressed here) is that MDA may have unexpected or detrimental effects on drug-resistance evolution. Using a previously developed individual-based stochastic mathematical model for *P. falciparum*, we evaluate certain drug-resistance consequences when dihydroartemisinin-piperaquine (DHA-PPQ) is used for MDA in populations of 40,000 and 300,000 individuals, with approximately 80% population participation in the MDA. The first-line drug for uncomplicated falciparum malaria in this population is assumed to be artemether-lumefantrine. We highlight three results from our study. First, MDA creates a genetic bottleneck, which introduces a large amount of unpredictability for genotype frequencies associated with drug resistance. This bottleneck tends to promote genotypes resistant to DHA-PPQ, but real-world results in small populations like this will always be stochastic and highly variable. Second, importation of artemisinin-resistant *kelch13* genotypes is associated with MDA failure. With low importation of *kelch13* resistant genotypes, the bottleneck effect is weak and MDA can succeed in low-transmission regions; with high levels of importation, *kelch13* mutants tend to fix in the period immediately following the bottleneck, leading to scenarios with 100% fixation of artemisinin-resistant genotypes. Third, the simplest solution to avoiding uncertainty during the post-MDA bottleneck is to repurpose the public health infrastructure put into place for the MDA and increase treatment access for the general population post-MDA. With increased treatment access (ITA), the probability of local elimination of *P. falciparum* increases in low-transmission regions ($PfPR_{2-10} < 2\%$); this result holds under high and low drug-resistance importation scenarios.

1663

THE ROLE OF DRUG QUALITY IN THE EMERGENCE AND TRANSMISSION OF ANTIMALARIAL RESISTANCE

Aleisha Brock[1], Joshua V. Ross[1], Adrian Esterman[1], Sunil Parikh[2]

[1]*University of South Australia, Adelaide, Australia,* [2]*Yale School of Public Health, New Haven, CT, United States*

The emergence and transmission of antimalarial resistance is hampering malaria eradication efforts and shortens the useful therapeutic life of currently available antimalarials. Drug selection pressure has been identified as a contributing factor; in particular, the population treatment coverage and subtherapeutic concentrations of active pharmaceutical ingredient (API) in the bloodstream. Poor quality antimalarial medicine can be a source of subtherapeutic doses of antimalarial API(s). The aim of this research was to investigate the impact of poor-quality antimalarial treatments and non-recommended quality assured monotherapies on the (i) emergence and/or (ii) transmission of the double *dhfr* mutant, associated with low-to-moderate levels of pyrimethamine resistance. Deterministic mathematical models were developed, with sensitivity analyses and validation methods used to test the models. The use of full and half dose pyrimethamine were used as a proxy to represent poor quality antimalarial use, and artemether-lumefantrine was used as a proxy to represent good quality antimalarial use. Key findings identified that the use of poor quality antimalarials provide a favourable environment for the emergence of resistance, and increase its transmission. The shortest time to the emergence of resistance occurred with the exclusive use of half dose pyrimethamine 193 days after the introduction of pyrimethamine treatment - a 54% reduction compared to baseline. The largest impact on the transmission of resistance was identified with the exclusive use of full dose pyrimethamine, resulting in 777% more malaria cases and an increase in the proportion of resistant infections by 18%, compared to baseline. In comparison, the exclusive use of half dose pyrimethamine resulted in 559% more malaria cases and 14% more resistant infections, compared to baseline. Findings for the triple *dhfr* mutant will additionally be presented. These models are the first to explore the impact of poor-quality antimalarial medicines on the emergence and transmission of resistance, providing insight into this important area of malaria control.

1664

QUANTIFYING MALARIA ACQUIRED DURING TRAVEL AND ITS ROLE IN MALARIA ELIMINATION ON BIOKO ISLAND

Daniel T. Citron[1], Carlos A. Guerra[2], Guillermo A. García[2], Sean L. Wu[3], Su Yun Kang[4], Katherine E. Battle[4], Harry S. Gibson[4], David L. Smith[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Medical Care Development International, Silver Spring, MD, United States,* [3]*University of California, Berkeley, CA, United States,* [4]*University of Oxford, Oxford, United Kingdom*

Over the past fifteen years, malaria burden on Bioko Island in Equatorial Guinea (EG) has decreased significantly. Despite extensive ongoing control and elimination efforts on Bioko, further progress towards elimination appears to have slowed in recent years. This includes apparently persistent malaria in the capitol city of Malabo, an urban area with few viable vectors. Past studies have shown that people traveling from mainland EG to Bioko are infected at a significantly higher rate than people traveling from Bioko to mainland EG, suggesting that infections acquired off-island may be an important factor explaining the persistence of malaria on Bioko. Beginning with data assembled from four island-wide malaria indicator surveys conducted in 2015-2018, we used a combination of mapping methods and mechanistic models to estimate the geographical distribution of malaria infections; estimate the influence of infections acquired during off-island travel; and investigate whether deploying additional interventions could reduce on-island prevalence to zero. We first mapped malaria prevalence and its relationship with recent off-island travel using a Bayesian geostatistical framework, discovering an elevated malaria risk among those who had recently traveled to mainland EG. We supplemented this initial analysis with a mechanistic model of malaria transmission to quantify the influence of off-island transmission. We discovered that infections occurring off-island were sufficient to explain a large fraction of cases mapped on Bioko, particularly among the residents of Malabo. Lastly, we used a simulation model of malaria transmission and human travel behavior, calibrated to the survey data and geostatistical maps, to evaluate the potential impact of adding mass treatment or vaccination to the current package of interventions on Bioko Island. We found that the influence of off-island transmission was too strong for additional on-island treatments to be effective in further reducing malaria prevalence, but that interventions to reduce off-island transmission could be more effective in the long term.

1665

THE ECONOMIC IMPACT OF SUBSTANDARD AND FALSIFIED ANTIMALARIAL MEDICATIONS IN NIGERIA

Sarah Laing[1], Sarah Beargie[1], Colleen Higgins[1], Daniel Evans[2], Daniel Erim[3], Sachiko Ozawa[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Duke University, Durham, NC, United States,* [3]*RTI International, Durham, NC, United States*

Nearly 20% of antimalarials in low- and middle-income countries (LMICs) have been found to be substandard or falsified (SF). SF antimalarials can have a detrimental impact on health, with the potential to contribute to antimalarial resistance (AMR) and impose adverse economic burden on patients and the health system. Nigeria has the highest burden of malaria in the world, accounting for 19% of malaria mortality largely among children. We estimated the health and economic impact of SF antimalarials on children under five in Nigeria by developing an agent-based SAFARI (Substandard and Falsified Antimalarial Research Impact) model. The model simulated children with background characteristics, malaria infections, patient care-seeking, disease progression, treatment outcomes, and estimated their incurred costs. Model inputs for Nigeria were informed by epidemiologic, demographic, cost, and pharmaceutical data from the literature. We estimated the impact of SF malaria medicines, antimalarial resistance, as well as possible interventions and policy scenarios such as improving adherence, educating more people to seek care or removing stock-outs from different sectors. Our results demonstrated that SF antimalarials contribute to 16% (12,300) of malaria deaths and 11% (US $892 million) of annual costs in Nigeria. If AMR were to develop, we simulated that the burden of malaria could increase by 7,700 deaths (10%) and add $839 million (11%) in costs. In our regional analysis, both the health and the economic impact of SF medicines in the northern region (9,700 deaths, $698 million) were more than triple that of the southern region (2,700 deaths, $193 million). Interventions that produced the greatest cost-savings were the elimination of stock-outs in all sectors ($1.1 billion) and removing SF antimalarials ($892 million). The results highlight the significant health and economic burden of poor quality antimalarials in Nigeria. In order to reduce the burden of malaria and prevent antimalarials from developing resistance, policymakers and donors must recognize the threat and implement interventions to combat the impact of SF antimalarials.

1666

SPATIAL COVARIATE-BASED CONSTRAINED RANDOMIZATION OF MALARIA INTERVENTIONS

Kathryn Colborn[1], Thomas Eganyu[2], Humphrey Wanzira[2], Ronald Mulebeke[2], Fred Bukenya[2], Richard Elliot[3], Adoke Yeka[4], Dorothy Echodu[2]

[1]*University of Colorado Denver, Aurora, CO, United States,* [2]*Pilgrim Africa, Kampala, Uganda,* [3]*Boise State University, Boise, ID, United States,* [4]*Makerere University, Kampala, Uganda*

A two-year cluster-randomized comparative effectiveness trial of two different community case management interventions in NE Uganda employs a novel technique for cluster randomization of spatially constrained areas. The potential for contamination of treatments due to spatial proximity of villages and the potential for vector flight between different intervention areas required us to develop a spatially explicit cluster randomization methodology for this study. We describe a design framework for randomizing 55 villages in a rural area of Uganda to one of two interventions, proactive integrated community case management (ProCCM) or integrated community case management (iCCM). We first assigned villages to geographically contiguous clusters that could then be randomized to receive either the ProCCM or iCCM intervention using *k*-means clustering. We used the sum-of-squares of Euclidean distances between the village centroids to determine the ideal number of clusters. This yielded 8 total clusters, which resulted in 70 possible randomizations. We then applied the covariate-based constrained randomization (CCR) of clusters methodology described by Moulton *et al*. CCR restricts the choices of randomizations to those that yield acceptable balance between the two arms with respect to important cluster-level confounders. The confounders we considered in this randomization included: baseline malaria prevalence by microscopy, household density, population density, whether or not the cluster bordered lake Bisina, and sub-county (there were 2 sub-counties in this study area). We used a cut-off of the top 12 randomizations according to balance scores and chose one of these randomizations at random. This randomization achieved high balance with respect to baseline confounders across the arms. We are exploring other types of spatial clustering methods for this framework as well as optimizing CCR using variable weighting. This methodology is important for spatially correlated outcomes, such as vector-borne disease prevalence, where human and vector mixing could potentially contaminate the interventions.

1667

A MODEL-BASED ASSESSMENT OF MALARIA IN VENEZUELA SUGGESTS THAT THE EPIDEMIC IS REVERSIBLE

John Huber[1], Luis Chaves[2], Amir Siraj[1], Jorge Moreno[3], Maria Villegas[4], Leonor Pocaterra[5], Leopoldo Villegas[4], T. Alex Perkins[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Instituto Costariccense de Investigación y Enseñanza en Nutrición y Salud, Tres Ríos, Costa Rica,* [3]*Centro de Investigación de Campo Francesco Vitanza,*

Tumeremo, Bolivarian Republic of Venezuela, [4]Global Development One, Silver Spring, MD, United States, [5]Universidad Central de Venezuela, Caracas, Bolivarian Republic of Venezuela

Coincident with accelerating economic decline, the incidence of malaria in Venezuela has risen sharply over the last decade, from approximately 50,000 cases in 2010 to more than 500,000 cases in 2017. As a result of internal migration to the Bolívar state for subsistence illegal gold mining and emigration to neighboring countries for better economic opportunities, the malaria crisis in Venezuela threatens to destabilize and reverse regional progress towards malaria elimination. Although incidence patterns themselves are clear about the severity of this situation, it is unclear what these patterns imply about the feasibility of reversing the dramatic rise of this epidemic. To provide a quantitative basis for the development of plans to confront this epidemic, we used a dynamic transmission model to estimate the reproduction number of the disease, *R*, over time and across space. The effect size–1/*R*–provides an estimate of the level of effort required to push *R* below 1 and the parasite toward local elimination. To characterize the trend in *R* and the effect size over time and across space, we fitted our transmission model to weekly incidence data in each municipality of the Bolívar state from 1995 to 2018. After accounting for effects of seasonality, human mobility and migration, and recrudescing and relapsing infections, our fitted model estimated that *R* has risen only slightly above 1 over the 24-year time period, despite the sharp increase in incidence. The relatively high effect sizes in Bolívar state, which is the epicenter of the epidemic, suggest that appropriately targeted control efforts could result in a proportionally large reduction in transmission. Based on this data-driven approach, our results indicate that the malaria epidemic in Venezuela can be reversed, should the resources and effort to confront this crisis be mobilized in a timely manner.

1668

MOLECULAR SURVEILLANCE AND MODELING REVEAL SPATIO-TEMPORAL TRENDS OF MALARIA TRANSMISSION IN THIÈS, SENEGAL

Albert Lee[1], Stephen F. Schaffner[2], Rachel F. Daniels[3], Yaye Die Ndiaye[4], Awa B. Deme[4], Aida S. Badiane[5], Bronwyn MacInnis[2], Sarah K. Volkman[6], Dyann F. Wirth[6], Daouda Ndiaye[5], Daniel L. Hartl[7], Edward A. Wenger[1], Joshua L. Proctor[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States, [2]Broad Institute of MIT and Harvard, Cambridge, MA, United States, [3]Harvard T.H. Chan School of Public Health, Boston, MA, United States, [4]Dantec Teaching and Research Hospital, Dakar, Senegal, [5]Cheikh Anta Diop University, Dakar, Senegal, [6]Harvard T.H. Chan School of Public Health, Cambridge, MA, United States, [7]Harvard University, Cambridge, MA, United States*

In the last decade, the availability of new genetic data, driven both by advances in sequencing technology and surveillance from operational efforts, has greatly furthered our understanding of the evolutionary processes and transmission dynamics exhibited by *Plasmodium falciparum*. We combine genomics with epidemiology in a mechanistic model designed to both simulate changes in diversity at the population level and to track the movement of individual strains. We apply this model to genetic data obtained from malaria infections in Thiès, Senegal, over the past 11 years to evaluate spatial and temporal changes in infections. Thiès is a malaria-endemic setting that has seen an overall decline in malaria incidence since 2006 from > 100/1000 to < 10/1000. We corroborate previous results and extend the model to incorporate GPS data and timestamps. The model is calibrated via approximate Bayesian computation, using a kernel density estimator that allows us to evaluate non-Gaussian likelihoods. We analyze the simulations to extract spatiotemporal features that can inform intervention strategies. In particular, we show that a decline in the complexity of infection over a season is a natural consequence of the seasonal variation in transmission. We also use principal component analysis to reveal spatiotemporal correlations between related infections and show that there is a characteristic speed at which strains can propagate in an endemic city. These two insights indicate that iterative and targeted genetic surveillance can greatly increase the informational content of resource-limited data. We build on these results to construct a new model for malaria in Senegal at the national scale and across multiple levels of transmission, which will enable us to identify potential gaps in surveillance strategies. Furthermore, through this work, modeling transmission dynamics and patterns of parasite infections will facilitate intervention stratification and targeting toward malaria elimination.

1669

A METHOD FOR SELECTIVE ENRICHMENT OF *PLASMODIUM FALCIPARUM* GENOMIC DNA FROM DRIED BLOOD SPOTS OF PATIENTS WITH MALARIA INFECTIONS FROM THE PERUVIAN AMAZON

Andry Mavila[1], Paulo Manrique[2], Juan Carlos Castro[3], Christopher Delgado-Ratto[4], Dionicia Gamboa[5], Oscar Nolasco[2]

[1]*Centro de Investigaciones de Recursos Naturales de la Amazonía (CIRNA), Iquitos, Peru, [2]Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Centro de Investigaciones de Recursos Naturales de la Amazonía (CIRNA)/Departamento Académico de Ciencias Biomédicas y Biotecnología, Facultad de Ciencias Biológicas, Universidad Nacional de la Amazonía Peruana, Iquitos, Peru, [4]Global Heath Institute, University of Antwerp, Antwerp, Belgium, [5]Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía/Instituto de Medicina Tropical "Alexander von Humboldt," Universidad Peruana Cayetano Heredia, Lima, Peru*

Malaria is one of the most important public health problems in Peru specially in the Loreto region which encloses most of the cases (97% of all malaria cases in 2016). Current malaria control efforts are challenged to the high prevalence of asymptomatic and sub-microscopic infections (low parasitemia not detectable by microscopy). Infections with low parasitemia are commonly missed by current gold standard (light microscopy) and even using molecular approaches. The objective of this work was to standardize a method for selective enrichment of *P. falciparum* genomic DNA retrieved from dried blood spots of malaria patients. Genomic *P. falciparum* DNA samples with variable range of parasitemia were enriched through the selective whole genome amplification (SWGA) method using the phi29 polymerase and sets of primers 6A and 8A. Through SWGA, the initial DNA of *P. falciparum* in simulated clinical samples was increased ~126 times (>5,000 molecules/μL). Using the *Fsp*EI enzyme the whole human genome DNA was eliminated and the parasitic genomic DNA was increased ~38 times. Furthermore, in clinical samples of parasitized patients the *P. falciparum* genomic DNA was increased ~ 29,126 times (from > 60,000 molecules/μL). In conclusion, the SWGA method produces the selective enrichment of *P. falciparum* genomic DNA in a wide range of concentrations, from simulated clinical samples and clinical samples of patients with malaria. Hereby, we provide a useful strategy to improve malaria research involving low parasitemia isolates.

1670

FROM PILOTS TO AN ELIMINATION PROGRAM: HOW MUCH DO MALARIA INTERVENTIONS COST

Katya Galactionova[1], Mar Velarde[1], Thomas A. Smith[1], John Miller[2], Melissa A. Penny[1]

[1]*Swiss Tropical and Public Health Institute; University of Basel, Basel, Switzerland, [2]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Lusaka, Zambia*

Countries with areas of very low malaria transmission require evidence on how best to achieve elimination given the limited resources available. Malaria elimination pilots in Ethiopia, Zambia, and Senegal generated evidence on costs and effectiveness of different intervention strategies. To inform decisions at country level there is a need to generalize these findings to implementation by programs. Using a standardized methodology we produced costs for a fixed setting and under an implementation modality representative of programs in Africa for

interventions recommended as part of an elimination strategy. We first evaluated intervention costs within the pilots. We then extrapolated from trial to program by revising implementation assumptions with expert opinion from MACEPA teams, country programs, and the literature. For each intervention we developed a prospective costing model around the reference implementation. Informed with the operational insight, translation of trial implementation to programmatic mode entailed imputing resource requirements at above district level, scaling down intensity of training and supervision, updating partner wages with those of the program, and scaling down incentives at community level. For the reference implementation and setting we estimated cost of RR at $0.18, cost RACD at $0.84, cost of MDA at $4.44, and cost of IRS at $5.42 per person per year. Here we illustrate a strategy to overcome the limited transferability of the costing data and highlight the scope for implementation science research and economic evaluation of scalable intervention models for malaria elimination. We show that differences in costs between the pilot sites were primarily driven by differences in intervention design and implementation of interventions. Differences in prices were a minor driver. Generalizing from pilots to program requires a careful re-assessment of resource requirements across the delivery pathway. As supportive activities and incentives are scaled-down outside the pilot, reflecting capacity constraints on the ground, implications for effectiveness of the tools should be further re-examined.

1671

CHANGES IN VENDOR MOUSE GUT MICROBIOTA HINDERS EXPERIMENTAL REPRODUCIBILITY

Rabindra K. Mandal[1], Joshua E. Denny[2], Morgan L. Duff[1], Nathan W. Schmidt[1]

[1]Indiana University, Indianapolis, IN, United States, [2]University of Louisville, Louisville, KY, United States

An inability to reproduce previous experiments has a tremendous impact on the biomedical enterprise, at both the individual and corporate level. Many factors contribute to this growing concern that has garnered attention both within, and outside of, the scientific community. While using the murine model of malaria, a profound and lasting change in the severity of disease was noted within mice obtained at different time points from a specific commercial vendor. Based on our prior work we hypothesized a change in gut microbiota composition contributed to the temporal change in severity of malaria. Using 16S rRNA gene sequencing and analysis, we identified a distinct and lasting shift in gut microbiota from mice obtained over time within a defined production suite from the commercial vendor, which impacted reproducibility of two infectious disease model systems. Germ-free mice colonized with cecal content from mice within the same production suite before and after this discrete change followed by *Plasmodium yoelii* infection provided a direct demonstration that the change in gut microbiota profoundly impacted the severity of malaria. We also observed differences in fecal bacterial burden following acute *Salmonella enterica* serovar Typhimurium infection. Given the growing recognition that gut microbiota affect diverse states of health and disease, this change in gut microbiota likely impacted experimental reproducibility of diverse research groups. These results also demonstrate the need for commercial vendors to frequently monitor gut microbiota within production rooms, and provide that information to the scientific community.

1672

USING BEHAVIORAL ECONOMICS TO UNDERSTAND THE DETERMINANTS OF PROVIDER ADHERENCE TO MALARIA CASE MANAGEMENT GUIDELINES

Faraz Haqqi[1], Julie Chambers[2], Angela Acosta[3], Sriram Sridharan[2], Patricia Rowland[4], Eno Idiong[5], Abba Otene Emmanuel[5], Ademola Oyewusi[5], Aderonke Popoola[5], Lucy Okolo[5], Ernest Obaseki[5], Justin DeNormandie[5], Bolatito Ayenigba[5], Stella Babalola[3], Ian Tweedie[5], Karina Lorenzana[1], Foyeke Oyedokun-Adebagbo[6]

[1]Breakthrough ACTION, ideas[42], Washington, DC, United States, [2]Breakthrough ACTION, ideas[42], New York, NY, United States, [3]Breakthrough ACTION, Johns Hopkins Center for Communications Programs, Baltimore, MD, United States, [4]Breakthrough ACTION, ideas[42], Abuja, Nigeria, [5]Breakthrough ACTION, Johns Hopkins Center for Communication Programs, Abuja, Nigeria, [6]President's Malaria Initiative and United States Agency for International Development, Abuja, Nigeria

Testing and treating fevers appropriately is critical to reducing child mortality and morbidity in Nigeria, where the prevalence of malaria parasitemia in children under 5 years (CU5) of age is 27%, according to microscopy data (2015 Nigeria Malaria Indicator Survey [NMIS]). When patients seek care for fever in health facilities, health providers may not test, diagnose, and treat malaria cases appropriately. To understand the determinants of health providers' fever case management practices, we conducted a formative research study using behavioral economics (BE) theory to identify potential factors. BE draws on social psychology, microeconomics, and other social sciences to understand how context shapes behavior, and why, for example, providers may know the national treatment guidelines, yet do not comply. From Oct-Nov 2018, Breakthrough ACTION Nigeria conducted site visits and interviews with 31 providers and 24 clients in 12 primary and secondary health facilities in Akwa Ibom (NMIS CU5 malaria prevalence: 23%), Kebbi (NMIS CU5 malaria prevalence: 64%), and Nasarawa (NMIS CU5 malaria prevalence: 36%) states. Results were coded and analyzed using BE theories and segmented by cadre and facility type. 74% (23/31) of providers indicated that they distrusted RDTs, citing various shortfalls or malfunctions. 42% (13/31) of provider responses suggest that they operate under a "scarcity" mindset, in which inadequate resources (including time) may cause providers to "tunnel," or intently focus, on seeing as many clients as possible instead of following protocol; compliance is further impeded by hassles (such as coordination with other departments). Providers additionally exhibited "base rate neglect," where they overestimated the probability of severe malaria and malaria in general compared to other potential causes of fever, and they seemed to view tests as tools designed to complement their own opinions, tending to favor their own clinical experience/judgment when tests appeared to contradict initial suspicions. BE provides a useful lens for identifying barriers related to fever case management in Nigeria.

1673

USE OF MALARIA SERVICES AND DATA QUALITY IMPROVEMENT TOOL IN CASCADED SUPERVISION APPROACH IMPROVED QUALITY OF MALARIA SERVICES: EXPERIENCE FROM MWANZA REGION, TANZANIA

Emmanuel Lesilwa[1], Goodluck Tesha[1], Jasmine Chadewa[2], Agnes Kosia[3], Zahra Mkomwa[1], Bayoum Awadhi[3], Rita Noronha[3], Dunstan Bishanga[3], Frank Chacky[4], Abdallah Lusasi[5], Ally Mohamed[5], Chonge Kitojo[6], Erik Reaves[6]

[1]USAID Boresha Afya Lake and Western Zone - PATH, Oyster Bay, Dar Es Salaam, United Republic of Tanzania, [2]USAID Boresha Afya Lake and Western Zone -Jhpiego, Oyster Bay, Dar Es Salaam, United Republic of Tanzania, [3]USAID Boresha Afya Lake and Western Zone -Jhpiego, Oyster Bay, Dar Es Salaam, United Republic of Tanzania, [4]National Malaria Control Programme, Dar Es Salaam, United Republic of Tanzania, [5]National Malaria Control Programme, Oyster Bay, Dar Es Salaam, United Republic

of Tanzania, [6]US President's Malaria Initiative/United States Agency for International Development, Oyster Bay, Dar Es Salaam, United Republic of Tanzania

Poor quality of malaria data is the challenges that National Malaria Control Program (NMCP) in Tanzania is addressing by innovations. In 2017, NMCP developed malaria services and data quality improvement (MSDQI) tool to guide supervisors. The tool comprises of seven modules addressing performance of Malaria Case Management with indicators weighed against a standard score. Facilities scoring below 50% of the overall score was deemed poorly performing, 50%-75% moderate and above 75% good performance. The supervisor provides mentorship, on job training (OJT) to poor performing facilities during supervision and develops action plan for improvement. The supervision was conducted through cascaded supervision approach where supervisors from high levels supervised lower level facilities using MSDQI tool. The project in collaboration with NMCP trained 190 supervisors selected from hospitals and health centers in Mwanza Region on malaria case management and data quality. After the training, the supervisors conducted supervision using the cascaded approach. Overall 207 facilities were assessed and 50 (24%) were classified as poor performers. Mentorship and OJT was provided to 430 health care providers with knowledge and skill gaps, between July and September 2018. Performance scores showed improvement in the percentage of providers demonstrating compliance to case management testing guidelines and adherence to treatment results. Cases tested for malaria rose from 75% in 2016 to 88% in 2018. There was a decline in proportion of malaria cases treated without a confirmatory diagnostic test from 1.5% in 2016 to 0.02% in 2018. Provision of IPTp2 (37.6%) and IPTp3 (37.6%) in 2016 rose to 72.4% for IPTp2 and 48.4% for IPTp3 in 2018. Issuing of bed nets to pregnant women increased from 4.9% 2016 to 75.6% in 2018, and from 2.9% 2016 to 65% in 2018 for infants according to DHIS2. During the period, the prevalence of malaria has decreased from 14% in 2016 to 8.1% in 2018 in Mwanza. Cascaded supervision approaches appeared to have contributed to improved quality of malaria service provision and hence improved malaria indicators.

1674

IMPROVEMENT OF HEALTHCARE PROVIDER ATTITUDE TOWARDS DOCUMENTATION THROUGH ON-SITE TRAINING AND SUPPORTIVE SUPERVISION OTSS AND CASE MANAGEMENT TRAINING IN OSUN STATE, SOUTHWEST NIGERIA

Victoria Erinle[1], Chinedu Chukwu[1], Faith Benebo[1], Isaac Adejo[1], Adeyinka Onikan[1], Thomas Hall[2], Mariah Boyd-Boffa[3], Bala Mohammed Audu[4], Shekarau Emmanuel[4], Nnenna Ogbulafor[4], Sonachi Ezeiru[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*Management Sciences for Health (MSH), Medford, MA, United States,* [4]*National Malaria Prevention Program, Abuja, Nigeria,* [5]*Catholic Relief Services, Abuja, Nigeria*

One of the key issues identified in Nigeria regarding malaria case management is the lack of adherence to national guidelines in respect to proper documentation of individual cases by frontline health workers, leading to frequent unconfirmed diagnosis and misuse of antimalarial medicines. In collaboration with the National Malaria Elimination Program and with funding from Global Fund, Management Sciences for Health carried out on-site training and supportive supervision (OTSS) and case management training in the 30 local government authorities (LGAs) in Osun state, southwest Nigeria between July and November 2018. The purpose of this study was to compare percentages of unclassified malaria diagnoses and proper documentation of cases before and after OTSS and case management training. National and state level experts facilitated a three days' case management training of 887 frontline health workers in 821 public health facilities in the state. An OTSS team comprising of subject matter specialists from the national, state, and LGA levels visited 120 public health facilities twice using a national OTSS checklist to assess case management practices and to mentor health workers on proper malaria case management and documentation according to national guidelines. A total of 120 health facilities (comprising 95% public primary healthcare and 5% secondary) were visited by the OTSS team and 600 case notes from the health facilities were reviewed by the OTSS team for documentation of signs and symptoms, evidence of parasitological test, and definitive diagnosis. After OTSS and case management training, unclassified diagnosis of malaria in patient case notes decreased from 61% to 36%, and satisfactory documentation improved from 27% to 32%. The quality of documentation improved following the case management training and the OTSS visits in Osun state. It is important that this is sustained so as to contribute to the national goal of pre-elimination of malaria as contained in the National Malaria Strategic Plan 2014-2020.

1675

UTILITY OF MALARIA CASE CLASSIFICATION CALCULATOR FOR CASE CLASSIFICATION AND RESPONSE

Saw Naung Naung, Ye Hein Naing, Phyoe Yarzar, Thant Zin Aung, Ei Ei Win Aung, Hein Htet Linn Nyan, Kyaw Myint Tun

University Research Co., Myanmar, U.S. President's Malaria Initiative (PMI) Defeat Malaria Project, Yangon, Myanmar

In January 2018, the PMI-supported Defeat Malaria project began working with the Myanmar National Malaria Control Program to pilot elimination activities in 3 Townships of Rakhine State. A malaria case classification calculator (MC3) was developed as an innovative tool to improve case investigation and response. This paper-based tool collects several types of information including date of onset of fever, history of previous malaria infections and blood transfusions, treatment completion according to national guidelines, and other relevant epidemiological linkages for appropriate classification of malaria cases. By using the MC3 to classify malaria cases, investigators can quickly identify the appropriate response and reduce the need for an expert epidemiologist or medical doctor's review. From April to September 2018, Defeat Malaria assessed the ease of use and effectiveness of MC3 through 7 key informant investigator reports, and reviewed 61 malaria case records from 41 out of 241 villages of Toungup Township. All key informants agreed that the MC3 tool was easy to use and helpful, in particular because it led to higher confidence and independence in case classification and calculation of appropriate timing for response, due to visualization of objectively verifiable epidemiological information. However, the application of the tool to mixed infections was more difficult. Of 61 malaria cases investigated, 58 (95%) were classified without difficulty (28 imported, 29 locally contracted, and 1 relapsed); only 3 cases (5%) could not be classified due to asymptomatic malaria, because the appearance of fever is a key starting point for the calculator utilization. MC3 has potential for classifying malaria cases and guiding appropriate timing for response, but it is not useful in classifying asymptomatic cases. Based on success thus far, the MC3 tool will be expanded to a mobile platform and introduced to other partners through the national malaria program.

1676

KNOWLEDGE, ATTITUDES AND PRACTICES OF MALARIA IN STABLE COMMUNITIES IN THE INTERIOR OF SURINAME

Roxana M. Hijlaard, Reana Burke, Bianca Jubitana, Edward van Eer

Medical Mission Primary Health Care, Paramaribo, Suriname

The malaria situation in the interior of Suriname has changed significantly over the last decades reaching near elimination levels in 2009. Both the absence of malaria and decreased interaction of health services for malaria interventions in stable populations might have changed the communities' perceptions and practices towards malaria. To inform future intervention strategies, this study aimed to determine and compare the current level of knowledge, attitudes and practices of malaria in stable communities with a variety of malaria exposure levels. A total of 567 households were included in a community based cross-sectional survey between January and March

2018 in selected villages in the interior of Suriname. The study area was categorized into low versus high annual parasite index (API) according to malaria transmission intensity history and currently being at risk of malaria transmission versus not at risk. Descriptive and analytical statistics were used to summarize data. Respondents from areas at risk of malaria had significant more knowledge about malaria transmission (84%) than areas not at risk (56%). When comparing knowledge regarding malaria transmission between areas with high and low API, significant differences were found (75% in high API and 61% in low API). A positive attitude to the use of mosquito net use was observed without significant differences between areas. Sleeping under a mosquito net was perceived as the best malaria prevention method and used by 80% in total. Mosquito net use was significantly lower in areas current not at risk (71%), compared to areas current at risk (84%). 20% of all respondents were exposed to malaria information messages in the past 5 years, exposure to malaria information was significantly higher in areas current at risk of malaria compared to areas not at risk. Although the study population has fair knowledge about malaria, misconceptions still occur. Poor practices in terms of protection against mosquitoes can stagnate the elimination of malaria. This suggests the need of health education incorporated in comprehensive behavior change interventions to raise the community's awareness.

1677

IMPROVING THE QUALITY OF MALARIA CASE MANAGEMENT AND PREVENTION DURING PREGNANCY IN PUBLIC HEALTH FACILITIES IN BURKINA FASO

Thierry Ouedraogo, Ousmane Badolo, Mathurin Dodo, Bonkoungou Moumouni, Youssouf Sawadogo, Blami Dao, Stanislas Nébié

Jhpiego, Ouagadougou, Burkina Faso

In 2017, malaria was the leading cause of medical consultation (43.34%), of hospitalization (44.05%) and of death (16.13%) in Burkina Faso. The disease mostly kills children under five years and pregnant women. One objective of the National Malaria Control Program (NMCP) is to contribute to improving the health of the population by reducing malaria burden by 2020. In order to achieve that, the NMCP revised the malaria treatment guidelines in 2014 and conducted training in primary health care. NMCP implemented a one-day orientation training in district and regional hospitals with the support of the PMI Improving Malaria Care Project. A cross-sectional study was conducted in 2015 to assess the quality of malaria prevention and treatment during pregnancy in public health facilities. Data were collected in all 13 health regions from 28 primary health centers, 12 district hospitals, 8 regional hospitals and 2 university teaching hospitals (UTHs). A total of 2,282 patient records were randomly selected and reviewed and 208 providers were interviewed. The assessment focused on the prevention, diagnosis, treatment and reporting of malaria data. A comparative analysis of 2015 and 2017 data was done to understand trends and to identify challenges in program performance. The results showed an increase in the provision of adequate care in 2017 versus 2015 at all levels of the health system, particularly at UTHs. Provision of adequate care at primary health care facilities was 64% in 2015 and 67.1% in 2017. Likewise, adequate care increased at district hospitals from 39.9% in 2015 to 43% in 2017 and from 50% in 2015 to 62.5% in 2017 in regional hospitals. Finally, UTHs showed an increase from 31% to 64% in 2015 and 2017 respectively. Despite the existence of some challenges like lack of central and regional regular supervision, improvement in the quality of care and prevention of malaria in pregnancy have been observed in the health facilities in Burkina Faso between 2015 and 2017. It is important to carry out regular quality assessment or analysis to identify the causes of persistent problems in diagnosis, treatment and prevention of malaria during pregnancy.

1678

RETENTION OF TECHNICAL AND TRAINING KNOWLEDGE AND SKILLS BY MASTER AND GENERAL TRAINERS IN THREE STATES/REGIONS IN MYANMAR

Ni Ni Aye

Jhpiego, Myanmar, U.S. President's Malaria Initiative (PMI) Defeat Malaria Project, Yangon, Myanmar

The NMCP of Myanmar aims to achieve malaria elimination through equitable and universal access to effective preventive and curative services to all at-risk populations in coordination with communities, national and international non-governmental organizations and other stakeholders. The PMI-funded Defeat Malaria project supports the National Strategic Plan's objective to reduce the malaria burden and contribute to elimination in part through capacity development of integrated community malaria volunteers (ICMVs), a new type of cadre introduced in 2017. Defeat Malaria aims to improve this new cadre's knowledge and skills in malaria epidemiology, prevention, and case management through a community-based intervention approach. Seventeen State/Regional (S/R) level master trainers (MTs) were trained from Kayin and Rakhine States and Taninthary Region, 13 of whom then trained 55 general trainers (GTs) in the same S/Rs. The GTs then trained 776 ICMVs. In order to assess MTs and GTs retention of knowledge and skills related to malaria and training methodology, and thus their ability to adequately train ICMVs, in December 2018 Defeat Malaria supported an evaluation of 11 MTs at 9 months after completion of their training using standardized tools in 2 of the 3 S/Rs. Forty-five percent of MTs achieved a passing score on knowledge of malaria technical areas (passing is defined as a score of ≥80% on knowledge and skills assessments). Twenty-seven percent achieved ≥80% on knowledge of training methodology. Regarding training skills, 73% of MTs achieved a passing score on facilitation skills, 82% on demonstration skills and 36% on coaching skills. All MTs achieved ≥80% on skills related to patient history taking, RDT use and treatment of malaria. In addition, 19 GTs were assessed for retention of training skills from 2-7 months after training. Thirty-one percent scored ≥80% on facilitation skills, 42% on demonstration skills and 10% on coaching skills. This evaluation will inform efforts to improve the curriculum, provide continuous mentoring of all trainers after training, especially to the GTs who train and provide supportive supervision to ICMVs.

1679

HOW ACCURATE ARE HOUSEHOLD SURVEYS IN ESTIMATING INDOOR RESIDUAL SPRAYING COVERAGE?

Olivier Tresor Donfack[1], Charity Okoro Eribo[1], Liberato Motobe[1], Wonder P. Phiri[1], Carlos A. Guerra[2], Guillermo A. Garcia[2]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Medical Care Development International, Silver Spring, MD, United States*

Indoor residual spraying (IRS) and malaria indicator surveys (MIS) have been conducted every year since the start of the Bioko Island Malaria Control Project (BIMCP). All households on Bioko Island have been uniquely identified and geo-referenced and this database represents the backbone for all malaria interventions on the island. During IRS rounds, sprayed households are tagged accordingly so that true IRS coverage can be accurately estimated. Households surveyed during MIS are also linked through the same unique identifier and the survey questionnaire enquires whether the house had been sprayed in the last round. Based on this information, we assessed the performance of the MIS in estimating spray coverage relative to the IRS data. To this end, households surveyed during the 2018 MIS were matched to those targeted for IRS in the round earlier in the same year to assess the sensitivity, specificity and accuracy of the survey responses. Out of 4,774 households that were surveyed during the MIS, 1,772 belonged to communities that were targeted during the corresponding IRS round. Only 1,557, however, provided an unambiguous response to the question (*i.e.* "yes" or "no") and were included in the analyses. Amongst these, 1,339 (86%) were sprayed during the IRS round. The MIS responses identified 1,277 true positives (95.4%), 127

true negatives (58.7%), 90 false positives (41.3%) and 62 false negatives (4.6%). Thus, the sensitivity and specificity of the MIS to determine IRS coverage were estimated at 95.4 and 58.7%, respectively. Though the overall accuracy was 90.2%, the high proportion of false positive responses rendered the MIS an unreliable source for estimating true IRS coverage. Our findings raise concern on the use of household surveys for this purpose. Similar assessments are recommended for other settings to improve the understanding of the reasons for response biases.

1680

IMPROVING BIMONTHLY MALARIA LOGISTICS DATA REPORTING ACROSS 459 HEALTH FACILITIES SUPPORTED BY THE GLOBAL FUND: A CASE STUDY OF STRENGTHENING COORDINATION AND GOVERNANCE SYSTEMS IN JIGAWA STATE, NORTHWESTERN NIGERIA

Melis Esi[1], Danny Camlavi[1], Thomas Hall[2], Isaac Adejo[1], Mariah Boyd-Boffa[3], Emmanuel Nfor[2], Olumide Elegbe[1], Bala Mohammed Audu[4], Mohammed Shaibu[4], Olukayode John[4], Sonachi Ezeiru[5], Chukwudi Uche[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*Management Sciences for Health (MSH), Medford, MA, United States,* [4]*National Malaria Elimination Program, Abuja, Nigeria,* [5]*Catholic Relief Services, Abuja, Nigeria*

Bimonthly facility stock reporting (BFSR) is a major component of the Nigeria health system, facilitating the timely and accurate resupplying of key, life-saving commodities, including those for malaria. Prior to 2018, facility staff in Jigawa state had received no training on stock reporting and there was inconsistent follow up from the local government authority (LGA) level, contributing to stock outs of key malaria commodities. Across 459 health facilities, the reporting rate was below 80% in 2017, including late submissions, leading to inaccurate Last Mile Delivery (LMD) plans. Management Sciences for Health in collaboration with the Global Fund, Catholic Relief Services and the National Malaria Elimination Program (NMEP), strengthened the Jigawa state and LGA logistics coordination units by training them to use onsite training and supportive supervision (OTSS) to build facility-level capacity to complete and submit timely and accurate BFSR. This technical assistance was coupled with support to reinforce the coordination platform called the Malaria Procurement and Supply Chain Technical Working Group. To facilitate this capacity building process, the NMEP and partners established new reporting and planning timelines, developed tools including a BFSR checklist used by both supervisors and facilities, and an OTSS form used during supervisory visits to facilities, while BFSR validation rules were used during BFSR review meetings and LMD plan generation. After the introduction of this method, the BFSR reporting rate increased to 94% in April 2018 and improved to 100% in each of the reporting periods since, up to March 2019, leading to the generation of timely and accurate LMD plans and allowing for timely resupply of malaria commodities in all 459 health facilities. Provision of technical assistance to state and local logistics management units to strengthen coordination, supportive supervision, and constructive feedback to facilities during bi-monthly review meetings improve reporting rates and planning, leading to improved resupply of malaria commodities and greater potential for proper management of malaria cases.

1681

WHAT DOES SELF-REPORTED BEDNET USE MEAN? EVIDENCE FROM REMOTE ADHERENCE MONITORING IN UGANDAN HOUSEHOLDS

Paul Joseph Krezanoski

University of California San Francisco, San Francisco, CA, United States

Long-lasting insecticide treated bednets (LLINs) have contributed to the decrease in malaria illness in recent decades, but new evidence suggests LLINs may not be as effective as previously. One critical component of LLIN effectiveness is how LLINs are used. Only recently have remote electronic LLIN use monitors been developed that can provide a more accurate and precise quantification of real-life LLIN use compared to traditional methods like self-reported use. In 2017, our group deployed electronic LLIN use monitors for 6 weeks each in 10 households in rural Uganda. We have previously used this data to develop new metrics of LLIN use such as duration of use, nights missed and times of use. In this study, we focus on how self-reported use might correlate with LLIN use measured by a remote adherence monitor. To achieve this, we used the electronic record to simulate 100 LLIN use assessments by self-report. Self-reported use was defined as nights where the electronic record detected at least 15 minutes use. Each assessment trial consisted of randomly chosen nights, one each from the 10 households. Self-reported use was high across the simulations, an average of 96%. According to the electronic record, average adherence between 0000-0600 hours was also high at 95%, and duration of use was 10 hours per night. However, coverage from 2100 to 0600 hours was 11% lower than self-reported use (85%), and 12% of households used their LLINs less than 5 hours per night. These findings demonstrate the imprecision of self-reports: even high self-reported LLIN use may harbor a wide variation in intra-night LLIN use behaviors with risk for malaria exposure. Combining this with findings of social desirability bias in self-reports, estimated at 13% in a recent paper, LLINs may be used less than surveys based on self-reports suggest. Given the remarkable impact of LLINs on malaria incidence, LLINs may be even more effective than previously thought. In conclusion, more studies using these tools for understanding how LLINs are used in real-life are indicated so that we can better understand the optimal role of LLINs in malaria prevention.

1682

HEALTH SYSTEM STRENGTHENING SUPPORT TO NATIONAL MALARIA PROGRAM ON DATA MANAGEMENT LEADS TO IMPROVEMENT IN THE QUALITY OF DATA REPORTED INTO THE NATIONAL HEALTH MANAGEMENT INFORMATION SYSTEM DATABASE (DHIS2) IN TARABA STATE, NORTHEAST NIGERIA

Chinedu Chukwu[1], Jerry Mbaka[1], Isaac Adejo[1], Adeyinka Onikan[1], Thomas Hall[2], Mariah Boyd-Boffa[3], Nnaemeka Onugu[4], Sonachi Ezeiru[4], Perpetua Uhomoibhi[5], Bala Mohammed Audu[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*Management Sciences for Health (MSH), Medford, MA, United States,* [4]*Catholic Relief Services, Abuja, Nigeria,* [5]*National Malaria Elimination Program, Abuja, Nigeria*

Management of health data is a significant challenge in Nigeria, impacting the performance of health providers, quality of care and the overall health system. Health care providers and data managers in Taraba have not received any training or consistent supervision in over five years, leading to weak adherence to data management guidelines. Though there is routine data submission to the state from the facility and local government authority (LGA) levels, these reports are frequently sent with "zero data elements", or data entry errors, and thus no malaria indicator performance measures. In collaboration with the Global Fund in Nigeria, Management Sciences for Health, in partnership with the National and State Malaria Elimination Programs, builds capacity of state-level data managers and health care providers on malaria services documentation and indicator reporting according to national guidelines. Capacity is built by providing trainings and by linking the state level with the most up-to-date tools, leading to improved analyses and use of malaria data for decision making. Capacity and performance are reinforced with routine, onsite supportive supervision and with the introduction of data validation meetings at the LGA level. In Taraba, this intensive support led to a 6% improvement in data quality, demonstrating a reduction in the number of data entry errors. The data quality rate improved each quarter in 2018, from 87% at baseline in the first quarter to 93% by the end of the year in the fourth quarter. Building capacity and knowledge in data management, indicator definitions, and national DHIS2 reporting requirements, with regular supportive supervision, are key to data quality improvement, and improved analysis and use of data for decision making.

1683

MALARIA DURING PREGNANCY AND NEWBORN OUTCOME AMONG INPATIENTS IN NON CRITICAL OBSTETRIC UNITS OF PUBLIC HOSPITALS OF THE PERUVIAN AMAZON

Priscilla Magno-Muro[1], Freddy Valera-Gallegos[2], Nataly Atarama[1], Raul Chuquiyauri[3], Wilma Casanova[4], Stalin Vilcarromero[5]

[1]*Sociedad Científica de Estudiantes de Medicina de la Amazonia Peruana (SOCIEMAP), Universidad Nacional de la Amazonia Peruana (UNAP), Iquitos, Peru,* [2]*Sociedad Cientifica de Estudiantes de Medicina de la Amazonia Peruana (SOCIEMAP), Universidad Nacional de la Amazonia Peruana (UNAP), Iquitos, Peru,* [3]*Sanaria Inc., Rockville; Medical Care Development International, Silver Spring, Malabo, Mexico,* [4]*Universidad Nacional de la Amazonia Peruana (UNAP), Iquitos, Peru,* [5]*Sociedad Científica de Estudiantes de Medicina de la Amazonia Peruana (SOCIEMAP), Medical Care Development International, Department of Medicine, Division of Infectious Diseases, Stony Brook University, Stony Brook, NY, United States*

Annual malaria incidence in the Peruvian Amazon increased by 21.5% from 2013 (43744 cases) to 2017 (53163 cases), and its impact among pregnant women has not been well described. We aimed to describe the clinical and epidemiological characteristics of pregnant women hospitalized in non-critical care units and its implications on the fetus or newborn. We reviewed the clinical charts of all pregnant women with malaria diagnosed by thick smear from 2010-2016, along with the clinical charts of their newborn. Cases admitted in critical care were not included. A total of 131 pregnant women met the criteria, and according to WHO criteria: 42 (30.4%) of the women were considered to have severe malaria, and 78 (56.5%) and 13 (9.4%) were 16-25 and <16 years old respectively. 71 (51.4%) lived in urban and peri-urban settings, 71 (51%) were in their third trimester, 120 (87%) reported no previous history of malaria and perception of temperature elevation (109, 79%), headache (93, 68%) and chills (66, 47.8%) were the most common symptoms. Only four were asymptomatic. The vast majority (126, 91.3%) were housewives, and only 47% (65) studied elementary school or less. For 61.6% (85) of women, this was their first or second pregnancy. *Plasmodium falciparum* was the most common species identified (78, 56.5%), and 73% (101) harbored parasite densities less than 3+. The most frequent maternal complications were anemia (74.6%, 106) and thrombocytopenia (46.4%, 58). 43.5% (62) received only oral therapy. Artesunate and Clindamycin were the most commonly prescribed medication. Only in 62 cases (45%) was it possible to obtain the newborn's clinical data. 83.9% (52/62) of the neonates presented an adequate weight at birth, 8% (11/62) were premature, 7.2% (10/62) were small for the gestational age, 4.3% (6/62) intrauterine growth restriction and 7/62 were considered congenital malaria (6 asymptomatic). The high sensitivity of WHO guidelines may explain why severe malaria cases were managed in a non-critical care setting. Despite malaria being endemic to rural communities, this data shows how impactful it is to urban/peri-urban settings.

1684

PILOTING A CASE-BASED SURVEILLANCE TOOL TARGETED AT PHARMACEUTICAL PRIVATE PROVIDERS (PSPS) IN LAGOS, NIGERIA.

Tayo Olaleye[1], Olukunle Adewusi[1], Luke Baertlein[1], Chizoba Fashanu[1], Omotayo Giwa[1], Abimbola Osinowo[2], Deepa Pindolia[3], Owens Wiwa[1]

[1]*Clinton Health Access Initiative (CHAI), Abuja, Nigeria,* [2]*Lagos State Malaria Elimination Program, Lagos, Nigeria,* [3]*Clinton Health Access Initiative (CHAI), Nairobi, Kenya*

Nigeria's 2015 malaria indicator survey confirms malaria prevalence in Lagos state to be 0% and ~2%, based on microscopy and mRDTs respectively. Prevalence however remains heterogeneous within the state. The private sector, specifically the Patent and Proprietary Medicine Vendors and Community Pharmacists collectively called PSPs, is the first point of care providing treatment for up to 67% of care-seeking fever cases. However, reliable data on diagnosis and treatment practices is lacking, preventing informed resource planning and effective government oversight. The scale-up of a surveillance system could contribute to efficient programming and providing a wholesome view of true malaria prevalence in Lagos; thus accelerating progress towards elimination. An innovative case-based surveillance system was developed and piloted in 387 PSPs across Lagos in March 2019. The system included configuration of DHIS2 data collection tool for mobile reporting and a retail private sector specific DHIS2 instance for hosting and analysis of reported data. The trained PSPs use a case-based reporting system via a mobile app; the data is presented in analytics to support decisions on case management practice and supportive supervision. On average, each month, 42% of the trained PSPs reported at least once. Case management indicators, such as test uptake and adherence, are calculated and displayed in dashboards and routine reports used by government officers and PSP supervisors to target supervision visits based on PSP performance. Data review meetings are held routinely to facilitate the use of the data for program and supervision planning. By July 2019, 111 targeted supervision visits using PSP-specific reported data, were conducted. Although reporting rate is low, this pilot clearly indicates that the retail private sector can collect and report malaria-specific case-based data which can be used to guide state malaria programming and operations. Routine reporting in this manner, scaled up to all the PSPs in the state, region and country, will provide a more representative data of malaria diagnosis and treatment practices in the private sector.

1685

MALARIA PREVENTIVE PRACTICES AMONG UNDER-FIVES IN RIVERS STATE, NIGERIA

Nsirimobu Ichendu Paul, Omosivie Maduka, Chijioke Adonye Nwauche, Ibinabo Laura Oboro, Terhemen Kasso, Lucy Eberechukwu Yaguo-Ide, Abimbola Temitayo Awopeju, Godly Otto, Ifeyinwa Nwogo Chijioke-Nwauche, Carol Iyalla

University of Port Harcourt, Port Harcourt, Nigeria

Malaria is a life threatening protozoan infection especially in under 5 children and strong malaria preventive practices is key to reducing its associated mortality. The study assessed malaria preventive practices among under-five children in Rivers State, Nigeria. This was a cross sectional study in public and private health facilities in Rivers state. Ethical approval for the study was obtained from the Research and Ethics committee of the University of Port Harcourt Teaching Hospital, while an informed written consent was obtained from the parents or caregivers of the participants. Stratified sampling method was used to select the health facilities and the subjects for the study. A pretested interviewer administered questionnaire was used to harvest relevant information on socio demographic characteristics of the subjects and informants and malaria preventive practices. Obtained data was analysed using SPSS version 22. A total of 1138 children participated in the study constituting of 613 (53.9%) male and 525(46.1%) female Mean age of participants was 1.74±1.08 years. Mothers, accounted for majority 1012 (88.9%) of the informants. Most of the informants had tertiary degree; 605 (53.4%) and 697 (61.8%) among mothers and fathers respectively. Among the occupations of fathers, public servants, civil servants and the self-employed were more represented, constituting 242 (21.4%), 200 (17.7%) and 149 (13.2%) respectively. Traders/business women, the self-employed and civil servants were most represented among the occupations of mothers in the study and these accounted for 444 (39.7%), 181 (16.2%) and 137 (12.3%) respectively. Malaria preventive practices included protective window nets, bed nets, indoor residual spraying and use of drugs which constituted 970 (85.2%), 605 (53.2%), 483 (42.4%) and 133 (11.7%) respectively. Protective window net use rate was high (85%) among children in Rivers state. Health education on the integrated approach to malaria prevention is advocated.

1686

KNOWLEDGE, ATTITUDES, AND PRACTICES REGARDING MALARIA TRANSMISSION AND PREVENTION AMONG THE MAIJUNA COMMUNITY: A QUALITATIVE STUDY IN THE PERUVIAN AMAZON

Kathryn M. Hogan[1], Michael von Fricken[1], Michael Gilmore[1], Graziella Pagliarulo McCarron[1], Brian Griffiths[1], Guillermo Garcia[2]

[1]*George Mason University, Fairfax, VA, United States,* [2]*Medical Care Development International, Silver Spring, MD, United States*

Over 90% of incident malaria cases in Peru are localized in the Department of Loreto, and many who reside there live in rural, subsistence farming communities that are geographically isolated from existing healthcare services. Therefore, the emphasis on preventive behaviors as well as early treatment seeking is vital to decreasing transmission in the area. The uptake of these behaviors is often dependent upon local malaria knowledge, beliefs, and overcoming the idea that malaria is embedded and unavoidable in society. The objective of this study was to explore the history of malaria transmission among the Maijuna people, an indigenous community of the Peruvian Amazon, and understand the impact that current knowledge and attitudes have on preventive behaviors in Sucusari. Knowledge, attitudes, and practices were assessed using semi-structured interviews, and CareStart Rapid Daignostic Tests were used to test for malaria. Individuals were sampled using purposive sampling, and each adult in the community was asked to take a malaria test. Thirty-four individuals were interviewed between January and April 2019, and 35 were tested for malaria out of a total 65 adults in the community. Themes emerged from interviews as follows: confusion about the transmission mechanism of malaria, the feeling that malaria is unavoidable, and the adoption of preventive behaviors as a direct results of local health programming. Following interviews, there was a community meeting to debrief and clarify any questions about transmission, prevention, and treatment. The results indicate that previous community-based programming may have been presented in a manner that was unclear to large portions of the community, or did not reach the whole intended audience. The results also show that while there was confusion about the mode of transmission of malaria, this was not directly related to the practice of preventive behaviors. Information from this study can act as a foundation for local health organizations to evaluate and alter community-based programming in indigenous communities in Sucusari, as well as other Peruvian indigenous communities.

1687

ASSOCIATION BETWEEN PLACENTAL MALARIA AND THE INCIDENCE OF MALARIA IN INFANTS BORN TO HIV-UNINFECTED UGANDAN MOTHERS LIVING IN A HIGH MALARIA TRANSMISSION SETTING

Abel Kakuru[1], Sarah Staedke[2], Daniel Chandramohan[2], Richard Kajubi[1], Teddy Andra[1], Harriet Adrama Harriet Adrama[1], Miriam Nakalembe[3], Tamara D. Clark[4], Theodore Ruel[4], Diane V. Havlir[4], Moses R Kamya R. Kamya[3], Grant Dorsey[4], Prasanna Jagannathan[5]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Makerere University College of Health Sciences, Kampala, Uganda,* [4]*University of California San Francisco, San Francisco, CA, United States,* [5]*Stanford University, San Francisco, CA, United States*

Although placental malaria (PM) has been associated with adverse birth outcomes, data on the effect of PM on the incidence of malaria during infancy remain inconclusive. We evaluated associations between PM and other maternal factors on the incidence of malaria during the first year of life in a birth cohort of 611 infants born to HIV-uninfected mothers. The study was conducted in Busia, Uganda, an area with high malaria transmission intensity. Mothers were enrolled during the second trimester of pregnancy, and PM was categorized based on the proportion of high-power fields with malaria pigment seen by histology as none (n=348); mild (<10%, n=135); moderate (10-<30%, n=100); and severe (30-60%, n=28). A total of 1008 episodes of malaria were diagnosed in infants (incidence 1.84 episodes per person year.) We observed significant effect modification between several maternal factors and infant sex, and therefore report stratified analyses. Among male infants, those born to mothers with severe PM had a higher incidence of malaria compared to those born to mothers with no PM (aIRR 1.87, 95% CI 1.03-3.37, p=0.04). Male infants born to a gravida ≥3 mother (aIRR 1.65, 95% CI 1.25-2.18, p<0.001) and those with either submicroscopic or microscopic parasitemia at enrollment (aIRR 2.56, 95% CI 1.73-3.81, p<0.001) had a higher incidence of malaria during infancy compared with those born to a gravida ≤2 mother or those without parasitemia at enrollment, respectively. Among female infants, only those born to mothers with submicroscopic or microscopic parasitemia at enrollment (aIRR 1.89, 95% CI 1.33-2.69, p<0.001) had a higher incidence of malaria during infancy. Together, these data suggest that the association between second trimester parasitemia and incidence of malaria during infancy reflects a shared exposure to malaria transmission. However, infant sex may modify associations between PM, gravidity and infant malaria incidence, resulting in important, sex-specific consequences of *in utero* malaria exposure in male, but not female, infants.

1688

MAINTAINING UNIVERSAL COVERAGE OF LONG LASTING INSECTICIDAL NETS THROUGH DISTRIBUTIONS IN SCHOOLS IN UGANDA

JohnBaptist Bwanika[1], Ruth Kigozi[1], Emily Godwin[1], Patrick Bukoma[1], Peter Thomas[2], James Tibenderana[3], Sam Siduda[1], Gloria Sebikaari[4], Belay Kassahun[4]

[1]*USAID's Malaria Action Program for Districts, Kampala, Uganda,* [2]*US President's Malaria Initiative, Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA, Kampala, Uganda,* [3]*Malaria Consortium, London, United Kingdom,* [4]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda*

There is strong evidence that regular use of a long lasting insecticidal net (LLIN) substantially lowers one's risk of contracting malaria. Mass distribution campaigns seek to achieve universal coverage (a net for every 2 individuals), an outcome that may be improved by distributing additional LLINs in schools. More data are needed on whether continuous distribution via this channel can improve universal coverage of LLINs in a general population. To evaluate this question, the US President's Malaria Initiative Malaria Action Program for Districts piloted a school distribution program in June 2018. LLINs were distributed in schools in 26 Ugandan districts that had below 70% LLIN coverage (intervention arm), and longitudinal data were collated - before distribution and six months after - from two representative population samples of 2,420 households in the intervention arm and 550 households in the control arm. To control for possible confounding, the proportion of pregnant women who received LLINs during ANC was checked and ascertained not to be significantly different at 65.2% (95%CI: 63.2%, 67.3%) in intervention and 63.1% (95%CI: 60.7%, 65.5%) in control areas. Households' socio-demographic and net ownership data were collected and analyzed; LLIN coverage was assessed by asking heads of households about the number of resident individuals and LLINs present in their households. Analysis revealed that the proportion of households with universal coverage of LLINs was maintained from 68.5% (95%CI: 66.6%, 70.4%) to 70.7% (95%CI: 68.5%, 72.9%) in the intervention arm, and fell, significantly, from 78.2% (95%CI: 75.3%, 81.1%) to 69.6% (95% CI: 65.0%, 74.1%) in the control arm. Households from the highest wealth quintile were more likely to have universal coverage than those from the lowest wealth quintile (OR 1.40, 95% CI 1.10-1.78). Likewise, households whose heads had university education were more likely to have universal coverage than those with household heads with no formal education (OR 2.08, 95% CI 1.39-3.11). These findings suggest that LLIN replenishment through schools can sustain universal coverage in the general population.

1689

EFFECT OF FOUR YEARS OF SEASONAL MALARIA CHEMOPREVENTION ON THE ACQUISITION OF ANTIBODIES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN OUELESSEBOUGOU, MALI

Almahamoudou Mahamar[1], Djibrilla Issiaka[1], Ahamadou Youssouf[1], Sidi Mohamed Niambele[1], Harouna M. Soumare[1], Oumar Attaher[1], Amadou Barry[1], David L. Narum[2], Patrick E. Duffy[2], Brian Greenwood[3], Michal Fried[2], Alassane Dicko[1]

[1]*Malaria Research and Training Center (MRTC), Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Rockville, MD, United States,* [3]*London School of Hygiene & Tropical Medicine (LSHTM), London, United Kingdom*

More than 200 million people live in areas of highly seasonal malaria transmission, where seasonal malaria chemoprevention (SMC) with SP and AQ was recommended in 2012 by WHO. The strategy is now widely implemented and protecting more than 17 million children in 2018. We previously reported that SMC reduced seropositivity to MSP-1_{42} and CSP (but not AMA1), and the duration of SMC did not further reduce seropositivity. In this study we assessed the impact of four years of SMC on the acquisition of antibodies to malaria antigens. A cross-sectional survey was carried out one month after the last dose of SMC in 2017 in children aged 4-5 years randomly selected from areas where SMC was given for 2 or 4 years during the malaria season. Antibody extracted from dry blood spots was used to measure IgG levels to CSP, MSP-1_{42} and AMA1 by ELISA. The prevalence of antibodies to MSP-1_{42} were similar in children who received SMC for 4 years when compared to those who received SMC for 2 years (85.1 vs 86.0%, p = 0.80). The prevalence of antibodies to AMA-1 and to CSP were not lower in children who received SMC for 4 years compared to those who received SMC for 2 years (95.3 vs 88.8%, p = 0.01 for AMA-1; 91.2 vs 81.9%, p =0.001 for CSP). Similarly to the seropositivity prevalence, the anti-MSP-1_{42} IgG median levels did not significantly differ between children that received SMC for four or two years (0.88 IQR: 0.64-1.15, 0.95 IQR: 0.68-1.15; p = 0.15) and anti-AMA-1 and anti-CSP IgG median levels were not inferior in children who received SMC for four years vs. two years (1.45 IQR: 1.24-1.68, 1.41 IQR: 1.17-1.64; p = 0.02) and (1.30 IQR: 1.00-1.56, 1.17 IQR: 0.87-1.47; p = 0.0005) for AMA1 and CSP respectively. In this area of high seasonal malaria transmission, children who had received SMC for four years did not had higher seropositive and antibody titers to CSP and AMA1 compared to children who had received SMC for two years suggesting that children may not be at higher risk of malaria after the cessation of SMC.

1690

COMMUNITY-BASED APPROACH TO REACH MALNOURISHED INFANTS FROM 6 MONTHS TO 5 YEARS DURING A SEASONAL MALARIA CHEMOPREVENTION (SMC) CAMPAIGN IN REMOTE AREAS IN NIGER

Hortense Angoran-Benié[1], Dr Hadiza Jackou[2], Chrestien Yameni[1]

[1]*Catholic Relief Services, Baltimore, MD, United States,* [2]*MNCP program, Niamey, Niger*

Malaria & acute malnutrition are major public health problems in Niger and Children 3 - 59 months are mostly victim. In 2016 National Data showed malaria incidence rate at 120,75 case per 1 000 habitants and moderate and severe malnutrition at 12.53% and 9.56%. Both diseases coincide with the lean rainy season. Since 2016, The National Malaria Control program (NMCP) and the Nutrition Directorate decided to integrate nutritional screening by trained community health workers during SMC campaigns. Infants were reached in the communities using MUAC, scored card and daily summary sheets through fixed, mobile and door to door approaches. Community workers were identified, trained and equipped by the national nutritional Directorate and its partners. In addition to training received on SMC campaign, community workers were also trained on: carrying out interpersonal communication with the mothers; the infant classification based on the color observed screening of edema; filling out data tools and proper infant referrals (yellow and red). Joint planification, supportive supervision and data validation processes by both the NMCP and the Nutrition Directorate were conducted in the communities. For 2018, the national target for Malnutrition screening was 3,750,315 enfants. 3,514,747 were screened for malnutrition. 3,349,058 (95,29) were healthy (arm circumference >125mm); 131,508 (3,74%) were moderately malnourished (arm circumference between 115 and 125; and 34,181 (0,9%) were deemed severely malnourished (arm circumference <115mm with or without lower extremity edema). In conclusion, the integration of malnutrition screening into seasonal malaria chemoprevention campaign using community volunteers is possible and is cost effective in the fight against children under five years who are vulnerable to malaria and malnutrition in low income country. However, management of referred malnourished children remains a great challenge.

1691

IMPROVED UPTAKE OF MALARIA IN PREGNANCY INDICATORS: A CASE FROM USAID LAKE AND WESTERN ZONE, TANZANIA

Zipporah Wandia[1], Jasmine Chadewa[2], Agnes Kosia[2], Goodluck Tesha[1], Lusekelo Njoge[2], Zahra Mkomwa[3], Dunstan Bishanga[2], Rita Noronha[2], Bayoum Awadhi[2], Gaudiosa Tibaijuka[2], Chonge Kitojo[4], Erik Reaves[4], Abdallah Lusasi[5]

[1]*Jhpiego, Dar es Salaam, United Republic of Tanzania,* [2]*USAID Boresha Afya Project -Jhpiego Tanzania, Dar es Salaam, United Republic of Tanzania,* [3]*USAID Boresha Afya Project –Path Tanzania, Dar es Salaam, United Republic of Tanzania,* [4]*President's Malaria Initiative/United States Agency for International Development, Dar es Salaam, United Republic of Tanzania,* [5]*National Malaria Control Program-Tanzania Ministry of Health, Community Development, Gender, Elderly and Children, Tanzania, Dar es Salaam, United Republic of Tanzania*

Malaria in pregnancy (MiP) has been recognized as a major public health concern contributing to poor maternal and newborn health outcomes. In Sub-Saharan Africa, up to 20% of stillbirths are attributable to MiP and contributes to an estimated 10,000 maternal deaths and 100,000 infant deaths. Tanzania implements a three-pronged approach to prevent the adverse effect associated with MiP as recommended by WHO: use of long lasting insecticide-treated bed nets (LLINs), intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine, and prompt diagnosis and treatment. Tanzania has improved uptake of IPTp and MiP indicators in seven regions supported by the USAID Boresha Afya Project in collaboration with the National Malaria Control Program and regional and council health management teams. The supported regions used the malaria data dashboard, a platform where all malaria data are displayed for easy access by all malaria stakeholders, to identify health facilities with low uptake of MiP indicators and poor documentation in Health Management Information System Book 6, the antenatal care (ANC) register used in Tanzania's health facilities. The project completed quarterly follow-up and mentorship for health care workers at ANC between 2016 and 2018 in 1817 (100%) health facilities. IPTp2 increased from 49% to 74% and IPTp3 increased from 2% to 49%, both approaching the national targets of 80% for IPTp2 and 60% for IPTp 3 in all seven regions. ANC visits <12 weeks increased from 14% to 37% and ANC 4th visits increased from 39% to 62%, pregnant women tested for malaria at 1st ANC visit increased from 68% to 98%, and ferrous folic supplementation uptake increased from 68% to 78%. The improvements in MiP indicators in the supported regions may be attributed to commitment among health care workers, mentorship, and proper documentation. Continued and expanded mentorship to reinforce proper documentation and use of the malaria data dashboard might further increase IPTp uptake, thereby reducing maternal and neonatal deaths associate with malaria in pregnancy.

1692

ACCEPTABILITY AND USE OF LONG-LASTING INSECTICIDAL NETS (LLIN) AND TOPICAL REPELLENTS AMONG FOREST-GOERS INVILLAGES OF TANINTHARYI REGION AND KAYIN STATE IN MYANMAR

Kaung Myat Thu[1], Nay Min Shein[1], Feliciano Monti[2], Zar Ni Htun[1], Bo Bo Thet Ko[1], Htin Lin Thaw[3], Sway Minn Htet[1], Kyaw Myint Tun[1]

[1]*University Research Co., Myanmar, U.S. President's Malaria Initiative (PMI) Defeat Malaria Project, Yangon, Myanmar,* [2]*United States Agency for International Development, Yangon, Myanmar,* [3]*American Refugee Committee, Yangon, Myanmar*

One of the malaria elimination challenges in Myanmar is that the disease primarily affects marginalized populations such as forest-goers. In 2018, Defeat Malaria project implemented a pilot intervention to explore the acceptability and utilization of preventive measures among forest-goers in 9 villages in 4 townships of Tanintharyi Region and Kayin State. In November-December 2017, about 950 forest-goers received a prevention kit comprised of a long-lasting insecticidal net (LLIN) and a 100g tube of a topical mosquito repellent, replaceable on demand, containing 12% N, N-diethyl-benzamide. The acceptability, utilization and perceived usefulness of both LLINs and repellent were assessed after 6 months, in June-July 2018, by reviewing activity data, interviewing beneficiaries with a structured questionnaire, and conducting focus group discussions. All forest-goers who received the kit were invited to participate in the assessment; 286 engaged in structured interviews, and 6 focus group discussions were conducted. Seventy eight percent of the forest-goers were male, with median age 41 years, an average monthly income of 60 USD, with 45% having an income of less than 1 USD/day. The most common types of forest-related work were farming (91%), followed by bamboo cutting/logging (41%), and hunting (22%). Sixty three percent worked at times from 6pm to 6am, while 85% of hunters hunted during the night. Although acceptability of LLINs was more than 90%, their utilization varied from 75% among farmers and wood cutters to 50% in hunters. The utilization of the repellent was very high among all types of forest-goers with 90% reported during the last night-time visit to the forest. Seventy eight percent reported using the repellent every night in the forest. According to the qualitative results, most of the forest-goers highly appreciated the usefulness of the repellent in reducing mosquito bites. Although acceptability of LLINs was high, their utilization during night was difficult. The high use of repellent is encouraging and warrants conducting further studies on its effectiveness in reducing malaria transmission among forest-goers.

1693

HEARING TO UNDERSTAND: ASSOCIATIONS BETWEEN HEARING MALARIA HEALTH MESSAGING AND MALARIA KNOWLEDGE, AWARENESS AND PRACTICE OF PREVENTATIVE MEASURES IN THE 2018 MALARIA INDICATOR SURVEY FOR BIOKO ISLAND, EQUATORIAL

Tammy Cavanzos[1], Matthew Rossheim[1], Olivier Tresor Donfack[2], Wonder P. Phiri[2], Guillermo A. Garcia[3], Michael E. von Fricken[1]

[1]*Department of Global and Community Health, George Mason University, Fairfax, VA, United States,* [2]*Medical Care Development International, Malabo, Equatorial Guinea,* [3]*Medical Care Development International, Silver Spring, MD, United States*

The Bioko Island Malaria Control Project (BIMCP) started in 2004 to reduce morbidity and mortality caused by malaria on Bioko Island. A Malaria Indicator Survey (MIS) is conducted annually to measure the impact of the project. This study uses the MIS household data to examine the associations between individual's reception of the malaria communication messaging with knowledge of malaria preventative measures and practice of malaria preventative measures. The sample size of this analysis is 4,444. 50% of the population heard a malaria message within 6 months. The use of Indoor Residual Spraying (IRS) was among the most popular malaria messages heard (10% recalled hearing it) while 9% recalled hearing the need to eliminate breeding sites/clean up trash. Messages regarding LLIN use were recalled by less than 1%. Household knowledge of preventive measures was 77% for using LLINs, 18% for using IRS, and 6% for prophylaxis. 58% of the population slept under an LLIN the night before and 43% received IRS within 12 months. After adjusting for potential confounders, the only factor associated with sleeping under an LLIN was knowledge of IRS as a preventative measure (OR=0.77, 95% CI: 0.65, 0.90). After adjusting for potential confounders, factors associated with indoor residual spraying within 12 months included knowledge of mosquito nets as a preventative measure (OR=0.64, 95% CI: 0.55, 0.74) and hearing a malaria-related message regarding sleeping under an LLIN (OR=0.78, 95% CI: 0.67, 0.91). Health-related messages are reaching the population. However, improvements are needed to increase malaria knowledge regarding preventative measures, as this affects acceptance and use of preventive measures.

1694

THE EFFECT OF EDUCATION ON MALARIA PREVENTION BEHAVIOR

Kevin Croke

Harvard T.H. Chan School of Public Health, Boston, MA, United States

Health-seeking behavior by individuals and improved socioeconomic status are key contributors to successful malaria control programs, and both may be affected by education levels. Yet there is relatively little causal evidence of impact of education on malaria prevention behavior and ownership of preventive commodities. We leverage education policy changes that induced plausibly exogenous shifts in cohort-level educational attainment in several Sub-Saharan African countries to examine the impact of increased education on bednet ownership and usage, and on treatment seeking behavior for children. We find that increases in education lead to significant increases in bed net ownership and usage, especially when it is female household members who gain additional education. Impact on treatment seeking behavior for febrile illness is more limited.

1695

INCREASING COVERAGE AND USE OF INSECTICIDE-TREATED NETS IN ZAMBIA: RESULTS FROM THE ZAMBIA MALARIA INDICATOR SURVEY 2018

Maya Fraser[1], Caterina Guinovart[2], Busiku Hamainza[3], Elizabeth Chizema-Kawesha[3], Kafula Silumbe[4], Mercy Mwanza-Ingwe[3], Hawela Moonga[3], Anthony Yeta[3], Mutinta Mudenda[3], Fred Masaninga[5], John M Miller[4]

[1]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States,* [2]*PATH/ISGlobal, Barcelona, Spain,* [3]*National Malaria Elimination Centre, Lusaka, Zambia,* [4]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [5]*World Health Organization, Lusaka, Zambia*

Universal coverage of vector control, defined as every household having adequate coverage of insecticide treated nets (ITNs) or indoor residual spraying (IRS), is a key piece of Zambia's National Malaria Strategic Plan for 2017–2021. From 2017–2018, in support of this goal, a mass distribution campaign of more than 10 million total nets was conducted across all provinces. The Zambia National Malaria Indicator Survey (MIS) 2018, a nationally representative household survey designed to assess key malaria indicators, was conducted in April–May of 2018 during peak transmission season, and provided an opportunity to assess coverage and usage of ITNs following mass distribution. The 2018 MIS was a two-stage cluster sample of 4,475 households selected from 179 standard enumeration areas, with 4,177 completed household interviews. Household members were asked a series of standardized questions about malaria interventions, including vector control. A net roster module listed each net that households owned, and which family members slept under it. Results showed that nationally Zambia achieved 80% coverage of at least one ITN per household. Eight of ten provinces had greater than 80% coverage.

Increases in coverage were especially notable in rural areas, where the household ownership of at least one ITN increased from 78% in 2015 to 87% in 2018. Forty-five percent of households reported having at least one ITN for every two household members (up from 42% in 2015), a measure of full coverage of ITNs within households. Among all household members, 65% reported using an ITN the previous night. For those in households with at least one ITN, 84% reported usage the previous night. Among children under age five and pregnant women, these figures were 69% and 71%, respectively. These findings show an increase among most all ITN indicators since 2015 and indicate that usage is high among those who have access to nets. Further results will be presented on the characteristics of households who did not receive ITNs and of individuals in households with nets that did not use them.

1696

COMMUNITY APPROACH TO FIGHT AGAINST MALARIA THROUGH THE USE OF DIGITAL HEALTH IN THE HEALTH DISTRICT OF NIORO DU RIP (SENEGAL)

Malick Anne[1], **Abdoulaye Ndione**[1], Diarga Mballo[1], Ibrahima Diankha[1], Mouhamed Gueye[1], Youssoupha Ndiaye[2]

[1]*Senegal Health Ministry and Social Action, Kaolack, Senegal,* [2]*Senegal Health Ministry and Social Action, Dakar, Senegal*

Worldwide, 219 million cases of malaria were recorded in 2017 and 435,000 deaths. Nearly 90% of malaria cases and 92% of deaths occur in sub-Saharan Africa. In Senegal, the evolution of parasite prevalence from 2009 to 2015 still ranks Senegal among the countries of sub-Saharan Africa (SSA) where malaria is endemic and constitutes a health problem. However, this burden dropped significantly by more than 50% between 2009 and 2015. The health district of Nioro, thanks to the numerous actions to fight against malaria and especially the indoor sprinkling, experienced a decrease in the incidence of malaria that increased from 2014 to 2017 from 34 ‰ to 3 ‰. In the drive to maintain this incidence of pre-elimination of malaria, the district has put in place a strategy in 2018 whose goal is to get communities to engage in the fight against malaria through the use of social networks with the concept group "Zero Malaria". A descriptive study of this strategy was carried out and the choice of villages was reasoned based on the incidences of malaria recorded in the health structures. The initiative is based on the mentoring of young volunteers named "ambassador of fight against malaria" who through the network whatsapp carry out activities of awareness and mobilization against the malaria at the level of their village. Thus 20 cine buses were made and 20 "zero malaria" groups created. 200 ambassadors had to carry out awareness activities against malaria by sharing audio and video made by the ambassadors via whatsapp. 3340 people were sensitized including 2461 women and 879 men. The strategy has strengthened knowledge about malaria and increased community ownership of malaria.

1697

PHARMACOKINETIC/PHARMACODYNAMIC MODELING TO IDENTIFY OPTIMAL DIHYDROARTEMISININ-PIPERAQUINE INTERMITTENT PREVENTIVE TREATMENT REGIMENS FOR YOUNG UGANDAN CHILDREN

Erika Wallender[1], Emma Hughes[1], Abel Kakuru[2], Prasanna Jagannathan[3], Mary Kakuru Muhindo[2], Bishop Opira[2], Meghan Whalen[1], Moses Kamya[4], Grant Dorsey[1], Francesca Aweeka[1], Philip J. Rosenthal[1], Rada M. Savic[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*Stanford University, Palo Alto, CA, United States,* [4]*Makerere University, Kampala, Uganda*

Intermittent preventive treatment (IPT) with dihydroartemisinin-piperaquine (DP) is highly protective against malaria in young children, but concerns remain about optimal dosing, toxicity, and selection for drug resistance. We use pharmacokinetic/pharmacodynamic (PKPD) modeling to identify the optimal DP IPT regimen for Ugandan children as young as 2 months of age by describing the PK in this population and quantifying relationships between piperaquine pharmacokinetics and protective efficacy, toxicity, and markers associated with drug sensitivity. Clinical data, PQ concentrations ([PQ)]), and *P. falciparum* genotypes associated with decreased PQ sensitivity in Africa (*pfmdr1* 86Y, *pfcrt* 76T) were obtained longitudinally from Ugandan children randomized to receive standard dosing of DP as IPT every 12 weeks (n=184) or every 4 weeks (n=96) from age 2-24 months. We developed a population PK model using nonlinear mixed effects modeling and used logistic regression to evaluate the relationship between [PQ] and probability of parasitemia. From 280 children, 4,509 [PQ]s (3,627 troughs, 882 intensive), 120 ECGs, 142 malaria episodes, and 82 asymptomatic parasitemia episodes were analyzed. Median [PQ] was lower for children compared to adults, lower at age 24 months compared to 3 months (2.0 vs 4.2 ng/mL 12-week DP, 4.4 vs 4.9 ng/mL 4-week DP), and lower with asymptomatic parasitemia compared to not detected (1.3 vs 2.6 ng/mL). With symptomatic malaria the median [PQ] was 3.7 ng/mL (below the limit of quantification-12.5 ng/mL; 2.5-97.5% percentile). After allometric scaling, age-dependent enzyme maturation and weight for age z-score were significant covariates for PQ clearance: older or malnourished children had faster PQ clearance, thus require higher DP doses. PKPD analysis revealed a significant [PQ]-effect relationship, with 10 ng/mL PQ associated with 95% protection from parasitemia. Ongoing analysis is evaluating the relationships between PK and ECG QTc length and resistance markers. The similar protective [PQ] identified for children and pregnant women in Uganda is a key target to guide DP IPT optimization.

1698

MIDLINE RESULTS FROM A MALARIA INTENSIFICATION PLAN IN HIGH BURDEN AREAS OF CAMBODIA

Dr. Siv Sovannaroth[1], Michelle Pahl[2], Kimhong Gove[2], Chelsea Hanlon[2]

[1]*National Center for Parasitology, Entomology and Malaria Control in Cambodia., Phnom Penh, Cambodia,* [2]*Clinton Health Access Initiative, Phnom Penh, Cambodia*

Malaria cases increased in Cambodia by 289% between January and March 2018, compared to the same period in 2017. 80% of all reported cases were concentrated in 10 out of 21 Operational Districts (ODs), with over 90% occurring in the forested areas among migrant and mobile populations (MMP) and forest goers. In response, the Cambodia National Center for Parasitology, Entomology and Malaria Control (CNM) and partners established an Intensification Plan (IP) to accelerate the prevention of malaria infection and improve case management by health centers (HCs) and village and mobile malaria workers (VMWs/MMWs) for September 2018 - August 2019. CNM analysed cases reported through the Malaria Information System at all points of care to identify the 30 highest burden HCs in 10 target ODs. CNM conducted a mass screen and treat exercise in 4 villages per HC to identify transmission hotspots and at-risk populations. The first IP objective aimed to strengthen a core package: case management, commodity tracking, and data-driven supervision with HC and VMWs. The second objective aimed to dramatically reduce cases and increase testing by MMP by recruiting 95 MMWs to conduct active case detection twice per month, introduce behaviour change activities, and provide services 24 hours. CNM launched monthly Data Review and Action meetings with all partners to track key indicators and solve operational challenges. Success at the midpoint is due to rapid implementation of the two objectives and a collaborative M&E and problem solving process. In February 2019 there were 2,478 total cases in the IP areas, a 28% drop in total cases and a 60% drop in Pf cases compared to this time the prior year. 15,102 total tests were conducted in February 2019 in the 10 IP ODs (average 16% positivity rate), compared to 6,512 tests (average 53% positivity rate) in February 2018. Launching an IP required strong coordination between partners and within multiple units at CNM. Through this coordination and data-driven decision-making, impact on case burden has been demonstrated. CNM continues to implement and track the IP to determine its full effects at endline and potential for expansion.

1699

BUILDING AN EVIDENCE BASE FOR COMMUNITY ENGAGEMENT DURING URBAN MALARIA OUTBREAKS: A QUALITATIVE STUDY IN SANTO DOMINGO, DOMINICAN REPUBLIC

Hunter Keys[1], Gregory Noland[2]

[1]*University of Amsterdam, Amsterdam, Netherlands,* [2]*The Carter Center, Atlanta, GA, United States*

Community engagement (CE) is considered vital to disease control and elimination. Haiti and the Dominican Republic (DR), which share the Caribbean island of Hispaniola, aim to eliminate malaria by 2020. In the DR, malaria has shifted from a rural to a mostly urban disease: the capital Santo Domingo accounted for 73% of all cases nationwide in 2018. Concurrent to these outbreaks, a policy of decentralization transferred primary responsibility for malaria control from the national to the local level. Malaria's changing epidemiology, the vulnerability of poor, peri-urban populations, and the need to strengthen local capacity have highlighted the need for effective CE, yet there has been no formal investigation of current CE practices in this setting. Using grounded theory, this qualitative study examined CE processes and outcomes in areas of Santo Domingo recently affected by malaria. Interviews and observational data were collected from July 2017 to March 2019 among national malaria program staff, local field teams, community members, and malaria patients. Findings suggest two predominant themes. First, while decentralization seeks to "empower the periphery" by training and supervising community volunteers, decision-making and knowledge-production are concentrated in sites of political and scientific power, creating missed opportunities for dialogue. Second, understandings of malaria's root cause and its elimination differ between public health and community spheres. Partly to avoid appearing too political, the malaria program focuses on the biological basis of disease: the parasite and its vector. In communities, however, malaria is understood to result from social, economic, and infrastructural inequalities: stagnant water, healthcare obstacles, and ineffective or corrupt institutions. These results reveal a disconnect in understanding between public health agencies and affected communities, and suggest that effective CE will likely require a more ground-up, politically-engaged approach.

1700

DEVELOPING AND PILOTING A SUITE OF DIGITAL SOLUTIONS FOR MALARIA ELIMINATION

Vivek Agrawal[1], Anne Liu[1], Lakshmi Balachandran[1], Pedro Pagalday Olivares[1], Sameen Babur[1], Juan Manuel Acosta[2], Jose Garcia Munoz[2], Karoline Tufte Lien[2], Marta Vila[2], Rodolfo Melia[2], Derek Treatman[3], Pierre Dane[3], Anna Winters[4], Annie Martin[4], Matt Berg[5], Craig Appl[5], Abdisalan Noor[6], Mwalenga Nghipumbwa[6], Arnaud Le Menach[1]

[1]*Clinton Health Access Initiative, Boston, MA, United States,* [2]*University of Oslo, Oslo, Norway,* [3]*Vital Wave, Palo Alto, CA, United States,* [4]*Akros, Lusaka, Zambia,* [5]*Ona, Burlington, VT, United States,* [6]*World Health Organization, Geneva, Switzerland*

The Digital Solutions for Malaria Elimination project commenced in 2017, by identifying gaps in existing malaria surveillance systems across three regions - Greater Mekong Sub-region, Sub-Saharan Africa and Mesoamerica. The identified gaps produced software requirements that could improve information systems geared towards strengthening surveillance systems - including more user-friendly mobile interfaces in areas of low connectivity, integrated dashboards for decision support across levels, support for resource allocation and task management, and targeted visualizations of case and *foci* data on maps. Since then, a suite of tools and tool enhancements have been developed to cater to these requirements. These include enhancements to the DHIS2 Web platform (the de-facto malaria information system in many countries), which has expanded its data model to now accommodate relationships between different records (e.g. cases to *foci*), and mapping functionality to draw and update geographic boundaries and color code cases. A new Case Notification, Case Investigation and Focus Investigation application, powered by the DHIS2 Android Application, has been built to register cases and record field investigation data through an intuitive user interface with offline functionality. A new Focus Investigation and Response Intervention application, powered by a mobile tool named "Reveal", has also been built to conduct focus investigation and record field intervention data, through a map-based interface for effective tracking and analysis. Finally, a set of common goods have been developed to be accessible by any digital tool, including geospatial tools to improve accuracy and access to geographic data, a data dictionary to provide a standard set of definitions for malaria data, and an implementation guide for supporting digital tool operationalization. The various tools are in the process of being field-tested across ten focus countries, in the form of structured pilots with actual users. User feedback from these pilots will be incorporated into future software development. A full-fledged roll-out is expected to commence in late 2019.

1701

MAPPING MALARIA HOTSPOTS IN OUTBREAKS FOR TARGETING INTERVENTIONS IN CAMBODIA IN 2018

Pengby Ngor[1], Siv Sovannaroth[2], Lisa J. White[1], Po Ly[2], Richard J. Maude[1]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*

Cambodia aims to eliminate malaria using a phased approach stratified by province for *P. falciparum* by 2020 and *P. vivax* by 2025. Efforts to achieve these targets are threatened by recent outbreaks with a 42% increase in reported malaria cases from 2017 to 2018, with a 28% reduction in *P. falciparum* and 137% increase in *P. vivax*. The Cambodia National Center for Parasitology, Entomology and Malaria Control (CNM) has adopted an intensification plan in 7 provinces which have around 80% of the cases. There is a need to identify areas within these provinces to target with additional resources to interrupt transmission during the outbreaks whilst minimising costs of implementation. To help with this, detailed maps of malaria hotspots were produced at village level using routine surveillance data from the CNM malaria information system (MIS) for 2018. The MIS in Cambodia includes individual level reports from all health facilities and village malaria workers in the country with data collected via a combination of paper and smartphone based reporting. In order to ensure maximum accuracy and completeness of the maps, locations of villages in the highest incidence provinces were verified, corrected and, where needed, collected by local healthcare workers using a combination of satellite images and field visits. These locations were then incorporated into the MIS and the maps produced using ArcGIS software. This poster will describe the process of collecting the data, producing the maps and give up to date information on how the results are helping CNM to plan the targeted delivery of interventions.

1702

REDUCTION IN MALARIA PREVALENCE IN SENTINEL POPULATIONS FOLLOWING INTRODUCTION OF A PACKAGE OF INTERVENTIONS FOR MALARIA ELIMINATION: RESULTS FROM EASY ACCESS GROUP SURVEYS IN 2017 AND 2018, GRANDE-ANSE (HAITI)

Thomas Druetz[1], Gillian Stresman[2], Vena Joseph[1], Ruth Ashton[1], Matt Worges[1], Lotus van den Hoogen[2], Bernadette Fouche[3], Eric Rogier[3], Michelle A. Chang[3], Jean F. Lemoine[4], Chris Drakeley[2], Thomas P. Eisele[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Programme national de lutte contre la malaria, Port-au-Prince, Haiti*

In 2017, a total of 8,627 confirmed malaria cases were reported in the Grande-Anse Department - about 45% of all confirmed cases in Haiti. While incidence was approximately 1.8 cases per 1,000 at the national level, it was ten times higher in Grande-Anse. Thus, aggressive interventions are necessary to achieve malaria elimination in this Department. In a pilot area of 5 communes, the following interventions were implemented in the latter half of 2018: improved surveillance using case based reporting, mobilization of communities to help eliminate malaria, community case management, and indoor residual spraying (IRS) and mass drug administration (MDA), using single-dose sulfadoxine-pyrimethamine + primaquine, targeted to the 12 highest transmission *foci* only. A pre-post study using easy access groups surveys was used to assess changes in malaria prevalence in the 5 target communes before and after implementation of the intervention package. Surveys were conducted in November-December 2017 and 2018 (pre- and post-intervention, respectively) in the same venues - 25 primary schools selected in 2017 by stratified random sampling, and all 16 functioning health facilities in the area. In each venue type, 2,500 participants were sampled each year. Participants were administered a questionnaire to assess self-reported exposure to IRS and MDA. Participants were tested for a current *Plasmodium falciparum* infection using an HRP2-based rapid diagnostic test and for previous malaria exposure by screening for a panel of antibodies by Luminex. A total of 614 households reported having been sprayed, and 834 individuals reported having received MDA (acceptance rate: 90% and 85%, respectively). Preliminary results suggest a reduction in the overall RDT-based malaria prevalence following the piloted interventions (from 6.8% in 2017 to 1.4% in 2018). Declines were observed both in schools (from 3% to 0.4%) and in health facilities (from 10.5% to 2.5%). Difference-in-differences estimators will be used to assess the effectiveness of the intervention package to reduce malaria transmission, and ultimately to inform the national elimination strategy.

1703

TIME SERIES ANALYSIS OF MALARIA IN BOTSWANA TO FORECAST FUTURE CASES

Refilwe Y. Senyatso[1], Erica P. Berlin[2], Tjantilili Mosweunyane[1], Mooketsi Molefi[3]

[1]*Botswana National Malaria Programme, Gaborone, Botswana,* [2]*Clinton Health Access Initiative, Westport, CT, United States,* [3]*University of Wits, Gaborone, Botswana*

Botswana is one of the countries that are targeted for malaria elimination by year 2020 by the World Health Organization E2020 Initiative and the Southern African Development Community Elimination 8 (SADC E8) Initiative. Botswana reoriented its malaria program towards elimination in 2010 due to a drastic reduction in confirmed malaria cases from 8056 in 2000 to 885 in 2009. The country has been recording incidence of less than 1 per 1000 population since 2011. However, upsurges of cases and incidence during outbreaks in 2014 and 2017 prevented Botswana from reaching earlier elimination targets in 2015 and 2018. This study uses ARIMA models to gain insight into which factors contribute most to driving malaria case trends in Botswana. Routine district level data on malaria cases, rainfall, temperature and Indoor Residual Spraying coverage were collected from January 2010-May 2018. We seek to find the impact these variables have on the number of malaria cases. We also aim to make projections to determine when Botswana will have zero local malaria cases to assess the feasibility of the 2020 elimination target. Preliminary analysis of the data of malaria cases compared with IRS coverage is not conclusive. This indicates that the changes on the number of cases are not affected by one variable. Notwithstanding this, further analysis indicates that malaria cases are declining beyond 2017. The findings of the study will inform strategies for accelerating malaria elimination in Botswana.

1704

ESTIMATING MALARIA PARASITE MOBILITY IN MOZAMBIQUE USING MOBILE PHONE RECORDS

Jessica R. Floyd[1], Pedro Rente Lourenço[2], Nick W. Ruktanonchai[1], Andrew J. Tatem[1], Nuria Oliver[2]

[1]*University of Southampton, Southampton, United Kingdom,* [2]*Vodafone Research, London, United Kingdom*

Despite large reductions in burden, malaria remains one of the primary causes of death in Mozambique. Human-mediated parasite movement combined with spatial and seasonal changes in transmission threatens the success of interventions by reintroducing parasites to areas targeted for elimination. This is a particular concern in southern Africa, where relatively high transmission in Mozambique poses a challenge for the low-transmission countries nearby. Call detail records (CDRs) provide a unique insight into human movements in real time and have previously been used to identify sinks and sources of malaria in other countries. In this study we used pseudonymized and aggregated call detail records (CDRs) from a sample of over 8.5 million subscribers of a mobile phone operator in Mozambique to quantify human movements for four months in 2017. We combined these data with high-resolution malaria prevalence maps to calculate parasite importation and exportation rates and to identify potential net sinks and sources of malaria across districts. We used a community structure algorithm to explore the connectedness of provinces in Mozambique through human mobility, and we calculated parasite mobility between urban and rural areas at a high spatial resolution. We identified seasonal differences in parasite mobility between the months, with parasite exportation and importation rates varying in certain districts from month to month. We found that the districts in southern Mozambique are more connected to each other through human mobility than to districts in northern Mozambique, suggesting that current efforts to eliminate malaria from the southernmost provinces of Mozambique by 2020 could have lasting effects on reducing the risk of cross-border exports into neighbouring countries. We also observed differences in parasite mobility between rural and urban areas of the country. This work will help provide evidence to inform malaria elimination strategies regionally. Controlling malaria at parasite sources would benefit both the populations living in the source areas and the populations that suffer from malaria exported from those areas.

1705

IMPROVING MALARIA ELIMINATION PLANNING BY ACCOUNTING FOR SEASONAL POPULATION DENSITY AND MOBILITY

Nick Warren Ruktanonchai[1], Victor Alegana[2], Elisabeth zu Erbach-Schoenberg[1], Andrew Tatem[1]

[1]*University of Southampton, Southampton, United Kingdom,* [2]*Kenya Medical Research Institute-Wellcome Trust Research Programme, Nairobi, Kenya*

In malaria elimination settings, programmatic activities are often focused on targeting likely high transmission areas and at-risk populations before transmission ramps up. In many cases, however, population, mobility, and risk data used to plan elimination are often either based on the period data collection took place (such as the census period, for movement or population densities), or an annual average (for risk). In reality,

demography and mobility can vary seasonally. This means disease spread and the population densities that determine exposure and intervention effort can differ between high and low transmission seasons. New data, including call detail records (CDRs) from mobile phones, could help quantify how population densities and mobility varies between seasons, and therefore help optimize intervention effort each month. Here, we present an analysis of seasonal mobility and population densities in Namibia, linked with seasonal maps of malaria transmission. We quantify how connectivity of Namibia changes each month, and how high risk locations and exportation rates change when seasonal estimates are used. Within an R Shiny-based interface, we demonstrate how this relates to intervention effort, by optimizing community health worker travel to simulated settlements across malaria-endemic regions of Namibia. We find that optimal travel paths and community health worker catchment areas change significantly when accounting for seasonal mobility and risk, and in some areas, catchment areas varied dramatically from one month to the next during the early high transmission season of Namibia. We also find that transmission dynamics themselves change dramatically when using a seasonal picture of transmission and risk, implying significantly different maps of sources and sinks nationwide compared to a model where the annual average of mobility and risk is used. These results underscore the importance of using data from the appropriate month when dealing with seasonally-variable diseases like malaria, as important factors such as human mobility can vary as well as the intrinsic rates of infectious risk by month.

1706

GENETIC RELATEDNESS OF MALARIA INFECTIONS IN SENEGAL REVEALS DISTINCT TRANSMISSION PATTERNS

Sarah K. Volkman[1], Stephen F. Schaffner[2], Rachel F. Daniels[1], Timothy Farrell[2], Yaye Die Ndiaye[3], Awa B. Deme[3], Aida S. Badiane[4], Fatou Ba Fall[5], Medoune Ndiop[5], Alioune Badara Gueye[5], Ibrahima Diallo[5], Yakou Dieye[6], Caterina Guinovart[7], Bronwyn MacInnis[2], Daniel L. Hartl[8], Doudou Sene[5], Daouda Ndiaye[4], Dyann F. Wirth[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Broad Institute, Cambridge, MA, United States,* [3]*Dantec Teaching and Research Hospital, Dakar, Senegal,* [4]*Cheikh Anta Diop University, Dakar, Senegal,* [5]*Senegal National Malaria Control Program, Dakar, Senegal,* [6]*PATH MACEPA, Seattle, WA, United States,* [7]*PATH MACEPA/ISGlobal Collaboration, Barcelona, Spain,* [8]*Harvard University, Cambridge, MA, United States*

Genetic analysis detected highly related Sénégal *Plasmodium falciparum* infections across time and space in both high burden and pre-elimination settings. We hypothesized that genetic signatures of parasite relatedness reflect transmission patterns and can be useful for targeting intervention and evaluating impact. We genotyped 4,397 malaria clinical samples collected from 2006 to 2018 representing distinct and dynamic transmission settings, including: Richard Toll (< 1/1000), Thiès (from > 100/1000 to < 10/1000), Kédougou (> 450/1000), and additional sentinel sites (n = 730). We analyzed data from 24 independent, high minor-allele-frequency single-nucleotide polymorphisms for relatedness using identity by descent (IBD) and identity by state (IBS). Only samples with a pairwise relatedness (IBD or IBS) of > 0.95 were included. Analysis revealed (1) significant temporal declines in IBS > 0.95, (2) persistent parasite lineages across geographic and temporal space, and (3) parasite relatedness that distinguished local from imported infections. In Thiès (n = 2,190) we detected 60 distinct parasite lineages persisting for more than one transmission season and up to 11 years duration. Epidemiological modeling of genetic patterns revealed transmission declines and rebounds, with significant differences ($p = 0.001$) in the fraction of IBS > 0.95 from 2006 to 2018. In Kédougou (n = 714) we observed increases in IBS > 0.95 and emergence of highly related and clonal parasites. In Richard Toll (n = 649), we found evidence for imported infections, with more than 21% of parasites sharing a genotype with a parasite from Thiès, as well as local infections with 10 parasite lineages persisting over multiple transmission seasons and highly related infections predominantly found within households of nontravelers ($p = 0.01$). Modeling spatiotemporal connectivity and simulations of parasite movement using genetic signatures of relatedness and gene flow are now being used to reveal patterns of malaria transmission to assess intervention strategies, stratification, and implementation.

1707

REDUCTIONS IN MALARIA BURDEN THROUGH THE USE OF A SCALABLE INTERVENTION PACKAGE (SIP) IN ACCORDANCE WITH THE ZAMBIA NATIONAL MALARIA ELIMINATION STRATEGIC PLAN 2017-2021: THE CASE OF MULOBEZI DISTRICT IN WESTERN PROVINCE

Kafula Silumbe[1], Javan Chanda[1], Ketty Ndhlovu[2], Marie-Reine Rutagwera[1], Busiku Hamainza[2], Anthony Yeta[2], Mutinta Mudenda-Chilufya[2], John M Miller[1]

[1]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [2]*National Malaria Elimination Centre, Lusaka, Zambia*

The Zambian Ministry of Health's National Malaria Elimination Strategic Plan (NMESP) 2017-2021 calls for a package of malaria interventions to reduce malaria transmission through the use of proven vector control tools, expanding access to malaria treatment through case management, clearing parasites through selective use of mass drug administration (MDA), and improved surveillance including at both facility and community levels. Mulobezi District in Western Province, Zambia, is one of the few districts that has received the full scalable intervention package (SIP) as prescribed by the NMESP across all 13 health facility catchment areas (HFCA) within the district. Insecticide-treated nets were distributed to all households in January 2018 as part of a national mass distribution and indoor residual spraying campaigns have been conducted consistently since 2016; in addition, the roll out of surveillance activities in communities that utilize community health workers who follow up cases has been conducted since June 2016. To complement these interventions, four rounds of MDA were carried out in Mulobezi from late 2017 to early 2019. With the deployment of this SIP, a marked reduction in malaria incidence has been noted in the district. Reviewing the data across the months of February from 2017 to 2019, passive confirmed cases have reduced from 65% (Feb 2017) to 43% (Feb 2019). Active confirmed cases have also exhibited a downward trend from 1,707 (Feb 2017) to 152 (Feb 2019). The parasite reservoir is largely characterized by afebrile infections and with previous studies carried out noting that nearly half of the parasite reservoir consistently resides in children age 5 to 14 who are essentially school-age children. More targeting with parasite-clearing-specific activities is essential to address, the parasite reservoir. Therefore, data driven timely deployment of interventions is key in maintaining parasite-free communities. Ensuring adequate coverage of vector control and treatment combined with surveillance will enable optimal malaria burden reduction and help this district achieve its targets.

1708

FEASIBILITY AND ACCEPTABILITY OF A PEER NAVIGATOR-LED MALARIA FOCAL TEST AND TREAT INTERVENTION TARGETING HIGH-RISK POPULATIONS IN SOUTHERN LAO PDR

Emily Dantzer[1], Andrew A. Lover[2], Bouasy Hongvanthong[3], Khampheng Phongluxa[4], Francois Rerolle[1], Sophia Hocini[5], Rattanaxy Phetsouvanh[6], Adam Bennett[1]

[1]*University of California San Francisco, Malaria Elimination Initiative, San Francisco, CA, United States,* [2]*Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA, United States,* [3]*Lao PDR Centre for Malariology, Parasitology, and Entomology (CMPE), Vientiane, Lao People's Democratic Republic,* [4]*National Institute of Public Health/Lao Tropical Public Health Institute, Vientiane, Lao People's Democratic Republic,* [5]*University*

of California Los Angeles, Los Angeles, CA, United States, [6]Department of Communicable Disease Control (DCDC), Ministry of Health, Lao PDR, Vientiane, Lao People's Democratic Republic

Populations at higher risk (HRPs) of malaria in southern Lao PDR share similar occupational, behavioral, and social characteristics that increase their exposure to outdoor-biting mosquitoes. Based on a series of formative assessments in Champasak Province, shared characteristics include routine forest- and field-based work, sleeping outdoors, and frequent travel to local worksites. To engage these often hard-to-reach populations and improve their access to care, we implemented a focal test and treat (FTAT) intervention using peer navigators to target and test HRPs in forests and rice fields. The FTAT intervention comprised a demographic and malaria risk factor survey, testing using standard and ultra-sensitive RDTs, dried blood spot collection, treatment of positive cases, malaria IEC/BCC, and GPS tracking of HRP movement patterns. Peer navigators conducted FTAT continuously from March-November 2018 as part of a larger randomized controlled trial assessing the effectiveness of different targeted test and treat strategies to reduce *Plasmodium falciparum* transmission among village residents and HRPs in 14 health center catchment areas in Champasak. A mixed-method design was used to assess the capacity, feasibility, and acceptability of peer navigators to implement FTAT over time. Data collection methods included key informant interviews (KIIs) and focus group discussions (FGDs) with a diverse set of stakeholders including HRPs, peer navigators, formal health staff, and national and local malaria officials, as well as direct observations of peer navigators conducting all related FTAT tasks. A total of 4 FGDs, 38 KIIs, and 20 peer navigator observations were conducted over the course of the study, and both qualitative and quantitative findings generally support that FTAT is feasible, acceptable, and capable of being implemented by peer navigators. As more countries progress towards elimination, identifying evidence-based, community-led strategies to target HRPs and improve their access to malaria services is a priority, and if found to be effective, feasible, and acceptable, FTAT represents one such approach to bridging this gap.

1709

A SURVEY OF COMMUNITY HEALTH WORKERS CONDUCTING MALARIA COMMUNITY SURVEILLANCE IN ZAMBIA

Travis Porter[1], Elizabeth Chiyende[2], Todd Jennings[2], Marie-Reine Rutagwera[2], Christopher Lungu[2], Michael Hainsworth[3], Busiku Hamainza[4], Thomas P. Eisele[1], John M. Miller[2]

[1]*Tulane University, New Orleans, LA, United States, [2]PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia, [3]PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States, [4]National Malaria Elimination Centre, Ministry of Health, Lusaka, Zambia*

As the burden of malaria has continued to decline in the southern regions of Zambia, the National Malaria Elimination Center (NMEC) has begun to further emphasize steps needed for achieving sub-national elimination. The National Malaria Elimination Strategic Plan calls for improved surveillance, community case management, and reactive case investigation to uncover remaining sources of transmission, in addition to vector control and other interventions that have been shown to substantially reduce malaria prevalence. To improve community access to malaria services and the health system's capacity to follow-up individual malaria cases, the NMEC began introduction of malaria focused community-based volunteer health workers (CHWs) in 2012. A community-led, decentralized recruitment approach has been instrumental in successfully expanding this network of CHWs across Southern and into Western and Central provinces, with over 3,400 CHWs introduced as of late 2018. However, little is known about the background, motivations, or support needs of this workforce which could help to improve program performance and sustainability, as well as ensure CHWs are receiving support needed to carry out their duties. We designed a cross-sectional survey to assess the professional experience, workload, perceptions of health system and community support, job satisfaction, and motivation of CHWs. In February and March of 2019, questionnaires were administered over the phone to a random sample of 480 CHWs—stratified by years of experience and province—operating within Southern, Western, and Central provinces. Early results from 351 completed questionnaires show that average scores on overall motivation and job satisfaction were high among program CHWs (4.2 and 4.6 out of 5, respectively), despite 40.7% reporting that CHW duties at least sometimes interfered with income earning or household responsibilities. Further analysis will explore associations between health system and community support and motivation on CHW performance, as well as the composition and predictors of motivation and job satisfaction among CHWs.

1710

WINDBORNE LONG-DISTANCE MIGRATION OF MOSQUITOES AND PATHOGENS: IMPLICATIONS FOR MALARIA ELIMINATION

Tovi Lehmann[1], Alpha Yaro[2], Zana Lamissa[2], Samake Djibril[2], Moussa Diallo[2], Ousman Yossi[2], Diana L. Huestis[1], Yvonne M. Linton[3], Reed Mitchell[4], Ben Krajacich[1], Roy Faiman[1], Laura Veru[1], Jason W. Chapman[5], Don R. Reynolds[6], David Weetman[7], Martin J. Donnelly[7], Adama Dao[2]

[1]*National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center (MRTC)/Faculty of Medicine, Pharmacy and Odonto-stomatology, Bamako, Mali, [3]Walter Reed Biosystematics Unit, Smithsonian Institution Museum Support Center, Suitland, MD, United States, [4]Smithsonian Institution - National Museum of Natural History, Suitland, MD, United States, [5]Centre for Ecology and Conservation, and Environment and Sustainability Institute, University of Exeter, Cornwal, United Kingdom, [6]Natural Resources Institute, University of Greenwich, Kent, United Kingdom, [7]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Mosquitoes are potent spreaders of some of the most impactful human diseases as is exemplified by malaria and dengue. Over the last century the most effective tools for combatting these diseases have targeted the vector. Long distance migration has been dismissed in mosquitoes since evidence for it has remained anecdotal and infrequent. To assess the potential for long distance mosquito migration, we sampled insects flying between 40 and 290 m above ground level in four Malian villages over three years using sticky nets mounted on tethered helium-filled balloons. Approximately 3,000 mosquitoes were collected amongst half a million other insects, and control nets confirmed that the insects were not captured near the ground. Over forty mosquito species, including members of the genus *Anopheles, Aedes,* and *Culex* were intercepted. Ten species, including primary and secondary malaria vectors were identified among >200 anophelines. Importantly, females accounted for >80% of all mosquitoes., and of these, 90% had taken a blood meal before their migration. This implies that pathogens will be transported by migrating females. The likelihood of capturing Anopheles increased with altitude and during the wet seasons. Simulated trajectories of mosquito flights indicated mean nightly travel of up to 300 km for 9-hour flight durations. Annually, the estimated number of mosquitoes at altitude crossing a 100 km line perpendicular to the winds were 6x10^6, and 4.4x10^7, for the vector species *An. coluzzii* and *An. squamosus*, respectively. These results provide compelling evidence that millions of previously blood-fed, malaria vectors frequently migrate over hundreds of kilometers across Mali, and thus almost certainly spread malaria over such distances. Malaria elimination success may, therefore, depend on whether sources of migrant vectors can be identified and controlled.

1711

EVALUATION OF COMMUNITY CASE MANAGEMENT AND REACTIVE CASE DETECTION (COMPONENT D) ON *PLASMODIUM FALCIPARUM* PARASITE PREVALENCE IN WESTERN PROVINCE, ZAMBIA

Travis Porter[1], Maya Fraser[2], Kafula Silumbe[3], Busiku Hamainza[4], Hawela Moonga[4], Joshua O. Yukich[1], Adam Bennett[5], Caterina Guinovart[6], Kammerle Schneider[2], Michael Hainsworth[2], Laurence Slutsker[2], John M. Miller[3], **Thomas P. Eisele**[1]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States,* [3]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [4]*National Malaria Elimination Centre, Ministry of Health, Lusaka, Zambia,* [5]*Global Health Sciences, University of California San Francisco, San Francisco, CA, United States,* [6]*Barcelona Institute for Global Health, Barcelona, Spain*

Zambia's current National Malaria Strategic Elimination Plan (NMESP) calls for high coverage with a comprehensive package of interventions to achieve malaria elimination. In addition to vector control and improved surveillance, the NMESP calls for improved access to malaria diagnosis and treatment through community case management (CCM). The CCM strategy enlists trained volunteer community health workers (CHWs) to provide malaria diagnosis and case management in their communities, and to report confirmed cases into the surveillance system. When feasible, CHWs also perform household testing and treatment for malaria infections around index cases within their community (aka, reactive case detection). Following successful scale-up of CCM in Southern Province by the end of 2014, the Zambia National Malaria Elimination Centre began a non-randomized district-level rollout of CCM in Western Province, starting with 7 districts in 2016, followed by 4 additional districts in 2017, 2 in 2018, with the remaining 3 districts slotted to receive CCM in the latter half of 2019. To evaluate the effectiveness of CCM at reducing *Plasmodium falciparum* parasite prevalence (*Pf*PR) in children <10 years old, 3 cross-sectional household surveys (2-stage cluster sampling design, proportional to cluster size) were conducted in Western Province during the peak malaria transmission seasons (April-May) in 2017-2019. Each survey was stratified by district to capture both those with and those yet to receive CCM. Preliminary results from the 2017 and 2018 surveys show *Pf*PR to have declined from 51.3% to 33.7% in districts which received CCM, while *Pf*PR declined from 40.6% to 24.9% over this period in districts absent CCM; 90.2% of households reported at least one form of vector control. A difference-in-difference estimator from random effects logistic regression showed the decline in *Pf*PR to be greater, although not statistically significantly, in the CCM districts as compared to non-CCM districts. Full results will be presented on the effect of CCM on *Pf*PR and confirmed malaria case incidence through mid-2019, with the inclusion of data from the ongoing 2019 survey.

1712

A COORDINATED EFFORT: THE INTEGRATION OF MALARIA SURVEILLANCE FOR ELIMINATION INTO THE NATIONAL ELECTRONIC COMMUNICABLE DISEASE REPORTING SYSTEM IN VIETNAM

Thanh Duong Tran[1], Van Hoang Ho[2], Thanh Dong Le[3], Quang Thieu Nguyen[1], Quy Anh Nguyen[1], Huu Toan Trinh[2], Thi Yen Nguyen[3], Thi Thanh Thuy Cao[4], Jillian Dunning[4], Charlene Chinda Barina[4], Ha Le Phan[4], Quang Tan Dang[5]

[1]*National Institute of Malariology, Parasitology, and Entomology (NIMPE), Hanoi, Vietnam,* [2]*Institute of Malariology, Parasitology, and Entomology (IMPE), Quy Nhon, Vietnam,* [3]*Institute of Malariology, Parasitology, and Entomology (IMPE), Ho Chi Minh City, Vietnam,* [4]*Clinton Health Access Initiative, Boston, MA, United States,* [5]*General Department of Preventive Medicine, Hanoi, Vietnam*

Vietnam is committed to achieving malaria elimination by 2030 and has experienced a 70% reduction in the number of confirmed malaria cases since 2015, reporting a total of 4,813 in 2018. Elimination requires enhanced efforts toward timely and complete case-based surveillance and rapid response. The largest reporting system is the Ministry of Health's electronic communicable disease system (eCDS), a national platform used for 43 notifiable diseases. Several reportable communicable diseases have standalone systems that do not integrate with the larger reportable disease requirements, though sustainability requires close integration with systems that will continue to be maintained over time. The Malaria Management System (MMS), an electronic case-based reporting system capturing information on malaria diagnostics, case management, and epidemiology, is the first effort to integrate any reportable disease management system with the eCDS. Under the combined leadership of the General Department of Preventive Medicine (GDPM), the National Institutes for Malariology, Parasitology, and Entomology (NIMPE), Institutes of Malariology, Parasitology and Entomology (IMPE) in Quy Nhon and Ho Chi Minh City, and Viettel, Vietnam's largest mobile network operator, a surveillance roadmap for system integration was developed, costed, and approved by all. This was followed by a process to operationalize national surveillance guidelines, coordinate a user-centric approach to developing the electronic system based on user needs at multiple levels, and provide a comprehensive, scalable implementation plan with multiple international donors' support. The MMS pilot trained 80 staff in three provinces that represent 60% of Vietnam's malaria case burden, and results showed 97% of all reported cases(n=147) were classified, identifying local cases at the commune level. This integrated systems approach involved innovative coordination efforts that provide critical insights for other countries' efforts towards malaria elimination, and provides an example of surveillance management in elimination settings that is efficient and sustainable.

1713

OUTER MEMBRANE PROTEIN COMPLEX AS A CARRIER FOR MALARIA TRANSMISSION BLOCKING ANTIGEN, PFS230

Puthupparampil V. Scaria[1], Christopher G. Rowe[1], Beth B. Chen[1], Olga V. Muratova[1], Elizabeth R. Fischer[2], Emma K. Barnafo[1], Charles F. Anderson[1], Irfan U. Zaidi[1], Lynn E. Lambert[1], Bob J. Lucas[3], Debbie D. Nahas[3], David L. Narum[1], Patrick E. Duffy[1]

[1]*Laboratory of Malaria Immunology and Vaccinology/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States,* [2]*National Institutes of Health, National Institute of Allergy and Infectious Diseases/Hamilton, MT, United States,* [3]*Merck, Kenlinworth, NJ, United States*

Malaria Transmission Blocking Vaccines (TBV) that target mosquito-stage development of the *Plasmodium* parasite by passive immunization of mosquitos during the blood meal from a vaccinated human, can play a significant role in malaria eradication. Pfs230 is a gametocyte and gamete surface antigen currently being evaluated as a TBV candidate. Chemical conjugation of subunit antigens to carrier proteins has been shown to be a good strategy to improve the immunogenicity of poorly immunogenic antigens. A 22kDa N-terminus domain of Pfs230 conjugated to a carrier protein EPA enhances its immunogenicity and this conjugate is currently under evaluation in clinical trials. Here, we assessed Outer Membrane Protein Complex (OMPC), a membrane vesicle derived from *Neisseria meningitidis*, as a carrier for Pfs230. We have synthesized Pfs230-OMPC conjugates with different levels of antigen load and examined their immunogenicity in mice. Chemical conjugation of Pfs230 to OMPC enhanced immunogenicity and functional activity of Pfs230 antigen, and OMPC conjugates achieved 2-20-fold higher antibody titer than Pfs230-EPA/ AdjuPhos® at different doses. OMPC conjugates were highly immunogenic even at low dose in mouse immunogenicity studies, suggesting a dose sparing effect. EPA conjugates generated an IgG subclass profile biased towards Th2 response in mice whereas OMPC

conjugates gave a strong Th1 biased immune response with high levels of IgG2, which can benefit Pfs230 functional activity that depends on compliment activation. OMPC appears to be a promising carrier for Pfs230 vaccines.

1714

GENERATION OF GENETICALLY MODIFIED MALARIA *PLASMODIUM FALCIPARUM* PARASITES EXPRESSING *PLASMODIUM VIVAX* CIRCUMSPOROZOITE PROTEIN FOR MALARIA VACCINE DEVELOPMENT

Yukiko Miyazaki[1], Catherin MarinMogollon[1], Takashi Imai[1], Fiona J. A. van Pul[1], Shinya Miyazaki[1], Jai Ramesar[1], Hans Kroeze[1], Séverine Chevalley-Maurel[1], Ahmed M. Salman[2], Arturo ReyesSandoval[2], António M. Mendes[3], Miguel Prudêncio[3], Blandine FrankeFayard[1], Chris J. Janse[1], Shahid M. Khan[1]

[1]*Leiden Malaria Research Group, Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands,* [2]*The Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [3]*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal*

Chimeric rodent malaria parasites where the gene encoding circumsporozoite protein (CSP) has been replaced with different *csp* genes from the human malaria parasites, *Plasmodium falciparum* or *P. vivax*, are being actively used as pre-clinical tools to evaluate CSP vaccines *in vivo* in mice. In addition, chimeric rodent parasites expressing *P. falciparum* CSP have been evaluated as a whole sporozoite (Wsp) vaccine in preclinical studies. We have shown that these chimeric rodent parasites produce sporozoites in *Anopheles stephensi* mosquitoes, which are capable of infecting rodent and human hepatocytes. The availability of comparable chimeric *P. falciparum* parasites expressing *P. vivax* CSP would not only open up possibilities to test *P. vivax* CSP vaccines in small scale clinical trials using controlled human malaria infections but also to examine immune responses to heterologous antigens expressed by genetically attenuated WSp vaccines. We will describe the creation using CRISPR/Cas9 methodologies of several different chimeric *P. falciparum* parasites where either the *csp* gene has been replaced by one of the two major *P. vivax csp* alleles, VK210 and VK247, or these *P. vivax* alleles have been introduced into the genome as additional copy genes. We will present data on sporozoite production in *An. stephensi* mosquitoes, CSP expression and infectivity and immunogenicity of these chimeric *P. falciparum* sporozoites expressing *P. vivax* CSP.

1715

PFS230D1M-EPA/AS01 TRANSMISSION BLOCKING VACCINE AGAINST *PLASMODIUM FALCIPARUM* IN MALIAN ADULTS: ASSESSMENT OF DURABILITY AFTER 1 YEAR

Issaka Sagara[1], Sara A. Healy[2], Mamady Kone[1], Mahamadoun H. Assadou[1], Abdoulaye Katile[1], Bruce Swihart[3], Jennifer Kwan[4], Mahamadou S. Sissoko[1], Merepen A. Guindo[1], M'Bouye Doucoure[1], Daman Sylla[1], Adama Sacko[1], Danielle Morelle[5], Marc Lievens[5], Charles Anderson[2], Kelly M. Rausch[2], David L. Narum[2], Puthupparampil Scaria[2], Nicholas J. MacDonald[2], Daming Zhu[2], Olga Muratova[2], Mamadou Coulibaly[1], Agnes Mwakingwe-Omari[2], Jen C.C. Hume[2], Amagana Dolo[1], Sekou F. Traore[1], Ogobara K. Doumbo[1], Patrick E. Duffy[2]

[1]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [4]*Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [5]*GlaxoSmithKline, Inc., Wavre, Belgium*

Transmission blocking vaccines prevent mosquito infections but require durable functional antibody responses for malaria elimination. A phase 1 double-blind, 1-1 randomized, comparator-controlled trial (NCT02942277) in Mali assessed safety, tolerability, and functional immunogenicity of Pfs230D1M conjugated to ExoProtein A (EPA) and formulated with GSK's AS01 adjuvant vs. comparator ENGERIX-B. In March 2017, 236 healthy 18-50 yo volunteers from Bancoumana and Doneguebougou villages started doses on Days 0, 28, 168, and booster on Day 476. Volunteers received three full doses (40μg Pfs230D1M, n=56) or two full plus a third fractional dose (8μg Pfs230D1M, n=61), followed by full dose booster (n=82). Comparators received 3 ENGERIX-B doses then Menactra® on Day 476. Most AEs were mild injection site pain, headache and pyrexia (38-38.4°C). A single serious AE not related to vaccine occurred in ENGERIX-B group. Local reactogenicity was higher in Pfs230 arms (full dose n=111, fractional dose n=90) compared to comparator ENGERIX-B (n=42), $p<0.0001$, although mostly grade 1 (mild). After booster dose, 50/82 (61%) participants experienced local reactogenicity vs. 13/81(16%) comparators ($p = 0.0001$). Frequency of solicited systemic reactions was not significantly more frequent in the full (n=10) vs. fractional dose arm (n=4, $p= 0.1406$). A fraction (12.7%) of subjects had pre-existing antibodies to Pfs230D1M. ELISA titers post-dose 3 were significantly higher after the full (geometric mean= 1471.7 EU) vs. fractional dose (geometric mean=560.5 EU) , $p<0.0001$, then increased after the booster (4^{th}) but did not significantly differ between full and fractional arms. Standard membrane feeding assays (SMFA) 2 weeks post dose 3 showed significant functional activity ($p<0.0001$) in TBV arms (Median TRA 95.8; CI: 92.7, 97.4) versus comparator (53.6; 46.9, 62.3), while TRA after full dose Pfs230D1M (96.9; 94.1, 98.8) vs. fractional dose Pfs230D1M (93.6; 82.6, 97.3) did not differ ($p=0.2$). Detailed data on safety, immunogenicity, and functional activity by SMFA as well as by direct skin mosquito feeding (DSF) assays will be presented.

1716

SCREENING FOR CANDIDATE DOMAINS WITHIN PFS230 THAT ELICIT TRANSMISSION-BLOCKING ANTIBODY RESPONSE

Mayumi Tachibana[1], Kazutoyo Miura[2], Eizo Takahima[3], Masayuki Morita[3], Hikaru Nagaoka[3], Luwen Zhou[2], Carole A. Long[2], C. Richter King[4], Motomi Torii[5], Takafumi Tsuboi[3], Tomoko Ishino[5]

[1]*Ehime University, Toon, Japan,* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*Ehime university, Matsuyama, Japan,* [4]*PATH's Malaria Vaccine Initiative, Washington, DC, United States,* [5]*Ehime university, Toon, Japan*

A malaria transmission-blocking vaccine is considered a vital tool for malarial eradication. Pfs230 expressed on the surface of gametes is one of the promising transmission-blocking vaccine antigens, as antibodies against Pfs230 have been shown to inhibit parasite development in mosquito midguts by standard membrane-feeding assay (SMFA). Since Pfs230 is a large protein consisting of 14 cystein motif (CM) domains which have hampered its production as an intact protein, most studies focused on the region including the first CM domain at the N-terminal. In this study, we aimed to screen all possible domains in Pfs230 that efficiently elicit transmission-blocking antibody response. Recombinant proteins containing single-CM-domain, 2-CM-domain, or 4-CM-domain were produced by the wheat germ cell-free system to obtain specific mouse antibodies. Western blotting and indirect immunofluorescence assay demonstrated that about half of the mouse antisera against Pfs230 fragments specifically recognized parasite Pfs230. Their transmission-blocking activity was evaluated by SMFA using *P. falciparum* NF54 strain. Of the antibodies, only those against protein fragments containing the CM domain 1 showed strong transmission-blocking activity. The results strongly support the concept that future Pfs230-based vaccine development should focus on the Pfs230 CM domain 1.

1717

COMPARATIVE ANALYSIS OF THE PARASITE NEUTRALIZING ACTIVITY OF ANTIBODIES RAISED AGAINST REGION II AND REGION III-V OF THE *PLASMODIUM FALCIPARUM* ERYTHROCYTE BINDING ANTIGEN-175

Kritika Chaddha[1], Gaurav Anand[1], Syed Yusuf Mian[1], Enna Dogra Gupta[2], Deepak Gaur[1]

[1]*Jawaharlal Nehru University, New Delhi, India,* [2]*Indian Council of Medical Research, New Delhi, India*

Blood-stage malaria vaccine development has focused on targeting critical ligand-receptor interactions that mediate RBC invasion by *Plasmodium* merozoites. In this receptor blocking approach, the goal has been to identify the RBC binding regions of different parasite ligands & analyze the invasion inhibitory activity of Abs raised against these binding regions. In this regard, the receptor binding domain, Region II of EBA-175 was one of the first Ags to attract attention. The binding of EBA-175 to Glycophorin A is mediated by RII. Recently, the non-binding region III-V has been reported to induce potent strain-transcending parasite neutralizing Abs that suggests a structural or functional role that is yet not fully elucidated. In order to validate the vaccine potential of the different regions of EBA-175, we conducted a head to head comparative analysis of the invasion inhibitory activity of Abs raised against RII & RIII-V individually & in combination with Abs against other essential antigens (PfRH5, CyRPA) that constitute a crucial multiprotein complex. The invasion inhibitory activity of the Abs were evaluated by standard FACS based growth inhibition assays (GIA). In head to head comparison of the GIA activity of RII & RIII-V Abs, we found RIII-V Abs to be significantly more potent than RII Abs (RIII-V 80% vs RII 30%) consistent with previous reports. Importantly, only RIII-V Abs exhibited strain transcending invasion inhibition further confirming previous reports. We further evaluated & compared the efficacy of RII & RIII-V Abs in combination with Abs against other essential merozoite Ags (RH5 & CyRPA). In all combinations, RIII-V outperformed RII, with RIII-V based dual combination (RIII-V+CyRPA) & triple combination (RIII-V+CyRPA+RH5) inhibited invasion by 80-90% in a synergistic/additive manner. Our study establishes Region III-V of EBA-175 as a more potent & efficacious vaccine target compared to Region II. Thus, our study endorses a paradigm shift to malaria vaccine research by validating the vaccine potential of a non-binding region of EBA-175 & supports its inclusion in a multi-component malaria vaccine against *P. falciparum*.

1718

CLINICAL SAFETY AND PROTECTIVE EFFICACY AFTER IMMUNIZATION WITH GENETICALLY MODIFIED *PLASMODIUM BERGHEI* SPOROZOITES EXPRESSI*NG P. FALCIPARUM* CIRCUMSPOROZOITE PROTEIN IN A FIRST-IN-HUMAN PHASE1/2A TRIAL

António M. Mendes on behalf of the PbVac consortium

Instituto de Medicina Molecular - Universidade de Lisboa, Lisboa, Portugal

Highly efficacious immunization strategies against malaria are needed that elicit high level and durable protection against infection by Plasmodium parasites. Whole-parasite vaccination approaches employing attenuated Pf sporozoites appear as a promising alternative to subunit vaccine candidates, but are still in relatively early stages of clinical development. We have recently established the pre-clinical proof-of-concept of a new immunization strategy based on the use of rodent P. berghei (Pb) parasites genetically engineered to express antigens of their human-infective counterparts. Here, we describe the first-in-human phase $^{1}/_{2}$a clinical evaluation of PbVac, the first malaria vaccine candidate of this type, consisting of Pb parasites that express the Pf circumsporozoite protein. Vaccine administration by infected mosquito bites was safe and well-tolerated by a total of ^{24} healthy trial participants. The vaccine elicited cross-species cellular immune responses and functional PfCS-dependent antibody responses, which can efficiently inhibit Pf sporozoite invasion of liver cells. Although sterile protection against a Pf challenge was not observed, the vaccine displayed a clear biological effect in all immunized individuals. A significant delay in patency as measured by qPCR and a marked decrease in parasite density at the onset of parasitemia were observed in all vaccinated volunteers, which translates into a > ^{90}% reduction in their liver Pf burden, relative to non-immunized controls. This study supports the proof-of-concept of a new paradigm in malaria vaccination.

1719

LOWER VACCINE DOSE ASSOCIATES WITH ANTI-CSP ANTIBODY DURABILITY AND FUNCTION: PHASE I TRIALS OF R21/MATRIX-M IN EUROPE AND AFRICA

Georgina Bowyer[1], Navin Venkatraman[1], Alfred B. Tiono[2], Duncan Bellamy[1], Daniel Silman[1], Amy Flaxman[1], Mehreen Datoo[1], Shahid M. Khan[3], Jenny M. Reimer[4], Sodiomon B. Sirima[5], Adrian V. Hill[1], **Katie J. Ewer**[1]

[1]*The Jenner Institute, University of Oxford, Oxford, United Kingdom,* [2]*CNFRP, Ouagadougou, Burkina Faso,* [3]*Leiden University Medical Centre, Leiden, Netherlands,* [4]*Novavax, Uppsala, Sweden,* [5]*Groupe de Recherche Action en Santé (GRAS), Ouagadougou, Burkina Faso*

R21 is a virus-like particle (VLP)-based vaccine displaying the immunodominant repeat region of the circumsporozoite protein (CSP) of *Plasmodium falciparum*. We assessed R21/Matrix M in Phase I clinical trials in malaria-naïve volunteers to assess the immunogenicity of different doses of vaccine and determine the effect of a fractional third dose of vaccine on the quality of the immune response. R21 is novel in that CSP epitopes cover the particle surface, in contrast to RTS,S where surface-exposed HBsAg epitopes predominate, and R21 induces a comparable titre of anti-CSP antibodies with a 5-fold lower dose. When decreasing doses of R21 were compared, lower doses elicited more durable antibody responses and induced a qualitatively different phenotype of peripheral memory T follicular helper cells. A fractional third dose regime (50,50,10) was compared with three fractional doses (10,10,10), and durability of antibody response was significantly improved in the group receiving three reduced doses. Lower doses also induced a larger memory B cell population, including switched memory B cells, and increased the proportion of IgG+ cells within the memory B cell pool. Persistence of antibody responses at 3 months after vaccination was associated with the proportion of switched memory B cells, suggesting that dose optimization is important for induction of durable immunity. We also characterized functional activity in an inhibition of sporozoite invasion assay employing transgenic *P. berghei* parasites encoding the *P. falciparum* CSP antigen, demonstrating these antibodies are capable of binding the sporozoite and potently blocking infection *in vitro*. In a subsequent Phase I study in a holo-endemic region of Burkina Faso, we observed significant boosting of naturally-acquired anti-CSP antibody levels and, importantly, substantial boosting of low-dose vaccine-induced antibody responses after the malaria season. Optimal deployment of CSP-based malaria vaccines may require careful dose optimization with the associated benefit of reduced costs using dose-sparing regimes.

1720

PRIOR EXPOSURE TO BLOOD STAGE MALARIA IMPAIRS PROTECTIVE EFFICACY OF A *PLASMODIUM YOELII* 17XNL PRE-ERYTHROCYTIC VACCINE CANDIDATE

Miranda S. Oakley, Pallavi Malla, Winter A. Okoth, Victoria Majam, Sanjai Kumar

Food and Drug Administration, Silver Spring, MD, United States

Immunogenicity and efficacy of pre-erythrocytic malaria vaccine candidates, such as RTS,S and radiation attenuated sporozoites (RAS), has been shown to be severely compromised in clinical trials conducted in endemic regions compared to the United States. In this study, we measured the effect of prior exposure to blood stage malaria on pre-erythrocytic vaccine efficacy using a circumsporozoite surface protein (*Py*CSP) vaccine candidate in the *Plasmodium yoelii* 17XNL murine model of malaria. Immunization with *Py*CSP delivered in Montanide ISA

51 adjuvant induced antibody-dependent sterilizing immunity against sporozoite challenge in C57BL/6 mice. However, prior exposure of *Py*CSP vaccinated mice to blood stage malaria resulted in a significant increase in intrahepatic parasite burden after sporozoite challenge demonstrating that blood stage malaria impaired the protective efficacy of *Py*CSP. This loss of vaccine efficacy caused by prior exposure to blood stage malaria was accompanied by a threefold reduction in IgG1 antibody titers. Results of an ELISpot assay performed 14 weeks after vaccination indicate that prior exposure to blood stage malaria causes a significant contraction of the memory B cell response to *Py*CSP. This model can be used to identify novel immune regulatory pathways induced by blood stage malaria that compromise pre-erythrocytic vaccine efficacy.

1721

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL ANTIGEN PFCDPK5 AS A MALARIA VACCINE

Dipak K. Raj, Brett Sherman, Anup Jnawali, Gerald Cham-Kpu, Jonathan D. Kurtis

Brown University, Providence, RI, United States

Malaria is among the leading causes of mortality in children under five years of age worldwide, with most of these deaths resulting from *Plasmodium falciparum* infection. Despite decades of research, no vaccine candidate has been shown to confer significant protection to children. In an ongoing antigen discovery studies, we pioneered a high-throughput differential whole proteome screening method to identify targets of antibodies that protect children from severe malaria or malaria-specific mortality and identified Schizont Egress Antigen-1 (Raj et al. Science 2014). In a parallel screening experiment, We identified antibodies against several clones of PfCDPK5 protein only in malaria resistant children's sera. The immediate goals of this study are to gain an immunological understanding of anti-PfCDPK-5 antibodies in preventing parasite maturation and egress. The localization study and growth inhibition activity were evaluated as per our published methods. The immunoblot shows PfCDPK5 expressed in ring and schizont stage of parasites. Immunolocalization studies demonstrate that the protein localization on the plasma membrane of ring stage parasite and on the merozoite of the rupturing schizont. The polyclonal antibodies generated by recombinant PfCDPK5 by protein or DNA vaccine shows significant growth inhibition activity in *in vitro* growth inhibition assay. Immunization of full length PbCDPK5 the mice orthologs of human malaria parasite gene (PfCDPK5) shows significant protection from parasitemia and survival against a lethal parasite strain. In the present study, we validate a rationally identified vaccine candidate; PfCDPK-5 using integrated translational approaches that harness high-throughput molecular techniques and *in vitro* and *in vivo* functional assays.

1722

SCREENING FAILURES: HOW TO IMPROVE HEALTH PROMOTION AND COVERAGE OF VACCINATION?

Marta A. Owono[1], Antonio Ngua Sama Roca[2], Esther Eburi[2], Juan C. Momo[2], Vicente Urbano[1], Fortunata Mochomuemue[1], Maria L. Gozo[1], Ali Mtoro[3], Ali Hamad[3], Said Jongo[3], Kamaka Ramadhani[3], Jose Raso[1], Maximilian Mpina[4], Elizabeth Nyakarungu[3], Carlos Cortes[5], Guillermo A. Garcia[6], Matilde Riloha Rivas[7], Bonifacio Manguire[8], Raul Chuquiyauri[9], LW Preston Church[10], Peter Billingsley[10], Claudia Daubenberger[11], Thomas Richie[10], Salim Abdulla[12], Stephen L. Hoffman[10]

[1]*Ministry of Health and Social Welfare, Equatorial Guinea Malaria Vaccine Initiative, Malabo, Equatorial Guinea,* [2]*Equatorial Guinea Malaria Vaccine Initiative, Medical Care Development International, Malabo, Equatorial Guinea,* [3]*Equatorial Guinea Malaria Vaccine Initiative, Ifakara Health Institute, Malabo, Equatorial Guinea,* [4]*Equatorial Guinea Malaria Vaccine Initiative, Ifakara Health Institute, Swiss Tropical and Public Health Institute, Malabo, Equatorial Guinea,* [5]*Medical Care Development International, Malabo, Equatorial Guinea,* [6]*Medical Care Development International, Silver Spring, MD, United States,* [7]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [8]*Marathon EG Production Limited, Malabo, Equatorial Guinea,* [9]*Equatorial Guinea Malaria Vaccine Initiative, Medical Care Development International, Sanaria Inc., Malabo, Equatorial Guinea,* [10]*Sanaria Inc., Rockville, MD, United States,* [11]*Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [12]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania*

Evaluation of the PfSPZ Vaccine has been conducted in Equatorial Guinea (EG) since 2015. Phase I and II trials have been completed. The EG Malaria Vaccine Initiative (EGMVI) team is planning to conduct a Phase III trial next year in Bioko Island, EG. Vaccine coverage is at the cornerstone for a malaria vaccine deployment in mass administration campaigns. This study is an overall assessment of the proportion of potential study volunteers who are excluded from enrollment based on the selection criteria set for the Phase II EGSPZV3 study. We aimed to analyze the causes of 'screening failures' in volunteers in the ongoing trials for PfSPZ Vaccine in Equatorial Guinea with the purpose of identifying potential obstacles for volunteer's enrolment. From a total of 376 participants that were screened for EGSPZV2 and EGSPZV3 studies, there were 142 (37.8%) among them that were screen failures according to the protocol eligibility criteria. The main causes of screen failures in both studies were: problems related to a functional/available phone 32(8.5%), hepatitis B virus infection 28(7.4%), hepatitis C virus infection 6(1.6%), other medical history 27(7.2%), pregnancy 11(2.9%), abnormal ECG 3(0.8%), travel history 7(1.9%), abnormal body mass index 16(4.3%), high risk of tuberculosis 3(0.8%), HIV infection 4(1.1%), and not willing to use depoprovera 5(1.3%). These EGSPZV trials invited subjects who considered themselves as being healthy. To explore the reasons for screening failures is important and allowed us to identify baseline health profile of the targeted study population. Scattered evidence is suggesting that these screening failures, which are costly and can delay the onset of the trial, can be easily prevented by our screening tests but the rate of viral hepatitis infections is a major concern for reviewing current operational challenges for vaccination coverage and health promotion activities.

1723

STRUCTURAL BASIS FOR DEVELOPMENT OF A *PLASMODIUM FALCIPARUM* TRANSMISSION BLOCKING VACCINE TARGETING THE 6-CYSTEINE RICH PFS230 OR THE PFS230-PFS48/45 PROTEIN COMPLEX

Kavita Singh[1], Martin Burkhardt[2], Raul Herrera[2], Apostolos Gittis[1], Sofia Nakuchima[2], Olga Muratova[2], Emily Higbee[2], Karine Reiter[2], Margery Smelkinson[3], Bruce J. Swihart[4], Baoshan Zhang[5], Richard Shimp[2], Vu Nguyen[2], Nicholas J. MacDonald[2], Patrick E. Duffy[2], David Garboczi[1], David L. Narum[2]

[1]*Structural Biology Section, Research Technologies Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Biological Imaging Section, Research Technologies Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [4]*Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [5]*Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

A transmission blocking (TB) malaria vaccine would lessen the burden of human disease. Two vaccine candidates from *Plasmodium falciparum* are derived from the sexual-stage proteins Pfs230 and Pfs48/45, which together form a membrane-bound complex on gametes. Currently, the leading TB vaccine employs the first cysteine rich domain (D1) of Pfs230 and induces TB antibodies in humans. However, little is known about Pfs230(D1) with regards to its structure, subcellular arrangement, functional epitope(s), role of complement or benefit of a combination vaccine targeting both proteins. Here, we present the crystal structure of the recombinant domain 1 of Pfs230 (Pfs230D1M) in complex with a recombinant Fab fragment of a TB monoclonal antibody (mAb) 4F12

which binds Pfs230D1M using mainly hydrophilic interactions. Using mAb 4F12 and a panel of four new mAbs, we characterized their binding to Pfs230, their capacity to block transmission, and further evaluated the role of complement in TB activity. With confocal microscopy we observed that the structural arrangement of Pfs230 on the surface of macrogametes differed from that on microgametes and that some Pfs230 appeared to be uncomplexed with Pfs48/45. Studies using an *ex vivo* feeding assay demonstrated that mixing mAbs against different epitopes of Pfs230D1, or against Pfs230D1 and Pfs48/45 domain 3 (D3 also identified as Pfs48/45 6C) significantly increased TB activity compared to single mAbs alone. These structure and function studies provide insight on the mechanism of action of the Pfs230D1M vaccine, model the functional activity induced by a polyclonal antibody response and support the continued development of TB vaccines targeting Pfs230 and the Pfs230-Pfs48/45 protein complex.

1724

MANUAL DISSECTION OF MOSQUITO SALIVARY GLANDS WITH SHORTENED TRAINING TIMELINES

Alyssa Arnheim, Urvashi Rai, Hajar Hazime, Stephen L. Hoffman, B. Kim Lee Sim, Sumana Chakravarty

Sanaria Inc., Rockville, MD, United States

Plasmodium falciparum (Pf) Sporozoite (SPZ)-based vaccines are the only malaria vaccines to have demonstrated >90% protective efficacy against controlled human malaria infection. All PfSPZ products use the stage of the malaria parasite that is transmitted from mosquitoes to humans. Therefore, in Sanaria's manufacturing process, the mosquito behaves as a bioreactor, from which parasites are then extracted by microdissection of the insects' salivary glands ensuring several thousand-fold purification away from irrelevant mosquito constituents. The extraction of salivary glands by microdissection of mosquitoes involves four distinct steps performed by skilled personnel a) mosquito alignment b) decapitation c) gland extrusion and d) gland collection. In earlier iterations all steps in dissection were performed by individual trained dissectors, whereas in late 2015, altered configurations led to increases in efficiency and throughput of fully manual dissection, adding at least 100 mosquitoes to the average rate of dissection per hour within just 2 months of being instituted, and a 2-3 fold increase in the total number of mosquitoes processed in a single day. In addition, ergonomic set up of the work stations reduced dissector fatigue and allowed longer working hours. Sanaria is currently in the process of scaling up production to meet the needs of upcoming Phase 3 clinical trials which is very feasible with the manual dissection setup. We have implemented a new training program that brings on part-time dissectors to participate in GMP dissection for manufacturing. Over the course of six months, we have developed and implemented this program, and finalized 12 trainees from a pool of 82 applicants. In the past three months, we have trained two cohorts of dissectors and are prepared to bring on a third. Each dissector spends an average of 4 hours and 50 minutes training per day and on average, trains four days a week. Though the dissectors had minimal prior experience with dissection and microscopy, they are on track to be fully trained within three months.

1725

CHALLENGES FACING NATIONAL MALARIA CONTROL PROGRAM VECTOR SURVEILLANCE

Tanya Russell[1], Robert Farlow[2], Tom Burkot[1]

[1]*James Cook University, Cairns, QLD, Australia,* [2]*R Farlow Consulting LLC, Burkeville, TX, United States*

Further gains in controlling malaria will require preserving the effectiveness of our present recommended vector control interventions while integrating new control strategies based on the susceptibility of local vectors to specific interventions. As the number of recommended interventions expands, the need for vector surveillance will grow to enable selection of appropriate interventions based on the ecology and biology of the vectors in specific epidemiological contexts. In order to assess the current status of vector surveillance by National Malaria Control Programs, an online survey gathered information from 35 national malaria control programs or their partner organizations. Few countries collected all the World Health Organization recommended surveillance data, and the underlying weaknesses that compromise national programs to implement fully effective vector surveillance are analyzed. The national programmatic limitations/weaknesses of vector surveillance self-identified by respondents all fell into three broad categories: lack of capacity, weak strategic planning and poor techniques. Overwhelmingly the countries provided responses that related to a lack of capacity within the Ministry of Health, with the main themes identified being limited spatial or temporal vector surveillance data, inadequate manpower and insufficient training opportunities. This has implications for the ability of National Malaria Control Programs to collect and utilize vector surveillance data that will support effective vector control and elimination.

1726

ONE YEAR OF MONITORING INSECTICIDAL DURABILITY OF LONG LASTING INSECTICIDAL NET IN MAII

Moussa Bm Cisse[1], Ibrahim Traore[1], Abdourhamane Dicko[2], Lansana Sangare[1], Yacouba Dansoko[1], Alice Dembele[1], Jean Marie Sanou[1], Jean Bedel Evi[3], Jules Mihigo[4], Aliou Diallo[4], Erin Eckert[5], Ousmane Koita[1]

[1]*Laboratoire de Biologie Moléculaire Appliquée/ Université des Sciences Techniques et des Technologies de Bamako, Bamako, Mali,* [2]*Programme National de Lutte contre le Paludisme, Bamako, Mali,* [3]*US Agency for International Development Global Health Supply Chain Program Procurement and Supply Management, Bamako, Mali,* [4]*President's Malaria Initiative US Agency for International Development, Bamako, Mali,* [5]*President's Malaria Initiative US Agency for International Development, Washington, DC, United States*

The objective of this study was to assess the insecticidal durability of long lasting insecticidal nets (LLIN) (75 denier polyester and 100 denier polyester) 6 and 12 months after a mass bed net distribution campaign in southern Mali in December 2017. We conducted the study in two sites that participated in the campaign. Random samples of 30 campaign nets were collected from the two districts Kenieba (Site 1) and Kita (Site 2) and we collected information about bed net use and washing practices. We used the World Health Organization (WHO) cone bioassay to assess the insecticidal effectiveness of the LLINs. A laboratory colony of *Anopheles gambaie* Yaounde was used to conduct the bio-assay following the recommendation of WHO for phase III testing of LLIN. The bio efficacy assessment is underway. Preliminary results indicate similar bed net usage patterns in the two sites although there was a tendency toward less usage in site 1. The proportion of bed nets used the night before the survey was lower in Site 1 (57%) than in Site 2 (90%). Similarly, the bed nets were used during both the rainy and dry seasons 53% of the time in Site 1 and 73% of the time in Site 2. The proportion of nets washed was similar in Sites 1 (70%) and 2 (83%) although the mean number of washings over the last 6 months (if washed) was 4.5 times in Site 1 in contrast to 2.9 times in Site 2. The mains soap used at both sites were detergent or bleach.

1727

LLIN EVALUATION IN UGANDA PROJECT (LLINEUP) - OWNERSHIP AND USE OF LONG-LASTING INSECTICIDAL NETS WITH, AND WITHOUT, PIPERONYL BUTOXIDE IN UGANDA

Agaba Katureebe[1], Samuel Gonahasa[1], Grant Dorsey[2], Catherine S. Maiteki[3], Mary Kyohere[1], Adoke Yeka[1], Jimmy Opigo[3], Amy Lynd[4], Janet Hemingway[4], Moses R. Kamya[5], Martin J. Donnelly[4], Sarah G. Staedke[6]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*University of California San Francisco, San Francisco, CA, United States,* [3]*Ministry of Health, Kampala, Uganda,* [4]*Liverpool School of Tropical Medicine,*

Liverpool, United Kingdom, [5]School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom

To achieve and maintain universal coverage of long-lasting insecticidal nets (LLINs), the World Health Organization (WHO) recommends that countries distribute nets free-of-charge through mass campaigns repeated every 3 years. However, a growing body of evidence calls the 3-year lifespan of LLINs into question. Together with the Ugandan Ministry of Health, we embedded a cluster-randomized trial to evaluate the impact of LLINs delivered in a mass distribution campaign in 2017-18. A total of 104 clusters (health sub-districts) in Eastern and Western Uganda were included, covering 48 of 121 (40%) districts. Using adaptive randomisation driven by the number of LLINs available, clusters were assigned to receive one of 4 types of LLINs, including 2 brands with PBO: (1) PermaNet 3.0 [n=32] and (2) Olyset Plus [n=20]; and 2 without PBO: (3) PermaNet 2.0 [n=37] and (4) Olyset Net [n=15]. We are conducting cross-sectional community and entomology surveys at baseline, and 6, 12, 18 and 24 months after LLIN distribution. Net survivorship will be assessed in the community surveys and net durability and bio-efficacy will be evaluated in nets withdrawn from households with replacement at 12 and 24 months. The 18-month follow-up surveys will be complete in September 2019. Our baseline survey highlighted the major problem of net attrition, with only 65.0% of households owning at least one LLIN and only 17.9% adequately covered (at least one LLIN per 2 residents) approximately 3 years after the last mass distribution campaign carried out in Uganda in 2013-14. We will present preliminary results from the first three follow-up surveys and discuss LLIN survivorship and attrition, and changes over time. The results of this innovative, large-scale trial embedded within a routine national distribution campaign will make an important contribution to malaria control policy in Uganda, and throughout Africa.

1728

LLIN EVALUATION IN UGANDA PROJECT (LLINEUP) - IMPACT OF LONG-LASTING INSECTICIDAL NETS WITH AND WITHOUT, PIPERONYL BUTOXIDE ON MALARIA INDICATORS IN UGANDA: A CLUSTER-RANDOMIZED TRIAL

Samuel Gonahasa[1], Moses R. Kamya[1], Grant Dorsey[2], Catherine Maiteki - Sebuguzi[3], Agaba Katureebe[1], Mary Kyohere[4], Adoke Yeka[1], Amy Lynd[5], Jimmy Opigo[3], Janet Hemingway[5], Martin Donnelly[5], Sarah G. Staedke[6]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda, [2]University of California San Francisco, San Francisco, CA, United States, [3]National Malaria Control Division - Ministry of Health, Kampala, Uganda, [4]Makerere University - John Hopkins University Research Collaboration, Kampala, Uganda, [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom*

Long-lasting insecticidal nets (LLINs) that combine pyrethroids with piperonyl butoxide (PBO) are a promising new tool to reduce the impact of pyrethroid resistance on malaria control. The World Health Organization has issued a preliminary endorsement of PBO LLINs, but additional epidemiological evidence of the effect of PBO LLINs is urgently needed. Together with the Ugandan Ministry of Health, we embedded a cluster-randomized trial to evaluate the impact of LLINs delivered in a mass distribution campaign in 2017-18. A total of 104 clusters (health sub-districts) in Eastern and Western Uganda were included, covering 48 of 121 (40%) districts. Using adaptive randomisation driven by the number of LLINs available, clusters were assigned to receive one of 4 types of LLINs, including 2 brands with PBO: (1) PermaNet 3.0 [n=32] & (2) Olyset Plus [n=20]; & 2 without PBO: (3) PermaNet 2.0 [n=37] & (4) Olyset Net [n=15]. We are conducting cross-sectional community surveys in 50 randomly selected households per cluster (5200 households per survey) & entomological surveillance for insecticide resistance in up to 10 randomly selected households enrolled in the community surveys per cluster (1040 households per survey), at baseline, & 6, 12, 18 & 24 months after LLIN distribution; the 18-month follow-up surveys will be complete in September 2019. The primary trial outcome is parasite prevalence as measured by microscopy in children aged 2-10 years in the follow-up surveys. The results of this innovative, large-scale trial embedded within a routine national distribution campaign will make an important contribution to malaria control policy in Uganda, & throughout Africa, where pyrethroid resistance in malaria vectors has increased dramatically. We will present the preliminary results from the first three follow-up surveys & discuss the impact of PBO LLINs on malaria control. We will also highlight this study design as a model paradigm for future assessment of malaria control interventions.

1729

THE LONG ROAD TO A MAINSTREAMED NATIONAL ENTOMOLOGICAL SURVEILLANCE INFORMATION SYSTEM IN UGANDA

Catherine Maiteki Sebuguzi[1], Charles Ntege[1], Daniel J. Kyabayinze[1], Damian Rutazaana[1], Paul Mbaka[2], Charles Katureebe[2], Bayo S. Fatunmbi[2], Mike Okia[3], Josephat Shililu[3], Henry D. Mawejje[4], Moses Kamya[4]

[1]*Ministry of Health, Kampala, Uganda, [2]World Health Oganisation, Kampala, Uganda, [3]PMI VectorLink, Kampala, Uganda, [4]Infectious Diseases Research Collaboration, Kampala, Uganda*

The Global Technical Strategy for malaria provides guidance to countries to ensure universal access to malaria prevention (Pillar 1) and transform malaria surveillance into a core intervention (Pillar 3) to accelerate progress towards malaria elimination. For the past decade, the Ministry of Health has embarked on programmatic scale up of vector control interventions mainly; the use of long lasting insecticidal treated nets and Indoor residual spraying aimed at reducing the vector density and preventing man-mosquito bites. The effectiveness of vector control tools is threatened by the development of insecticide resistance, inappropriate use of tools and poor quality of deployment. Furthermore, the scale up of vector control has contributed to reduction in malaria morbidity and mortality with increased likelihood of epidemics. In Uganda, entomological surveillance is conducted by partners, and entomological data is not routinely captured in the Health Management Information System (HMIS) to aide timely decision making. From 2016-2019, Uganda embarked on a systematic multifaceted approach to strengthen the national entomological surveillance information system to monitor the effectiveness of vector control tools, insecticide resistance, and for early epidemic detection. We present the processes of mainstreaming entomological surveillance. First, we conducted a situation analysis of the current entomological surveillance system. Findings guided the implementation phase including: Development of Integrated Vector Management strategy, guidelines, and Insecticide Resistance management plan; Capacity building at various levels; Inclusion of entomological indicators in the HMIS; and Development of an entomological surveillance framework. The framework spells out stakeholders roles and responsibilities, coordination mechanisms, surveillance methods, data management, flow, analysis, and use for decision making. Partners' were mapped to identify and define their activities and geographical scope. The accountability for decision-making and their follow-up will rest with the National Malaria Control Division.

1730

LONG-LASTING INSECTICIDAL NETS INCORPORATING PERMETHRIN AND PIPERONYL BUTOXIDE REDUCE RISK OF *PLASMODIUM* INFECTION IN WESTERN KENYA: A CLUSTER RANDOMIZED CONTROLLED TRIAL

Noboru Minakawa[1], James Kongere[2], George O. Sonye[3], Beatrice Awuor[3], Jinping Hu[1], Hitoshi Kawada[11], Kyoko Futami[1], Rie Isozumi[4], Sammy M. Njenga[5]

[1]*Nagasaki University, Nagasaki, Japan,* [2]*Centre for Research in Tropical Medicine and Community Development, Nairobi, Kenya,* [3]*Ability to Solve by Knowledge Project, Mbita, Kenya,* [4]*Osaka City University, Osaka, Japan,* [5]*Kenya Medical Research Institute, Nairobi, Kenya*

The increase of pyrethroid-resistance in vectors of *Plasmodium falciparum* has become a threat to the vector control program using long-lasting insecticidal nets (LLINs). Olyset®Plus is a LLIN incorporating pyrethroid permethrin and piperonyl butoxide (PBO). PBO is a synergist inhibits the activities of the enzymes that metabolize the pyrethroid. We examined whether Olyset®Plus reduces *P. falciparum* infection in children aged between 0 to 10 years in Gambe East, western Kenya. The main vectors in this area are *Anopheles arabiensis* and *An. funestus* s.s that have developed resistance to pyrethroid insecticides. The study area was divided to 12 sub-areas, and Olyset®Plus was distributed to residents in randomly selected four sub-areas. Olyset®Net (permethrin only LLIN) was also distributed in randomly selected other four sub-areas for the control arm. The PCR based pre-intervention infection rates of *P. falciparum* were 61.0% in the area covered with Olyset®Plus and 59.0% in the Olyset®Net area. After six months, the rates were reduced to 30.6% and 44.8% in the Olyset®Plus area and the Olyset®Net area, respectively, and 34.7% and 46.6% after 12 months, respectively. The differences in the post-intervention rates were statistically significant (permutation test at 5% significant level after adjusting for the confounding factors). Olyset®Plus was more effective for reducing *P. falciparum* infection.

1731

SURVIVAL OF 8 LLINS TYPES 6, 12, 24 AND 36 MONTHS AFTER A MASS DISTRIBUTION CAMPAIGN IN RURAL AND URBAN SETTINGS IN SENEGAL

Mbaye Diouf[1], Roger Clément Tine[1], Demba Anta Dione[2], Olivier Briet[3], Babacar Thiendella Faye[4], Isma Sow[5], Abdoulaye Konate[1], Abdoulaye Kane Dia[1], El Hadji Diouf[1], El Hadji Amadou Niang[1], Lassana Konate[1], Ousmane Faye[1]

[1]*University Cheikh Anta Diop, Dakar, Senegal,* [2]*Health and Development Solution, Dakar, Senegal,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [4]*University Cheikh Anta DIOP, Dakar, Senegal,* [5]*Service de Lutte Anti-Paludisme, Thiès, Senegal*

Long Lasting Insecticidal Nets (LLINs) are one of the core components of global malaria prevention and control. The lifespan of LLINs varies widely depending on the population, AND randomized studies are required to compare net types in households under different field condition. This study evaluated the survival of LLINs in Senegal. 12,608 LLINs were distributed in 5 regions each stratified by rural and urban setting. As part of the longitudinal follow-up, 2222 nets were randomly sampled and monitored from 6 to 36 months. Using random effects for households, Bayesian model were allowed to estimate independent survival by net type and by area (rural/urban) with a coefficient of variation superior to 0 in the 95%CI. The complement of survival, attrition and median survival time of each net type, was determined as those nets that were missing because they were reported destroyed AND thrown, or repurposed. Three net types had a proportion of survival above 80% after 24 months: Interceptor®87.8% (95%CI 80-93.4); conical PermaNet® 2.0 86.9% (95%CI 79.3-92.4) and LifeNet 85.6% (95%CI 75-93). At 36 months, conical PermaNet® 2.0 maintained a good survival rate, 79.5% (95%CI 65.9-88.8). The odds of survival was 2.5 times higher in rural settings than in urban settings (OR 2.5; 95%CI 1.7-3.7). The attrition due to redistributed nets showed that the two conical net types (PermaNet® 2.0 and Interceptor®) were more often retained by households AND their median survival time was well above three years(Tm=3.5 years for PermaNet® 2.0 and Tm=4 years for Interceptor®). Despite this good retention, Interceptor® had a weak physical integrity and its median survival due to wear and tear was below three years (Tm= 2.4 years). Differences in survival among LLINs types maybe driven by brand, shape or environmental setting. It appears that in Senegal, conical nets may survive longer. Conical nets also are retained longer by households due to shape preference and they also benefit from increased effective lifespan.

1732

LEVERAGING THE US PRESIDENT'S MALARIA INITIATIVE AND GLOBAL FUND RESOURCES TO IMPROVE THE OUTCOMES OF THE 2018 MASS CAMPAIGN OF LONG LASTING INSECTICIDE-TREATED NETS TO COMBAT MALARIA

Jocelyn Razafindrakoto [1], Hasina Harinjaka Ramiandrisoa[2], Soza Andriamarovesatra[3], Emery Nkurunziza[3], Laurent T. Kapesa[1], Aline Mukerabirori[4], Fanjanirina Randrianarivony[5], Mauricette Andriamananjara Nambinisoa[2], Cecilia Vitale[6]

[1]*USAID/PMI, Antananarivo, Madagascar,* [2]*Programme National de Lutte Contre le Paludisme, Antananarivo, Madagascar,* [3]*PSI, Antananarivo, Madagascar,* [4]*MSH, Antananarivo, Madagascar,* [5]*Consultant, Paris, France,* [6]*The Global Fund, Geneva, Switzerland*

Madagascar's National Malaria Control Program began instituting mass distribution campaign of long-lasting insecticide-treated nets (LLIN) since 2009, in an effort to combat malaria. Funding for these mass campaigns came primarily from the Global Fund (GF) and the US President's Malaria Initiative (PMI). During the 2009/2010, 2012/2013 and 2015 campaigns, each donor was assigned different implementation zones, and used different approaches and discordant timing based on funding availability, which resulted in coordination and reporting challenges. For the most recent 2018 mass campaign, implementation challenges became more profound, due to the transition from one Global Fund grant to another. Fifteen months prior to the campaign, the in-country Roll Back Malaria committee (RBM) including NMCP, PMI and GF agreed on unified campaign with one implementation plan, M&E plan and a strategy based on task attribution rather than on zonal attribution between donors. The decision was made to implement majority of campaign activities prior to June 30, 2018 with GF funds, while both GF and PMI funded activities between July-December 2018. Under this agreement, GF support to activities covered the pre-campaign census, development and reproduction of campaign register books, training of community agents training and transportation and storage of LLINs. PMI funds were used to support the campaign monitoring, training, training and stipends of data clerks, supervision, stipends for community health agents, and regional support teams. This task arrangement by donor enabled a cost savings, and allowed PMI to distribute an additional 745,000 GF-procured LLINs to cover gaps. In total, 13 million LLINs have been distributed to 5.9 million households by 103,000 community health agents through 7,701 distribution sites. Donor flexibility around resource mobilization and geographic areas of support, along with a unified and transparent mass distribution plan and budget contributed to the success of the distribution of 13 million of LLIN in Madagascar.

1733

INCREASED BITING RATE OF INSECTICIDE-RESISTANT *CULEX* MOSQUITOES AND COMMUNITY ADHERENCE TO IRS FOR MALARIA CONTROL IN URBAN MALABO, BIOKO ISLAND, EQUATORIAL GUINEA

Godwin Fuseini[1], Raul Ncogo Nguema[1], Wonder P. Phiri[1], Olivier Tresor Donfack[1], Carlos Cortes[1], Michael E. von Fricken[2], Jacob I. Meyers[3], Immo Kleinschmidt[4], Guillermo A. Garcia[5], Carl Maas[6], Christopher Schwabe[5], Michel A. Slotman[3]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Department of Global and Community Health, George Mason University,*

Fairfax, VA, United States, [3]Department of Entomology, Texas A&M University, College Station, TX, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom, [5]Medical Care Development International, Silver Spring, MD, United States, [6]Marathon EG Production Limited, Malabo, Equatorial Guinea

Sustaining high levels of indoor residual spraying (IRS) coverage (≥85%) for community protection against malaria remains a challenge for IRS campaigns. We examined biting rates and insecticide resistance in *Culex* species and *Anopheles gambiae* s.l., and their potential effect on community adherence to IRS. The average IRS coverage in urban Malabo between 2015 and 2017 remained at 80%. *Culex* biting rate increased 6.0-fold between 2014 and 2017, reaching 8.08 bites per person per night, whereas that of *An. gambiae* s.l. remained steady at around 0.68. Although *An. gambiae* s.l. was susceptible to carbamates and organophosphates insecticides, *Culex* spp. were phenotypically resistant to all four main classes of WHO recommended IRS insecticides. Similarly, the residual activity of the organophosphate insecticide used since 2017, ACTELLIC 300CS, was 8 mo for *An. gambiae* s.l., but was almost absent against *Culex* for 2 mo post-spray. A survey conducted in 2018 within urban Malabo indicated that 77.0% of respondents related IRS as means of protection against mosquito bites, but only 3.2% knew that only *Anopheles* mosquitoes transmit malaria. Therefore, the increasing biting rates of *culicines* in urban Malabo, and their resistance to all IRS insecticides, is raising concern that a growing number of people may refuse to participate in IRS as result of its perceived failure in controlling mosquitoes. Although this is not yet the case on Bioko Island, communication strategies need refining to sensitize communities about the effectiveness of IRS in controlling malaria vectors in the midst of insecticide resistance in non-malaria vector mosquitoes.

1734

EFFECTS OF POST INDOOR RESIDUAL SPRAYING ON MALARIA ENTOMOLOGICAL PARAMETERS OF MALARIA TRANSMISSION IN MALI

Moussa Keita, Ibrahim Sissoko, Sory Ibrahim Diawara, Drissa Konate, Sékou F. Traore, Seydou Doumbia, Nafomon Sogoba

West African International Center for Excellence in Malaria Research (ICEMR-WA), University of Sciences, Techniques and Technologies of Bamako, Mali), Bamako, Mali

In Mali, insecticide treated long lasting nets (LLINs) distribution and indoor residual spraying (IRS) in selected districts are the key interventions for vector control. Koulikoro has been one of the selected districts for IRS for about nine consecutive years (2008-2016). In 2016, the National Malaria Control Program (NMCP) decided to stop the IRS in Koulikoro district. In this study we are assessing the potential effect of post IRS on entomological parameters of malaria transmission in previous and No-IRS areas in the districts of Koulikoro and Banamba, respectively. Mosquitoes were collected using pyrethrum spray-catch. *An. gambiae s.l.* was morphologically identified. Enzyme-linked immunosorbent assay (ELISA) was used to determine sporozoite infection rates. Monthly entomological inoculation rates (EIR) were estimated by combining the PSC data mosquito sporozoite infection rates. *An. gambiae s.l.* was the only malaria vector collected in both areas. A total of 1787 specimen of *An gambiae s.l.* was collected previous IRS area after IRS stopping against 68 specimens during IRS period. In the No-IRS area 1438 specimen against 1040 were collected after stopping and during IRS period, respectively. The mean density of *An. gambiae s.l.* per room after stopping the IRS was 11.9 times (7.4/0.62) higher than during the IRS. In the No-IRS area and after stopping the IRS, the mean density was only 1.5 times lower (8.7/6.0) than during the IRS. During the IRS campaign in previous IRS area, data collected did not allow us to detect a transmission. During the post IRS in the same area we observed 3.86 infective bites person month. In No-IRS area the mean EIR was 0.17 infective bite person month during IRS against 1.58 after stopping IRS. There was an increase in both areas probably because of the inter-annual variation in transmission. But the magnitude in the increase in the previous IRS area is certainly due to the effect of stopping IRS. IRS reduces malaria transmission where it is implemented and also positively impact on neighborhood areas. Stopping IRS increase the risk of transmission in both areas with higher magnitude in the IRS area.

1735

A GOOD SPRAY: ENTOMOLOGICAL SURVEILLANCE RESULTS FROM A CLUSTER RANDOMIZED TRIAL TO EVALUATE THE IMPACT OF A THIRD GENERATION INDOOR RESIDUAL SPRAY PRODUCT ON MALARIA TRANSMISSION IN MOZAMBIQUE

Joseph Wagman[1], Aklilu Seyoum[2], Stephen Magesa[3], Kenyssony Varela[3], Rodaly Muthoni[3], Christelle Gogue[1], Kenzie Tynuv[1], Carlos Chaccour[4], Francisco Saute[5], Rose Zulliger[6], Abuchahama Saifodine[7], Baltazar Candrinho[8], Jason Richardson[9], Christen Forndel[9], Laurence Slutsker[10], Molly Robertson[1]

[1]*PATH, Washington, DC, United States, [2]Abt Associates, Bethesda, MD, United States, [3]Abt Associates, Maputo, Mozambique, [4]ISGlobal/Centro de Investigação em Saúde de Manhiça, Barcelona, Spain, [5]Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique, [6]President's Malaria Initiative, Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention, Maputo, Mozambique, [7]President's Malaria Initiative, US Agency for International Development, Maputo, Mozambique, [8]Programa Nacional do Controlo da Malaria, Maputo, Mozambique, [9]IVCC, Liverpool, United Kingdom, [10]PATH, Seattle, WA, United States*

The Mopeia District of Zambezia Province in Mozambique has a high malaria burden despite high levels of household coverage with long-lasting insecticidal nets (LLINs). In this context, a two-year, two-armed cluster-randomized trial (CRT) was conducted to evaluate the impact of indoor residual spraying (IRS) with a third-generation IRS product when combined with a 2017 mass LLIN distribution campaign. One study arm received pyrethroid-impregnated LLINs and the other arm received two annual rounds (late 2016 and again in late 2017) of IRS with a microencapsulated formulation of pirimiphos-methyl (Actellic®300CS) in addition to LLINs. After the 2017 mass distribution campaign, LLIN use measured in an active cohort of over 1500 children was consistently greater than 85% for 15 months across both study arms. Based on entomological surveillance, the primary vector species throughout all of Mopeia was *Anopheles funestus* s.s., though *An. gambiae* s.s. and *An. arabiensis* were also collected at much lower densities. Field-collected *An. funestus* populations demonstrated emerging resistance to multiple pyrethroids and to bendiocarb (between 80% – 90% mortality in standard WHO tube tests) but were 100% susceptible to pirimiphos-methyl. During the six months after each IRS campaign, monthly *An. funestus* densities were substantially reduced at sentinel sites in IRS clusters compared to non-IRS clusters: 63% fewer specimens were collected in indoor light traps after the 2016 campaign, and 85% fewer after the 2017 campaign. Similar reductions in the numbers of *An. funestus* landing on human landing collectors were also recorded from houses in IRS clusters, both indoors and outdoors, following each spray campaign. Taken together with concurrent significant reductions in malaria infection and case incidence rates in the IRS clusters during the trial, these vector surveillance results provide compelling evidence of the additional impact of combining a third generation IRS campaign with a universal LLIN coverage campaign in an area of high transmission, moderate pyrethroid resistance, and high LLIN usage rates (>85%).

1736

COULD REPORTING HOUSE-LEVEL INDOOR RESIDUAL SPRAY COVERAGE IN URBAN SETTINGS IN AFRICA BE MISLEADING?

Liberato Motobe[1], Lucas Ondo[1], Jordan M. Smith[1], Jose Antonio Mba Nlang[1], Wonder P. Phiri[1], Carlos Cortes[1], Godwin Fuseini[1], Carlos A. Guerra[2], Guillermo A. Garcia[2]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Medical Care Development International, Silver Spring, MD, United States*

The effectiveness of indoor residual spraying (IRS) in providing community protection against malaria largely depends on achieving high coverage. The World Health Organization prescribes that ≥85% of all structures that are potential resting places for anopheline mosquitoes be sprayed. These include internals walls of structures, eaves, ceilings, doors/windows and furniture. A structure in this context is defined as a sprayable three or four walled space with a roof. Traditional housing in rural settings in Africa may or may not have wall partitions, hence a structure could be defined as an entire house. However, in urban areas, houses generally have multiple internal walls separating habitable spaces, some of which, like kitchens, are not sprayable. Reporting IRS coverage at house level could be misleading in urban settings if not all the structures in the houses are sprayed. We reviewed IRS data on Bioko Island where the average number of structures per house is five. From 2016 and 2018, it we found that between 78.6 and 82.0% of houses were sprayed. However, coverage based on number of structures sprayed was only between 75 and 77%. Our analyses aim to describe the discrepancy between the two methods of quantifying IRS coverage in an urban setting and to assess its implications on the effectiveness of this intervention at the community level.

1737

COMBINING LONG-LASTING INSECTICIDAL NETS (LLINS) WITH THIRD GENERATION-INDOOR RESIDUAL SPRAYING (IRS) PROVIDES SIGNIFICANT ADDED PROTECTION COMPARED TO LLINS ALONE IN CHILDREN UNDER FIVE YEARS OF AGE IN A HIGH-TRANSMISSION AREA OF MOZAMBIQUE

Carlos Chaccour[1], Rose Zulliger[2], Joseph Wagman[3], Aina Casellas[4], Abuchahama Saifodine[5], Baltazar Candrinho[6], Jason Richardson[7], Molly Robertson[3], Francisco Saute[8]

[1]*ISGlobal/Centro de Investigação em Saúde de Manhiça, Barcelona, Spain,* [2]*President's Malaria Initiative, Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention, Maputo, Mozambique,* [3]*PATH, Washington, DC, United States,* [4]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [5]*President's Malaria Initiative, US Agency for International Development, Maputo, Mozambique,* [6]*Programa Nacional do Controlo da Malaria, Maputo, Mozambique,* [7]*Innovative Vector Control Consortium, Liverpool, United Kingdom,* [8]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique*

The most recent World Malaria Reports suggest we are not on track to reach the WHO's 2030 global goals for morbidity and mortality reduction. However, new tools or better coverage with existing ones can help us get back on track. There is growing interest in combining indoor residual spraying (IRS) with long-lasting insecticidal nets (LLINs) as an insecticide resistance management strategy, and a pressing need for additional evidence on the public health impact and cost-effectiveness of this approach. A cluster-randomized trial comparing the impact of LLINs vs LLINs + IRS (Actellic®300 CS) was conducted in Mopeia, a district of Mozambique with high malaria transmission, over two consecutive transmission seasons (November 2016 to October 2018). The district also received a mass distribution of standard pyrethroid-only LLINs in June 2017. The main outcome was malaria infection incidence in a prospective cohort of 1500 children under five years of age. Secondary outcomes included malaria prevalence at peak of transmission season and confirmed malaria cases reported from health facilities with strengthened reporting. The incidence cohort showed a significantly lower malaria infection rates in the IRS+LLIN arm compared with the LLIN-only arm throughout the 2-year follow up - overall incidence rate ratio (IRR) 0.83 (95%CI: 0.79-0.86; p<0.001). A reduction in cases reported from health facilities was also observed - overall case IRR of 0.72 (0.71-0.73; p<0.001). No difference in prevalence between arms was found in the cross-sectional survey conducted at baseline in 2017 prior to the LLIN distribution, (OR 1.08: 0.82-1.43; p=0.585). In the 2018 survey a significantly lower prevalence was observed in the IRS+LLINs arm, OR 0.70 (95%CI: 0.53-0.93; p=0.015); this was most evident in the under-five population, OR 0.54 (95%CI: 0.36-0.8; p=0.002). In the high transmission setting of Mopeia, the combination of LLINs with IRS was associated with significantly lower malaria incidence than LLINs alone. These findings, along with ongoing cost-effectiveness analyses, provide additional evidence for guidance on combining vector control interventions.

1738

COST AND COST-EFFECTIVENESS OF COMBINING LONG-LASTING INSECTICIDAL NETS (LLIN) WITH THIRD-GENERATION INDOOR RESIDUAL SPRAYING (IRS) IN A HIGH-TRANSMISSION AREA OF MOZAMBIQUE

Rose Zulliger[1], Sergi Alonso[2], Carlos Chaccour[3], Baltazar Candrinho[4], Joseph Wagman[5], Abuchahama Saifodine[6], Molly Robertson[5], Francisco Saute[7]

[1]*U.S. President's Malaria Initiative and Malaria Branch, Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention, Maputo, Mozambique,* [2]*ISGlobal/Centro de Investigação em Saúde de Manhiça/Centre for Primary Care and Public Health, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London, United Kingdom,* [3]*ISGlobal/Centro de Investigação em Saúde de Manhiça, Barcelona, Spain,* [4]*Programa Nacional do Controlo da Malaria, Maputo, Mozambique,* [5]*PATH, Washington, DC, United States,* [6]*U.S. President's Malaria Initiative, US Agency for International Development, Maputo, Mozambique,* [7]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique*

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLIN) are cornerstones of malaria prevention, but there are limited data on the cost-effectiveness of IRS in combination with LLINs. This cluster randomized controlled trial determined the costs of IRS with Actellic®300 CS and of malaria in a high burden area of Mozambique with high household coverage with standard LLINs to help calculate the incremental cost effectiveness of IRS. The direct (e.g. medical costs to family) and indirect (e.g. lost productivity) costs and burden of malaria were collected through a community cohort, cross-sectional surveys, health facility (HF) data and questionnaires on health system expenditures from 2016-18. All IRS costs were collected prospectively. Disability-adjusted life years saved will be modeled using the trial effect estimates to calculate incremental cost effectiveness ratios. In cross-sectional surveys (n=805) the median all-age household cost of uncomplicated malaria was US$3.46 (IQR $0.07–22.41) and of severe malaria was $81.08 ($39.34–88.38). Household costs for children (n=1536) were $1.63 (IQR $0.00–7.79) for uncomplicated malaria and $64.90 ($49.76–80.96) for severe. The median health system cost was $3.55 ($3.50–3.60) for uncomplicated malaria and $26.56 (IQR $18.03–44.09) for severe. Cohort LLIN use after a 2017 mass distribution campaign was consistently >85% in both study arms. The 2016 and 2017 IRS campaigns sprayed homes of 76,669 and 70,988 people at a total cost of $563,212 ($7.35/person protected) and $658,259 ($9.27/person protected), respectively. There were 960 fewer cohort infections in the IRS arm, a crude incidence rate ratio of 0.79 (CI_{95} 0.76-0.82; p=0.0007). Individuals from IRS clusters had 19% fewer HF visits and the protective effect was greater (27%) in children. Despite the provision of free malaria services, households incur important malaria expenses. While IRS was associated with substantial costs, it was also associated with significant reductions in malaria burden, underscoring the importance of considering both the effectiveness and costs of vector control interventions.

1739

EFFICACY OF SUMISHIELD®50WG FOR INDOOR RESIDUAL SPRAYING AND SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* S.L. TO CLOTHIANIDIN IN NORTHERN GHANA

Sylvester Coleman[1], Yemane Yihdego[1], Frank Gyamfi[1], Edem K. Obum[1], Lena Kolyada[1], Jon Eric Tongren[2], Kristen George[3], Jennifer Armistead[3], Sixte Zigirumugabe[4], Dominic Dery[4], Samuel Dadzie[5], Maxwell Appawu[5], Daniel Boakye[5], Daniel Szumlas[6], Dereje Dengela[7]

[1]*U.S. President's Malaria Initiative Vectorlink Project, Accra, Ghana,* [2]*U.S. President's Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Accra, Ghana,* [3]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Washington, DC, United States,* [4]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Accra, Ghana,* [5]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana,* [6]*Armed Forces Pest Management Board, Silver Spring, MD, United States,* [7]*U.S. President's Malaria Initiative Vectorlink Project, Abt Associates Inc., Bethesda, MD, United States*

In 2018, the U.S. President's Malaria Initiative (PMI) VectorLink Ghana Project piloted indoor residual spraying (IRS) with SumiShield 50WG® (SS), a newly available insecticide product with clothianidin as an active ingredient, in Mamprugu Moaduri district. Six other districts were sprayed with Actellic 300CS® (AC), for which pirimiphos-methyl is the active ingredient. Clothianidin was introduced as a first step towards operationalization of an insecticide resistance management strategy through insecticide rotation with the aim of delaying potential emergence of pirimiphos methyl resistance after six years of spraying AC in PMI project districts. Using standard World Health Organization (WHO) cone wall bioassays, residual bio-efficacy of SS sprayed in Mamprugu Moaduri was compared with that of AC sprayed in two districts, West Mamprusi and Kumbungu. *Anopheles gambiae* s.l. susceptibility to clothianidin was assessed across 11 sentinel sites, eight that received IRS and three that did not, using Centers for Disease Control and Prevention (CDC) bottle assays. WHO tube assays were also conducted to measure the susceptibility of *An. gambiae* s.l. to pirimiphos methyl and clothianidin. Actellic 300CS® showed a residual life of 6-8 months on mud, cement and wood surfaces, when tested with *An. gambiae* Kisumu, and up to six months was recorded for wild *An. gambiae* s.l. after a 24-hour holding period. SumiShield 50WG® remained effective for eight months and seven months in cone assays with *An. gambiae* Kisumu and wild *An. gambiae* s.l. respectively, with >80% mortality, when mosquitoes were held for up to 3 days post exposure. The residual bio-efficacy of SS was comparable to that of AC. *An. gambiae* s.l. was susceptible (> 98% mortality) to clothianidin at all sites. The general susceptibility of the vector to clothianidin and the 6-8 months residual life of SS indicate that this insecticide is a potential option for rotation in subsequent IRS campaigns in Northern Ghana. Continuous field assessments of the susceptibility of the vector and monitoring of biomarkers for early detection of resistance is recommended.

1740

COVERAGE OF INDOOR RESIDUAL SPRAYING (IRS) AND IMPACT OF IRS IN *ANOPHELES* POPULATIONS IN SITES OF ELEVATED MALARIA TRANSMISSION IN GRANDE ANSE, HAITI

Daniel Impoinvil[1], Rodrigue Anagonou[2], Ffyona Patel[2], Djenam Jacob[2], Amber M. Dismer[1], Jean Baptiste Merilien[3], Karen E. Hamre[1], Kathleen Holmes[1], Willy Lafortune[3], Jean Frantz Lemoine[3], Michelle A. Chang[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Abt Associates Inc., Cambridge, MA, United States,* [3]*Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti*

Indoor Residual Spraying (IRS) for malaria control has not been used in Haiti for about 3 decades, despite the WHO recommendation that IRS be a core malaria intervention. The Malaria Zero Consortium included IRS in its pilot of a targeted interventions package to accelerate malaria elimination in Haiti. IRS with a capsule suspension formulation of pirimiphos-methyl was applied in 11 zones of elevated malaria transmission in the communes of Dame Marie, Anse d'Hainault and Les Irois. One round of IRS was done during the rainy season from 15 October to 2 November 2018. Entomological monitoring was done in sentinel sites in the 3 IRS areas and 3 non-IRS areas. Mosquitoes were collected using human landing catches, UV light traps and pyrethrum spray catches, morphologically identified to species, and assessed for parity. While, in total, 11,557 households with 35,469 people were enumerated in the 11 zones, spray teams identified 9,497 eligible for IRS; the operational coverage rate was 92% (8,709 of 9,497), while the total effective coverage rate was 75% (8,709 of 11,557). Reasons that eligible houses were not sprayed included refusals (34%), unmovable sick individuals (18%), closed houses (8%), head of household unavailable to consent (16%), and other reasons. Some specific reasons for refusals were concerns about insecticide smell or side effects, refusal to move furniture, or being busy. Using the WHO wall bioassay to assess insecticide residual activity, susceptible field-collected *Anopheles albimanus* exposed to sprayed walls monthly from October 2018 to January 2019 had a 100% mortality after 24 hours. Approximately 99.3% (2,230 of 2,246) of *Anopheles* collected across all six sentinel sites were identified as *A. albimanus*, 0.5% as *A. pseudopunctipennis* (n = 11) and 0.2% as *A. vestitipennis* (n = 5). *Anopheles* abundance fell by 85% following IRS (pre-spray: n = 1049; post-spray: n = 154) and by 7% in non-IRS areas (pre: n = 94; post: n = 87). Parity rates of *A. albimanus* decreased from 79% (pre) to 49% (post) in the IRS areas, but increased in non-IRS areas from 73% (pre) to 77% (post). IRS reduced *Anopheles* populations; IRS may be an important intervention to reduce malaria rates in Haiti.

1741

ANOPHELES DYNAMICS: BITING ACTIVITIES IN JABI-THENAN DISTRICT IN NORTHWESTERN ETHIOPIA

Alemnesh H. Bedasso[1], Habte T. Maasho[2], Sisay D. Lemma[1], Eliningaya J. Kweka[3]

[1]*Ethiopia Public Health Institute, Addis Ababa, Ethiopia,* [2]*Addis Ababa University, Addis Ababa, Ethiopia,* [3]*Division of Livestock and Human Disease Vector Control, Tropical Pesticides Research Institute, Tanzania, United Republic of Tanzania*

Distribution, abundance, feeding behavior and human-biting are among the medical entomological parameters evaluated when determining the vector capacity of mosquito species. Despite a tremendous expansion in the financing and coverage of malaria control tools provision that has led to a wide-scale reduction in malaria incidence and mortality, the disease continuous to be a global health threat. Generating evidence on the mosquito species composition, abundance, resting behavior, biting pattern is important in entomological studies monitoring disease vectors, especially in malaria endemic regions and helps to design effective malaria vector control strategy. From September 2016 to August 2017, adult mosquitoes were collected twice monthly using CDC light traps, pyrethroid spray catches (PSC), pit fall shelters (PFS) and human landing catches (HLC) in each village. During the 12-month sampling period, a total of nine thousand two hundred fifty-eight (9,258) *Anopheles* and *Culex* mosquitoes were using CDC, HLC, PSC and PFS methods. Overwhelming majority, 7,519(81.2%) was composed of *Culex spp* followed by *Anopheles gambiae* s.l. 1,183(12.8%), *An. pharoensis* 281(3%), *An. funestus* 151(1.6%) and *An. coustani* 124(1.3%). The mean density comparison of primary vector *An. gambiae* s.l. showed no significance difference between indoor and outdoor resting sites for both CDC and HLC collections. The peak biting time for major malaria vector *An. gambiae* s.l. was found to be between 22:00 to 00:00 hours with 54.8% of total collections were caught in the time interval. Malaria vectors from Jabi-Tenhan district showed both endophagic/exophagic and endophilic/exophilic behaviors. Thus, in addition to the indoor based interventions (LLINs and IRS) in place, additional vector control methods should be designed to address the problem of outdoor biting mosquitoes.

1742

MORE RESOURCES REQUIRED FOR DEFINITIVE DIAGNOSIS OF CHOLERA IN UGANDA

Peterson stephen Kyebambe[1], Godfrey Bwire[2], Timothy Kiggwe[1], Stephen Alele[1], Francis Ongole[3], Douglas Kizito Makanga[1], Pross Ingabire[1], Julius Kabali Kuule[1], Robert Isabirye[1]

[1]*Naguru Referral Hospital, Kampala, Uganda,* [2]*Ministry of Health, Kampala, Uganda,* [3]*Uganda National Health Laboratory Services, Kampala, Uganda*

According to the World Health Organization, Cholera is entwined with consumption of contaminated water. In areas of the world where universal access to safe water has been achieved the disease has been eliminated but in Africa and parts of Asia as well as the Caribbean plus Latin America, it remains endemic. From early January to late February 2019 there was an outbreak of Cholera in Kampala,Uganda. A total of 51patients,26 male and 25 female, were admitted for treatment at the Naguru Hospital isolation unit.The first cases to be identified were residents of Rubaga,one of the five divisions of the city.Later on the disease spread to other areas. In an effort to get a definitive laboratory diagnosis for each case, we whenever possible, subjected their stool samples to the tests of *Vibrio cholera* Rapid Diagnostic Test (RDT), culture and Polymerase Chain Reaction(PCR). The tests were carried out at the national reference laboratory,UNHLS, in Butabika , Kampala.This undertaking met several challenges, mainly inadequate supply of test kits and reagents. Thirty two RDTs were done of which 9 were positive,23 negative and 19 were not tested. Thirty one had culture of which only 3 showed growth of V. Cholera. Of the 23 who were RDT and culture negative only 9 were subjected to PCR and of these 8 were positive indicating they were falsely identified as negative by the other two tests. There was one case of positive RDT but negative culture and PCR. The results indicate the need to scale up availability of cholera test facilities to enable definitive diagnosis, treatment and follow up of cases thus preventing mortality and propagation of the disease in the community as some sufferers of the illness end up becoming carriers of the bacterium and can contaminate more water sources.

1743

TO COMPARE ENVIRONMENTAL SAMPLE COLLECTION POINTS FOR ACUTE FLACCID PARALYSIS (AFP) SURVEILLANCE FOR POLIO BURDEN VERSUS COLLECTION POINT FOR *SALMONELLA TYPHI* CULTURE POSITIVE CASES BURDEN STRATIFIED BY TOWNS IN KARACHI, PAKISTAN

Abdul Momin Kazi, Ayub Khan, Mohammad Tahir Yousafzai, Zabin Wajidali, Farah Naz Qamar

Aga Khan University, Karachi, Pakistan

Pakistan is currently facing Extensively Drug Resistant (XDR) *Salmonella typhi* epidemic with confirmed large outbreaks in Hyderabad and Karachi cities. In November 2016, the first case of ceftriaxone resistant bacillus was reported from Hyderabad. An outbreak investigation was then conducted to identify the determinants, burden and geo spatial distribution of the outbreak using GIS mapping analysis. Simultaneously there was spread of cases in all towns of Karachi. Since Nov 2016 to March 2019, 1801 *S. typhi* resistant culture positive cases have been confirmed by Aga Khan University Hospital main lab and collection point in Karachi and using geospatial mapping techniques location based GIS maps have been plotted according to all towns of Karachi. To order to understand the environmental factors for *S. typhi* resistant culture positive cases burden, water samples in Karachi are being taken from same points where polio AFP samples are being collected. However, comparing *S. typhi* resistant culture positive cases disease burden according to Karachi town versus AFP sample collection points are quite different. In this study we have shown high resolution maps comparing high risk areas for polio versus *S. typhi* resistant culture positive cases density areas where environmental samples are being collected. We identified that areas for XDR cases are not same as the areas from where polio AFP sampling is done according to disease burden from disease perspective. In conclusion, prevalence of *S. typhi* resistant culture positive cases is geographically different from polio AFP surveillance. Therefore, the location for environmental samples should be different according to disease burden.

1744

MICROBE LITERACY: A NOVEL STRATEGY FOR INCREASING VACCINATION COVERAGE IN SINDH PAKISTAN

Farah N. Qamar[1], **Mohammad T. Yousafzai**[1], Sultan Karim[1], Hina Memon[1], Amber Kashif[1], Ed Higgins[2]

[1]*The Aga Khan University, Karachi, Karachi, Pakistan,* [2]*Microbe Literacy, New York, NY, United States*

According to the latest Pakistan Demographic Health Survey 2017-18, acceptance for all routine immunization offered through expanded program of immunization (EPI) was very low in Sindh (49%). In the recent outbreak response in Hyderabad using Typhoid conjugate vaccine (TCV), caregivers' stated reasons for refusing TCV as, unfamiliarity with infectious disease; knowledge of adverse effects of other vaccines, and rumours about vaccines. We believe that misconceptions about the infectious disease process are abstract to people with limited education. We piloted the effectiveness of a 2-hour microbe literacy (ML) workshop in improving vaccination coverage for TCV in Hyderabad. We did a cluster randomized trials in two locations in Hyderabad. Each cluster comprised of 40 households and 11 workshops on ML were conducted in the intervention clusters. Participants included caregivers, parents, grandparents, political leadership and religious stakeholders of the cluster. Opportunity was given to the participants to collect biological samples from their household and visualize on a LCD connected to a microscope. At the end of the workshop clear messages that diseases due to these microbes could be avoided by hand washing, boiling of water and vaccination. There were 11 clusters consisting of 420 households whose parents participated in the ML workshops. Of the 1265 children in the target age group for TCV, in the intervention cluster, 1235 agreed to receive TCV (98% acceptance). In the control cluster there were, 320 households of which 203 households accepted vaccination (63% acceptance). The ML workshop is a simple and novel strategy for improving vaccine acceptance in areas of low literacy.

1745

REDEFINING TYPHOID DIAGNOSIS: WHAT SHOULD A BETTER TEST LOOK LIKE, AND WHAT INNOVATIONS ARE AVAILABLE TO MEET THE NEEDS?

Richard Mather[1], Peter J. Dailey[2], Heidi Hopkins[1], **Sabine Dittrich**[2]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland*

Typhoid fever is a bacterial infection caused by *Salmonella enterica* serovar Typhi (*S.* Typhi*)*. It is a infectious disease, with ~10.9 million new cases worldwide and ~116,800 deaths in 2017. The disease is most common in Asia and sub-Saharan Africa, with children predominantly affected. Various aspects of *S.* Typhi biology make diagnosis challenging. While simple tests are available (e.g., Widal), a recent Cochrane review highlighted the poor diagnostic accuracy of currently available tools. These diagnostic deficiencies result in over- and under-estimations of disease prevalence, as well as over- and under-treatment of patients. An accurate typhoid test could reduce morbidity/mortality through faster diagnosis, improve targeting of antibiotic use, and support global vaccine strategies. In recent years, novel approaches have been described to develop typhoid diagnostics aiming to overcome the biological challenges. Building on the recent momentum around improved typhoid surveillance and potential advances in typhoid identification, a target product profile (TPP) was developed using a Delphi process with test users and key opinion leaders. The TPP contains 36 test characteristics scored by 19/40 stakeholders (48%), ranging from physicians/laboratory technologists working in endemic areas, to global health researchers and implementers. The final "optimal" scope focuses on a point-of-care test to improve care for *S.* Typhi and Paratyphi. In addition, to inform R&D, a detailed technology

landscape is being prepared to link possible innovations with technology solutions. This presentation will focus on the overall stakeholder vision for new tests, and the discordant Delphi process results that require additional discussion in targeted meetings. Mapping if and how recent advances in serological, molecular, metabolomic, proteomic, and transcriptomic research could be translated into product development efforts will be explored through a developer's lens. The TPP and the landscape will support the global typhoid agenda, and is a necessary step to support the roll-out of new vaccines and safeguarding of global antibiotics.

1746

NATIONAL FOOD SECURITY AND ANNUAL CHOLERA INCIDENCE RATE: A MULTI-DIMENSIONAL ANALYSIS OF 30 COUNTRIES FROM 2012-2015

Aaron Richterman[1], Andrew S. Azman[2], Georgery Constant[3], Louise C. Ivers[4]

[1]*Department of Medicine, Brigham and Women's Hospital, Boston, MA, United States,* [2]*Division of Infectious Disease Epidemiology, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Partners in Health/Zanmi Lasante, Cange, Haiti,* [4]*Center for Global Health, Massachusetts General Hospital, Boston, MA, United States*

Individual and household-level evidence suggests a relationship between food insecurity and cholera risk. The relationship between national food security and the size of cholera outbreaks is unknown. We performed an analysis from 2012-2015 estimating the relationship between national food security and annual cholera incidence rate. As our measure of food security we used components of the Global Food Security Index (GFSI), a composite measure of food security at the country level which is reported annually, incorporates 28 unique indicators, and allows for inter-dimensional and cross-national comparisons. We included countries with available GFSI reporting cholera during the study period, excluding high-income countries. We developed multivariable zero-inflated negative binomial models with cholera incidence rate as the outcome, GFSI components as the exposure of interest, fixed effects for country and year, and time-varying controls related to water, sanitation, and hygiene, oral cholera vaccine deployment, health care expenditure, conflict, and extreme weather. 30 countries were included, reporting 550,106 cases of cholera 2012-2015 with a median annual incidence rate of 3.1 cases per 100,000 people (IQR 0.3-9.9). Included countries were, in general, food insecure - among all countries with available GFSI, they had a median rank of 90 (IQR 77-103) out of 113 countries for Overall GFSI during the study period and included eleven of the thirteen most food insecure countries. We found an independent relationship between cholera incidence rate and Overall GFSI (IRR 0.57, 95% CI 0.43-0.78), GFSI-Availability (IRR 0.81, 95% CI 0.70-0.95), and GFSI-Affordability (IRR 0.76, 95% CI 0.62-0.92). In the context of prior evidence at the individual and household level, this suggests there is a linkage between food insecurity and cholera at the national level that should be further explored and considered when evaluating the risk and consequences of outbreaks. Food security is not currently considered in any programmatic guidance for cholera control, and the impact of cholera outbreaks on food security is rarely discussed.

1747

CLINICAL PREDICTORS FOR VIRAL ETIOLOGIES OF ACUTE DIARRHEA IN RESOURCE-LIMITED SETTINGS

Benjamin J. Brintz[1], Benjamin Haaland[1], Joel Howard[1], Andrew Pavia[1], Tom Greene[1], Dennis Chao[2], Joshua Proctor[2], Adam Levine[3], James Platts-Mills[4], Karen Kotloff[5], Daniel Leung[1]

[1]*University of Utah, Salt Lake City, UT, United States,* [2]*Institute of Disease Modeling, Seattle, WA, United States,* [3]*Brown University, Providence, RI, United States,* [4]*University of Virginia, Charlottesville, VA, United States,* [5]*University of Marland, College Park, MD, United States*

Non-laboratory methods to more accurately assess etiology are needed for appropriate management of pediatric diarrhea in low and middle income countries (LMICs). In LMICs, etiological diagnosis is rarely made, and a large number (up to 70%) of patients with acute diarrhea are prescribed antibiotics. With the goal of antibiotic stewardship, we use clinical and quantitative molecular etiologic data from the Global Enteric Multicenter Study (GEMS) to develop predictive models for etiology of diarrhea in young children. Given the lack of testing done in LMICs for infectious diarrhea, the attributable pathogen data from GEMS presents a unique opportunity to determine if a child's clinical information can help determine the etiology of the diarrhea. We aimed to build an implementable model of virus-only etiology for use in LMICs given the lack of efficacy of antibiotics in this context. In order to build a parsimonious model, we first screened potential clinical predictors from the GEMS survey via random forest variable importance. In addition to the clinical information, we constructed a season variable to represent rainy/dry and hot/cold weather patterns near the health centers, based on climate data from nearby weather stations. Variables predictive of virus-only etiology include age, vomiting, BMI, bloody diarrhea, breastfeeding, and season. We assessed performance using the receiver operating characteristic (ROC) curve and area under the curve (AUC) with 10-fold cross-validation of various parameter sizes for both random forest regression and logistic regression. Both the variable screening and model fitting were nested within the cross-validation. The cross-validation showed that with a small number of variables, both types of models can achieve AUCs of between .825 and .850. Results also show that the model will perform better in some locations than others and that location specific information is helpful. Our model's performance suggests that implementing a clinical prediction rule in LMICs can assist providers in managing infectious diarrhea treatment and reduce unnecessary and damaging prescription of antibiotics.

1748

DETECTION OF *SALMONELLA* TYPHI IN BILE BY QUANTITATIVE REAL-TIME PCR

Sharon M. Tennant[1], Ellen Higginson[1], Joseph Nkeze[1], Jasnehta Permala-Booth[1], Irene Kasumba[1], Rosanna Lagos[2], Juan Carlos Hormazabal[3], Gad Frankel[4], Myron M. Levine[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Hospital de Ninos Roberto del Rio, Santiago, Chile,* [3]*Instituto de Salud Publica, Santiago, Chile,* [4]*Imperial College, London, United Kingdom*

Typhoid fever remains an important cause of febrile illness in many areas of Africa, Asia and Oceania. Short-cycle transmission of typhoid occurs via ingestion of food contaminated by short-term or chronic carriers of *Salmonella* Typhi who have lapses in personal hygiene (failure to wash hands with soap) after defecation. Chronic gallbladder typhoid carriers, who typically have gallstones, constitute the long-term reservoir of infection. One way to estimate the prevalence of chronic biliary carriers is to test bile collected at the time of cholecystectomy. Herein we describe detection of *S.* Typhi in bile by real-time PCR. A quantitative PCR protocol for bile is especially important where antibiotics are routinely given to patients just before cholecystectomy. We evaluated probesets that target *oriC* (all *Salmonella*), *viaB* (Vi-expressing *Salmonella*), *fliC*-d (*Salmonella* with Phase 1 flagellin type 'd'), STY0201 (*S*. Typhi only) and *stoD* (*S*. Typhi only). We evaluated sensitivity and specificity of each probeset. As expected, the *oriC* probeset was able to detect all *Salmonella* serovars tested, the *viaB* probeset was able to detect serovars that possess Vi capsule, the *fliC-d*, STY0201 and *stoD* probesets were specific for *S*. Typhi. The *oriC* probeset was the most sensitive followed by the STY0201 probeset. Using an optimized DNA extraction technique, we detected *S.* Typhi in spiked human bile samples down to a concentration of $7.4x10^2$ CFU/ml. Human bile samples were spiked with *S*. Typhi and cephalosporins. *S*. Typhi in suspensions as low as 10^2 CFU/ml were detectable by real-time PCR in samples containing cefazolin, cefotaxime or ceftriaxone but could only be detected by culture in samples containing cefazolin. This real-time PCR detection method for *S.* Typhi in bile is also useful where bile-containing duodenal fluid is obtained by string capsule

from household contacts of acute typhoid cases, in attempts to find chronic carriers. These techniques will be helpful for efforts to control typhoid disease in endemic regions.

1749

PRODUCTION OF THE FECAL INFLAMMATION MARKERS AFTER ORAL CHALLENGE WITH SHIGELLA SONNEI 53G IN A CONTROLLED HUMAN INFECTION MODEL

K. A. Clarkson[1], K. T. Lerner[1], R. W. Frenck[2], M. Dickey[2], A. E. Suvarnapunya[1], L. Chandrasekaran[1], M. McNeal[2], K. Detizio[3], S. Parker[2], A. Hoeper[2], C. K. Porter[3], N. Maier[4], A. Fix[4], A. L. Bourgeois[4], M. Venkatesan[1], R. W. Kaminski[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*CCHMC, Cincinnati, OH, United States,* [3]*Naval Medical Research Center, Silver Spring, MD, United States,* [4]*PATH, Washington, DC, United States*

Shigella is a leading cause of diarrhea-associated morbidity and mortality in children under the age of 5 living in low to middle income countries (LMICs) where *Shigella* species are endemic. Repeated non-fatal enteric infections with pathogens such as *Shigella*reduces gut permeability, resulting in reduced intestinal absorption of nutrients. In children, the reduction in nutrient absorption contributes to a constant state of malnutrition manifesting as physical and cognitive stunting. Recently, a cGMP lyophilized strain of *S. sonnei,* 53G was produced and used in a Controlled Human Infection Model (CHIM) in North American adults to determine a dose that safely and reproducibly achieved a 60-70% attack rate. The *S. sonnei,* 53G CHIM was developed to provide a more standardized model for use in evaluating the efficacy of interventions, such as vaccines or immunoprophylactics. The model also provides a unique opportunity to investigate the intestinal inflammatory response post-infection. Fecal samples were collected prior to challenge and 3, 7 and 14 days post-challenge and the concentrations of calprotectin and myeloperoxidase were quantified by ELISA. Calprotectin and myeloperoxidase concentrations peaked 3 days post-challenge and returned to baseline by day 14. Both calprotectin and myeloperoxidase levels were highly correlated with multiple disease outcomes including diarrhea severity, disease severity score and dysentery. In addition, calprotectin and myeloperoxidase concentrations were predictive of the adaptive mucosal immune response in that subjects with high levels of fecal inflammatory markers also had robust *Shigella*-specific mucosal immune response, as measured by IgA secreting $\alpha4\beta7$ positive gut-homing B cells. These results suggest that the evaluation of intestinal inflammation markers may be used as an additional measure of vaccine efficacy to help further differentiate the impact of vaccination on *Shigella* disease.

1750

AN EVALUATION OF LOW-COST SPECIMEN PRESERVATION FOR CHARACTERIZATION OF ETEC AND SHIGELLA AMONG CHILDREN WITH DIARRHEA AND/OR DYSENTERY IN TWO REGIONS OF CAMEROON

Amanda K. Debes

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Worldwide, more than 600,000 children under 5 years of age die annually from diarrheal diseases; nearly one-third of pediatric diarrheal deaths are due to Enterotoxigenic Escherichia coli (ETEC) and *Shigellae*. There is a need for information on the strains and serotypes in endemic areas which crucial to the development of effective vaccines targeting the most vulnerable populations. Classical microbiological methods are often not practical in resource constrained settings where future vaccine efficacy trials need to be conducted. We hypothesized that ETEC and *Shigellae* cause a large proportion of moderate-to-severe (MSD) diarrheal illnesses which may be controlled with vaccines currently being developed. We nested a pediatric cohort into on-going multi-site surveillance efforts in Cameroon. Children and adolescents <18 years of age were enrolled between 1/1/15 and 12/31/17 and were recruited based on >3 loose stools within 24hours, presence of dehydration and/or blood and/or mucus. A total of 3522 persons were enrolled, 2222 meet the enrollment criteria. 198 specimens were randomly selected and screened using the Taqman Array Card (TAC). We found 34.0% (N=67) ETEC positive, of which 35.8% (N=24) are LT-STh positive, and 10.4% (N=7) are LT-STp positive. 32.8% (N=22) of ETEC positive were in patients >5 years. CFs were found present in 41.8% of ETEC positive samples. 34.0% (N=67) of persons presenting with MSD and/or bloody and/or mucoid stool are IpaH positive, of which 31.3%(N=21) >5 years of age. 77 (85.6%) of 90 cases had one diarrhea-attributable pathogen detected and 13 (14.4%) cases had two or more. *Shigellae* and rotavirus are most strongly associated with diarrhea in children with mixed infections. The 5 most common attributable pathogens are *Shigellae*, rotavirus, ST-ETEC, *V. cholerae,* and Norovirus GII; accounting for 85.5% of all attributable diarrhea. This study identified areas where the rates of *Shigellae* and ETEC were higher than hypothesized, warranting the need for further research investigating these high-risk areas. Such research may identify potential field sites for evaluation of vaccine candidates being developed.

1751

TEMPORAL TRENDS IN NON-TYPHOIDAL *SALMONELLA* GASTROINTESTINAL INFECTIONS IN CHILDREN UNDER FIVE FROM THREE COUNTRIES IN SUB-SAHARAN AFRICA

Irene N. Kasumba, Helen Powel, Anna Roose, Sunil Sen, Shamima Nasrin, Jasnehta Permala-Booth, Sharon M. Tennant, VIDA Consortium

University of Maryland Baltimore, Baltimore, MD, United States

Non-typhoidal *Salmonella* (NTS), an etiologic agent of gastroenteritis, is a risk factor for morbidity and mortality in young children. The two most common serovars that cause gastroenteritis worldwide are *S*. Typhimurium and *S*. Enteritidis. Additionally, variants of these two serovars also cause invasive disease in infants in sub-Saharan Africa. We evaluated the serovars, antimicrobial sensitivity and genotypes of *Salmonella* isolated from stools of children < 5 years old with moderate-to-severe diarrhea (MSD) or matched community controls at sites in The Gambia, Mali and Kenya during the Vaccine Impact on Diarrhea in Africa (VIDA) study (2015-2018) and compared data to isolates from the Global Enteric Multicenter Study (GEMS; 2007-2010) and the follow-up study GEMS-1a (2011). Approximately, 44.7%, 1.1% and 54.2% of *Salmonella* spp. were recovered from VIDA stools collected from The Gambia, Mali and Kenya, respectively. The most common isolates were serogroup B (18.4%), serogroup C_2-C_3 (12.3%), serogroup F (11.7%) and G (11.2%). In comparison to GEMS, a lower frequency of *S*. Typhimurium (38.2% versus 5.0%) and a higher frequency of *S*. Enteritidis (6.3% versus 9.5%) isolates were recovered during VIDA. During VIDA, we observed that 80% of *S*. Typhimurium were multi-drug resistant (MDR) like GEMS and GEMS1a. However, MDR for *S*. Enteritidis decreased from 69% during GEMS to 14.3% during VIDA. Importantly, we detected pan-susceptibility to ceftriaxone and ciprofloxacin during GEMS, however, 10.5 and 33.3% of serogroup B and *S*. Typhimurium isolates, respectively, were resistant to ceftriaxone in VIDA. All 74 *S*. Typhimurium isolates from GEMS stools were sequence type (ST) 313. Out of seven *S*. Typhimurium from VIDA stools, five were ST313 and all were MDR (100%), and two were ST36 and were pan-susceptible. *S*. Typhimurium ST313, which is responsible for most NTS-related bacteremia in Africa, and the only genotype of *S*. Typhimurium from GEMS stools, was also the dominant genotype in VIDA stools from Kenya. Ongoing studies should monitor causes of the changing landscape of *S*. Typhimurium and *S*. Enteritidis in Africa.

1752

ISOLATION OF BACTERIOPHAGES FROM WATER SOURCES IN THE AMAZON RIVER BASIN OF PERU WITH LYTIC ACTIVITY AGAINST CLINICALLY RELEVANT MDR *ACINETOBACTER BAUMANNII*

Brian Drury[1], Dylan Stephens[1], Emma Baker[1], Alexandra de la plante[1], Dallas Hamlin[1], David Craft[1], James M. Regeimbal[2], Ricardo Abadie[2]

[1]*Pennsylvania State Milton S. Hershey Medical Center and College of Medicine, Hershey, PA, United States,* [2]*Naval Medical Research Unit No. 6, Lima, Peru*

The WHO classifies MDR bacteria as an imminent threat to human health. The reinvestigation of lytic bacteriophages is a promising therapeutic approach to treat these infections that requires tailoring therapeutic cocktails from diverse phage libraries. Here we continued to expand a clinically relevant library of phages obtained from sewage water in Iquitos, Peru. Clinically sourced MDR *Acinetobacter baumannii* (MDRAB) strains isolated in Iquitos hospitals between 2011-2017 were used to harvest and characterize newly isolated phages. Collected sewage water was used to generate 3% w/v TSB, and was subsequently left as TSB/waste water or 7.4% FBS v/v or 5% v/v sheep's blood (FBS/SB) were added. The 3% TSB/waste water was inoculated with 5 MDRAB strains and grown overnight at 37°C. The FBS/SB was inoculated with 5 MDRAB strains and grown overnight at room temperature (RT). All three supernatants were then cleared by centrifugation and sterile filtration. Serial dilutions were spotted onto individual lawns of the initial 5 MDRAB strains. 3% TSB lawns were incubated overnight at 37°C and FBS/SB lawns were incubat3ed overnight at RT. Agar plugs of well-isolated plaques were suspended in PBS and filter sterilized. Initial phage isolation steps were repeated using different MDRAB strains and sewage water sources. Phage host-ranges were evaluated against 20 clinical isolates of MDRAB using sport-plates across all growth conditions and temperatures. 21 lytic phages were isolated with only one phage demonstrating specificity beyond the original host strain. That phage demonstrated activity against four other MDRAB strains and in all three growth conditions. All other phage isolates demonstrated activity against the host strain only but showed activity in all growing conditions. As demonstrated in other studies, we found it difficult to harvest and isolate phages that exhibited a wide range of activity against multiple MDRAB strains. Future efforts will continue to explore method modifications to yield a more diverse library of phages with broad specificity that could be used as tailored cocktails for the therapeutic management of patients with MDR infections.

1753

MULTI-VALENT ORAL VACCINE AGAINST ENTEROTOXIGENIC *ESCHERICHIA COLI* AND ENTERIC FEVERS (ETEC)

Tint Wai[1], MingLin Li[1], Sumana Chakravarty[2], Eric R. James[2], Bruce Liberi[1], Weiping Zhang[3], David Sack[4], Stephen L. Hoffman[2], B. Kim Lee Sim[1]

[1]*Protein Potential LLC, Rockville, MD, United States,* [2]*Sanaria Inc., Rockville, MD, United States,* [3]*Kansas State University College of Veterinary Medicine, Manhattan, KS, United States,* [4]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

We aim to develop an oral, bivalent vaccine protective against enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* Typhi. ETEC is estimated to cause 2.8-4.0x10^8 diarrheal cases in children <5 years of age, 10^8 cases in children >5 years and >1.5x10^5 deaths annually, and infect nearly every child living in a developing country. Typhoid causes ~2.1x10^7 million clinical cases and >10^5 deaths annually. Our recombinant live oral ETEC vaccine (Ty21a-ETEC) is composed of Ty21a, the oral typhoid vaccine, expressing both heat-labile (LT) and heat stable enterotoxin (STa) and 7 adhesins [CFA/I, CFA/II (CS1-CS3) and CFA/IV (CS4-CS6)] that facilitate colonization of host intestines and binds GM1. Thus, our vaccine candidate targets ETEC's key virulence antigens, toxins and adhesion factors. Further, our 7 adhesins comprise a multi-epitope fusion antigen (MEFA) that has shown to have broad spectrum anti-adhesin activity. We report that our oral bi-valent vaccine candidate Ty21a-ETEC, expresses our target toxins, MEFA antigens as well as the O-antigen of *Salmonella* Typhi. Intranasal (IN) immunization of BALB/c mice, that mimicked a mucosal/oral route of immunization, induced antibodies against LTB and MEFA that blocked binding to GM1, showing the induction of anti-toxin activity. We further showed that antibodies induced by IN immunization induced antibodies that blocked adhesion of ETEC to Caco-2 cells. Further, Ty21a-ETEC is stabilized at room temperature by foam drying. This room temperature stable, orally administered vaccine has the potential to provide enormous public health benefits for residents of developing countries, protect travelers and deployed military personnel to developing countries, and potentially serve national biodefense programs against ETEC-mediated biowarfare and bioterrorism threats.

1754

EVALUATION OF THE ANTIMYCOBACTERIAL AND ANTIMYCOLACTONE EFFICACY OF KOMBUCHA TEA

Adiza Abass[1], Elizabeth Gyamfi[2], Regina Appiah-Opong[3], WSK Gbewonyo[2], Phyllis Addo[3], Lydia Mosi[2]

[1]*Tokyo Medical and Dental University, Tokyo, Japan,* [2]*University of Ghana, Accra, Ghana,* [3]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana*

Buruli ulcer is caused by mycolactone (toxin) produced by Mycobacterium ulcerans. The treatment of the disease and early diagnosis is hampered by the lipid nature of the toxin and emerging bacterial resistance to currently used antimycobacterials. Kombucha tea, a health drink consumed worldwide has been shown to exert some medicinal properties. This study was aimed at assessing the antimycobacterial and mycolactone inactivating potential of Kombucha tea. Probiotic microbial isolates from the tea were identified using culture dependent and independent methods for the identification and characterization of bacteria and yeasts, respectively. Phytochemical analysis was conducted on the tea to determine the active organic compounds, total phenolic content and antioxidant properties. Increasing concentrations of the tea were co-incubated with mycolactone for various time points to observe for toxin attenuation. The presence of intact or inactivated mycolactone was detected using TLC and cytotoxicity assays on cultured human fibroblasts. The antimicrobial potency of increasing concentrations of the tea was tested against S. aureus and M. ulcerans by pre-incubation prior to microscopy and culture to observe morphological changes and viability respectively. Yeasts in the Kombucha tea were identified as *Dekkera bruxellensis, Brettanomyces bruxellensis, Rhodotorula mucilaginosa and Lachancea fermentati* and the major bacteria as Acetobacter sp, Paenibacillus lactis, Bacillus licheniformis and Lactobacillus amylolyticus. Phytochemicals detected in the tea were saponins, flavonoids, alkaloids, and phenols. The antioxidant property and phenolic content of Kombucha was significantly higher compared to unfermented tea with the latter being 2-fold less potent in total phenolic content than Kombucha tea. The fermented tea possessed antimicrobial activity against S. aureus but not against M. ulcerans as viable bacilli of M. ulcerans were observed after Kombucha treatment. Mycolactone treated with Kombucha tea retained its potency against human fibroblast cells suggesting that Kombucha tea had no toxin inactivating capacity.

1755

FIRST REPORTED HUMAN CASE OF *RICKETTSIA FELIS* IN GUATEMALA

Beatriz Lopez-Castellanos[1], Maria R. Lopez[2], Arlyn N. Gleaton[3], Ida H. Chung[3], Cecilia Y. Kato[3], Paige A. Armstrong[3], Manuel Sagastume[4], John P. McCracken[2], Andres Espinosa-Bode[5]

[1]*TEPHINET/Centers for Disease Control and Prevention, Guatemala City, Guatemala,* [2]*Universidad del Valle de Guatemala, Guatemala City, Guatemala,* [3]*Centers for Disease Control and Prevention, Atlanta, GA,*

United States, [4]Ministerio de Salud Publica y Asistencia Social, Guatemala City, Guatemala, [5]Centers for Disease Control and Prevention, Guatemala City, Guatemala

Rickettsia felis, from the spotted fever group *rickettsiae*, is an emerging pathogen that has been reported worldwide. The first human cases were documented in 1994 in the United States and in 2000 in Mexico. Early clinical presentation of *R. felis* infection can resemble that of dengue and malaria, which makes differentiating it from other acute febrile illnesses (AFI) difficult. The real burden of *R. felis* rickettsiosis may be underestimated. To our knowledge, no human cases of *R. felis* infection have been reported in Guatemala. However, *R. felis* has been isolated from fleas from cats and dogs in the country. During 2013-2018, 241 blood samples were collected from participants enrolled in an AFI sentinel surveillance implemented by the Guatemala Ministry of Health, the Centers for Disease Control and Prevention (CDC) and the Universidad del Valle de Guatemala. Five of these samples were positive for *Rickettsia sp.* by PCR assays for the detection of spotted fever group *rickettsia* (SFGR) and typhus group *rickettsia* DNA. The samples were sent for confirmation to the CDC Rickettsial Diagnosis Laboratory and one sample was confirmed to be positive for *R. felis* by DNA sequencing with the SFGR 17kDa target. The confirmed *R. felis* sample was collected from a 3 year-old boy screened in December 2017 in Nueva Santa Rosa, Department of Santa Rosa. The case presented with fever that started 2 days before screening. Other reported symptoms were cough, difficulty breathing, vomiting, abdominal pain and fatigue. No additional information was collected because the case was lost to follow-up after enrollment. This is the first identified human case of R. *felis* detected via AFI surveillance efforts in Guatemala. Its detection provides evidence that it may be an under-appreciated cause of illness in Guatemala and possibly all of Central America. Continued surveillance for *R. felis* and other emerging rickettsiosis is imperative to better understand the magnitude and public health impact of these infections in the region.

1756

INCIDENCE OF MENINGOCOCCAL MENINGITIS SEROGROUP C IN TWO NORTHWESTERN STATES OF NIGERIA

Olaiya Paul Abiodun[1], Zachary Gwa[2], Olumide Ajani[1], Felix Olaniyi Sanni[3], Abiodun Ogunniyi[4], Abiola Abiodun[5]

[1]*Department of National Integrated Specimen Referral Network, AXIOS International, Utako, FCT, Abuja, Nigeria,* [2]*Department of Business Development, AXIOS Foundation, Utako, FCT, Abuja, Nigeria,* [3]*Department of Global Health, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria,* [4]*Department of Prevention and Program Coordination, Nigeria Centre for Disease Control Utako, FCT, Abuja, Nigeria,* [5]*General Hospital North Bank, Makurdi, Nigeria*

The localized outbreaks of meningitis caused by a new strain of Neisseria meningitidis serogroup C (NmC) were reported in the northern parts of Nigeria in 2013, 2014 and 2015. From December 2016 to June 2017, an outbreak caused by the same novel NmC strain also occurred in several States over a wider geographical area, similar in characteristics to the 2015 outbreaks. We report the incidence of the outbreak in Sokoto and Zamfara using the case definition for cerebrospinal meningitis (CSM) as designed by Médecins Sans Frontières (2008), UNICEF and WHO. From week 51 2016 to week 19, 2017, data on cerebrospinal meningitis (CSM) cases and deaths were recorded on standardized line-lists from case management sites. Cerebrospinal fluid (CSF) samples collected from suspected cases during the outbreak were tested using rapid Pastorex® latex agglutination to determine causative serogroup. A total of 5,372 suspected cases of MNC were reported in Zamfara and Sokoto states. CSF was collected from 281 (5.2%) suspected cases (190 from Sokoto and 91 from Zamfara), there were 277 deaths in Sokoto and 81 in Zamfara, making a total of 358 deaths, 5,188 probable cases and 184 confirmed cases from both states. Out of 5,372 suspected cases of NmC seen, 57.2% were males and 42.8% were females (M: F = 1.3:1). The most affected age in both states was 6-15 years with 49.3% for Sokoto, 53.9% for Zamfara and overall 49.8%. The peak of meningitis cases was observed at week 7, 2017 in Zamfara while the peak was observed in Sokoto at week 15. Marudun local government recorded the highest incidence (146) in Zamfara while in Sokoto state, Sokoto North and South LGAs accounted for the highest incidence (1016; 21.2%). The outbreak was one of the largest caused by NmC documented in Nigeria. Reactive vaccination in the affected areas may have helped curtail the epidemic. A vaccination campaign against NmC with a long-lasting conjugate vaccine should be considered in the northern parts of Nigeria. Keywords: Meningitis belt, outbreak, cerebrospinal fluid, reactive vaccination, epidemiology

1757

SEROLOGICAL EVIDENCE AUGMENTED BY NEXT-GENERATION SEQUENCING IDENTIFIES *ORIENTIA TSUTSUGAMUSHI* AS A CAUSATIVE AGENT OF SEPSIS IN CAMBODIA

Amitha Fitkariwala[1], Dennis Faix[1], Tin Som[1], Pichit Pin[1], Sokhun Song[1], Daraden Vang[1], Heng Bun[1], John Brooks[1], Te Vantha[2], Logan Voegtly[3], Regina Z. Cer[4], Kimberly A. Bishop-Lilly[3], Casandra Philipson[5], Chien-Chung Chao[6], Kevin L. Schully[7], Danielle V. Clark[8]

[1]*Naval Medical Research Unit-2, Phnom Penh, Cambodia,* [2]*Takeo Provincial Referral Hospital, Takeo, Cambodia,* [3]*Genomics and Bioinformatics Department, Biological Defense Research Directorate, Naval Medical Research Center-Frederick, Fort Detrick, MD, United States,* [4]*Leidos, Reston, VA, United States,* [5]*Defense Threat Reduction Agency, Fort Belvoir, VA, United States,* [6]*Viral and Rickettsial Diseases Department, Naval Medical Research Center-Silver Spring, Silver Spring, MD, United States,* [7]*Austere Environments Consortium for Enhanced Sepsis Outcomes Department, Biological Defense Research Directorate, Naval Medical Research Center-Frederick, Fort Detrick, MD, United States,* [8]*The Henry M Jackson Foundation, Bethesda, MD, United States*

Diagnosis of *Orientia tsutsugamushi*, the agent of scrub typhus, is typically accomplished by serological analysis of acute and convalescent samples using ELISA and IFA. However, in cases where patients succumbed to their infections or failed to follow-up, the absence of a convalescent sample makes serological diagnosis unreliable. Next Generation Sequencing (NGS) offers a comprehensive and complementary method for unbiased profiling of sepsis pathogens, and its integration into clinical and surveillance programs is increasingly more common. As part of an observational trial of sepsis in Takeo Province, Cambodia, we enrolled 200 patients hospitalized under the suspicion of infection. In addition to serum, blood was collected directly into PAXgene RNA stabilization tubes. Using standard serological techniques, we identified serological evidence of *O. tsutsugamushi* infection in 8 patients based on a four-fold increase in antibody titer. However, for patients who succumbed to infection or were lost to follow up, confirmation of seroconversion was impossible due to the lack of convalescent sera. To detect potential etiological agents of sepsis in a broad and unbiased manner, RNAseq was performed on all acute whole blood samples. By targeting RNA, the sequence space was focused transcripts indicative of actively replicating bacteria. Microbes were identified using read-based taxonomy tools embedded in EDGE bioinformatics software. *O. tsutsugamushi* was identified as the predominant microbe in 8 additional samples of which 7 were from patients exhibiting elevated acute IgM titers but nonexistent convalescent samples. This identification was further confirmed by aligning reads that mapped to the *O. tsutsugamushi* genome to the 16S rRNA and 23S rRNA regions of *O. tsutsugamushi*. While standard microbiological techniques are essential to disease surveillance, they are not always effective surveillance tools for unculturable bacteria, viruses, nor novel pathogens. The *in silico* identification of *O. tsutsugamushi*, a bacterium not culturable by standard culture methods, demonstrates the utility of NGS as a biosurveillance tool.

1758

STENOTROPHOMONAS MALTOPHILIA: SELDOM ALONE, NON-INVASIVE

Don W. Kannangara, Priya Patel, Dhyanesh Pandya

St Luke's University Health Network, Phillipsburg, NJ, United States

Stenotrophomonas maltophilia (SM) is known for inherent resistance to multiple antibiotics and bio-film formation.We studied 316 isolates of *S. maltophilia* from wounds, sputum, bronchial secretions, urine, blood and sinus cultures reported by our network lab during the last 3 years. Majority of the isolates were from respiratory (107), wound (71), urine (58), blood (10) and other sites. In every site including blood, the organism was present as part of a polymicrobial infection excluding a small number of cases. Of 316 isolates only 10 were isolated from blood. Only 2 out of 10 Blood cultures were monomicrobial infections. The organism was present only in 1 out of 2 blood cultures drawn in all 10 patients and was always absent in the repeated blood cultures. None of the patients with urinary or wound isolates had positive blood cultures. One patient with advanced sarcoidosis on high dose prednisone had 1positive blood and sputum cultures with SM and *Enterobacter cloacae*. 5 bacteremic patients were narcotic dependent. Two were iv drug users and 3 had chronic pain syndromes. Three were line infections. Majority of isolates from wounds were from the lower extremity. Majority of patients with urine and wound isolates were above the age 50. All isolates were susceptible to Bactrim. All but 2 were susceptible to levofloxacin. About half the isolates were susceptible to ticarcillin/clavulanate or ceftazidime. This study shows a remarkable lack of invasive infections by SM. Detailed statistics will be presented. The present study was limited by absence of burn wound or organ transplant patients.

1759

UTILITY OF NEXT GENERATION SEQUENCING (NGS) BASED METHODS AND THE GENE MEDIATED ANTI-MICROBIAL RESISTANCE (AMR) IN DEFINING MICROBIAL COMMUNITIES IN CHRONIC DIABETIC FOOT ULCERS IN RURAL SRI LANKA

Sandani Yasara Weerasundara, Harshika Sachini Welgama, Hiruni Shermila Weerasingha, Iruni Weerathunga, Kusal Dulanjala Weerakkody, Sudaraka Harindu Wageesha, Suneth Buddhika Agampodi

Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka

Rapidly developing molecular and NGS methods are usually not accessible to healthcare institutions in rural middle and low income country settings. We investigated the utility of these techniques in defining the microbial communities of chronic Diabetic Foot Ulcers (DFUs), the leading cause for non-traumatic lower extremity amputation and an emerging epidemic in even rural Sri Lanka. Using a commercially available sequencing service, we evaluated the microbial communities of tissue samples of patients with DFUs admitted to the Teaching Hospital, Anuradhapura. Conventional culture methods were also used for comparison of NGS based bacterial 16S and fungal ITS rRNA sequencing using Ion Torrent. The presence of AMR genes in common infecting organisms was evaluated by quantitative Polymerase Chain Reaction (qPCR) with previously validated, specific primers for bla_{VIM} and bla_{IMP} genes. Of the 50 patients selected, NGS was available for 20. The majority of the bacteria detected by 16S rRNA sequencing were aerobes and facultative anaerobes (90%) but anaerobic *Bacteroides fragilis*(20.0%) was the commonest species detected, followed by *P. aeruginosa* (15.0%), *Streptococcus spp*. Group B(15.0%), *Arcanobacterium haemolyticum* (15.0%), *Corynebacterium diphtheriae*(15.0%), *Corynebacterium simulans*(15.0%), *Klebsiella pneumoniae*(10.0%), *Enterococcus faecalis*(10.0%). Fungal ITS rRNA sequencing identified a single growth of *Candida albicans.* Of the 50 wound swabs, 47 were culture positive and 35 (70.0%) had mixed growths with a single sample with fungal growth. The most frequent culture isolate was *Pseudomonas spp.*(n=27,54.0%) followed by *Staphylococcus spp.* (n=21,42.0%), *Proteus spp.*(n=10,20.0%), *Streptococcus spp.*(n=6,12.0%), *Escherichia spp.*(n=3,6.0%) and *Klebsiella spp.*(n=1,2.0%). None of the *Pseudomonas* isolates exhibited AMR through bla_{VIM} or bla_{IMP} genes. NGS based methods are complementary to traditional methods and cannot be replaced due to differences in detection. We haven't observed the AMR through the selected genes in this cohort of patients.

1760

A CLUSTER OF MELIOIDOSIS CASES FOLLOWING HEAVY RAINS IN BATTICALOA, SRI LANKA

Aruna D. De Silva[1], Himali S. Jayasinghearachchi[1], Enoka M. Corea[2], Vaithehi R. Francis[3], Shivankari Krishnananthasivam[4], Harindra D. Sathkumara[4]

[1]*General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka,* [2]*Faculty of Medicine, University of Colombo, Colombo, Sri Lanka,* [3]*Faculty of Health-Care Sciences, Eastern University, Batticaloa, Sri Lanka,* [4]*Genetech Research Institute, Colombo, Sri Lanka*

Burkholderia pseudomallei is a Gram-negative environmental bacterium that causes melioidosis, a potentially life-threatening infectious disease. There was a cluster of 10 cases (with 4 deaths) that occurred in Batticaloa in the Eastern Province of Sri Lanka in October/November 2015 following heavy rains. The deaths included three female patients with severe community-acquired bronchopneumonia, suggesting acquisition via inhalation. The *B. pseudomallei* strains isolated from these patients had MLST and whole-genome sequencing carried out to understand the phylogeography, transmission and also help deduce evolutionary relatedness between isolates from this cluster of cases. Eight isolates (BPs110, BPs111, BPs112, BPs114, BPs115, BPs116, BPs122 and BPs 133) of *B. pseudomallei* obtained from these melioidosis patients were confirmed by real-time polymerase chain reaction (PCR) assay, amplifying *lpxO*, Yersinia-like fimbrial (YLF) and *B. thailandensis*-like flagellum and chemotaxis (BTFC) gene clusters of *B. pseudomallei* using a multiplex SYBR green real-time PCR assay. The molecular diversity of strains was assessed using the *B. pseudomallei* multilocus sequence typing (MLST) schema. Seven housekeeping genes (*ace, gltB, gmhD, lipA, narK, lep* and *ndh*) were amplified for MLST and the PCR products were subjected to automated Sanger sequencing. The genomic DNA of each isolate was independently subjected to whole-genome sequencing (WGS) from a paired-end with a ~301-bp insertion size using the Illumina MiSeq 2000 platform at Agiomix, FZ LLC, UAE. SPAdes Version 3.10.1 was used for the assembly with *B. pseudomallei* K96243 as reference. There is a substantial diversity of ST types in the areas where the case cluster of melioidosis was reported following the heavy rains. ST 594 is the predominant ST type and other ST types detected were ST1152, ST1364, ST1442, ST1413, ST1179. This matches with previous reports of case clusters resulting from severe weather events such as cyclones, in which a single infectious disease is caused by strains of diverse molecular types, probably due to widespread aerosolization of multiple strains.

1761

DETERMINATION OF AREAS WITH POTENTIAL RISK OF HUMAN LEPTOSPIROSIS IN THE DEPARTMENT OF CÓRDOBA, COLOMBIA

Virginia C. Rodríguez, Eidy Martinez, Ana M. Castro, Alfonso Calderón, Misael Oviedo

Universidad de Córdoba, Monteria, Colombia

Leptospirosis is an emerging zoonotic bacterial disease, which has become a global public health problem. A descriptive study was designed by means of a convenience sampling, and the registries of patients were selected (and the patients retroactively notified) from the National Surveillance System of Colombia as a case of leptospirosis, from 2012 to 2015. Active surveillance was carried out in the Lending Institutions of Health Services of the department of Córdoba. Patients with clinical symptoms of leptospirosis during the years 2016-2018 were included. After signing the informed consent form and completing the epidemiological record, blood

and urine samples were taken. The cases were confirmed by PCR and Microagglutination Test (MAT). Association between cases of leptospirosis and risk factors through univariate and bivariate analysis of information was established. A multivariable logistic regression analysis was carried out for the elaboration of the predictive model. The distribution of cases of the disease was analyzed by means of the Bernoulli model. Were identified 532 patients as possibly infected, of these 40 were confirmed with leptospirosis. The majority were men, aged 12-26 years old, and no significant differences were found regarding sex and age. Social risk factors were identified such as: living in urban areas, working as a farmer, having contact with domestic and synanthropic animals, lacking inadequate disposal of solid waste and participating in recreational activities or sports in dams. No association was established with the confirmed cases of leptospirosis regarding the environmental risk factors. The results of the predictive model showed that patients with fever, headache, myalgia, jaundice and conjunctival bleeding are more likely to have complications during the disease. Coming into contact with horses represents a risk factor for acquiring the bacteria. The disease is widely distributed in a homogeneous way, not concentrated in the different subregions of the department.

1762

ANTIBACTERIAL EFFECT OF *CORRYOCACTUS BREVISTYLUS* (SANKY) AGAINST *ACINETOBACTER BAUMANII*

Hugo Carrillo-Ng[1], Ronald Aquino-Ortega[2], **Miguel A. Aguilar-Luis**[1], Wilmer Silva-Caso[1], Luz M. Paucar-Menacho[3], Juana M. del Valle[4]

[1]*Investigation Center and Innovation of the Health Sciences Faculty, Universidad Peruana de Ciencias Aplicadas (UPC), Av. San Marcos cdra* [2] *Cedros de Villa, Lima, Peru,* [2]*Instituto de Investigacion Nutricional, Lima, Peru,* [3]*Universidad Nacional del Santa, Av Universitaria, Nuevo Chimbote, Peru,* [4]*Universidad Peruana de Ciencias Aplicadas, Lima, Peru*

Acinetobacter baumanii is an oportunistic nosocomial pathogen with an increasingly rate of antibiotic resistance worldwide. In 2017, the WHO published a group of bacteria for which new research in antibiotics is urgently needed. *Acinetobacter baumanii* was included in first group among the bacteria that require the development of new antibiotics. *Corryocactus brevistylus*, is a peruvian cactacea with traditional medicinal use that grows in the Andes, however, its antibacterial effect agaisnt *Acinetobacter baumanii* has not been studied yet. The aim of this study was to determine the antibacterial effect of the methanol extract of the *Corryocactus brevistylus* fruit (Sanky) against *Acinetobacter baumanii (ATCC 19606).* The fruits of *Corryocactus brevistylus* were pulverized, soaked with methanol (1:2, w/v) and stored for 7 days. The antibacterial effect against *Acinetobacter baumanii* were evaluated using the cup-plate agar diffusion method by preparing wells with the experimental solutions cultivated in aerobic conditions for 24 h at 37 °C . Six independent tests were performed using ampicillin-sulbactam, tigecicline and tetracycline as positive controls. The MIC was determined using the microdilution method as described by the CLSI. Antibacterial effect of the methanol extract was observed with inhibition halos of 25.12 ± 0.52 mm. Meanwhile, ampicillin-sulbactam, tigecicline and tetracycline showed inhibition halos of 21.97 ± 0.18 mm, 22.36 ± 0.45 mm, 21.44 ± 0.28 mm, respectively. The minimum inhibitory concentration of the fruit extract was 101 mg/L. The *Corryocactus brevistylus* methanol extract showed a favorable antibacterial effect against *Acinetobacter baumanii*. These findings contribute to the potential development of new treatment options based on natural products. However, further studies are required to determine the bioactive compound responsible for this effect and the cytotoxicity of the extract.

1763

REPORT OF HIGH PREVALENCE OF *ACINETOBACTER BAUMANNII* INFECTION IN PEDIATRIC PATIENTS IN PERU

Miguel A. Aguilar-Luis[1], Isaac Peña-Tuesta[2], Juana del Valle-Mendoza[1], Victor Zavaleta- Gavidia[3]

[1]*Investigation Center and Innovation of the Health Sciences Faculty, Universidad Peruana de Ciencias Aplicadas (UPC), Lima, Peru,* [2]*Instituto de Investigacion Nutricional, Lima, Peru,* [3]*Dirección Regional de Salud de Cajamarca, Cajamarca, Peru*

Acinetobacter baumanni is an opportunistic nosocomial pathogen that has the ability to develop mechanisms of antibiotic resistance, including resistance to broad-spectrum β-lactamases and carbapenemases. The most common clinical manifestations are acute pneumonia and bacteremia, *A. baumannii* is ranked 1st in the critical priority category in the list of priority pathogens for research published by the World Health Organization (WHO). The objective of this study was to determine the prevalence of A. baumannii in hospitalized children under 1 year of age in Peru. A cross-sectional study was conducted in 286 children under 1 year of age with a clinical diagnosis of whooping cough admitted in 5 hospitals in Peru during January 2010 - July 2012. Samples of nasopharyngeal swab were collected with the help of a calcium alginate swab and samples They were immersed in phosphate buffer solution. The detection of A. baumannii was performed by Real Time PCR using primers specific for the OXA-51 gene. The amplified products were sequenced for confirmation. The presence of A. baumannii was detected in 19.1 % (48/252) of the samples. Children from 29 days to 3 months were the most affected, with a prevalence of 52.1 % (25/131), followed by 25% (12/71) in children whose age is 3 to 5 months. The most frequent symptoms in the positive cases were paroxysmal cough (85.4 %, 41/48), respiratory difficulty (79.2 %, 38/48), redness (68.8 %, 33/48), cyanosis (62.5 %, 30/48) and difficulty in breastfeeding (58.3 %, 28/48). It was found that 56.3 % (27/48) of the positive cases showed coinfection with *Bordetella pertussis*, 4.2 % (2/48) with *Mycoplasma pneumoniae* and respiratory syncytial virus type A and 2.1 % (1/48) with parainfluenza I. In conclusion, a high frequency of A. baumannii was detected in children recently admitted to hospitals in Peru. This result supports the report of the increase in infections of *A. baumannii* worldwide. It is concluded that it is necessary to carry out more studies on the infection of *A. baumannii* acquired in hospitals in order to determine the real prevalence in the Peruvian population.

1764

IDENTIFICATION OF THE INTESTINAL MICROBIOTA OF TYPE 2 DIABETIC PATIENTS CONTROLLED METABOLICALLY AND UNCONTROLLED

Katherine Cóndor-Marín[1], Angie J. Hamasaki-Matos[1], Ronal Aquino-Ortega[2], Hugo Carrillo-Ng[2], **Miguel A. Aguilar-Luis**[3], Juana M. del Valle-Mendoza[3]

[1]*School of Nutrition, School of Medicine. Research and Innovation Centre of the Faculty of Health Sciences, Universidad Peruana de Ciencias Aplicadas, Lima, Peru,* [2]*Instituto de Investigacion Nutricional, Lima, Peru,* [3]*School of Medicine. Research and Innovation Centre of the Faculty of Health Sciences, Universidad Peruana de Ciencias Aplicadas, Lima, Peru*

The increasing incidence of patients with the diagnosis of Diabetes Mellitus type 2 (DM2) has shifted the focus of new research on preventative therapeutic approaches. Recent evidence suggests an important association between the prognosis of patients with DM2 and their gastrointestinal bacterial microbiota linked to modifications that may positively or negatively change the host's metabolism. The study included 26 patients diagnosed with Diabetes Mellitus Type 2 in the Endocrinology service of a tertiary referral hospital, between August 2016 and February 2017. Stool samples were collected from each patient as well as their food consumption frequency record and relevant clinical data. The fecal bacterial microbiota was analyzed by conventional PCR to identify and characterize 13 different genus of gastrointestinal bacteria.

The mean body mass index (BMI) for the uncontrolled diabetic patients' group was 28.8 kg/m^2, considered as obesity for a mean age of 65 years. These patients had the highest incidence of associated comorbidities. We identified at least one genus of bacteria in 71.4 % and 57.9 % of cases in the controlled and uncontrolled group of diabetic patients respectively. In conclusion, the bacterial microbiota of a host is unique to each patient and varies constantly. Some differences were observed according to the state of the disease between the controlled and uncontrolled group.

1765

THE BURDEN OF AND RISK FACTORS FOR TRACHOMA IN SELECTED DISTRICTS OF ZIMBABWE: RESULTS OF 16 POPULATION-BASED PREVALENCE SURVEYS

isaac Phiri[1], Portia Manangazira[1], Nicholas Midzi[2]

[1]*Ministry of Health- Zimbabwe, Harare, Zimbabwe,* [2]*University of Zimbabwe, Harare, Zimbabwe*

Trachoma, a leading cause of blindness, is targeted for global elimination as a public health problem by 2020. In order to contribute to this goal, countries should demonstrate reduction of disease prevalence below specified thresholds, after implementation of the SAFE strategy in areas with defined endemicity. Zimbabwe had not yet generated data on trachoma endemicity and no specific interventions against trachoma have yet been implemented. Two trachoma mapping phases were successively implemented in Zimbabwe, with eight districts included in each phase, in September 2014 and October 2015. The methodology of the Global Trachoma Mapping Project was used. Our teams examined 53,211 people for trachoma in 385 sampled clusters. Of 18,196 children aged 1-9 years examined, 1526 (8.4%) had trachomatous inflammation-follicular (TF). Trichiasis was observed in 299 (1.0%) of 29,519 people aged ≥15 years. Of the 16 districts surveyed, 11 (69%) had TF prevalences ≥10% in 1-9-year-olds, indicative of active trachoma being a significant public health problem, requiring implementation of the A, F and E components of the SAFE strategy for at least 3 years. The total estimated trichiasis backlog across the 16 districts was 5506 people. The highest estimated trichiasis burdens were in Binga district (1211 people) and Gokwe North (854 people). In conclusion, implementation of the SAFE strategy is needed in parts of Zimbabwe. In addition, Zimbabwe needs to conduct more baseline trachoma mapping in districts adjacent to those identified here as having a public health problem from the disease.

1766

QUALITY ASSURANCE THROUGH POSTOPERATIVE FOLLOW-UP OF OPERATED TRACHOMATOUS TRICHIASIS (TT) CASES IN BURKINA FASO AND CAMEROON

Whitney Goldman[1], Assumpta Lucienne Bella[2], Clarisse Bougouma[3], Emilienne Epée[2], Martin Kabore[3], Issouf Bamba[4], Fanny Yago-Weinne[4], Jean-Paul Djiatsa[4], Albert Kiemde[4], Phylippe Bayala[4], Marc Sepama[4], Julie Akame[5], Jules Patrick Evenga[5], Michel Hendji[5], Yannick Nkoumou[5], Carine Fokam Tagne[5], Stephanie Parker[1]

[1]*Helen Keller International, Washington, DC, United States,* [2]*Ministry of Health, Cameroon, Yaounde, Cameroon,* [3]*Ministry of Health, Burkina Faso, Ouagadougou, Burkina Faso,* [4]*Helen Keller International, Burkina Faso, Ouagadougou, Burkina Faso,* [5]*Helen Keller International, Cameroon, Yaounde, Cameroon*

Ensuring high-quality trachomatous trichiasis (TT) surgery is essential for trachoma programs. As one of several quality assurance activities, the U.S. Agency for International Development (USAID)'s Morbidity Management and Disability Prevention Project has collaborated with ministries of health in Burkina Faso and Cameroon since 2016 to implement postoperative follow-up of individuals 3-6 months after they receive TT surgery. In 2017, the project revised its approach of a single 3-6 month follow-up activity targeting a random sample of operated cases to conducting two distinct activities: surgical audit and outcome assessment. Outcome assessments aim to provide clinical follow-up 3-6 months following TT surgery by organizing appointments at a centralized site. Surgical audits assess individual surgeons' performance across a sample of patients who are visited in their homes. In Cameroon, when surgical output did not allow for auditing the minimum recommended number of cases per surgeon, the project implemented a single activity that integrated surgical audit and outcome assessment principles. From 2017 onward, approaches to outcome assessment and surgical audit were iteratively improved through adjustments such as increasing time spent at each outcome assessment site and providing phone credit to increase contact between health staff and operated cases. Overall, the average proportion of operated cases receiving a 3-6 month follow-up exam increased following the shift from the original approach to the revised approach (from 39% to 57% in Burkina Faso and from 38% to 74% in Cameroon). Through the refinement of the approach to outcome assessment in Burkina Faso, specifically, the proportion of cases self-presenting increased from 16% to 53%. These results indicate that the shift to the revised strategy was followed by an improvement in the rates of follow-up within the 3-6 month postoperative window. In addition, iterative adjustments to the revised strategy implemented in Burkina Faso were followed by an increased proportion of operated cases self-presenting for follow-up 3-6 months after surgery.

1767

USING PHOTOS OF OPERATED TRACHOMATOUS TRICHIASIS (TT) CASES AS A TOOL TO FACILITATE SURGEON AND TECHNICAL SUPERVISOR DISCUSSION

Whitney Goldman[1], Assumpta Lucienne Bella[2], Clarisse Bougouma[3], Emilienne Epée[2], Martin Kabore[3], Issouf Bamba[4], Jean-Paul Djiatsa[4], Albert Kiemde[4], Phylippe Bayala[4], Marc Sepama[4], Julie Akame[5], Jules Patrick Evenga[5], Michel Hendji[5], Yannick Nkoumou[5], Carine Fokam Tagne[5], Lauren Johnson[1], Katherine Nerses[1], Stephanie Parker[1], Emily Gower[6]

[1]*Helen Keller International, Washington, DC, United States,* [2]*Ministry of Health, Cameroon, Yaounde, Cameroon,* [3]*Ministry of Health, Burkina Faso, Ouagadougou, Burkina Faso,* [4]*Helen Keller International, Burkina Faso, Ouagadougou, Burkina Faso,* [5]*Helen Keller International, Cameroon, Yaounde, Cameroon,* [6]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Since 2018 the U.S. Agency for International Development (USAID)'s Morbidity Management and Disability Prevention Project has collaborated with ministries of health in Burkina Faso and Cameroon to pilot photographing operated eyelids during trachomatous trichiasis (TT) surgery and during 3-6 month post-operative follow-up exams. Surgeon and technical supervisor feedback during the pilot suggested photos could potentially be useful to facilitate technical supervision and generate feedback to support surgical capacity strengthening. To explore this potential utility, each country held a photo review and feedback session in Nov.-Dec. 2018. Surgeons and supervisors followed a structured facilitator guide (with questionnaires) to conduct a technical review of a sample of photographed eyelids (22 eyelids in Burkina Faso and 41 in Cameroon). Surgeons independently assessed characteristics visible in the photos of eyelids they had previously operated; technical supervisors then independently assessed the same photos and provided feedback to the surgeons on the observed characteristics. Surgeons and supervisors had complete agreement in their assessment of the overall surgical quality for 64% of eyelids (14/22) in Burkina Faso and 56% of eyelids (19/34) in Cameroon. Among the six characteristics assessed, rates of agreement were highest for granuloma (98%) and post-operative TT (82%) and lowest for assessing eyelid margin regularity (65%). Surgeons more frequently failed to identify issues identified by supervisors. All completed questionnaire responses rated the technical review as "Very useful" and surgeons noted "Yes" when asked if they learned something about their surgical capacity. The structured review revealed supervisors and surgeons did not have complete agreement on the overall quality of the surgery. The facilitator guide and questionnaires for photo review provide a framework

for technical supervisors and surgeons to discuss surgical quality and post-operative complications to potentially support surgical capacity strengthening.

1768

TRACHOMA PREVALENCE FOLLOWING DISCONTINUATION OF MASS AZITHROMYCIN DISTRIBUTION

William W. Godwin[1], Paul M. Emerson[2], Pamela J. Hooper[3], Ana Bakhtiari[3], Michael Deiner[1], Travis C. Porco[1], Thomas M. Lietman[1], Catherine E. Oldenburg[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Emory University, Atlanta, GA, United States,* [3]*International Trachoma Initiative, Decatur, GA, United States*

Mass Drug Administration (MDA) regimens of azithromycin have proved to be effective in reducing the prevalence of trachomatous inflammation—follicular (TF) in children age 1-9 years. While there is evidence that the number of rounds of MDA can predict TF decline in locations of continuous MDA, little is known about how TF prevalence behaves in locations of discontinued MDA. Current guidelines suggest regular regimens of MDA in locations above 10% TF until reaching "control" (<5%). However, TF may decline in borderline prevalence areas without additional antibiotic intervention. Here, we evaluate the effect of discontinuation of antibiotic on TF prevalence in formerly endemic areas. We used district-level data from the International Trachoma Initiative database to assess how TF prevalence responds after discontinuation of MDA. Districts were included in the analysis if they had at least one year of MDA, followed by two annual surveys in which there was no MDA between the surveys. A total of 233 districts fit the inclusion criteria. Mean number of years between most recent MDA and initial survey was 1.5 and mean number of years between initial survey and follow-up survey were 3.2. Mean (SD) TF prevalence was 2.4% (2.0) and 2.1% (2.7) for initial and follow-up survey, respectively. Between initial and follow-up survey, TF prevalence decreased by >50% in 37.3% (87/233) of the districts. Of the 19 districts with baseline TF >5%, 15 districts achieved control, while 9.3% (20/219) of districts with TF control at baseline increased to >5% at follow-up. Sixteen of the 20 districts that increased from control status to above control status were in Ethiopia and Niger. In most districts, resurgence of TF in the absence of continued MDA does not appear to occur. However, in some districts, predominantly in Ethiopia and Niger, TF has increased above control status. This may be due to misclassification error or, less likely, true resurgence. Understanding the degree to which TF may display resurgence and factors associated with resurgence will be important to achieving global TF control in a timely, resource-efficient manner.

1769

HOW TO DETERMINE WHERE TO SURVEY FOR TRACHOMA: LESSONS LEARNED FROM THE DEMOCRATIC REPUBLIC OF CONGO

Bonaventure Ngoyi[1], Pitchouna A. Uvon[2], Janvier N. Kilangalanga[3], Felix Makangila[2], Katie Crowley[4], Raymond Stewart[1], Jeremiah M. Ngondi[4]

[1]*RTI International, Kinshasa, Democratic Republic of the Congo,* [2]*Ministry of Health, Kinshasa, Democratic Republic of the Congo,* [3]*Saint Joseph Hospital, Kinshasa, Democratic Republic of the Congo,* [4]*RTI International, Washington, DC, United States*

Until 2014, trachoma was suspected to be endemic in the Democratic Republic of Congo (DRC); however, evidence to support program initiation was limited. Health zones (HZ) were prioritized for surveys in a phased approach. Phase I (2014-2015) identified priority HZ based on a desk review of Global Atlas of Trachoma, local clinical reports on trachoma, results of a questionnaire-based survey of health care workers (HCW) enquiring about known trichiasis cases and a questionnaire-based community key informants' interviews (KII) during transmission assessment surveys (TAS) enquiring about the presence of trichiasis. Phase II (2016-2017) comprised HZs bordering those DRC HZ identified as endemic in the Phase I surveys. Phase III (2017-2018) HZ were selected based on the results of trachoma rapid assessments (TRA) conducted in HZ contiguous to those mapped in Phases I & II. We analyzed the distribution of surveys results by prioritization method. From 2014 to 2018, surveys were conducted in 111 HZ (21% of all DRC HZ) and TRA was conducted in 66 HZ. Survey results indicated that 51 HZ (45.9%) of the 111 surveyed HZ had trachomatous inflammation-follicular (TF) prevalence ≥ 5% and 55 HZ (49.5%) had trachomatous trichiasis (TT) prevalence ≥ 0.2%. By phase the number of HZ mapped were: 31 in phase I; 50 in phase II; and 30 in phase III. TF prevalence varied by prioritization method. The proportion of HZs with TF≥5% was as follows: review of health records and HCW trichiasis survey (100%); HZ bordering endemic district across countries (71.4%); HZs bordering endemic ones after Phase I mapping (44.4%); community trichiasis KII survey during TAS (35.3%); and TRA (33.3%). The results illustrate that a variety of methods can be employed to inform prioritization of investments for mapping in settings like DRC where vast areas are still un-mapped. This DRC case study provides important lessons for countries that have not yet started mapping, no completed mapping, and strategies to be considered in under-resourced settings.

1770

VALIDATING AND COSTING A TRACHOMATOUS TRICHIASIS "SUPER SURVEY"

Rebecca Mann Flueckiger[1], Rachel Stelmach[2], George Kabona[3], Alistidia Simon[3], Upendo Mwingira[3], Jeremiah Ngondi[4]

[1]*RTI International, Atlanta, GA, United States,* [2]*RTI International, Washington, DC, United States,* [3]*Tanzania NTD Control Programme, Dar es Salaam, United Republic of Tanzania,* [4]*RTI International, Dar es Salaam, United Republic of Tanzania*

As trachoma programs move towards elimination, the number of surveys necessary to evaluate the status of trachomatous trichiasis (TT) is growing. Currently, the World Health Organization recommends a district-level population-based prevalence survey for trachoma that involves a two-stage cluster design. Previous work has demonstrated that a random selection of 30 clusters within a district suffices for measuring TT prevalence with precision against the elimination threshold. In an effort to reduce survey costs, we explored the validity of an alternative survey design and the associated cost. There are scenarios where multiple districts are geographically contiguous and homogeneous. We determined homogeneity in terms of TT through grouping districts with consistent climatic, environmental and socioeconomic variables. We evaluated the loss of precision involved in combining these districts into single evaluation units (EUs) and modulating the sample size by running simulations on existing data sets. Preliminary findings from two scenarios in Tanzania show variability in when it is appropriate to implement this alternative survey design. In scenario A, which evaluated Babati DC and Mbulu DC, the mean TT prevalence estimate maintained the same likelihood to be above the elimination threshold (TT prevalence <0.2% in adults >=15 years old) when the districts were combined into a single EU and the sample size was decreased to 80% of the original sample. However, in scenario B, which evaluated Iringa DC, Kilolo DC and Mufindi DC, this was not the case. These preliminary findings stress the importance of determining climate, environmental and socioeconomic characteristics associated with classifying districts as homogeneous in terms of TT. Learnings from this first portion of work will be carried forward for testing in additional settings and the cost saving of this approach will be determined prior to ASTMH.

1771

USING DATA FROM DOOR TO DOOR (*RATISSAGE*) TRACHOMATOUS TRICHIASIS SURGERY CAMPAIGNS TO DEMONSTRATE ACHIEVING ELIMINATION CRITERIA IN MALI

Lamine Traoré[1], Modibo Keita[2], Benoit Dembele[2], Famolo Coulibaly[1], Mamadou Dembele[1], Boubacar Guindo[2], Dramane Traoré[2], Brehima Mariko[1], Seydou Goita[2], Abdoul Karim Sidibé[1], Fama Kondo[2], Mama Niele Doumbia[2], Mohamed Lamine Yattara[2], Yaobi Zhang[3], Steven Reid[4]

[1]*Ministère de la Santé et de l'Hygiène Publique, Bamako, Mali,* [2]*Helen Keller International, Bamako, Mali,* [3]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [4]*Helen Keller International, New York, NY, United States*

As Mali nears trachoma elimination, the Malian National Eye Health Program (PNSO) has conducted door-to-door (*ratissage*) outreach in each health district (HD) where the trichiasis (TT) prevalence remains above the WHO elimination threshold of 0.2% according to the latest survey results. Following these surgical outreach campaigns, to demonstrate that the elimination criteria have been attained, an additional survey or TT only survey can be performed. However, surveys provide a point estimate in TT prevalence in a limited number of people and often have wide confidence intervals. In Mali, Kayes region is one of the regions affected most by trachoma with a large backlog of patients who need TT surgery. Following WHO guidelines, trachoma surveillance surveys (TSS) conducted in 2017 in Diéma and Yélimané health districts (HDs) indicated that the prevalence of trachomatous inflammation-follicular (TF) in children aged 1-9 years in was 0.08% and 0.36% respectively. However, the TT prevalence was 0.53% in Diéma and 0.41% in Yélimané. The PNSO estimated the number of patients requiring TT surgery (the backlog) was 450 in Diéma and 241 in Yélimané. The PNSO conducted *ratissage* campaigns in these HDs to screen and find TT patients and offer them surgical services. During the ratissage campaigns, the PNSO screened 131 out of 146 (90%) villages in Diéma and 93 out of 98 (95%) villages in Yélimané. In Diéma 128,747 adults out of an estimated adult population of 131,846 were screened (97.6% coverage) and 248 new TT cases were detected (0.19% TT). In Yélimané 105,945 adults were screened out of an estimated adult population of 121,459 (coverage of 87.2%) and 110 new TT patients discovered (0.1%). These data indicate that, in contrast to the prevalence estimate from the TSS in 2017, where 1,501 and 1,456 adults were surveyed in Diéma and Yélimané respectively, the criteria for TT elimination has been reached. Given the larger number of people screened for TT, these ratissage data are more compelling evidence than a TSS or TT-only survey and therefore could be used in the trachoma elimination dossier as evidence of achieving the elimination criteria.

1772

TRACHOMATOUS TRICHIASIS MANAGEMENT IN TANZANIA: INVESTIGATION OF THE PRODUCTIVITY OF CASE FINDING AND REFERRAL OF PATIENTS TO SURGERY SERVICES

George Kabona[1], Jeremiah Ngondi[2], Alistidia Simon[1], Upendo Mwingira[1], Rebecca Flueckiger[3]

[1]*Tanzania NTD Control Programme, Dar es Salaam, United Republic of Tanzania,* [2]*RTI International, Dar es Salaam, United Republic of Tanzania,* [3]*RTI International, Atlanta, GA, United States*

Prolonged conjunctival infection with Chlamydia trachomatis leads to an inflammatory response, trachomatous inflammation follicular (TF). Overtime, repeat infection can progress to scarring of the conjunctiva causing the eyelid to turn inward, resulting in lashes rubbing against the cornea. This stage of the disease is called trachomatous trichiasis (TT). TT can damage the cornea, leading to vision impairment or blindness. However, TT can be managed through quality surgery. The continuum of care involves: (1) identification of a case by case finder, (2) identified case screened by eye care professional, (3) identified case confirmed by TT surgeon, and (4) identified case receives surgery. To initiate this continuum of care, district-level prevalence is estimated through population-based prevalence surveys (PBPS) and after a period of intervention another PBPS is conducted. The Tanzania NTD Control Programme has found high TT prevalence after intervention in several districts. To understand why targets are not being achieved and provide learnings for informing program adaptations to improve access to services a mixed method study is underway. The study involves a retrospective review and analysis of program data and implementation of key informant interviews (KIIs). The quantitative analysis will provide evidence around which factors influence success in a district. This information will be interpreted and used for identifying recommendations for improving equitable access to services, lessening the gap between linking to services and receiving surgery, and explore better strategies for creating service demand. The KIIs will provide contextual information around the quantitative findings. In addition, they will provide insight into how linkage and receival of surgery could be more equitable and what the current barriers/facilitators are. The KIIs among case finders, surgeons and particularly among district-level health officials will provide much needed insight into how TT case finding, and surgical delivery can be incorporated into existing disease control programs and service delivery platforms.

1773

HOUSE-TO-HOUSE CASE FINDING FOR TRICHIASIS SURGERY IN AMHARA REGIONAL STATE, ETHIOPIA: AN END GAME STRATEGY

Eshetu Sata[1], Yirga Bieza[1], Ayalew Shiferaw[1], Berhanu Melak[1], Sintayehu Aweke[1], Abebe Fissha[1], Mengesha Halefom[1], Mulat Zerihun[1], Temesgen Minas[1], Tedla Desta[1], Getachew Mekonnen[1], Demis Assegie[1], Scott D. Nash[2], Aisha E.P. Stewart[2], Zerihun Tadesse[1], E. Kelly Callahan[2], Melkamu Beyene[3]

[1]*The Carter Center, Addis Ababa, Ethiopia,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*Amhara Regional Health Bureau, Bahir Dar, Ethiopia*

Trachomatous trichiasis (TT) surgery is one of the WHO endorsed SAFE strategies to treat the blinding stage of trachoma. Since 2001, the Amhara Regional Health Bureau, in collaboration with The Carter Center, operated more than 680,000 TT cases throughout the region. As the backlog of TT declines, case finding has become increasingly challenging as cases often live in remote areas. To reach these cases, this study piloted an intensive house-to-house case finding approach and assessment of reasons for refusing surgery in 31 districts. The estimated TT backlog in the 31 districts was 52,387 cases, 32.7% of the regional backlog. In 23 of the districts case finding was done by trained case finders who were selected from the community, and in the remaining districts health extension workers (HEWs) served as case finders. The case finders moved from house-to-house district-wide, targeting 100% coverage of both households and adults 15 years and older. Between October and December 2018, 871,377 households (85% of the household target) were visited and 2,093,860 people above 15 years (82% of the population target) were examined for TT. 85,303 suspected TT cases (4% of the examined people) were registered; 70,222 (82% of suspect cases) were mobilized to surgery sites; and 15,129 (22%) were confirmed to have TT. Of the confirmed TT cases, 10,255 (68%) were operated, 2,779 (18%) refused the service and, for the remaining cases, surgery was postponed. The number of TT cases confirmed was considerably lower than the estimated TT backlog. The house-to-house case finding showed an estimated TT backlog of 18,536 in the 31 districts. The case detection rate by HEWs/health workers and case finders was 50% and 18%, respectively. The majority of the refusals were due to participation in harvesting and household tasks. Findings from the house-to-house case finding suggest the approach is an effective way to reach cases living in remote areas. Future plans include mop-up in areas with low mobilization, addressing refusals and examining the feasibility of applying the house-to-house strategy in other areas.

1774

MASS DRUG ADMINISTRATION OF AZITHROMYCIN FOR TRACHOMA ALONG THE CROSS-BORDER COMMUNITIES OF KENYA AND UGANDA IN MARCH/APRIL 2019

Getrude Nasike Barasa[1], Daniel Esimit Echakan[1], Samson Lokele Akichem[1], Hadley Sultani Matendechero[2], Peter Otinda[3]

[1]*Turkana County Government, Lodwar, Kenya,* [2]*Neglected Tropical Diseases Unit, Nairobi, Kenya,* [3]*Sight Savers Kenya, Nairobi, Kenya*

Trachoma an eye disease caused by infection with the bacterium Chlamydia trachomatis, is a public health problem responsible for irreversible blindness or visual impairment with 158 million people living in endemic areas being at risk (WHO). In Kenya, Turkana is one of 6 prevalent counties, with about 1.25 million people at risk. The multi-sectoral SAFE (Surgery, Antibiotics, Face washing and Environmental cleanliness) strategy is implemented in collaboration with Queen Elizabeth Trust. With six mass drug administration campaigns (MDA) within 2011-2019 and an average coverage of 82.9%, the lowest recorded in two sub counties bordering Uganda in Jan 2019, was attributed to migration of border populations in search for pasture. The objective for the cross-border MDA exercise was to ensure the missed pastoralists are followed into Uganda. This was an eight day ecological case study serving as the baseline for future cross border activities, targeting regions along the border in 6 districts with 3 of these bordering Turkana County. Data was compiled and presented in Ms. Excel. The activity was integrated with immunization and Trachoma trichiasis screening. Across 3 districts, a total of 15,497 (61.9%) people were reached and administered with Azithromycin (tablets or syrup) or Tetracycline ointment. A total of 30 children were immunized, 34 pregnant women given Ante-Natal Care services, 63 treated for other ailments and 2 Trachoma trichiasis cases identified and referred for surgery. This first cross border exercise, reassured good will between two countries for future collaborations. Challenges were: insecurity in some areas, unreachable hard to reach areas, inadequate time for mobilization and implementation, inadequate resources among others. Positively, it facilitated linkage between counterpart facilities along the border for communication and future planning especially integrated outreaches. Need to plan alongside these migratory patterns to ensure there are effective intervention measures that enable continuity of health services among these highly mobile populations was recommended.

1775

TRACHOMA ELIMINATION IN CAMEROON: RESULTS FROM A BASELINE MAPPING OF A REFUGEE CAMP IN MINAWAO

Emillienne Epée[1], Bella Assumpta[1], Georges Nko'Ayissi[1], Mahamat Fane[1], Julie Akame[2], Patrick Mbia[2], Carine Fokam[2], Steven D. Reid[3], Yaobi Zhang[4], Jean Jacques Tougué[5], Ismael Teta[2]

[1]*Ministry of Public Health, Yaoundé, Cameroon,* [2]*Helen Keller International, Yaoundé, Cameroon,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [5]*RTI International, Washington, DC, United States*

Baseline trachoma surveys conducted prior to 2012 in the Far North and North regions of Cameroon revealed that 21 health districts (HDs) were endemic. The Cameroon ministry of health, with financial support from USAID implemented mass drug administration (MDA) with azithromycin in these HDs where the trachomatous inflammation - follicular (TF) prevalence was >5%. Impact surveys carried out between 2014 and 2017 revealed that all 21 HDs had reached the criteria for stopping MDA (TF<5%). However, as a result of ongoing insecurity in the area, there was an influx of people, mostly from a trachoma-endemic HD of Nigeria, to a refugee camp in Minawao in the Far North region. As Cameroon nears trachoma elimination, trachoma baseline surveys in Minawao, and other refugee camps, were necessary to document any need for intervention. During August 2018, a baseline survey, was conducted, following WHO guidelines, to estimate the prevalence of TF in children 1-9 years and trachomatous trichiasis (TT) in adults ≥15 years in the Minawao camp. The prevalence of TF in 1,167 children aged 1-9 years, examined for trachoma, was 0% while the prevalence of TT among 3,070 adults examined was 0.71%. The survey results of this survey in the Minawao refugee camp in the Far-North region reveal that TF in children was not a public health problem. However, TT prevalence was above the WHO elimination threshold of 0.2%, which suggests that TT surgery intervention was needed. The National Program will plan be required interventions for trichiasis surgery outreach to achieve the goal of eliminating trachoma as a public health problem.

1776

TRACHOMA PREVALENCE IN REFUGEE CAMPS IN THE EAST REGION OF CAMEROON

Assumpta Bella[1], Sidi Coulibaly[2], Georges Nko'Ayissi[1], Bidjang Mathurin[1], Julie Akame[3], Carine Fokam[3], Patrick Mbia[3], Ismael Teta[3], Yaobi Zhang[4], Jean Jacques Tougué[5], Steven D. Reid[6]

[1]*Ministry of Public Health, Yaoundé, Cameroon,* [2]*Independent Consultant, Bamako, Mali,* [3]*Helen Keller International, Yaoundé, Cameroon,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [5]*RTI International, Washington, DC, United States,* [6]*Helen Keller International, New York, NY, United States*

Cameroon aims to eliminate trachoma as a public health problem by 2020 thus, reviewing trachoma epidemiology in areas previously not suspected to be endemic is critical. The National Blindness Control Program (PNLCé) carried out baseline mapping in six HDs from East region to estimate TF and trachomatous trichiasis (TT) prevalence as these HDs border highly endemic HDs (range 6.8%- 32.3%) in the Central African Republic (CAR). These HDs had been declared non-endemic and no baseline survey was carried out during initial mapping. Four of these HDs house five refugee camps which were established in 2014. The refugee camps were grouped as one evaluation unit (EU). Using a 2-stage cluster random survey design, refugee camps were subdivided into clusters, defined as camp blocks. Thirty clusters were randomly selected and 30 households were sampled in each cluster. Household residents aged 1 year and above were examined for signs of trachoma. The data were recorded using the Tropical Data (TD) software and then transferred to the TD platform for analysis. Prevalence estimates were adjusted for age and sex. Among 1,150 children aged 1 to 9 years examined, TF prevalence was 0.49% (95% confidence interval [CI] 0.1-1.0). Among 1365 adults aged ≥15 years examined, prevalence of TT unknown to the health system was 0.19% (95% CI 0.01-0.47). Of the surveyed households: 100% had an improved drinking water source; 95.5% has a water source less than 1km away; and 89.6% had improved sanitation facilities. Although the refugee camps have populations from trachoma endemic HDs of CAR, the lower than expected TF prevalence observed suggest that camps have lower risk of trachoma possibly due to good access to water and sanitation facilities. No trachoma control activities are warranted in these refugee camps.

1777

OUTCOMES WITHIN AN ANEMIA SCREENING AND TREATMENT SERVICE EMBEDDED IN A WELL-BABY CLINIC IN THE DOMINICAN REPUBLIC

John D. McLennan[1], Maria Mosquea[2]

[1]*Children's Hospital of Eastern Ontario - Research Institute, Ottawa, ON, Canada,* [2]*Servicio Nacional de Salud, Santo Domingo, Dominican Republic*

Despite the prevalence and importance of child anemia, there are few published reports on process and outcome indicators from child anemia management in primary care services. A few available reports from US clinics serving low-income communities suggest substantial quality care gaps. The aims of this study were to: (1) examine outcome indicators for child anemia management in a well-baby clinic in the Dominican Republic, and (2) determine whether a set of variables predicted change in hemoglobin (Hb) at follow-up. The study setting was a well-baby clinic in a low-income community in the Dominican Republic which offered free anemia screening and treatment with ferrous sulfate. Data available on children who were screened through a complete blood count (CBC)

between 11-23 months of age were analyzed. Baseline hematological parameters, treatment variables, pre-post changes in hematological parameters, and predictors of Hb change were determined. Of 135 eligible children, 80.7% (n=109) had Hb <11.0g/dl at the screening point. Among this anemic subgroup, 75.2% (n=82) had a follow-up CBC between 16-52 weeks later. Ferrous sulfate was provided to the child caregivers of 98.9% (n=81) of this group with an aim for a 12 week course of treatment. Only a modest mean increase in Hb (0.9g/dl) was found at follow-up for this group and many (72.0%) still had Hb<11.0g/dl. Lower Hb at baseline predicted greater Hb change at follow-up, but other hypothesized predictors were not significant. Although attainment on some key treatment processes were higher than values reported in some previous health services studies (e.g., fraction of anemic children receiving iron and follow-up), the amount of associated change in Hb was smaller than observed in clinical trials of iron treatment for anemia. Further determination of realistic outcomes that can be achieved with a simple anemia screen and iron provision approach is warranted, including consideration of more refined, but still cost-effective, strategies.

1778

MORBIDITY AMONG SPECIAL AIR SERVICE (SAS) PERSONNEL DURING THE MALAY EMERGENCY

David Adams, Valerie Adams

Point University, Midway, GA, United States

Morbidity among Special Air Service (SAS) Personnel during the Malay Emergency The Malay Emergency, a counterinsurgency effort mounted by British and Commonwealth troops between 1948 and 1960, proved to be one of the more anti-communist efforts by Western forces. The anti-colonial struggle, mounted by the Malayan Communist Party's militant Malayan National Liberation Army (MLNA), had begun on 1948 to attack British and Commonwealth-owned rubber plantations and mines. Ironically, the "emergency" was termed such—rather than calling it a "war"—because insurers would have refused to cover the damages had it been deemed anything other than an "emergency". Hence, the label stuck. This presentation, based on archival data, will examine morbidity among the SAS (Special Air Service) in the early 1950s. This case study highlights the extreme difficulty of maintaining combat-readiness among even the most highly trained troops of that time.

1779

ENHANCING VACCINE IMMUNOGENICITY AND STABILITY USING A GEL-DEPOT ADJUVANT

Vanessa Silva-Moraes[1], Lisa M. Shollenberger[2], Jessica C. Ramadhin[1], Ted M. Ross[1], Justine C. Shiau[1], Ashutosh K. Pathak[1], Demba Sarr[1], Courtney Murdock[1], Donald E. Champagne[1], Evelina Angov[3], Donald A. Harn[1]

[1]*University of Georgia, Athens, GA, United States,* [2]*Old Dominion University, Norfolk, VA, United States,* [3]*Walter Reed Army Institute for Research, Silver Spring, MD, United States*

Infectious diseases are the most common illnesses plaguing low- to middle-income countries. In this regard, vaccines offer the most reliable and cost-effective method for improving health outcomes. One of the challenges related to vaccine use is the maintenance of cold-chains, which limit reliable storage of vaccines in low resource settings. Freeze-dried vaccines have become attractive, but some antigens are sensitive to this process. Our lab has pioneered VacSIM (patent 9,566,338), a self-assembling matrix which uses the (RADA)4 polypeptide as a non-immunostimulatory adjuvant. VacSIM increases vaccine persistence, enhances immune responses, and eliminates local and systemic reactogenicity caused by molecular adjuvants. Herein, we evaluated maintenance of immunogenicity and efficacy after both lyophilization and long-term storage of protein-based vaccines mixed with VacSIM. Initial trials to study the feasibility of freeze-drying antigens mixed with VacSIM were performed using the model antigen ovalbumin (OVA). We found that we could store lyophilized OVA/VacSIM for up to 36 months at 37C with no loss of immunogenicity. We next evaluated the ability to freeze-dry malaria (*Plasmodium berghei* CelTOS and CSP) and influenza (N/C rHA) in VacSIM. Compared to conventional delivery of these 3 antigens, we found VacSIM enhanced the humoral response three-fold more after prime-boost, and lyophilization did not alter the immune response. To determine if freeze-drying influences vaccine efficacy, we have just initiated our challenge studies. For influenza HA vaccine, BALB/c mice were challenged with 1 x 10^7 pfu of influenza virus from N/C strain and for malaria CelTOS and CSP vaccines, mice were challenged with 1000 *P. berghei* sporozoites. We are currently evaluating vaccine efficacy. Future work includes assessing T-cell responses and determining the effect of long-term storage (1 to 36 months at 4, 20 and 37C) on these vaccines. If effective, these experiments and results will aid our long-term goal of generating functional thermostable subunit human vaccines in a cost-effective manner for global infectious diseases.

1780

INTEGRATING OPT-OUT HEPATITIS C SCREENING WITH EMERGENCY SERVICES FOR HIGH RISK POPULATIONS

Austin T. Jones[1], Lisa Moreno-Walton[2], Kanayo R. Okeke-Eweni[2], Jenna Miller[2], Dylan Soderstrum[2], Patricia Kissinger[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Louisiana State University, New Orleans, LA, United States*

Hepatitis C virus (HCV) chronically infects 71 million people worldwide. Updated World Health Organization (WHO) guidelines recommend a "Treat All" approach to eliminate HCV by 2030, calling for 90% of cases to be identified and 80% to be treated. The objective of the study was to evaluate the effectiveness of an emergency department opt-out HCV screening program at identifying HCV+ patients and linking them to health services. A retrospective cohort study was conducted. Patients who tested HCV antibody-reactive in the University Medical Center Emergency Department in New Orleans, LA from March to December 2015 were included. Outcomes measured include retention along the HCV care cascade and time to follow-up. A total of 902 patients screened HCV antibody-reactive. The majority had not been previously tested for HCV (67.5%). Of those antibody-reactive, 855 (94.8%) completed viral load testing. Median follow-up time was less than 1 day (IQR 7). Of those who received RNA testing, 633 (74.0%) were found to be chronically infected, while 222 (26.0%) had spontaneous viral clearance. Of those chronically infected, 294 (46.4%) followed up with radiology for hepatic ultrasound. Median radiology follow-up time was 183 days (IQR 287) post-screening. Primary care was attended by 192 patients (30.3%). Median primary care follow-up time was 136 days (IQR 253) post-screening. Viral hepatitis clinic was attended by 192 patients (30.3%). Median viral hepatitis clinic follow-up time was 173 days (IQR 261) post-screening. Treatment was initiated in 80 patients (12.6%). Median time to begin treatment was 302 days (IQR 403) post-screening. Sustained virologic response (SVR) was achieved in 59 patients (9.3%). Median time to SVR was 419 days (IQR 545) post-screening. Opt-out screening successfully captures previously undiagnosed patients and links them to curative treatment. Integration of HCV screening with emergency services is an effective delivery model, supporting the WHO recommendation to incorporate HCV screening with existing health services. Updated 2018 WHO guidelines for HCV and lessons applicable to resource-limited areas will be discussed.

1781

TRACHOMATOUS TRICHIASIS (TT) CASE FINDERS AND THEIR IMPACT IN PATIENT IDENTIFICATION AND UPTAKE OF SURGICAL SERVICES. CASE STUDY OF DODOMA AND LINDI REGIONS OF TANZANIA

Alistidia Simon[1], Hope Rusibamayila[1], Jeremiah Ngondi[2], Upendo Mwingira[1], Jennifer Harding[3], Harran Mkocha[4], Peter Kivumbi[5], Gosbert Katunzi[6], George Kabona[7], Andreas M. Nshala[8]

[1]*NTD Control Program, Dar Es Salaam, United Republic of Tanzania,* [2]*RTI, Washington, DC, United States,* [3]*HKI, Dar Es Salaam, United Republic*

of Tanzania, [4]Kongwa Trachoma Project, Dodoma, United Republic of Tanzania, [5]Sight Savers, Dar Es Salaam, United Republic of Tanzania, [6]Sightsavers, Dar Es Salaam, United Republic of Tanzania, [7]Iringa Regional Hospital, Iringa, United Republic of Tanzania, [8]Uppsala University, Uppsala, Sweden

The World Health Organization (WHO) targets global elimination of trachoma as a public health problem by the year 2020 through the SAFE (surgery, antibiotics, facial cleanliness, environmental change) strategy. Since inception of SAFE in 1999, Tanzania has implemented trichiasis surgical services based on WHO guidelines with the aim of achieving GET 2020 goals. According to WHO elimination of trachomatous trichiasis (TT) is considered when the prevalence of trichiasis cases un-known to the health system is <0.2% in people aged 15 years and above. In Tanzania TT surgical services are provided mainly through camps. Cases are identified through two different approaches: active Case finders; and Community sensitization. Case finders are trained to search for TT patients in their community and report back the list of patients identified to the nearest health facility. The Case finder searches for patients through house-to-house approach. Community sensitization on the other hand, is usually done through village meetings and road show announcements to invite all people with eye problems to show up during the planned camp at their designated health facility. Cultural leaders are very influential people in calling such village meetings. The Case finder approach has showed promising results with increased patient identification, increased surgical uptake and substantial declines in TT backlog. Since implementation of case finder approach Lindi and Dodoma regions in 2016 a total of 911,132 people have been screened and 9,064 were brought to the camp by case finders and 5,429 surgeries have been done. TT burden estimates suggest the backlog has been reduced from 5,150 cases in 2016 to 2,924 cases in 2018. The case finder approach was effective in identification and referral of TT cases for surgery and will facilitate elimination of trachoma in Tanzania.

1782

INTEGRATING CHAGAS DISEASE CARE INTO PRIMARY CARE: THE STRONG HEARTS/CORAZONES FUERTES/CORAÇÕES FORTES PROJECT IN BOSTON, MASSACHUSETTS

Jillian Davis[1], Jennifer Manne-Goehler[2], Juan Huanuco Perez[1], Ingrid Carmelo[3], Hong Sun Park[4], Katherine M. Collins[4], Natasha S. Hochberg[3], Davidson H. Hamer[3], Elizabeth D. Barnett[3], **Julia R. Köhler**[4]

[1]East Boston Neighborhood Health Center, Boston, MA, United States, [2]Brigham and Women's Hospital, Boston, MA, United States, [3]Boston Medical Center, Boston, MA, United States, [4]Boston Children's Hospital, Boston, MA, United States

In Massachusetts, there are over 3,000 estimated cases of Chagas disease (CD). Treatment in the asymptomatic interval before development of cardiac signs is the best chance for the 20-30% of patients who will otherwise progress to heart disease and death. Asymptomatic *T. cruzi* infection hence must be detected by serologic screening. The Strong Hearts (Corazones Fuertes/Corações Fortes) program intends to establish CD screening as a component of primary care for at-risk patients and to implement, as the standard of care, referral to specialized care including treatment as indicated. The program comprises 1. raising awareness among medical providers, 2. education of at-risk communities, 3. establishment of a robust screening program, and 4. referral for treatment and support of patients. In our local experience, logistical hurdles within the health care system that obstruct appropriate care include need for confirmatory testing of positive screening results at the CDC; need for an affirmative referral process by which each electronic referral receives follow-up; language barriers; and challenges in obtaining benznidazole from the manufacturer. For infants of *T. cruzi*-positive mothers, follow-up protocols had to be established between the tertiary care hospital and the health center. Systematic interdisciplinary efforts among clinician teams to address these hurdles are productive and ongoing, since not all problems have been solved. In the first 23 months of screening in one health center, 42 patients had positive confirmatory *T. cruzi* antibody testing at the CDC, among 4833 completed serologic tests (0.87%). All patients were referred for treatment, though barriers to medical care for persons at risk for CD are high. These barriers include lack of health insurance, lack of transportation, fear of asking the employer for time off work, fear of an unknown hospital and fear of persecution as Latin-American immigrants. Support for patients is crucial, as best possible with available resources. The Strong Hearts program shows that addressing CD can be part of standard care when attention is directed to the health needs of at-risk communities.

1783

ANALYTICAL PERFORMANCE OF THE FILMARRAY® GLOBAL FEVER PANEL

Jared R. Helm, Corike Toxopeus, Pascal Belgique, Lex Border, Olivia Jackson, Alex Kelley, Micah Mortenson, Cynthia Phillips

BioFire Defense, Salt Lake City, UT, United States

A large number of pathogens that include bacteria, viruses, and parasites can cause Acute Febrile Illness (AFI). BioFire Defense is developing the Global Fever (GF) Panel to be used on the FilmArray® System in collaboration with the U.S. Department of Defense[a] and NIAID[b]. The FilmArray is an *in vitro* diagnostic test platform that combines nucleic acid purification and nested multiplex PCR for the simultaneous identification of many infectious agents in under an hour using a closed, sample-to-answer system. The FilmArray GF Panel detects and identifies nucleic acid from chikungunya virus, CCHF virus, dengue virus (serotypes 1-4), Ebolavirus, Lassa virus, Marburgvirus, West Nile virus, Yellow fever virus, Zika virus, *Bacillus anthracis*, *Francisella tularensis*, *Leptospira* spp., *Salmonella enterica* serovar Typhi and Paratyphi A, *Yersinia pestis*, *Leishmania* spp., and *Plasmodium* spp. in venous blood specimens from individuals with signs and/or symptoms of AFI or recent AFI and with known or suspected exposure to target pathogens. The LoD studies demonstrate that the GF Panel is a sensitive system that can accurately detect multiple pathogens, including Category A biothreat pathogens, and is appropriate to use testing samples that may contain multi-analytes. Analytical specificity (exclusivity) testing demonstrates that the GF Panel is highly specific for the pathogens it is designed to detect in blood specimens. Assessment of the analytical reactivity and efficacy (inclusivity) show that the assays possess a high level of reactivity for their intended targets. A multiplex FilmArray panel could aid in rapid and actionable AFI diagnosis.

1784

SEVERE MUCOSAL LEISHMANIASIS: CLINICAL MANIFESTATIONS AND BRONCHOSCOPIC FINDINGS

Alejandro Elmer Llanos[1], Braulio Valencia[1], Cesar Colunche[2], Ana A. Ramos[1], Fiorela Alvarez[1], Oscar Gayoso-Liviac[1], Oscar Gayoso[1]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Servicio de Neumología y DEITD, Hospital Cayetano Heredia, Lima, Peru

Severe Mucosal Leishmaniasis: Clinical manifestations and bronchoscopic findings. Alejandro Llanos-Cuentas[1,3], Braulio Valencia[1], Cesar Colunche3, Ana Ramos[1], Fiorela Alvarez[1], Oscar Gayoso-Liviac[1] and Oscar Gayoso[2,3] 1 Instituto de Medicina Tropical *Alexander von Humboldt*, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru. 2 Facultad de Medicina, UPCH. 3 Servicio de Neumología y DEITD, Hospital Cayetano Heredia. Mucosal leishmaniasis (ML) is the most severe form of tegumentary leishmaniasis (TL). Most cases in the New World are caused by *Leishmania (V.) braziliensis* with a prevalence around 4% of total TL yearly cases. ML has no spontaneous cure, patients with severe disease can die due to invasion of the mucous membranes and underlying tissues. The severity of mucosal disease is the main parameter of the therapeutic response. In severe form, patients present compromise of the mucous membranes: nasal, oropharynx, larynx, trachea, and even bronchi. The cure rate of ML is <10% when treated with pentavalent antimonials, because of this

reason the therapy of choice is amphotericin B, either in the liposomal presentation or as deoxycholate. We report the results of 20 patients with clinical diagnosis of severe ML who underwent bronchoscopy prior to the start of therapy with amphotericin B. All patients were male, the mean age was 39 years (range 23-55 years),70% were infected in the department of Madre de Dios. Reported symptoms were hoarseness 90%, productive cough 80%, severe dysphagia 80%, dysphonia 75%, and dyspnea during physical activities 42%. The main clinical findings were wheezing 60%, stridor 45% and clubbing 30%. The bronchoscopy detected compromise of epiglottis and vocal chords 100%, subglottic 70%, upper 2/3 of the trachea 60%, lower third of the trachea 30%, subglottic stenosis 40% and compromise of the bifurcation of the major bronchi 14%. Only 15% ML patients had a normal spirometry. The remaining spirometries had an obstructive 70%, restrictive 5% and mixed 10% pattern. In summary, patients with the severe ML form present extensive and severe upper and lower airway involvement and need different therapeutic approach.

1785

RISK FACTORS AND TRENDS IN NEONATAL MORTALITY IN A SPECIAL CARE NEWBORN UNIT IN A TERTIARY CARE HOSPITAL

Ananya Kumar[1], Kyu Han Lee[1], Abu Faisal Pervez[2], Sanwarul Bari[3], Shams El Arifeen[3], Farzana Islam[3], Emily S. Gurley[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Faridpur Medical College Hospital, Dhaka, Bangladesh,* [3]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh*

South Asian countries accounted for 38% of the global burden of neonatal mortality in 2017; within the region, 60% of under-5 deaths were neonatal deaths. Bangladesh's neonatal mortality rate in 2017 was 18.4 deaths per 1000 livebirths - special care newborn units (SCANUs) have been established in multiple hospitals country-wide to improve neonatal survival. We conducted a retrospective cohort study in the SCANU of Faridpur Medical College Hospital, Bangladesh to profile the neonates admitted to the unit in 2018, describe trends in admissions and deaths, and establish risk factors associated with death within 48 hours of admission to the unit. Neonates (0-28 days old) who were admitted alive to the unit between January and November 2018 with admission dates available in the medical records and did not abscond in the first 48 hours from admission were included in the study population. We described demographic and clinical characteristics of the population as well as trends in admissions and deaths each month. Logistic regression models were generated to examine the relationship between death within 48 hours of admission to the unit and potential demographic, diagnostic and clinical risk factors. 646 neonates were included in the study population; 41% (n=263) died in hospital, 26% within 48 hours of admission. Sixty-one percent were admitted on the day of birth, 56% were male, and 82% were born prematurely (before the completion of 37 weeks of pregnancy). No seasonal clustering of admissions or deaths was observed. Each additional day of age was associated with a 5% reduction in the odds of death within 48 hours of admission to the unit (OR: 0.95, 95% CI: 0.9, 0.99); male sex (OR: 1.53, 95% CI: 1.04, 2.23), prematurity (OR: 2.5, 95% CI: 1.3, 4.7) and a perinatal asphyxia diagnosis (OR: 2.2, 95% CI: 1.4, 3.4) were all associated with an increased odds. The high mortality among neonates in this unit warrants further investigation to identify strategies to improve outcomes. Other SCANUs in the country with similar treatment gaps may also require attention and resources in order to improve child survival in these units.

1786

FACTORS ASSOCIATED WITH MORTALITY IN PRETERM NEONATES AND INFANTS WITHIN THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK

Navit T. Salzberg[1], Dianna M. Blau[2], Shams El Arifeen[3], Victor Akelo[4], Quique Bassat[5], Richard Chawana[6], Emily Gurley[7], Karen Kotloff[8], Shabir Madhi[6], Inacio Mandomando[9], Dickens Onyango[10], Samba O. Sow[11], Robert F. Breiman[1], for the CHAMPS Network Consotrium[1]

[1]*Emory Global Health Institute, Emory University, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*International Centre for Diarrhoeal Diseases Research, Bangladesh, Dhaka, Bangladesh,* [4]*United States Centers for Disease Control and Prevention-Kenya, Kisumu, Kenya,* [5]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [6]*Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa,* [7]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [8]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [9]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique,* [10]*Kisumu County Public Health Department, Kisumu, Kenya,* [11]*Center for Vaccine Development, Bamako, Mali*

While it is known that mortality is inversely proportional with gestational age at birth, the specific causes of death that are associated with the increased vulnerability among preterm infants is not well characterized in locations with high mortality. Through the collection of comprehensive cause of death data, including postmortem specimen testing, to determine the sequence of events leading to death, CHAMPS will help define specific causes of under-five mortality in Africa and South Asia. Causes of death among neonates (0-27 days) and infants (28 days to < 12 months), born preterm (n=245) and full term (n=205), from December 2016 –March 2019 were compared. Preterm was defined as a gestational age of less than 37 weeks (when information available) or as determined by a panel of experts using available clinical data. Over half of enrolled deaths were born premature and 27% of those died of complications unique to prematurity. With respect to those complications, the majority died of respiratory distress syndrome (71%), followed by pulmonary hemorrhage (9%), primary atelectasis of the lung (7%) and necrotizing enterocolitis (NEC) (3%). Among neonates and infants born preterm, 62% had a pathogen-associated illness identified in the causal chain of death, compared to 49% of neonates and infants born full term. While causes of death among infants born prematurely were similar to those among infants born full term (i.e. pneumonia, sepsis), the pathogens associated with death had different distributions; preterm infants were less likely to die of pneumococcal, *Haemophilus influenzae*, malaria, and HIV infections, whereas they were more likely to have had gram negative sepsis, many resulting from nosocomial infections acquired during long-term hospitalization needs. No significant differences in pathogen associated illness were found between preterm neonates and preterm infants. CHAMPS data supports and provides granularity to the role of infections in preterm mortality; this detail will help focus efforts on identifying and treating infection through the through the first year of life to increase the chances for survival.

1787

CHILDHOOD GROWTH AND NEUROCOGNITION ARE ASSOCIATED WITH DISTINCT SETS OF METABOLITES

G. Brett Moreau[1], Girija Ramakrishnan[1], Heather Cook[1], Todd Fox[1], Uma Nayak[1], Swapna Kumar[2], Jennie Ma[1], E. Ross Colgate[3], Beth Kirkpatrick[3], Charles Nelson[2], Rashidul Haque[4], William Petri, Jr.[1]

[1]*University of Virginia, Charlottesville, VA, United States,* [2]*Boston Children's Hospital, Boston, MA, United States,* [3]*University of Vermont, Burlington, VT, United States,* [4]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh*

Undernutrition is a serious global problem that contributes to increased child morbidity and mortality, impaired neurocognitive development, and decreased educational and economic attainment. Current interventions are only marginally effective, and identification of associated metabolic pathways can offer new strategies for intervention. Plasma samples were collected at 9 and 36 months from an undernourished child cohort in Bangladesh and targeted metabolomics was performed on bile acids, acylcarnitines, amino acids, phosphatidylcholines, and sphingomyelins. Metabolic associations with linear growth and neurocognitive outcomes at four years were evaluated using correlation and penalized-linear regression analysis as well as conditional random forest modeling. Analysis identified that different metabolites were associated with growth and neurocognitive outcomes. Improved growth outcomes were associated with higher concentrations of hydroxy-sphingomyelin and essential amino acids as well as lower levels of acylcarnitines and bile acid conjugation. Neurocognitive scores were largely associated with phosphatidylcholine species and early metabolic indicators of inflammation. All metabolites identified explain ~45% of growth and neurocognitive variation. Growth outcomes were predominantly associated with metabolites measured early in life (9 months), many of which were biomarkers of insufficient diet, environmental enteric dysfunction, and microbiome disruption. Hydroxy-sphingomyelin was a significant predictor of improved growth. Neurocognitive outcome was predominantly associated with 36 month phosphatidylcholines and inflammatory metabolites, which may serve as important biomarkers of optimal neurodevelopment. The distinct sets of metabolites associated with growth and neurocognition suggest that intervention may require targeted approaches towards distinct metabolic pathways. Further work is currently underway to identify the mechanisms underlying these metabolic associations.

1788

CLINEPIDB: THE CLINICAL EPIDEMIOLOGY DATABASE RESOURCE

Danica A. Helb[1], Cristina Aurrecoechea[2], John Brestelli[1], Brian P. Brunk[1], Danielle Callan[1], David Falke[2], Steven Fischer[1], Jay Humphrey[2], John Judkins[1], Jessica C. Kissinger[2], Brianna Lindsay[1], David S. Roos[1], Sheena Shah Tomko[1], Christian J. Stoeckert Jr[1], Jie Zheng[1]

[1]*University of Pennsylvania, Philadelphia, PA, United States,* [2]*University of Georgia, Athens, GA, United States*

Population-based epidemiological studies provide new opportunities for innovation and collaboration among researchers addressing pressing global health concerns, however access to study data pose many challenges. ClinEpiDB (https://clinepidb.org) is an open-access online resource that enables investigators to maximize the utility and reach of their research and to make optimal use of data released by others. Existing infrastructure for EuPathDB (https://eupathdb.org)--a collection of databases covering 170+ eukaryotic pathogens, relevant free-living and non-pathogenic species, and select pathogen hosts--was used to build the ClinEpiDB database, and enables complex interrogations of underlying data with a sophisticated search strategy system. When Integrating studies into the database, a unified semantic web framework is used to describe the data. Over 2400 different variables about participants, their associated anthropometry, demographics, and disease episodes were collected in the clinical and epidemiological studies hosted on ClinEpiDB to date. Query results can be analyzed and graphically visualized via interactive web applications launched directly in the ClinEpiDB browser, providing insight into distributions and exploratory associations with any covariates. ClinEpiDB currently hosts data from the Gates Foundation-supported Malnutrition and Enteric Diseases Network (MAL-ED) and the Global Enteric Multicenter Study (GEMS) projects, which studied the etiology, incidence and impact of childhood enteric disease in low-income countries. ClinEpiDB also hosts data from two NIH-supported International Centers for Excellence in Malaria Research (ICEMR) studies, which used comprehensive longitudinal data to elucidate interactions between malaria parasites, their mosquito vectors, and human hosts. The ClinEpiDB resource will continue to grow with enhanced tool development, significant user outreach and education, and integration of new datasets (such as Pneumonia Etiology Research for Child Health (PERCH) and several additional ICEMR studies, which will be released over the coming year).

1789

GAZELLE, A PROMISING POINT-OF-CARE DIAGNOSTIC FOR HEMOGLOBIN DISORDERS IN INDIA: BRIDGING THE GAP IN CONTROL PROGRAM

S. Rajasubramaniam[1], Rajat Kumar[1], Shweta Shrivas[1], Anil K. Verma[1], Priyaleela Thota[2], Praveen Bharti[1], Anne Rocheleau[2], Tyler Witte[2], R. Uikey[1], Muhammad Noman Hasan[3], Umut A. Gurkan[3], Aparup Das[1]

[1]*National Institute of Research in Tribal Health, Jabalpur, India,* [2]*Hemex Health, Portland, OR, United States,* [3]*Case Western Reserve University, Cleveland, OH, United States*

Sickle Cell Disease (SCD) and thalassaemias are the two prevalent genetic disorders causing anaemia and associated complications. The prevalence of both SCD and carriers varies from 5-35% among ethnic groups and Scheduled caste communities in 5 states namely Madhya Pradesh, Gujarat, Maharashtra, Odisha, Chhattisgarh and Andhra Pradesh in India. Mass screening involves solubility test followed by confirmation by high-performance liquid chromatography (HPLC) or haemoglobin electrophoresis. Absence of public health diagnostic facilities results in high morbidity and mortality among undetected patients. Evaluatory phase I studies on "Gazelle™", a microchip-based cellulose acetate electrophoresis device were conducted in tribal-dominated Madhya Pradesh and Chhattisgarh states of. Gazelle™ is a rapid (<10 minutes) and easy-to-use test that can be carried out using a finger-prick volume of blood. Blood samples were collected from 300 patients enrolled in a pilot study at a high prevalence setting (ICMR-NIRTH, India). Each sample was tested with Gazelle™, and the results were compared to electrophoresis and HPLC. A total of 295 patient samples were included in the analysis (3 samples were excluded due to incomplete runs; sequencing results are pending for 2 samples). Phase II testing with improved Gazelle™ is ongoing and results will be presented at the Conference. Of the 295 tested samples, 4% were HbSS and HbSβ-thal, 21% were sickle cell trait and 74.9% were of normal (HbAA) category. Gazelle™ showed high accuracy (100%), sensitivity and specificity in detecting SCD (HbSS) and β-Thalassemia, sickle cell trait (HbAS) when compared with standard screening tests. Microchip electrophoresis technology is a promising, low-cost ($2 per test), rapid, and accurate method for detecting hemoglobin disorders such as SCD in limited resource settings in India. Gazelle aims to bridge the gap in diagnosis of sickle cell and hemoglobin disorders in healthcare.

1790

SUCCESSFUL MANAGEMENT OF POISONING WITH IVERMECTIN (MECTIZAN®) IN THE OBALA HEALTH DISTRICT (CENTRE REGION, CAMEROON): A CASE REPORT

Hugues Nana Djeunga, Cyrille Donfo Azafack, Floribert Fossuo Thotchum, Joseph Kamgno

Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaoundé, Cameroon

The efficacy, safety and tolerability of ivermectin (IVM) have led to its large scale use for mass treatments, that is without any prior diagnosis. Data reporting the clinical presentation of poisoning to IVM are very scanty, even in experimental studies. We report the case of a 19 years old female patient who deliberately swallow about 400 IVM pills with self-murder intention after a family conflict. Of note, she opened a sealed box of 500 tablets of IVM 3 mg and when she was found in a state of obsession, less than 100 pills were remaining. The patient was admitted to the Obala district hospital the day after drug intake for obnubilations, asthenia and vomiting. Once admitted, a physical checkup was undertaken and revealed an alteration of her general state with asthenia and anorexia, a digestive disorder with nausea and marked sensitivity to of right iliac fossa, visual involvement with reactive bilateral mydriasis and visual acuity without correction of 1/10 for both eyes, heart involvement regular heartbeats, and neurological involvement with rotational vertigo, headache, kinetic ataxia with osteotinous hyperreflexia. Prior the unfortunate event, the patient reported a visual disorder that never required consultation, though a history of visual disorder was reported in her family. She had no known psychiatric pathology and no known allergy to a drug. She declared never received any IVM treatment before the event. The case management consisted in saline-based hyperhydration, antiulcer drug (Omeprazole 40mg daily), and Paracetamol 1g for headache. The clinical evolution was satisfactory from day 2 to day 4 post-poisoning, with a decrease of vertigo, asthenia, ataxia but persistent visual disturbances and asthenia. The patient was discharged at day 4 and totally recovered by one-month post-poisoning. This is the first report of a poisoning with ~100 times higher the recommended dosage. This case report confirms the safety and tolerability of IVM, even at exceptionally high dosage, and is supportive of the suspected mechanism underlying severe adverse events occurring post IVM among individuals heavily infected with *Loa loa*.

1791

IMPACT OF PRENATAL MATERNAL STRESS ON BIRTH ANTHROPOMETRICS AND PREGNANCY OUTCOMES IN RURAL GHANA

Kenneth Ayuurebobi Ae-Ngibise[1], Blair J. Wylie[2], Darby W. Jack[3], Felix B. Oppong[1], Seyram Kaali[1], Oscar Agyei[1], Patrick L. Kinney[4], Rosalind J. Wrigh[5], Kwaku Poku Asante[1], Alison G. Lee[5]

[1]*Kintampo Health Research Centre, Kintampo, Ghana,* [2]*Beth Israel Deaconess Medical Center, Boston, MA, United States,* [3]*Mailman School of Public Health, Columbia University, New York, NY, United States,* [4]*Boston University School of Public Health, Boston, MA, United States,* [5]*Icahn School of Medicine at Mount Sinai, New York, NY, United States*

In developed countries, prenatal maternal stress has been associated with impaired fetal growth, however this has not been evaluated in rural sub-Saharan Africa. We evaluated the effect of prenatal maternal stress on fetal growth and birth outcomes in rural Ghana. Leveraging a prospective, rural Ghanaian birth cohort, we ascertained prenatal maternal negative life events, categorized scores as 0-2 (low stress; referent), 3-5 (moderate), and >5 (high) among 353 pregnant women in the Kintampo North Municipality and Kintampo South District located within the middle belt of Ghana. We employed linear regression to determine associations between prenatal maternal stress and infant birth weight, head circumference, and length measured within 24 hours of birth. We additionally examined associations between prenatal maternal stress and adverse birth outcomes, including low birth weight, small for gestational age, and stillbirth using logistic regression. Models were adjusted for maternal age, weight, height, socioeconomic status, marital status, ethnicity, infant sex, and intervention cluster. . Effect modification by infant sex was examined. Compared with low prenatal maternal stress, high stress was associated with reduced birth length (=-0.91 cm, p=0.04; p-value for trend=0.04) for all infants. Among female infants, moderate and high prenatal maternal stress were both associated with reduced birth weight (=-0.16 kg, p=0.02; =-0.18, p=0.04 respectively; p-value for trend=0.04) and head circumference (=-0.66 cm, p=0.05; =-1.02, p=0.01 respectively; p-value for trend=0.01). In female infants, high prenatal stress increased odds of a composite of adverse birth outcomes (OR 2.41, 95% CI 1.01-5.75; p for interaction=0.04). In conclusion, in this rural Ghanaian cohort, our data suggests that prenatal maternal stress may negatively impact fetal growth and pregnancy outcomes in all infants, especially for female infants. Understanding risk factors for impaired fetal growth may help develop preventative health strategies for future public health interventions

1792

PREVALENCE AND CORRELATES OF STUNTING AMONG CHILDREN 1-59 MONTHS DISCHARGED FROM THREE HOSPITALS IN WESTERN KENYA

Hannah E. Atlas[1], Rebecca L. Brander[1], Kirkby D. Tickell[1], Christine J. McGrath[1], Susan K. Oongo[2], Ingred V. Bitengo[2], Grace C. John-Stewart[1], Barbra A. Richardson[1], Benson O. Singa[2], Judd L. Walson[1], Patricia B. Pavlinac[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Kenya Medical Research Institute, Nairobi, Kenya*

Stunting (length-for-age z-score {LAZ} < -2 SD) is associated with significant morbidity and mortality among children under age 5 in sub-Saharan Africa. We determined correlates of stunting among children 1-59 months upon discharge from 3 hospitals in Western Kenya enrolled in an ongoing clinical trial (Toto Bora Trial, NCT02414399), using log-binomial regression to estimate prevalence ratios (PRs), adjusting for child age and study site. Of the 991 children enrolled to date, the median age was 18 months (interquartile range: 9-32) and 24.2% were stunted. Among stunted children, 7.4% also had moderate acute malnutrition (-3 ≤ weight-for-length z-score [WLZ] -2) and 4.1% were severely acutely malnourished (WLZ < -3). Children whose caregivers had primary school education or less were also more likely to be stunted (aPR: 2.00 [95% CI: 1.54-2.61]), after additional adjustment for caregiver age. Having a flush toilet was associated with a lower prevalence of stunting relative to pit latrine (aPR: 0.47 [95% CI: 0.25-0.89]). Not exclusively breastfeeding in the first 6 months of life was associated with higher stunting prevalence compared to exclusively breastfeeding (aPR_{never}: 3.89 [95% CI: 1.50-10.04]; $aPR_{partial}$: 1.69 [95% CI: 0.80-3.56]). Children who were HIV-infected (1.6%) or HIV-exposed, uninfected (9.5%) were more likely to be stunted than HIV-unexposed children (84.7%) ($aPR_{HIVINFECT}$: 2.49 [95% CI: 1.27-4.88]); aPR_{HEU}: 1.76 [95% CI: 1.18-2.64]). Presence of diarrhea at presentation, discharge diagnosis and previous hospitalization in the last year were not associated with stunting. Among children hospitalized for an infectious condition, several routine sociodemographic factors, as well as HIV exposure, were important contributors to stunting. Hospital discharge may represent an important opportunity for administering targeted interventions to prevent further growth faltering, particularly among HIV-affected children.

1793

IMPACT OF TOXOPLASMOSIS AND CYTOMEGALOVIRUS ON PREGNANT WOMEN AND THEIR NEWBORNS IN LIMA, PERU

Grace Trompeter[1], José Camones Huerta[2], Alexander Cordero Campos[2], Mayra Ochoa Porras[2], Sonia Apaza Chayña[2], Andrea Diestra Calderón[2], Erasmo Huertas Tacchino[3], Mónica Pajuelo Travezaño[2], Maritza Calderón Sanchez[2], Robert Gilman[4]

[1]*Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, United States,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Instituto Nacional Materno Perinatal, Lima, Peru,* [4]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Toxoplasma gondii, the parasite that causes toxoplasmosis, and cytomegalovirus (CMV) are two pathogens with the ability to cause significant morbidity in the developing fetus, resulting in such problems as hearing or vision loss, brain calcifications, CNS abnormalities, and hepatosplenomegaly. Often times, infants are born with subclinical infection, which remains undetected until they develop sequelae later in childhood. Globally, it is estimated that these infections affect 0.5-2.0% of all births. While the seroprevalences of *T. gondii* and CMV among the general population are known to be higher in developing countries, the rates of vertical transmission in Latin America, and its subsequent effects on fetal development, remains understudied. Therefore, understanding the contribution of both CMV and *T. gondii* congenital infections will be important to help focus public health efforts. The primary goal of the initial phase of the study was to determine the rates of *T. gondii a*nd CMV congenital infection in a sample of newborns in Lima, Peru and to characterize the epidemiological characteristics of those subjects determined to be positive by either serology and/or nucleic acid testing. Women over age 18 who presented to Instituto Nacional Materno Perinatal in Lima, Peru were recruited prior to delivery. A brief questionnaire assessing maternal risk factors for infection was conducted and samples from the mother, newborn, and placenta were collected. ELISA was used to detect IgM antibodies to *T. gondii* in serum from cord blood of newborns. Real time PCR was used to detect CMV DNA in newborn saliva samples. IgG ELISA was used to determine the seroprevalence of CMV and *T. gondii* among participating mothers. This work is important in contributing to the understanding of the relative risks of CMV and *T. gondii* to public health and will allow communities across Peru to better direct public health efforts.

1794

OUR EXPERIENCE WITH HISTOPATHOLOGIC, MICROBIOLOGIC AND GENETIC CHARACTERISTICS OF THE PARASITIC OOMYCETE, *PYTHIUM INSIDIOSUM*

Dennis J. Rocheleau, Rupal M. Mody, Rebecca A. Smiley, Matthew J. Perkins

William Beaumont Army Medical Center, El Paso, TX, United States

Pythiosis, caused by *Pythium insidiosum*, is a very rare infection that mimics mucormycosis. Differentiating between the two infections is important as the efficacy of antifungal agents against P. insidiosum is unclear and there is a therapeutic vaccine for treatment of pythiosis. A case of pythiosis was identified at our institution. A report of this case, focusing on the clinical features, has previously been published. At the time of the patient's admission, histopathology of a lesion showed hyphae with rare septae on Grocott's methenamine silver (GMS) stain concerning for cutaneous zygomycosis. Samples of abnormal tissue were plated directly on to Sabouraud-dextrose agar plates. The organism was subsequently cultured in liquid media. A fine, white, filamentous organism grew on Sabouraud-dextrose agar plates three days after samples were plated. Vegetative growth adhered closely to the agar and was not characteristic of a "lid-lifter" with extensive upward growth as has been described with agents of mucormycosis. The organism was identified as *P. insidiosum* via ribosomal RNA sequencing. Phylogenetic analysis revealed clade-I genotype, the clade most typically identified in the North America. Liquid media culture was performed resulting in induction of motile zoosporogenesis. Histopathologic samples of skin and fascia revealed inflammation with areas of necrosis, abscess formation, granulomas and marked eosinophilic infiltrate. Hyphae stained most clearly with GMS stain but did not stain well on periodic acid-Schiff with hyphal structures 4-6μm in width. Our experience with pythiosis is illustrative of characteristics helpful to differentiate it from mucormycosis. Eosinophilic infiltrate is suggestive of a diagnosis alternative to mucormycosis. Agents of mucormycosis typically having hyphae 6-15μm in diameter and P. insidiosum hyphae ranging from 2-7μm. Induction of zoosporogenesis in liquid culture is diagnostic of *Pythium*. Identification of the organism utilizing PCR is most useful for diagnosis and, if suspected, samples of tissue or organism growing in culture should be sent to a laboratory with this capability.

1795

INCIDENCE OF ACUTE GASTROENTERITIS AND NOROVIRUS IN A COMMUNITY COHORT, CUSCO, PERU, 2015-2018

Giselle M. Soto[1], Candice Romero[1], Yeny Tinoco[1], Wesley Campbell[2], Patricia Galvan[1], Roxana Caceda[1], Laura Calderwood[3], Anita Kambhampati[3], Andrea McCoy[1], Aron Hall[3]

[1]*US Naval Medical Research Unit Six, Callao, Peru,* [2]*Walter Reed National Military Medical Center, Bethesda, MD, United States,* [3]*US Centers for Disease Control and Prevention, Atlanta, GA, United States*

Norovirus is the most common cause of sporadic and outbreak-associated acute gastroenteritis (AGE).The highest incidence of norovirus is reported among children <5 years of age but norovirus can cause disease at any age. Population-based data on norovirus epidemiology from tropical settings are limited and the impact of norovirus in low- and middle-income countries (LMIC) is poorly understood. In April 2015, we implemented active surveillance in 268 households in Cusco, a highland city in Peru, to assess the burden of AGE and norovirus. We defined AGE as either ≥3 loose stools, ≥2 vomiting episodes, or ≥1 vomiting episode and ≥1 loose stool within 24 hours. Stool samples from participants meeting the case definition were tested for norovirus by RT-PCR. Stool samples from household members of AGE cases and members of matched control households were also collected and tested for norovirus. During April 2015 – December 2018, overall incidence of AGE and of norovirus across all ages was 25 cases/100 person-years (PY) (95% confidence interval [CI], 23–27/100 PY) and 5/100 PY (95% CI, 4–6/100 PY), respectively. Children aged ≤2 years had the highest incidence of norovirus with 66/100 PY (95% CI, 51–86/100 PY). Norovirus prevalence was 11.8% among non-AGE household members of index AGE cases and 8.5% among members of healthy control households. Norovirus is an important etiology of AGE in this LMIC community. Further assessment of associated risk factors as well as circulating norovirus genotypes will help to better understand the burden of AGE and norovirus in this community and guide future interventions.

1796

UNBIASED METAGENOMIC SEQUENCING FOR MICROBIAL DETECTION AND IDENTIFICATION USING THE IDSEQ PLATFORM

Vida Ahyong[1], Maira Phelps[1], Michelle Tan[1], Rene Sit[1], Norma Neff[1], Joseph DeRisi[1], Cristina Tato[1], IDseq Engineering team Chan Zuckerberg Initiative[2]

[1]*CZ Biohub, San Francisco, CA, United States,* [2]*Chan Zuckerberg Initiative, Redwood City, CA, United States*

Metagenomic next generation sequencing (mNGS) has enabled the rapid detection and identification of microbes without the need for specialized microbiological tests, culturing, nor *a priori* knowledge of the microbial landscape. In a partnership between the Chan Zuckerberg Biohub and the Chan Zuckerberg Initiative, we have developed a cloud-based, open-access web portal, IDseq, to facilitate the computationally demanding analyses of mNGS data derived from a diversity of biological samples, for the purpose of unbiased interrogation of pathogens. By enabling bench scientists, clinicians, and bioinformaticians alike the ability to analyze these

large datasets, our aim is to bring this technology to bare in lower resource laboratory settings where advanced research or diagnostic tools are limited. In this talk, I will describe our training program which includes best practices for performing a successful metagenomic sequencing experiment from initial study design, sample collection, library preparation, and sequencing on a benchtop sequencer. Finally, I will demo the IDseq portal to demonstrate the ease of use and the strength of the tool to generate new hypotheses and to drive data-based decisions around clinically validated pathogens identified with this method.

1797

THE WEST AFRICAN CENTER OF EXCELLENCE FOR GLOBAL HEALTH BIOINFORMATICS TRAINING PROGRAM IN MALI, A MODEL FOR STRENGTHENING DATA SCIENCE CAPACITY BUILDING IN AFRICA

Mamadou Wele[1], Jian Li[2], Cheickna Cisse[1], Mahamadou Diakité[1], Alia Benkahla[3], Cheick Oumar Tangara[1], Darryl Hurt[4], Christopher Whalen[4], Doulaye Dembele[5], Donald J. Krogstad[2], Frances J. Mather[2], Seydou O. Doumbia[1], **Jeffrey G. Shaffer**[2]

[1]*University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Tulane University, New Orleans, LA, United States,* [3]*Institute Pasteur of Tunis, Tunis, Tunisia,* [4]*National Institutes of Health, Bethseda, MD, United States,* [5]*Institute of Genetics and Molecular and Cell Biology, Strasbourg, France*

Bioinformatics and data science research has boundless potential across Africa due to its high levels of genetic diversity and a disproportionate burden of the world's most devastating infectious diseases, including malaria, tuberculosis, HIV-AIDS, Ebola virus disease, and Lassa fever. The West African Center of Excellence for Global Health Bioinformatics Training program is a joint effort between the University of Sciences, Techniques and Technologies of Bamako, Mali (USTTB), Tulane University, and NIAID providing advanced bioinformatics and data science training to West African students and researchers. This work highlights the program's inaugural bioinformatics symposium and training workshop series funded by NIH/Fogarty International through its Human Health and Heredity in Africa (H3Africa) initiative. We designed a Bioinformatics for Global Health Symposium at USTTB uniting researchers and students from Mali and its neighboring countries. The inaugural symposium was held in March 2019 and included 106 attendees, 19 oral presentations, 8 student poster presentations, and 4 panel discussions. Workshop training was provided on geographic information systems (GIS), bioinformatics, and data science topics. The program participants and moderators included USTTB faculty and students and representatives from The African Society for Bioinformatics Computational Biology, and the National Institutes of Health. Home countries for the program participants included Mali, Tunisia, France, Burkina Faso, Morocco, and the U.S. Among n = 14 workshop participants only 21.4% (3/14) reported prior GIS or bioinformatics training. Enhanced bioinformatics and data science training and publication is vital in Mali as it is the epicenter for some of the world's deadliest diseases. Bioinformatics and data science symposiums provide a platform for nurturing research networks, sharing research ideas, and monitoring progress of training and research efforts. More literature on the topic of symposium development is needed to exhibit its utility and expand such efforts in developing countries.

1798

PREVALENCE OF ANTIMICROBIAL RESISTANCE IN COMMENSAL *E. COLI* FROM CHILDREN DISCHARGED FROM HOSPITAL IN WESTERN KENYA

Stephanie N. Tornberg-Belanger[1], Doreen Rwigi[2], Rebecca L. Brander[1], Kirkby D. Tickell[1], Christine J. McGrath[1], Michael Muraya[2], Lynnete Kitheka[2], Nancy Onamu[2], Derrick Ounga[2], Samuel M. Kariuki[2], Benson O. Singa[2], Judd L. Walson[1], Patricia B. Pavlinac[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Kenya Medical Research Institute, Nairobi, Kenya*

Children who have been discharged from hospital in sub-Saharan Africa are at high risk of death and re-hospitalization. This increased risk may be partially due to carriage of antimicrobial resistant (AMR) bacteria leading to treatment failure during the post-discharge period. The Toto Bora trial (ClinicalTrials.gov # NCT02414399) randomizes children at hospital discharge to a 5-day course of azithromycin or placebo to determine the effect of azithromycin on morbidity and mortality. Utilizing a random selection of commensal *Escherichia coli* (*E. coli*) isolates collected from children enrolled in this RCT prior to receiving the intervention, we determined susceptibility to azithromycin, imipenem, and antibiotics prescribed for common childhood illnesses in sub-Saharan Africa. Interpretations of resistance was based on the 2018 Clinical & Laboratory Standards Institute and intermediate zone sizes were considered as resistant. Resistance prevalence and 95% confidence intervals (CI) assuming a binomial distribution were reported. Among 991 children enrolled in the RCT, 88% had *E. coli* isolated. Of the 191 participants with *E. coli* who had AMR testing performed, the median age was 18 months (interquartile range: 9 - 32 months), the average length of hospitalization was 4 days, and 87% were prescribed at least one antibiotic during their hospital stay. The prevalence of resistance to ampicillin was 92% (95% CI: 87% - 96%), 33% (95% CI: 26% - 40%) to azithromycin, 29% to gentamicin (95% CI: 22% - 36%), 28% (95% CI: 22% - 35%) to ciprofloxacin, and 40% (95% CI: 33% - 47%) to ceftriaxone. Resistance to imipenem, a carbapenem not routinely available in this setting, was 1% (95% CI: 0% - 2%). Additionally, 39% (95% CI: 32% - 46%) of isolates were extended-spectrum beta-lactamase (ESBL) producing. Children discharged from hospitals demonstrate a high prevalence of resistance to commonly prescribed antibiotics and to azithromycin, despite the relatively infrequent use of azithromycin in the area. As children discharged from hospital are particularly vulnerable to death and re-hospitalization, high carriage of AMR may be contributing to poor outcomes.

1799

ANTIBIOTIC PRESCRIPTION IN FEBRILE PATIENTS ATTENDING AN EMERGENCY DEPARTMENT IN RIO DE JANEIRO, BRAZIL

José Moreira, Roxana Mamani, Patricia Brasil, Andre Siqueira

Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil

Fever is a common reason for seeking care in Emergency Departments (ED), and evidence on antibiotic prescription practices in febrile patients attending ED is limited.We aim to determine the proportion of antibiotic prescriptions given to febrile patients visiting an ED in Rio de Janeiro and its risk factors. We did a cross-sectional analysis ofconsecutive patients who presented to the ED over a total of 7-weeks throughout 2019 (14^{th}January - 28^{th}February) and were evaluated for fever. Eligible subjects had either a history of fever before arrival at the ED or axillary temperature ≥ 37.5 °C at arrival at the ED. Information related to antibiotic prescribed and source of infection were collected. Categorical variables were compared with Fisher's test and continuous with Mann-Whitney. Binary logistic regression was used to model the antibiotic prescription. 12.721 patients attended the ED during the study period, and we screened 1106 (8.69%) patients during the working hours. 239 patients presented to the ED with fever, corresponding to a cumulative incidence of 21.6 per 100 triaged patients over the seven weeks. The mean age was 32.7 years (± 14.3), and 147 (61.5%) were females. 94 (69%; 95% CI 61-77%)

patient presentations were prescribed at least one antibiotic. Infections of the upper respiratory tract (42.2%) were the most common indication for prescribing antibiotics, followed by urinary tract (14.8%), and skin infections (13.3%). The antibiotic drug class most prescribed were Beta-lactam (52.7%), followed by quinolone (18.3%), and macrolides (12.9%). Patient who were prescribed antibiotics had a higher proportion of prior antibiotic use (17.9% *vs*. 2.5%, p=0.02), temperature ≥ 37.5 °C at presentation (22.8% *vs*. 13.6%, p=0.11), and distinct source of infection (p<0.005), compared to untreated group, respectively. In the adjusted model, only the presumed source of infection remained statistically associated with antibiotic prescription (urinary infection aOR: 7.58 (1.21-47.5%). Antibiotics are frequently prescribed for febrile patients seeking care at ED and respiratory infection account for the predominantly type of infection.

1800

LEADING CAUSES OF DEATH IN INFANTS AND CHILDREN UNDER 5 FROM THE CHAMPS NETWORK

Claudia M. Moya[1], Dianna M. Blau[2], Shabir Madhi[3], Victor Akelo[4], Quique Bassat[5], Karen L. Kotloff[6], Shams E. Arifeen[7], Richard Chawana[3], Emily S. Gurley[8], Inacio Mandomando[9], Dickens Onyango[10], Samba O. Sow[11], Robert F. Breiman[1], for the CHAMPS Network Consortium[1]

[1]*Emory Global Health Institute, Emory University, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [3]*Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa,* [4]*United States Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya,* [5]*Institut de Salut Global de Barcelona (ISGlobal), Universitat de Barcelona, Barcelona, Spain,* [6]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [7]*icddr,b (International Centre for Diarrhoeal Disease Research), Bangladesh, Dhaka, Bangladesh,* [8]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [9]*Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique,* [10]*Kisumu County Public Health Department, Kisumu, Kenya,* [11]*Center for Vaccine Development, Mali, Bamako, Mali*

The Child Health and Mortality Surveillance (CHAMPS) Network aims to better identify and understand specific causes of under-5 mortality in areas of Sub-Saharan Africa and South Asia through postmortem minimally invasive tissue sampling (MITS), laboratory tests, clinical records, and verbal autopsies. From these gathered data, determination of cause of death (DeCoDe) expert panels review each case and assign a cause of death using WHO guidelines. As of April 2019, we analyzed 246 cases of infant and child deaths with cause of death determined from CHAMPS sites in Bangladesh (0.4%), Kenya (30.1%), Mali (6.6%), Mozambique (14.1%), and South Africa (48.8%). Across all five sites, the most common underlying causes of death among infants and children were congenital birth defects (13.7%), malnutrition (11.9%), lower respiratory infections (11.0%), HIV (10.6%), and neonatal preterm birth complications (9.7%). Main immediate causes of death included lower respiratory infections (36.1%), sepsis (28.6%), diarrheal diseases (6.2%), malaria (6.2%), and meningitis (4.0%). For those deaths due to lower respiratory infections, the most frequent etiologic pathogens were *Klebsiella pneumoniae* (8.5%), *Streptococcus pneumoniae* (7.3%), *Haemophilus influenzae* (3.7%), *Staphylococcus aureus* (3.5%), and Cytomegalovirus (3.3%), and for sepsis, the most common etiologies were *Klebsiella pneumoniae* (13.6%), *Acinetobacter baumanii* (5.8%), *Streptococcus pneumoniae* (4.8%), *Staphylococcus aureus* (3.0%) and *Pseudomonas aeruginosa* (2.7%). Of these infant and children deaths, approximately 71% were considered preventable by the panels. Early CHAMPS data show the importance of understanding specific conditions leading to death in infants and children across five sites so that actions can be developed and utilized for reducing under-5 mortality.

1801

UNDERSTANDING AND RELATING THE EFFECTS OF INFECTIOUS PARAMETERS WITH G6PD DEFICIENCY

Tina Marye Slusher[1], Sarayu Patturi[2], Troy Lund[1], Stephanie Lauden[3], Grace Edowohorhu[4], Kolade Ernest[5], Daniel Gbadero[4]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Wayzata High School, Plymouth, MN, United States,* [3]*Nationwide Children's Hospital, Columbus, OH, United States,* [4]*Bowen University Teaching Hospital, Ogbomoso, Nigeria,* [5]*University of Ilorin Teaching Hospital, Ilorin, Nigeria*

Glucose-6-Phosphate Dehydrogenase deficiency (G6PDD) is the most common enzymopathy worldwide, affecting approximately 400 million people and causes hemolytic anemia as well as oxidative stress. The largest prevalence rates are in Africa. A previous study in Cameroon suggested G6PD deficiency might be related to Hepatitis C and syphilis infections. This study was undertaken to Determine the relationship between G6PDD and infectious and demographic parameters while validating the local prevalence of G6PD deficiency. Leftover blood from potential blood donors was collected at a large referral hospital in southwest Nigeria. Demographic information, blood types, and infectious markers [Hepatitis B , Hepatitis C, VDRL (syphilis) all using LabACON™ Rapid Test Strips; and HIV using Allere Determine™ HIV-1/2 test strip] were collected per hospital screening protocol except for addition of syphilis (VDRL) screening which was not routine. Additionally, the prevalence of G6PD deficiency was determined using the Carestart® screening test. G6PDD was found in 21% of 1085 blood donors tested. Mean age, hematocrit, and Rhesus status did not differ between groups. Blood groups did differ with type O being more common in the G6PD sufficient group (56.4% vs 46.8%). The prevalence of Hepatitis B, Hepatitis C viruses and VDRL were not statistically significant with respect to G6PDD (p=0.19, 0.67 and 0.09 respectively). HIV was positivity statistically correlated with G6PDD (p values 0.02). In conclusion, we demonstrate a relationship between G6PDD and HIV with a trend toward a relationship between G6PDD and HIV. This suggests correlation between G6PDD and HIV. VDRL screening should be validated with RPR before correlation can be assumed. Both findings highlight the need for further studies, raise questions about G6PDD representing an underlying immunodeficiency, and suggest possible changes to routine screening practices.

1802

FACTORS ASSOCIATED WITH DELAYS IN DIAGNOSIS AND TREATMENT OF MALARIA IN RETURNED TRAVELERS

Ariella Goldblatt[1], Emily Shaffer[2], Adrienne Showler[3]

[1]*MedStar Georgetown University Hospital Department of Internal Medicine and Pediatrics, Washington, DC, United States,* [2]*Georgetown University School of Medicine, Washington, DC, United States,* [3]*MedStar Georgetown University Hospital Division of Infectious Disease, Washington, DC, United States*

Malaria is the leading cause of infection-related death in returned travelers, who are at risk for severe disease due to lack of pre-existing immunity. In non-endemic areas, significant delays in malaria diagnosis and treatment are common, and contribute to poor outcome. We aimed to identify patient and health care systems factors associated with delayed care in returned travelers. We retrospectively identified all patients with an ICD 9/10 code diagnosis of malaria presenting to two academic tertiary care hospitals in Washington, DC, United States between January 1, 2013 and December 31, 2017. Only patients with microscopically confirmed malaria, or a positive Rapid Diagnostic Test (RDT) were included. We recorded patient demographics, and timing of hospital triage, malaria diagnostics, and anti-malarial administration. Fifty-nine patients had laboratory-confirmed malaria, the majority with *Plasmodium falciparum* acquired in Africa. Median time from triage to malaria diagnosis was 3.9 hours [IQR 2.4-5.9]. Only 16% of patients were diagnosed within 2 hours, and 22% experienced prolonged diagnostic delays of >6 hours. There was no difference in time to diagnosis based on race, insurance status, RDT availability, season, presentation on a night or weekend, % parasitemia,

malaria species, or presence of fever at triage. The median time from malaria diagnosis to treatment with any anti-malarial was 2.4 hours [IQR 1.1-5.3], with severe delays of >6 hours in 12% of patients. Median time from triage to treatment was 6.6 hours [IQR 4.5-8.5]. Patients treated with artemether-lumefantrine received antimalarials twice as quickly as those treated with atovaquone-proguanil (p<0.04). The World Health Organization recommends that results of malaria diagnostics be available within 2 hours, followed by immediate anti-malarial treatment. Only a minority of patients in our study received timely care, with a median time from presentation to treatment of almost 7 hours. Future quality improvement initiatives are needed to enable rapid identification and treatment of malaria in returned travelers.

1803

CLINICAL EVALUATION OF THE FILMARRAY® GLOBAL FEVER PANEL

Brian W. Jones, David Rabiger, Mark A. Gurling, Wendy Smith, Madeline Veloz, Olivia Jackson, Nathan King, Marisssa Burton, Christa Shorter, Cynthia D. Andjelic, Cynthia L. Phillips

BioFire Defense, LLC, Salt Lake City, UT, United States

Acute Febrile Illness (AFI) is caused by a diverse set of pathogens. Standard testing for the causal agent is often difficult, slow, costly, and complicated by the overlapping differential diagnoses for each potential pathogen. Testing for multiple pathogens simultaneously would simplify and accelerate diagnosis. The FilmArray Global Fever (GF) Panel, developed by BioFire Defense in collaboration with the U.S. Department of Defense[a] and NIAID[b], uses an automated, multiplex PCR system to evaluate whole blood samples for 19 pathogens simultaneously in under an hour. Targets of the GF Panel are Chikungunya virus, CCHF virus, dengue virus (serotypes 1-4), Ebolavirus, Lassa virus, Marburgvirus, West Nile virus, Yellow fever virus, Zika virus, *Bacillus anthracis*, *Francisella tularensis*, *Leptospira* spp., *Salmonella enterica* serovar Typhi and Paratyphi A, *Yersinia pestis*, *Leishmania donovani* complex, *Plasmodium* spp., *P. falciparum*, and *P. ovale/vivax*[c]. BioFire Defense is conducting a prospective clinical study to evaluate the sensitivity and specificity of the GF Panel when used to test blood collected from subjects with fever or who have recently had fever. This multi-center study is being conducted at locations around the world. Comparator testing consists of in-house developed PCR assays followed by bidirectional sequencing. Here we report the positive percent agreement and negative percent agreement of the GF Panel versus comparator testing, the rate of positive detections, and the fraction of specimens with more than one detected infection. Our results show that the FilmArray GF Panel could aid in rapid and actionable AFI diagnosis.

1804

HIGH REACTIVITY FOR HEPATITIS B AND OTHER TRANSFUSION-TRANSMISSIBLE INFECTIONS IN THE PREDONATION SCREENING OF BLOOD DONOR BY RAPID TEST IN A PUBLIC HOSPITAL OF THE PERUVIAN AMAZON

Mary Jeanette Rios[1], Andrea Saavedra[1], Jessye Cubas[1], Maher Zapana[1], Stalin Vilcarromero[2], Graciela Meza[1], Amy Morrison[3], Jaime Ramos-Flores[4]

[1]*Universidad Nacional de la Amazonía Peruana (UNAP), Iquitos, Peru,* [2]*Department of Medicine, Division of Infectious Diseases, Stony Brook University, New York, NY, United States,* [3]*Department of Entomology and Nematology, University of California Davis, Davis., California, CA, United States,* [4]*Hospital Regional de Loreto, Iquitos, Peru*

Infectious risk associated with blood transfusion remains a major public health concern in Peru, especially in endemic areas for other blood borne disease. Iquitos, the largest city of the Peruvian Amazon, is endemic for malaria and HIV, but other infectious disease such hepatitis B and Chagas are more prevalent in rural setting. Donations in the Peruvian Amazon of Peru require screening for at least five infectious markers: surface antigen (HBsAg) and the core antibody of hepatitis B virus (HBcAb), antibodies against human immunodeficiency virus (HIV) type 1 and 2 (anti-HIV 1 and/or anti-HIV 2), antibodies against hepatitis C virus (HCV); Treponema pallidum(syphilis); human lymphotropic viruses (anti-HTLV-1/2) and markers for Chagas disease. Usually potential donor gives the total blood sample before it is screened by rapid test (Vircell kit or Chagas and Beijing Wantai Biological Pharmacy Enterprise, Beijing, China for others). During January 2008 and December 2016, Hospital Regional de Loreto, a reference center for blood transfusion, received 43,288 blood units for potential blood donors, from which 4003 (9.25%) units were rejected and discarded due reactivity or indeterminate rapid test result. 3160 (78.94%), 629 (15.7%), 95 (2.38%), 59 (1.47%), 49 (1.2%) and 35 (0.87%) were reactive for HBcAb, Syphilis, HTLV I & II, HBsAg, Chagas and VIH 1&2. Other 161 (4.2%) were undetermined with a 74% for HBcAb. 3441 (86%) were male, 2635 (65.8%) had 17-38 years old, 1330 (33.22%) were single, 3420 (85.43%) live in urban setting. HTLV 1&2 and HBsAg and syphilis shows a marked trend to increase from 2008 to 2016. Other markers show a flat curve suggesting an endemicity. Interestingly, similar to the other markers, for HBcAb, 2641/3130 (84.37%) of rejected blood donators lived in urban setting. In a region with deficits of blood units, and also endemic for several blood-borne pathogens, it is worrisome that almost 10% of donated units are discarded demanding time and high cost. This analyze would be reflecting an epidemiological change (from rural to urban setting) especially for hepatitis B. Recent studies of seroprevalence would be necessary.

1805

INTEGRATING COMMUNITY CASE MANAGEMENT (ICCM) PAST THE BEND IN THE RIVER IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC)

Kate E. Gilroy[1], Jocelyne Kibungu[2], Elizabeth Hourani[1], Jimmy Anzolo[3], Osée Lieke Likunda[3], Emmanuel Likunde[4], Papy Luntadila[2], Michel Pacque[1]

[1]*MCSP/JSI, Washington, DC, United States,* [2]*MCSP/JSI, Kinshasa, Democratic Republic of the Congo,* [3]*MCSP/JSI, Kisangani, Democratic Republic of the Congo,* [4]*MCSP/JSI, Kinsangani, Democratic Republic of the Congo*

In the DRC, remote and vulnerable communities have benefited from community case management (CCM) of malaria services through vertical programs. Health facilities also benefit from malaria programs, but often do not have other child treatments available. In 2017, with support from USAID's flagship Maternal and Child Survival Program (MCSP), Tshopo and Bas-Uélé provinces introduced a more complete package of integrated CCM services in 119 remote and underserved communities. Community volunteers already providing malaria CCM were trained to manage and treat diarrhea and pneumonia for children under five years of age. With USAID support, MCSP procured oral rehydration solution and zinc for treating diarrhea and dispersible Amoxicillin tablets for treating pneumonia free of charge in the 119 community sites and 106 linked health facilities. Using data from the national health management information system, we assessed if the introduction of a complete package of services in communities increased the number of sick children treated or if it remained the same—indicating that sick children who would have sought care at the facility were now accessing community-based services. In 2016, approximately 60,000 cases of malaria, 7,000 of pneumonia, and 6,400 of diarrhea were treated at 106 facilities and 54 community sites with complete data. In 2018, this rose to over 85,500, 37,800 and 26,800 cases treated for childhood malaria, pneumonia and diarrhea, respectively, at the same facilities and community sites. The number of cases of childhood diarrhea and pneumonia treated increased over four-fold between 2016 and 2018 at the facility level and eight-fold at the community level. Over 20% of new cases of sick children were seen in the community in 2018. Our results suggest that introducing integrated CCM can increase the number of sick children treated for malaria and other illnesses in remote areas. The provision of free drugs through donor support can substantially increase the number of cases of child illnesses treated at all levels, although it raises important challenges in balancing sustainability versus equity and reaching the underserved.

1806

SHARED PATHOGEN-SPECIFIC RESERVOIRS AND TRANSMISSION PATHWAYS ASSOCIATED WITH ENTERIC PATHOGEN CO-INFECTIONS AMONG CHILDREN FROM THE KOLKATA, INDIA SITE OF THE GLOBAL ENTERIC MULTICENTER STUDY

Kurt Z. Long[1], Suman Kanungo[2], Inong Gunanti[3], Johanna Sanchez[4], James P. Nataro[5], Dilruba Nasrin[6], Myron Levine[6], Karen Kotloff[7]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*National Institute of Cholera and Enteric Diseases, Kolkata, India,* [3]*Faculty of Medicine and Biomedical Sciences, University of Queensland, Brisbane, Australia,* [4]*, Faculty of Medicine and Biomedical Sciences, University of Queensland, Brisbane, Australia,* [5]*Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA, United States,* [6]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [7]*Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD, United States*

The implementation of WASH programs and the development of new vaccine concerned with reducing the burden of diarrheal disease among young children have not considered the contribution of co-infections to this burden. Enteric co-infections may lead to poorer clinical outcomes in children compared with single infections. Bayesian path analyses were used to model the effect of household pathogen transmission pathways on the risk of the most prevalence co-infections among children enrolled in the Indian component of the Global Enteric Multicenter Study (GEMS). Models were developed to test whether such potential pathogen reservoirs as water source, storage and treatment as well as sanitation facilities were associated directly with greater risk of enteric co-infections or had indirect effects mediated by hygiene behaviors. Twenty-eight percent of pathogen isolates among children were co-infections of which 20 percent of these were co-infections due to *Giardia* and *Cryptosporidium*. Unimproved sanitation facilities in the household was associated with a greater risk of infections by both pathogens but this risk was reduced when caretakers washed hands before nursing with a greater reduction found for *Cryptosporidium*. A household water source in the yard was negatively associated with infections by both pathogens indirectly among children from households with unimproved sanitation facilities when mediated by handwashing before nursing. Storage of water was directly associated with greater risk of *Giardia* infections but a reduced risk of *Cryptosporidium* infections. An indirect and negative association of water storage with infections by both pathogens was also found in households with unimproved sanitation facilities when mediated by handwashing before nursing. These results suggest that co-infections by *Giardia* and *Cryptosporidium* may result from shared transmission pathways involving water source and water storage in households with unimproved sanitation but that specific handwashing behaviors can block such transmission.

1807

IDENTIFYING POTENTIALLY PREVENTABLE UNDERLYING CIRCUMSTANCES THAT LED TO INFANT DEATH IN LUSAKA, ZAMBIA: AN EXTENDED APPROACH TO THE THREE DELAYS MODEL

Andrew William Enslen[1], Ronke Olowojesiku[1], Anna Larson[2], William Macleod[2], Rotem Lapidot[2], Christopher J. Gill[2]

[1]*Harvard TH Chan School of Public Health, Boston, MA, United States,* [2]*Boston University School of Public Health, Boston, MA, United States*

Infant mortality rates remain disproportionately high in the sub-Saharan African region. Studies suggest that many of these deaths may be preventable and correspond to types of three delays: delay in seeking care, delay in reaching care, and delay in receiving adequate care. The purpose of this study is to identify preventable structural and sociocultural factors contributing to infant death in Zambia by applying an extended approach to the three delays model using narratives from caregivers. We reviewed a community-based collection of 230 verbal autopsies of infants aged 0-6 months who died outside of the hospital in Lusaka, Zambia. The verbal autopsies were collected between August 2017 and October 2018 as part of the ongoing Zambia Pertussis-RSV Infant Mortality Estimation (Z-PRIME) study conducted at the University Teaching Hospital. We performed a retrospective qualitative analysis of free text narratives included in the verbal autopsies. We assigned themes to the narratives and analyzed the results using Nvivo v12. 14 qualitative themes were assigned to the 230 verbal autopsies. 190 deaths were associated with the following delays in care: seeking care (n = 127, 55.2%), reaching care (n = 10, 4.3%), receiving adequate care (n = 53, 23.0%). The most common locations of death were in the home (n = 111, 48.2%) and en-route/upon arrival to clinic (n = 74, 32.2%). Among those who died prior to reaching care, over one-third (65/185) were previously seen by a provider. Four of the most common symptoms associated with a delay in seeking care were weakness (20/25), poor feeding (50/69), fever (75/109), and fast breathing (44/88). Additionally, 32 of the cases were not inconsistent with a diagnosis of sudden unexpected infant death. The majority of infant deaths were associated with household or structural circumstances leading to delays in care. Although more work is needed to further characterize these factors, our study suggests several opportunities for future interventions, such as improving caregiver education of "danger signs" and instituting triage protocols and patient return policies.

1808

HIGHER TREATMENT TO DIAGNOSIS RATIO IN MALARIA CASE MANAGEMENT IN NIGERIA

Wellington A. Oyibo[1], **Wellington Oyibo**[2], Diwe Ekweremadu[3], Genevieve Eke[3], Chukwudi Uche[3], Victoria Erinle[3], Victor Adebayo[3], Temitope Ipinmoye[3]

[1]*College of Medicine of the University of Lagos, Nigeria, Lagos, Nigeria,* [2]*College of Medicine, University of Lagos, Department of Medical Microbiology and Para., Lagos, Nigeria,* [3]*Catholic Relief Services, Abuja, Nigeria*

The policy recommendation that all suspected cases of malaria be confirmed with malaria rapid diagnostic tests (RDT) or microscopy before treatment with artemisinin combination therapies (ACTs) is critical in the implementation of the World Health Organization's test, treat and track recommendation which has also been adopted as the diagnosis and treatment of malaria policy in Nigeria since 2010. Over diagnosis and over-treatment of malaria is a challenge and has been reported in several countries in the literature. However, assessment of tests performed and treatment given in the case management of malaria in public health facilities where malaria RDTs are supplied through the Global Fund Programme is yet to be fully highlighted in a larger scale, especially as it relates to commodity quantification in making procurement decisions. We assessed malaria RDT and ACT use in public health facilities in six Global Fund supported States in Nigeria: Kwara, Osun, kano, Bauchi, Imo and Cross River States. In the six States where Malaria case management was strengthened through on-the-job supervision, the ratio of testing to treatment ranged from 1:2 - 1:4 in Health Facilities while in the states, it was 1:2 - 1:3. Overall diagnosis to treatment ratio was 1:2, indicating that diagnosis was yet to catch up with treatment. This finding has great implications in effective malaria case management with diagnosis, the implementation of the mandatory testing prior to treatment policy, waste of malaria medicines due to overuse of ACTs, increased spending on ACT procurement that ought to be reducing with declining malaria prevalence, and likely pressure on parasites that could trigger resistance to the ACTs. On-the-job supervision of healthcare worker infused with on-site training is recommended for increased RDT utilisation and a more strengthened malaria case management practices.

1809

COMPARISON OF RISKS OF READMISSION AFTER BREAST CANCER RECONSTRUCTION PROCEDURES: AN ANALYSIS OF 2011 TO 2014 NATIONAL READMISSION DATABASE

Oumar Thiero[1], Meghan Garstka[2], Alan Stolier[2], Emad Kandil[2]

[1]*International Center of excellence in research (ICER-MALI), University of Sciences, Techniques and Technology of Bamako (USTTB), Bamako, Mali,* [2]*Tulane University, School of Medicine, New Orleans, LA, United States*

The psychosocial benefits of breast reconstruction are numerous, particularly for women wellbeing. Study of specific risks factors associated with the readmission after breast cancer reconstruction may steer and optimize the reconstruction strategies. From 2011 to 2014, we performed a retrospective analysis of adult patients in the National Readmission Database. Patients undergoing breast reconstruction were grouped as implant/tissue -expender or autologous reconstructions. We used a weighted descriptive, univariate and the survey logistic regression analysis to compare risk factors associated with the two reconstructions procedures. Of 12532, 12503, 12951, and 12789 patients included in this study, 9400(75.01%), 9514(76.09%), 9784(75.55%), and 9337(73.01%) respectively underwent Implant/Expender reconstructions in 2011, 2012, 2013 and 2014 respectively. The two groups experienced similar readmission rates/year (around 10%=implant/expender and 11%=autologous, p's>0.5). However, of 12 commons predictors of the adjusted model/year, 10 were significantly associated with the reconstruction types. Furthermore, the autogenous experienced high rates (up to 6 times) in *All_Patient_Refined DRGs_Severity* loss of function (ref=*minor_loss*), comorbidities(ref=no-comorbidity) etc.., while implant/expender experienced high rates (up to 12 times) in *All_Patient_Refined_DRGs _Mortality* risk (ref=*minor_risk*) and number of chronic conditions(ref=no-chronic). The two groups maintained similar *zip_income* rate/year. In conclusion, autologous and implant/expender breast reconstruction procedures were found to be strongly risk-specifics related, suggesting an appropriate risk-stratification and scrutiny before discharge may reduce the impact of readmission.

1810

LYMPHATIC FILARIASIS ENDGAME: UNDERSTANDING HOTSPOTS IN ELIMINATION PROGRAMS

Sellase A. Pi-Bansa[1], Joseph Harold Osei[1], Kwadwo Kyeremeh Frempong[1], Elisabeth Elhassan[2], David Agyemang[2], Samuel Dadzie[1], Maxwell Alexander Appawu[1], Michael David Wilson[1], Benjamin Guibehi Koudou[3], Dziedzom Komi de Souza[1], Jürg Utzinger[4], Daniel Adjei Boakye[1]

[1]*Noguchi Memorial Institute for Medical Research (N.M.I.M.R), Accra, Ghana,* [2]*SightSavers International, Accra, Ghana,* [3]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Lymphatic Filariasis (LF) is earmarked for elimination through mass drug administration (MDA). Six years was hypothesized to interrupt LF transmission. There have been at least 16 years of MDA in some districts of Ghana and yet there is ongoing low level transmission. Several factors which may be endemic area specific could be contributing to transmission in these districts termed LF "hotspot districts". These factors need understanding if elimination is to be achieved. The objective of this study was to identify possible factors contributing to persistent LF transmission and their impact on elimination in Ghana. The study was done in two hotspot (Ahanta West, Kassena Nankana West) and control (Mpohor, Bongo) districts in the Western and Upper East regions of Ghana respectively. Community vector collectors (CVCs) were trained in all districts to collect mosquitoes using human landing catches (HLC), pyrethrum spray catches (PSC) and window exit trap (WET). *Anopheles* (*An.*) *gambiae* were dissected from all districts and polymerase chain reaction (PCR) run for *An. gambiae* and *Wuchereria bancrofti* identification respectively. Questionnaires were administered in all districts to assess vector control activities. Mosquitoes collected by CVCs were 31,064 [HLC=27,739 (89.3%), PSC=2687 (8.7%) and WET=638 (2.1%)]. In all districts, *Aedes*, *An.coustani*, *An.gambiae* s.l., *An.pharoensis*, *Culex* and *Mansonia* mosquitoes were found. *An.melas* was found only in Ahanta West district and also infective therefore likely to be sustaining LF transmission (annual transmission potential =7.4). There was no infection in all other districts. Bednet usage was generally higher in control compared to hotspot areas (Mpohor=87.0%, Ahanta West=69.1%; and Bongo=91.1%, Kassena Nankana West=82.4%). It is feasible to use CVCs for xenomonitoring and/or post-MDA surveillance. We observed higher mosquito abundance and specific species may be involved in the ongoing transmission. Due to spatial heterogeneities, there should be unique intervention programs in LF endemic districts. Xenomonitoring should therefore be considered as part of the LF end time surveillance.

1811

MICROFILARIAE CIRCULATING IN DOGS FROM CALI, COLOMBIA

Luisa M. Nieto Ramirez[1], Tania Gaviria[1], Claudia L. Villegas[2], Isabel C. Garcia[2], Leidy L. Diaz[3], Beatriz E. Ferro[3]

[1]*Universidad Santiago de Cali, Cali, Colombia,* [2]*Laboratorio Zoolavet, Cali, Colombia,* [3]*Universidad Icesi, Cali, Colombia*

Different filarial species can be transmitted by vectors and affect mammals, including humans and dogs. In Cali, Southwestern Colombia, microfilariae cases in dogs is not well documented. A local diagnostic laboratory described an observational study that detected 61 microfilariae cases during 1999 to 2003, while 50 cases were detected in one-year period in 2017, suggesting a potential parasitic outbreak. However, the causing species were not identified for most of the cases. To study this potential outbreak and the circulating microfilaria species, we conducted a molecular characterization of positive cases identified from August 2018 to February 2019 in dogs from Cali. Microfilariae cases detected by blood smears stained with Wright were also evaluated by a standard immunoassay. In addition, DNA was extracted from EDTA-blood samples to perform a PCR test to amplify ribosomal DNA spacer sequences for different canine microfilaria species including: *Acanthocheilonema reconditum, Dirofilaria immitis* and *D. repens*. Sanger-sequencing of PCR products was used for the specie confirmation in a subset of samples. Clinical and demographic data were also collected. We detected 34 positive microfilariae cases. All except one were negative for *D. immitis* by the immunoassay. PCR test was positive for all the cases and negative for 15 negative DNA control samples obtained from healthy dogs. Partial sequencing results revealed the presence of *A. reconditum* in 11 cases tested. Mixed-breed (17/34, 50%), followed by poodle (6/34, 17.6%) were the most affected breeds, with a median age of 6 years (interquartile range 3-10 years). Male dogs represented 85.3% (29/34) of the positive cases. All cases ranged from no signs of disease to cutaneous lesions, anorexia, and anemia (50% of cases). The PCR test contributed to the further detection of the causing microfilariae specie in most of the studied cases, revealing a microfilaria specie, *A. reconditum,* that has not been widely identified in Colombia. The clinical significance of this specie in dogs as well as its zoonotic potential remains to be determined with a larger number of cases.

1812

MAPPING THE PRE-CONTROL PREVALENCE OF LYMPHATIC FILARIASIS ACROSS NIGERIA

Obiora A. Eneanya[1], Claudio Fronterre[2], Ifeoma Anagbogu[3], Chukwu Okoronkwo[3], Tini Garske[1], Jorge Cano[2], Christl Donnelly[1]

[1]*Imperial College London, London, United Kingdom,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Federal Ministry of Health, Abuja, Nigeria*

The pre-control endemicity profile of lymphatic filariasis (LF) is a key benchmark for planning control programmes, monitoring their impact on transmission and assessing the feasibility of achieving elimination. Presented in this work is the modelled serological and parasitological

prevalence of LF prior to the scale-up of mass drug administration (MDA) in Nigeria using a machine learning based approach. LF prevalence data generated by the Nigeria Lymphatic Filariasis Control Programme during country-wide mapping surveys conducted between 2000 and 2013 were used to build the models. The dataset comprised of 1,103 community-level surveys based on the detection of filarial antigenaemia using rapid immunochromatographic card tests (ICT) and 184 prevalence surveys testing for the presence of microfilaria (Mf) in blood. Using a suite of climate and environmental continuous gridded variables and compiled site-level prevalence data, a quantile regression forest (QRF) model was fitted for both antigenaemia and microfilaraemia LF prevalence. Model predictions were projected across a continuous 5 × 5km gridded map of Nigeria. The number of individuals potentially infected by LF prior to MDA interventions was subsequently estimated. Maps presented predict a heterogeneous distribution of LF antigenaemia and microfilaraemia in Nigeria. The North-central, North-west, and South-east regions displayed the highest predicted LF seroprevalence, whereas predicted Mf prevalence was highest in the southern regions. Overall, 8.7 million and 3.3 million infections were predicted for ICT and Mf, respectively. QRF is a machine learning-based algorithm capable of handling high-dimensional data and fitting complex relationships between response and predictor variables. Our models provide a benchmark through which the progress of ongoing LF control efforts can be monitored.

1813

DOSING POLE RECOMMENDATIONS FOR MASS DRUG ADMINISTRATION OF IVERMECTIN AND DIETHYLCARBAMAZINE FOR LYMPHATIC FILARIASIS ELIMINATION: A HEIGHT-WEIGHT QUANTILE REGRESSION MODELING APPROACH

Charles W. Goss[1], Katiuscia O'Brian[1], Peter U. Fischer[1], Myra Hardy[2], Purushothaman Jambulingam[3], Christopher L. King[4], Moses Laman[5], Jean Frantz Lemoine[6], Leanne Robinson[7], Josaia Samuela[8], Swaminathan Subramanian[3], Taniawati Supali[9], Gary J. Weil[1], Kenneth B. Schechtman[1]

[1]*Washington University, St Louis, MO, United States,* [2]*Murdoch Children's Research Institute, Melbourne, Australia,* [3]*ICMR-Vector Control Research Centre, Puducherry, India,* [4]*Case Western Reserve University, Cleveland, OH, United States,* [5]*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea,* [6]*Ministère de la Santé Publique et de la Population (MSPP), Port au Prince, Haiti,* [7]*Burnet Institute, Melbourne, Australia,* [8]*Fiji Ministry of Health and Medical Services, Suva, Fiji,* [9]*Universitas Indonesia, Jakarta, Indonesia*

Lymphatic filariasis (LF) is a debilitating parasitic disease that affects over 70 million people across the world. To eliminate this disease the World Health Organization (WHO) recommends treating entire endemic communities with drug combinations that include ivermectin (IVM) diethylcarbamazine (DEC). Ideally, the amount of drug administered should be determined by weight. However, this is often not feasible in rural, resource limited areas. The alternatives currently recommended by the WHO based on height (a dosing pole for IVM) and age (DEC) have maximal doses below those recommended by weight-based dosing. In this study we used data from a large multi-center LF community-based clinical trial (5 countries, >26,000 individuals) to evaluate current WHO age- and height-based dosing recommendations, and to develop and evaluate new height-based dosing (dosing poles) recommendations based on these data. Our analysis involved a 3-step modeling approach to develop and evaluate new dosing pole cutoffs for both IVM and DEC. We first analyzed data collected from the LF clinical trial using quantile regression to predict weight from height. Weight predictions from our models were then used to develop new dosing pole cutoffs. Finally, we compared our model-based dosing poles to recommended weight-based dosing to determine whether our models improved upon the current height and age-based dosing recommended by the WHO. Our results showed that the WHO methods would have resulted in 32% and 27% of individuals in the clinical trial dataset receiving below the recommended weight-based dosage of DEC and IVM, respectively. Underdosing would have been especially common in adult males (54% for DEC and 39% for IVM), who tend to have the highest LF prevalence in many endemic areas. Results from our statistical analyses showed that a model-based dosing pole would markedly reduce underdosing with 2% and 6% receiving below the recommended dosage for DEC and IVM, respectively. The dosing pole we propose has the potential to dramatically improve dosing and facilitate the elimination of lymphatic filariasis globally.

1814

COMPARISON OF MICROSCOPY TO REAL-TIME POLYMERASE CHAIN REACTION AND LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAYS IN MONITORING OF SKIN MICROFILARIAE OF *ONCHOCERCA VOLVULUS* WITHIN SIX MONTHS OF DIRECT OBSERVED TREATMENT WITH IVERMECTIN

Samuel Wanji[1], Raphael Awah Abong[1], Glory Amambo[1], Patrick W. Ndongmo[1], Abdel Jelil Njouendou[1], Manuel Ritter[2], Amuam Andrew Mbeng[1], Mathias Eyong Esum[1], Kebede Deribe[3], Jerome Fru[1], Fanny Fri Fombad[1], Theobald Mue Nji[1], Peter Ivo Enyong[1], Catherine B. Poole[4], Kenneth Pfarr[2], Achim Hoerauf[2], Clotilde K. Carlow[5]

[1]*University of Buea, Buea, Cameroon,* [2]*Institute of Medical Microbiology, Immunology and Parasitology, University Hospital, Bonn, Germany,* [3]*Global Health and Infection Department, Brighton and Sussex Medical School, Brighton, United Kingdom,* [4]*New England Biolabs, Ipswich, MA, United States,* [5]*New England Biolabs, Ipswich, MA, United States*

The low sensitivity of microscopy in the diagnosis of skin microfilariae of *Onchocerca volvulus* is a shortcoming for effective monitoring and evaluation of control programmes of onchocerciasis based on micro- or macrofilaricides. We compared the microscopy technique to two nucleic acid-based techniques in the detection of skin microfilariae of *O. volvulus* following ivermectin treatment at 1, 3 and 6 months. Identified microfilaridermic individuals in Bafia and Melong were treated with ivermectin and re-examined after 1, 3, and 6 months. Infection prevalence was compared for the three techniques. Microscopy was used as an imperfect gold standard to determine performance characteristics of real-time PCR and LAMP assay. The performances of the tests were evaluated using Bayesian Latent Class Models (LCM). From the 51 patients monitored, microscopy detected 21.6%, 23.5% and 45.8% prevalence of *O. volvulus* skin infection at 1, 3 and 6 months of screening, respectively. However, real-time PCR detected prevalence of 23.5%, 31.4% and 54.2%, whereas LAMP technique detected prevalence of 29.4%, 37.3% and 64.6%, respectively. There was a statistically significant difference in the prevalence of infection at the different time points using the three diagnostic methods ($P \leq 0.014$). The overall prevalence for the 150 samples collected during the six-month period was 30%, 36.3% and 43.7% for microscopy, real-time PCR and LAMP assay respectively and the difference between them was statistically significant ($P < 0.05$). LAMP assay had the best sensitivity (99.4%) compared to real-time PCR (98.1%) and microscopy (80.7%). This study has demonstrated that LAMP technique has superior sensitivity over microscopy and real-time PCR in detecting skin microfilariae of *O. volvulus* within six months following ivermectin treatment. The LAMP assay could be useful in monitoring *O. volvulus* microfilariae following treatment with filaricides.

1815

ACCOUNTING FOR PREFERENTIAL SAMPLING IN SPATIOTEMPORAL MODELS OF LYMPHATIC FILARIASIS PREVALENCE

Chris Schmidt, Kevin Kwong, Katie Donkers, Elex Hill, David Pigott, Shreya Shirude, Simon Hay, Elizabeth Cromwell

University of Washington, Seattle, WA, United States

Lymphatic filariasis (LF), a leading cause of long-term disability, has been targeted for global elimination as a public health problem. Programmatic decision-making for the initiation or cessation of mass drug administration

(MDA), the primary intervention for LF elimination, relies on estimates of infection prevalence obtained through a multi-year strategy of baseline mapping, longitudinal sampling in sentinel sites, transmission assessment surveys (TAS), and post-MDA surveillance. High-resolution geospatial models can leverage existing sources of LF prevalence data to predict prevalence in data-poor regions and identify priority locales for additional data collection. While LF monitoring programs may emphasize sampling in areas expected to have particularly high or low prevalence, depending on programmatic stage, standard geospatial models assume that data are collected non-preferentially, i.e., that survey sites are sampled independently of their expected prevalence. Failing to account for this preferential sampling may bias model inferences. In order to improve global estimates of LF prevalence, we test for evidence of preferential sampling in Bayesian spatiotemporal models of global LF prevalence. We compare inferences from three models: (1) a standard model assuming non-preferential sampling; (2) the addition of simple fixed effects for programmatic stage (e.g., mapping, sentinel site, or TAS); and (3) an explicit preferential sampling model employing Poisson point processes for spatial sampling probabilities. We compare model predictions in both heavily sampled and undersampled regions and discuss implications for LF elimination programs and for other neglected tropical diseases with similarly complex data generation.

1816

IMPACT OF REPEATED ANNUAL MASS DRUG ADMINISTRATION WITH IVERMECTIN THROUGH COMMUNITY DIRECTED TREATMENT ON THE ENTOMOLOGICAL INDICATORS OF *LOA LOA* TRANSMISSION IN CAMEROON

Patrick W. Ndongmo, Glory Ngongeh, Fanny Fri Fombad, Abdel Jelil Njouendou, Bertrand Ndzeschang, Mathias Eyong Esum, Peter Enyong, Samuel Wanji

University of Buea, Buea, Cameroon

Loiasis is a filarial infection endemic in the rainforest zone of west and central Africa. Repeated treatments with ivermectin have been delivered using the annual community directed treatment with ivermectin (CDTI) approach for several years to control onchocerciasis in some *Loa loa*-*Onchocerca volvulus* co-endemic areas. The impact of CDTI on *L. loa* transmission in those areas is not known. We, therefore, designed this cross-sectional study to assess the impact of several rounds of CDTI on entomological indicators of loiasis. The study was conducted in 3 CDTI projects of Cameroon. Two communities per CDTI project were selected. *Chrysops* were collected with sweep net and dissected using microscopy. A total of 7029 female *Chrysops* were collected from the 6 communities under study. *Chrysops* biting densities and parous rates were reduced significantly in the northwest and southwest sites post CDTI while in the east, biting densities were similar in CDTI and nonCDTI sites with higher parous rates in the nonCDTI area. Infection and infective rates in the East nonCDTI site were 4.4% and 1.8% respectively but 3.3% and 1.3% in the CDTI district with 8 ivermectin treatment rounds. In the Northwest site, significant reductions of *Chrysops* infection and infective rates from 10.2% and 4.2% respectively to 3.5% and 1.2 (after 9 ivermectin rounds) were registered post CDTI while in the southwest, infection rates significantly increased from 1.74% to 2.8% and infective rates remained statistically unchanged after 14 rounds of CDTI (0.45% - 0.40%). Similar trends in Mean Head L3 were observed in all but the east CDTI site. Globally, a negative relationship was observed between the number of CDTI rounds and *Chrysops* infection and infective rates. Monthly transmission potentials significantly decreased after CDTI only in the northwest sites. This study has for the first time demonstrated that in areas where onchocerciasis and loiasis are co-endemic, CDTI has reduced the number of *Chrysops* carrying infective larvae with concomitant decrease in *L. loa* monthly transmission potentials; but has not interrupted transmission of loaisis.

1817

INDIVIDUAL RISK OF POST-IVERMECTIN SEVERE ADVERSE EVENTS IN INDIVIDUALS INFECTED WITH *LOA LOA*

Cédric B. Chesnais[1], Sebastien D. Pion[1], Jacques Gardon[1], Nathalie Gardon-Wendel[2], Joel Fokom-Domgue[3], Joseph Kamgno[4], Michel Boussinesq[1]

[1]*Institut de recherche pour le Développement, Montpellier, France,* [2]*Antenne ORSTOM auprès du Centre Pasteur, Yaoundé, Cameroon,* [3]*The University of Texas MD Anderson Cancer Center, Houston, TX, United States,* [4]*Centre for Research on Filariasis and other Tropical Diseases, Yaoundé, Cameroon*

Implementation of onchocerciasis and lymphatic filariasis elimination programs has been delayed in Central Africa because of the risk of post-ivermectin severe adverse events (SAEs) in people with high *Loa loa* microfilarial densities (MFD). The incidence rate of SAEs (i.e. with a functional impairment requiring for at least one week full-time assistance) has been assessed in some settings and the relative risk, compared to subjects without *Loa* microfilariae (mf), of developing a SAE or a marked reaction (with functional impairment for several days) for increasing *Loa* MFD has been evaluated. However, the individual predicted risk of SAE for a given *Loa* MFD is unknown. To estimate this individual risk, as well as the MFD for which the predicted risk of SAE is 1/1,000 and 1/100, we used information from two trials conducted in Cameroon: one in 1997 in the Lekie division (Central region), and the other in 2005 in the Lom-et-Djerem division (East region). We performed mixed multivariable logistic models using fractional polynomials for age and pre-treatment *L. loa*, and *Mansonella perstans* MFD and category for sex. The models included a random effect on the village of residence. All possible interactions were tested. Among the 10,506 trial subjects treated with ivermectin (males: 48.9%, mean age: 35.4 years), 38 developed an SAE, including two cases of coma. A total of 2,792 (28.3%) subjects were microfilaremic for *L. loa*. The results showed a higher risk of SAE in males and in subjects with high *L. loa* MFD and no significant association with age and *M. perstans* MFD. This statistical modeling allows for predicting individual risk of SAE for a given *L. loa* MFD: subjects with 10,000 mf/ml and 27,000 mf/ml have a risk of 1/1,000 and 1/100, respectively, to develop a SAE following ivermectin treatment. These results can help to better predict the risk of post-ivermectin SAE in communities where the distribution of *L. loa* MFD has been assessed, for example during mapping activities.

1818

THE EFFECT OF ALBENDAZOLE TREATMENT ON *LOA LOA*: A SYSTEMATIC REVIEW, META-ANALYSIS AND MODELLING STUDY

Charles Whittaker[1], Joseph Kamgno[2], Amy Klion[3], Martin Walker[4], Sébastien D.S. Pion[5], Cédric B. Chesnais[5], Benjamin Lambert[1], Annette Kuesel[6], Maria-Gloria Basáñez[1], Michel Boussinesq[5]

[1]*Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom,* [2]*Centre for Research on Filariasis and Other Tropical Diseases, and Faculty of Médicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon,* [3]*Human Eosinophil Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [4]*Department of Pathobiology and Population Sciences, Royal Veterinary College, Hatfield, UK, Hatfield, United Kingdom,* [5]*Institut de Recherche pour le Développement (IRD), Montpellier, Montpellier, France,* [6]*UNICEF/UNDP/World Bank/World Health Organization Special Programme on Research and Training in Tropical Diseases (TDR), Geneva, Switzerland*

Increasing evidence suggests that loiasis (caused by the filarial nematode *Loa loa*) poses a significant public health threat to the estimated 10 million infected individuals across Central Africa. Treatment of the disease is complex: although the anti-parasitic drugs diethylcarbamazine and ivermectin are highly efficacious at clearing the infection, they cannot be administered to individuals with heavy microfilarial loads, due to the

risk of fatal encephalopathy associated with treatment. A 3-week course of daily albendazole was reported to safely and progressively decrease *L. loa* microfilaraemia, but shorter regimens would be more practically applicable. Previous trials on the effect of short-term regimens have shown mixed results, including significant inter-individual variability of their effect on *L. loa* microfilaremia: further clarification of their effect is crucial given that albendazole remains one of the few anthelmintics routinely administered to individuals with the highest microfilarial densities. We undertook a systematic review and meta-analysis of trials (n = 5, containing data for 226 patients) using various albendazole regimens (ranging from 1-21 days) and dosages (400-800mg daily) to better understand their effect on loiasis. We constructed a set of pharmacokinetic/pharmacodynamic models of treatment dynamics to integrate the available longitudinal individual-level data detailing microfilarial densities following treatment, and assessed the effect of the drug, dosage and treatment regimen on microfilaremia. Our results reveal substantial variation both between studies (in which dose and regimen have varied considerably) and between individuals (despite identical dosing and treatment regimen), and better resolves the extent of the effect of albendazole treatment on *L. loa*. This work highlights potential drivers of the pronounced heterogeneity observed in response to albendazole treatment, as well as possible strategies to optimise the use of albendazole in loiasis infected individuals moving forward.

1819

THE CURRENT EVIDENCE BASE FOR LYMPHATIC FILARIASIS ELIMINATION THRESHOLDS: IDENTIFYING THE KEY UNKNOWNS

Emma L. Davis[1], Lisa J. Reimer[2], Lorenzo Pellis[3], T Deirdre Hollingsworth[4]

[1]*University of Warwick, Coventry, United Kingdom,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*University of Manchester, Manchester, United Kingdom,* [4]*Big Data Institute, University of Oxford, Oxford, United Kingdom*

In the global drive for elimination of lymphatic filariasis, 15 countries have brought prevalence below the WHO-specified thresholds for halting mass treatment and achieved validation of elimination as a public health problem (EPHP). There is a strong mathematical and biological basis for the existence of a break-point for lymphatic filariasis: a threshold below which transmission cannot be sustained. The current thresholds for stopping MDA were set as practical targets with the expectation that they would lead to a high probability of transmission fading away in most settings (this is called elimination of transmission, or EOT). However, this assumption has been undermined by recent evidence of ongoing transmission in some regions. Here we show that the probability of EOT explicitly depends on key biological parameters, many of which have a poor evidence base. We calculated the probability of lymphatic filariasis transmission elimination, given a particular prevalence (e.g. 1%), by considering the probability a chain of transmission will die out to show that stochastic extinction is possible before the true break-point is reached. However, our results demonstrate that the existing experimental evidence does not support a high probability of elimination for the current WHO targets, partially due to uncertainties in parameters. We also demonstrate the importance of the annual biting rate in determining suitable threshold levels for targeting EOT. As more countries progress through MDA programs and begin to cease interventions it is vital that we ensure this process is well-informed, as prematurely halting control programs could pose a serious threat to global progress. Our findings demonstrate that we cannot be comfortably assured of EOT following validation of EPHP, according to the current thresholds for passing TAS, and that refining experimental estimates of key unknowns could be a vital step to greater understanding of the conditions that make elimination possible.

1820

ASSESSMENT OF LYMPHATIC FILARIASIS (LF) PREVALENCE TRENDS ALONG THE COASTAL REGIONS OF TANZANIA

Upendo John Mwingira[1], Denis Kailembo[2], Andreas Nshala[3], Veronica Kabona[4], Cecilia Uisso[1], Mwelecele Malecela[5]

[1]*National Institute for Medical Research, Dar Es Salaam, United Republic of Tanzania,* [2]*NTD Control Program, Dar Es Salaam, United Republic of Tanzania,* [3]*Uppsala University, Uppsala, Sweden,* [4]*IMA World Health, Dar Es Salaam, United Republic of Tanzania,* [5]*World Health Organization, Geneva, Switzerland*

Tanzania is endemic for all preventive chemotherapy Neglected Tropical Diseases NTDs and adopted the World Health Organization (WHO) resolutions for elimination and control of NTDs including elimination of Lymphatic Filariasis (LF). Mapping for LF reported 65% (120 out 185) of the districts in Tanzania to have >2% Circulating Filarial Antigen (CFA) levels thus necessitating MDA to be initiated. By 2019, over 80% of the endemic districts (96 out of 120) have met criteria for stopping MDA. However, the remain 20% (24 out of the 120 endemic councils) still require MDA. We assessed the transmission dynamics of *Wuchereria bancrofti* in the human population along the Pwani, Lindi and Mtwara regions. We gather evidence from mapping, baseline assessment, sentinel and spot-check sites to gauge impact of mass drug administration in the absence of the complete transmission assessment survey (TAS). Mapping in the 24 coastal districts had average CFA levels of 46% (0.0% to 72.0%). Following MDA CFA level have steadily declined to <1% in 62.5% (i.e. 15 of 24 districts), thus meeting the criteria to stop MDA. These coastal districts bear most of the burden of LF as 37.5% still require MDA compared the national average of 20%. Over 51 sentinel and spotcheck sites were assessed and individual aged 5 years and above tested for CFA- resulting in decrine over the years from 46% to an average of 4.8%. In stopping MDA, CFA levels and MDA coverage for 5 rounds are important for interruption of transmission, however, only 4% of the districts treated for five (5) rounds as recommended by WHO achieved criteria for stopping MDA. There is clear trend of persistent infection transmission in this coastal belt, with 46% of districts going up to nine (9) rounds of MDA before stopping MDA. Coverage monitoring is important. All districts had average epidemiological coverage ranging from 65% to 78% over the implementation period. In conclusion, a significant reduction of CFA prevalence is noted however, 37% of districts have persisted infections as CFSA is still <2% despite more than five annual rounds of MDA.

1821

THE ENVIRONMENTAL SUITABILITY OF ONCHOCERCIASIS IN AFRICA

Elizabeth Cromwell, Joshua Osborne, Kimberely Johnson, Elex Hill, Shreya Shirude, Katie Donkers, David Pigott, Simon I. Hay

University of Washington (IHME), Seattle, WA, United States

Recent evidence from the Americas, Sudan and Uganda suggest that onchocerciasis elimination may be feasible after 10-15 years of mass drug administration with ivermectin. A shift from control to elimination of onchocerciasis will require national programs in Africa to conduct new baseline data collection in implementation units (IUs) where prevalence of infection is unknown. Using Boosted Regression Trees with optimized hyperparameter selection, we implemented an environmental suitability analysis using available data on the presence of onchocerciasis in Africa to predict to what extent any of the approximately 2,400 IUs identified by WHO ESPEN as uncertain endemicity status were likely to be suitable for transmission. Covariates included elevation, enhanced vegetation index, tasseled cap brightness, tasseled cap wetness, minimum distance from rivers of 25m in width or larger, elevation grade, aridity, cumulative annual precipitation, land surface temperature, and urbanicity. Mean environmental suitability estimates were concordant with areas currently defined as endemic with 96% of IUs with at least one pixel >75% suitability. The majority of IUs with high environmental suitability are located in Angola (76 IUs), Ethiopia (82 IUs), DRC (167 IUs), Kenya (81

IUs) and Nigeria (72 IUs). Of the IUs considered for baseline surveys not currently under MDA for LF, model results suggest approximately 45% are highly suitable. We observed maximum pixel and minimum pixel values per IU ranging as high as .95, suggesting a high degree of variation within each IU at the fine spatial scale. Among IUs for which onchocerciasis endemicity status is unknown but are currently under MDA with ivermectin for the purpose of LF elimination, 57% were predicted to have environmental suitability exceeding 75%. The scale of baseline data collection required to complete mapping of onchocerciasis endemicity across the entire African continent presents an opportunity to use spatial data to facilitate survey planning. IUs most likely to sustain transmission of onchocerciasis could be prioritized for data collection, enabling earlier initiation of MDA programs.

1822

COVERAGE ASSESSMENT FOLLOWING MASS DRUG ADMINISTRATION OF THE NEW WHO-RECOMMENDED THREE-DRUG REGIMEN FOR LYMPHATIC FILARIASIS ELIMINATION IN AMERICAN SAMOA

Tara A. Brant[1], Rebecca J. Chancey[1], Lynette Suiaunoa-Scanlan[2], Tamara Buhagiar[2], Ryan E. Wiegand[1], Emily A. Dodd[1], Kimberly Y. Won[1], Emi Chutaro[3], Fara Utu[4], Motusa Tuileama Nua[4]

[1]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Pacific Island Health Officers' Association, Pago Pago, American Samoa,* [3]*Pacific Island Health Officers' Association, Honolulu, HI, United States,* [4]*American Samoa Department of Health, Pago Pago, American Samoa*

In 2017, the World Health Organization (WHO) introduced a three-drug regimen of ivermectin, diethylcarbamazine, and albendazole (IDA) for lymphatic filariasis (LF) mass drug administration (MDA). Models suggest that in most settings ≥75% coverage of IDA MDA is needed for two rounds to reach elimination targets. Despite efforts to eliminate LF from American Samoa (AS) from 2000-2015, results from a 2016 survey indicated widespread transmission. In 2018, AS became the second site globally to implement IDA MDA at program scale. The reported drug coverage based on the 2010 census data was approximately 55%. However, coverage in Manu'a District was >90% based on a community census conducted during MDA, bringing into question the accuracy of the 2010 census data. We conducted a coverage survey in February 2019 to assess program reach and validate reported drug coverage. We used systematic random sampling to identify 30 clusters in 24 of the 74 villages and interviewed all members of selected households about their participation in the MDA. Program reach and coverage were calculated based on the number of participants who were offered and who swallowed the drugs. Results indicated that among the 715 participants sampled, 88.7% (95% Confidence Interval (CI), 84.7%-78.0%) stated they were offered drugs during MDA. Coverage was 72.7% (95%CI, 66.5%-78.0%) and coverage estimates by village ranged from 48.6% to 100.0%. Of the 68 who were offered but did not take the drugs, 27.9% were ineligible and 22.0% were too busy. Based on coverage assessed by the survey, we estimate that 30,314 people were treated during the MDA and that 41,697 people were actually residing in AS during the MDA, approximately 25% fewer than recorded in 2010. Based on the program report of coverage, AS did not meet the current WHO threshold of ≥65%; however, surveyed coverage was close to the ≥75% threshold needed for effective IDA MDA. As monitoring and evaluation guidelines for IDA MDA are being refined, coverage will likely be an important indicator of impact of the new regimen. These data highlight the importance of coverage surveys, especially in the absence of accurate population estimates.

1823

DETECTION OF RESIDUAL *FOCI* OF LYMPHATIC FILARIASIS TRANSMISSION TWO YEARS AFTER STOPPING MASS DRUG ADMINISTRATION: CASE OF DANO HEALTH DISTRICT IN BURKINA FASO

Roland Bougma[1], Mamadou Serme[1], Christophe Nassa[1], Micheline Ouedraogo[2], Appolinaire Kima[1], Clarisse Bougouma[1], Dieudonné Nare[2], Jean-Paul Djiatsa[2], Fanny Yago-Wienne[2], Amy Veinoglou[3], Yaobi Zhang[4]

[1]*NTD Control Program, Ministry of Health, Ouagadougou, Burkina Faso,* [2]*Helen Keller International, Ouagadougou, Burkina Faso,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Burkina Faso has made great progress toward the elimination of lymphatic filariasis (LF) as a public health problem, with 61 of 70 originally endemic health districts (HDs) having stopped mass drug administration (MDA). Dano HD was mapped in 2000 and recorded a high prevalence (ICT: 72%). After more than 15 years of MDA, Dano was the only HD in the South West region to meet the criteria for stopping MDA during the transmission assessment survey (TAS1) using the Filariasis Test Strip (FTS) in 2016: 8 positive cases were detected (critical cut-off of 18). Two years after stopping MDA, TAS2 was conducted in December 2018 using FTS. A total of 1818 children of 6-7 years were surveyed with zero positives detected (critical cut-off of 18). A descriptive cross-sectional study was integrated with TAS2 to follow-up the positive cases originally detected during TAS1 in 2016. The objective was to detect possible LF transmission *foci* around the old positives. All individuals two years and older residing in the same compound as the 8 TAS1 positive cases were surveyed. The variables studied were sex, age, the FTS result, and the status of ivermectin and albendazole treatment. Each child was tested with at least two FTS tests. 81 people were surveyed around the 8 index cases in 68 households. The age ranged from 2 to 78 years (average 22.2 years) and 50.6% were female. 79.0% of the respondents had received treatment at least once. Four people were tested positive (4.9%). Among them, 3 index cases remained positive in 2018 and a new positive case was detected who was the grandmother of one of the 3 remaining positive index children sharing the same room. The positives and all persons within the compound were treated. The results of the TAS2 confirmed the sustained interruption of LF transmission. Five of the old 8 positives turned negative. It suggests that the risk of recrudescence of transmission around index cases is minimal. This study provided an example of following up positive cases combining with other surveys that would allow for the detection and selective treatment of positives and their families.

1824

EPIDEMIOLOGY OF LYMPHATIC FILARIASIS DURING MASS DRUG ADMINISTRATION IN DREIKIKIR, PAPUA NEW GUINEA

Daniel J. Tisch[1], Brooke Mancuso[2], Nelly Sanuku[3], Philip Lus[3], Estee Cramer[1], Willie Pomat[4], Christopher L. King[1], Peter A. Zimmerman[1], James W. Kazura[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Tulane University, New Orleans, OH, United States,* [3]*Papua New Guinea Institute for Medical Research, Maprik, Papua New Guinea,* [4]*Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea*

Lymphatic filariasis infection and transmission has been monitored in 14 communities of Dreikikir, Papua New Guinea since the 1990's during mass drug administration (MDA) and bednet interventions. This study characterizes serological markers of infection during current and prior interventions. In the past year, these communities have completed 4 rounds of annual mass drug administration (MDA) with DEC and Albendazole. Night-time finger prick blood samples were collected during repeated cross sectional surveys in 3,000 individuals before and after annual MDA from 2015 to 2018 (immediately before the 1st and 4th MDA, respectively). Samples were processed for microscopy to detect circulating microfilaria (MF) and serological markers of infection. The results are

analyzed according to geography, age, and estimates of entomological transmission. MF prevalence decreased from 18.0% to 1.1% a year after 3 MDAs in western communities and from 1.4% to 0% in eastern communities. Among individuals with longitudinal observations, no negative individuals acquired MF positivity during three annual MDAs. However, antigen prevalence detected by Filarial Test Strips (FTS) remained high across the study site (8.4% to 30.6%). Among 78 antigen positive individuals followed longitudinally, 76% did not clear antigen and 5% of individuals FTS negative at baseline became FTS positive after 3 rounds of MDA. These results suggest that elimination targets may have been met in communities geographically located in the eastern communities, but that the adjacent western communities may have ongoing transmission, despite uniformly high coverage of MDA and long lasting insecticide treated bednets (LLIN). Additional serological biomarkers provide additional context to understand the impact of these results on LF elimination efforts. The fact that MF prevalence remains above 1% in some communities located <5km away from communities with no detectable MF highlights the focal distribution of infection post-MDA in this region. The distribution of infection also highlights the potential reservoir of adults for MF in these communities.

1825

ILLUMINA SEQUENCING TO MONITOR *WUCHERERIA BANCROFTI* INFECTION DURING AND AFTER MASS DRUG ADMINISTRATION

Daniel J. Tisch[1], E. Ricky Chan[1], Krufinta Bun[1], Scott T. Small[2], James W. Kazura[1], Peter A. Zimmerman[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*University of Notre Dame, Notre Dame, IN, United States*

Lymphatic filariasis infection and transmission has been monitored in 14 communities of Dreikikir, Papua New Guinea since the 1990's across mass drug administration (MDA; DEC and Albendazole) and bednet interventions. During this time, these interventions have been associated with significant decreases in infection and transmission across numerous age groups. However, antigen prevalence detected by Filarial Test Strips (FTS) still remains high across the study site (8.4% to 30.6%), even after recently renewed MDA, suggesting that elimination targets have not been met. Furthermore, some communities likely to serve as an reservoir for transmission due to persistent microfilaria (MF) prevalence >1%. In order to understand how parasite population genetics may be used to monitor *Wuchereria bancrofti* (Wb) elimination, we studied "paired" historical samples from five individuals (ages 6 to 40 years) with MF density of 33 to 26,468 MF/ml. One ml night-time blood samples (10pm-2am) were collected during the initial MDA intervention in these communities (1996) and 10 years after the cessation of the intervention (2008); Wb diagnostics were performed by microscopy and serological assays. Here we performed targeted Illumina sequencing of the Wb mitochondrial gene, cytochrome oxidase 1 (CO1) to assess the complexity of infection during and after MDA. Overall coverage of Wb CO1 ranged from 4,777 to 46,825 (median=30,756). Analyses provide evidence of complex infections characterized by 55 haplotypes. By comparing specific haplotype proportions across these sampling time points we observed significant differences in the overall population complexity in all five infected individuals. We also observed a significant shift in haplotype distribution in individuals between 1996 to 2008 suggesting a change in population structure within infected individuals following MDA. Results provide a unique approach for assessing Wb infection complexity, provide perspective for monitoring the impact of new treatment strategies, identifying resilient parasite strains and assessing elimination of lymphatic filariasis.

1826

PROGRESS TOWARD ELIMINATION OF LYMPHATIC FILARIASIS (LF) AFTER IMPACT SURVEYS IN 11 HEALTH DISTRICTS (HDS) OF 3 REGIONS IN CAMEROON

Biholong Benajmin[1], Ebene Clarisse[1], Georges NKO'Ayissi[2], Julie Akame[3], Patrick Mbia[3], Carine Fokam[3], Michel Hendji[3], Yaobi Zhang[4], Steven D. Reid[5], Ismael Teta[3]

[1]*Ministry of Public Health, PNLO, Yaoundé, Cameroon,* [2]*Ministry of Public Health, NTD Coordination Unit, Yaoundé, Cameroon,* [3]*Helen Keller International, Yaoundé, Cameroon,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [5]*Helen Keller International, New York, NY, United States*

Cameroon is endemic for lymphatic filariasis (LF). The country aims to eliminate LF by 2020 and one of the steps towards elimination is that the health districts (HDs) must meet criteria for stopping mass drug administration (MDA). According to WHO recommendations, this is done through a transmission assessment survey (TAS) in HDs having completed at least 5 rounds of MDA with epidemiological coverage >65% and passed pre-TAS. In 2017, 11 out of 12 HDs from three regions successfully passed a pre-TAS, and were thus eligible for TAS1. One HD was not reached due to insecurity in the South West region. In 2018, this TAS1, conducted under the USAID-ENVISION project led by RTI International, in 11 HDs from Adamawa, Center and Eastern regions. The HDs were grouped into 8 evaluation units (EUs) according to their epidemiological profile and geographical location. The Survey Sample Builder (SSB) was used to calculate sample size and select the clusters. The sampled population consisted of children aged 6-7 years. The survey was conducted in schools in Center and East regions and in communities in Adamawa region where the school enrolment rate was <75%. The Filariasis Test Strip (FTS) was used to detect LF antigen. Data were captured on smartphones using ODK technology, stored on an ONA platform and processed through an electronic template with control measures. The teams performed day-time calibrated blood smears (CBS) in *Loa Loa* co-endemic areas. Children testing positive were all confirmed by a second FTS test. 11,548 children in 276 clusters were tested and the initial FTS was positive in 6 children. All of them were re-tested and only 1 child was positive with the second, confirmatory FTS in only one EU, well below the critical cut-off value. This TAS1 results showed recrudescence is unlikely to occur in these 11 HDs bringing the number of HDs having met the criteria to stop MDA to 136 (out of 137 endemic HDs). These results suggest that the National NTD Program should start preparing the LF surveillance phase across the country.

1827

SEROPREVALENCE AND DETERMINANTS OF TRANSFUSION TRANSMISSIBLE INFECTIONS AMONG VOLUNTARY BLOOD DONORS IN HOMABAY KISUMU AND SIAYA COUNTIES IN WESTERN KENYA

George Calleb Onyango[1], Lilian Ogonda[2]

[1]*Kenya Medical Training College, Kisumu, Kenya,* [2]*Maseno University, Kisumu, Kenya*

Since the implementation of a series of blood donation safety improvements in Kenya, information about seroprevalence and determinants of transfusion transmissible infections among voluntary blood donors especially in high HIV burden regions of Homabay, Kisumu and Siaya counties remain scanty. A cross-sectional study examining HIV, syphilis, hepatitis B and C virus sero-markers and associated determinants was conducted among voluntary blood donors. Their demographic characteristics and previous risk exposure were recorded in a pre-donation questionnaire, while blood samples collected were screened for hepatitis B, hepatitis C, human immunodeficiency viruses by ELISA and RPR (syphilis), then confirmed using CMIA. Overall TTIs seroprevalence was 114 (9.4%), distributed among HIV, HBV, HCV and syphilis at 14 (1.15%), 42 (3.46%), 39 (3.21%) and 19 (1.56%), respectively, with co-infections of 3 (0.25%). There were no significant differences in proportions distributions among

demographic variables. However, high risk sex was significantly associated with higher odds of HBV infections [> 1 partner vs. 0-1 partner; odd ratio (OR) 2.60; 95% confidence interval (CI) 1.098-6.86; p = 0.046]. In conclusion, a substantial percentage of blood donors still harbor transfusion transmissible infections despite recent safety improvements with greater majority cases caused by HBV infections arising from previous exposure to high risk sex.

1828

AWARENESS, ACCEPTABILITY AND WILLINGNESS TO USE A PROSPECTIVE HIV VACCINE AMONG HEALTHCARE WORKERS IN SOUTHEAST NIGERIA

Ikechukwu N. Dozie, Chiamaka C. Eluwa-Okonkwo, Chikere I. Ebirim, Uchechukwu M. Chukwuocha

Federal University of Technology, Owerri, Imo state, Nigeria

This study was undertaken to investigate the awareness, acceptability and willingness to use a prospective HIV vaccine by healthcare workers in Imo State, South east, Nigeria. Information was elicited using structured pretested questionnaire administered to 297 respondents drawn from the Federal Medical Centre (FMC) Owerri, Imo State, Nigeria. Data analysis was using Chi-square on Statistical Package for Social Sciences (SPSS) version 21. Majority of the respondents (81.3%) were aware of a prospective HIV vaccine, with the 50+ age group (95.8%) and Consultants (100.0%) being the most aware. Their main source of information was journal articles (24.5%), followed by conferences/workshops (22.1%) while the least source was radio (3.9%). About 260 (87.5%) of the respondents agreed that HIV vaccine can prevent spread of virus and the best time suggested for vaccination was at birth (60.8%) as against attaining puberty before first sexual activity (7.4%). Also 290 (97.6%) respondents showed willingness to accept vaccination and would encourage their patients to accept vaccination too. Very few respondents (13.5%) did not agree that vaccination should be free and the adduced reasons included misuse (45.0%), taken for granted (47.5%) and encouragement of unsafe sex practices (7.5%). Nonetheless, 92.3% of respondents would pay for vaccination which should cost between USD 30 and USD 50 per vaccine. Socio-demographic variables were not found to be statistically significant predictors of awareness while only gender(P=0.025) was statistically associated with acceptability. The study showed that awareness and acceptability of HIV vaccine is high amongst healthcare workers in Imo State, South east Nigeria. This underscores the probability of high uptake of a prospective vaccine in the area as a preventive measure against HIV infection.

1829

A CASE OF SEVERE CRYPTOCOCCAL IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME PRESENTING WITH BRAIN AND INTRADURAL ABSCESSES IN AN HIV PATIENT

Thomas Michael Kalinoski[1], Arthur Jeng[1], Jason Malenfant[2], Catherine Yim[1], Wenchang Guo[3]

[1]*Olive View - University of California Los Angeles Medical Center, Sylmar, CA, United States,* [2]*University of California Los Angeles, Los Angeles, CA, United States,* [3]*LAC+USC Medical Center, Los Angeles, CA, United States*

Clinical worsening or new manifestation of cryptococcal disease following initiation of anti-retroviral therapy (ART) in an HIV-infected individual is a hallmark of cryptococcal immune reconstitution inflammatory syndrome (C-IRIS). However, it can be difficult to distinguish IRIS from worsening infection or new pathogen, especially in the setting of severe disease and ART initiation following an adequate window period. Here, we present a case of severe C-IRIS, involving development of multiple cerebellar abscesses, spinal intradural abscesses, and spinal arachnoiditis seven months after ART initiation in an AIDS patient who had uncertain ART compliance before that time. He had a complicated history of multiple prior episodes of cryptococcal meningitis necessitating placement of a ventriculoperitoneal shunt (VPS), and had been on suppressive fluconazole when he developed worsening and new brain manifestations. The patient received empiric re-induction of anti-cryptococcal therapy without any improvement and was subsequently treated for tuberculous meningitis as well as possible VPS infection. During this presentation, all CSF cultures remained sterile, with negative *Cryptococcus* PCR testing, and his condition continued to worsen prior to corticosteroid initiation. Ultimately, C-IRIS was diagnosed by brain biopsy. This case documents both an extreme in severity of C-IRIS and in the timeline of presentation after ART initiation.

1830

HIGH FREQUENCIES OF TUMOR-INFILTRATING AND CIRCULATING ΓΔ T CELLS IN ENDEMIC BURKITT LYMPHOMA PATIENTS

Cecilia Smith-Togobo[1], Maria del Pilar Quintana[2], Michael F. Ofori[1], **Lars Hviid**[2]

[1]*University of Ghana, Accra, Ghana,* [2]*University of Copenhagen, Copenhagen N, Denmark*

Endemic Burkitt lymphoma (eBL) is a highly aggressive B-cell cancer that is only seen in children exposed to massive, early Epstein-Barr virus infection, and who are living in areas with stable and intense transmission of the malaria parasite *Plasmodium falciparum*. In such areas, eBL can be the most common pediatric cancer, which has a very poor prognosis in the absence of effective chemotherapy that is often not available. High frequencies (>5% of all circulating T cells) of Vδ1$^+$ γδ T cells have been reported in various infectious and neoplastic diseases, but also among healthy individuals from areas with stable transmission of *P. falciparum* malaria. The function of the Vδ1$^+$ T-cell subset is largely unknown, but it appears to be "adaptive-like" and quite distinct from that of the "innate-like" and largely complementary Vγ9Vδ2$^+$ γδ T-cell subset, which universally responds to non-peptide prenyl pyrophosphate metabolites (so-called phospho-antigens) produced by a variety of stressed cells and pathogens. We have previously proposed that the still quite enigmatic Vδ1$^+$ T-cell subset serves an adaptive auto-regulatory function in conditions characterized by massive and/or chronic B-cell activation. On the above basis, we set out to test the hypothesis that the Vδ1$^+$ T-cell subset subset is expanded in patients with endemic Burkitt lymphoma (eBL). We recruited a series of Ghanaian children with eBL, and compared the frequencies and phenotypes of Vδ1$^+$ T cell in fine-needle tumor aspirates and in the peripheral blood of the patients. Our preliminary evidence documents high frequencies of Vδ1$^+$ γδ T cells in both cell compartments. We will present results of our detailed multi-parameter phenotypic analysis of these cells, which will be the first detailed report of its kind in patients with this important tropical cancer.

1831

FIVE-YEAR VIH INCIDENCE, PREVALENCE AND MORTALITY IN CANADA, MEXICO AND USA: OBSERVATIONAL DESCRIPTIVE STUDY

Nina Mendez-Dominguez[1], Sabrina Fajardo-Ruiz[1], Peter Gulick[2], Andrea Cámara[1], Martin Inurreta[1]

[1]*Universidad Marista de Merida, Merida, Mexico,* [2]*Michigan State University, Michigan, MI, United States*

At the end of 2017, 940,000 people died due to HIV worldwide. In the absence of an etiologic cure for HIV, antiretroviral therapy (ART) can provide a better quality of life and survival and provide infected people a chance to live a healthy and productive live. Even when Mexico, USA and Canada share environmental and biological common aspects, differential access to preventive medicine services might might not be equal. The objective of the present study is to describe the epidemiologic profile of HIV and its relationship with mortality patterns in the three North American countries between 2012 to 2016. *Methods.* In this observational retrospective study, the VIH epidemiologic surveillance open access datasets from Canada, Mexico, and United States for years 2012-2016 is described. Data was analyzed by country considering gender, age

and region and presented in tables and figures. Chi squared tests and mean comparison tests followed by post hoc analyses were developed when comparing between groups. *Results.* In the USA, the yearly estimated incidence in population infected by HIV from the years 2012 to 2016 was 15.06, in Mexico for the same years it was 4.74 and in Canada 5.94 per 10,000 inhabitants. The highest rates of new HIV cases occurred in young adult men. Incidence increased in Mexico among women living in rural areas. The modal age group in Mexico is 25-34, in the USA the 45-54 group have the highest seroprevalence and in Canada, subjects aged 30-39 have the highest seroprevalence. The average age at death caused by HIV in Mexico is 39 years old, which could represent a shorter life expectancy among infected Mexicans when compared to Americans and Canadians. The HIV epidemiology vary between these three North American countries, while USA has a higher incidence of HIV, the average age of mortality in México is at a younger age. Epidemiology of HIV in Mexico might reflect that HIV diagnosis could be improved to implement ART treatment and achieve a longer survival.

1832

FACTORS ASSOCIATED WITH DIARRHEAL ILLNESS AMONG HIV PATIENTS IN AN OUTPATIENT CLINIC IN JAMAICA

Obinna Nnaemeka Nnedu[1], Rasheedah Godfrey[1], Clara Engmann[2], Alaa Mohammed[1], Tamara Thompson[3]

[1]*Ochsner Clinic Foundation, New Orleans, LA, United States,* [2]*Tulane University School of Medicine, New Orleans, LA, United States,* [3]*University of West Indies Mona, Mona, Kingston, Jamaica*

HIV infected individuals are at increased risk for developing diarrhea from both infectious and non-infectious causes. A previous study in Jamaica showed that 26.3% of HIV infected adults sought medical care in the prior 12 months for gastrointestinal complaints. We undertook a case-controlled study to determine the factors associated with diarrheal illness among HIV infected patients seeking care at an outpatient HIV clinic in Jamaica. The study period was January 1, 2011 to December 31, 2015. A total of 514 individuals met our inclusion criteria for the study. Basic demographic and clinical information were obtained via chart review. We performed univariate analysis to determine what factors to fit in a multivariate logistic regression model. In univariate analysis, patients on HIV medications were less likely to have diarrhea OR=0.27 (95% CI: 0.17-0.42) compared to those not on therapy. Looking at specific HIV medications, univariate analysis showed that any protease inhibitor use and use of ritonavir-lopinavir specifically may be associated with increased risk of diarrhea; OR=1.68 (95% CI: 1.05-2.68) and OR=2.58 (95% CI: 1.56-4.03) respectively. Use of efavirenz decreased the risk of diarrheal illness in univariate analysis; OR=0.41 (95% CI: 0.24-0.69). In multivariate logistic regression analysis, we found that use of ritonavir-lopinavir was associated with an increased risk of diarrhea, OR=1.9 (95% CI: 1.18-3.06) while use of efavirenz was associated with decreased risk of diarrhea, OR=0.5 (95% CI: 0.29-0.86). Our findings suggest that with increased access to HIV medications in developing countries, ritonavir-lopinavir use may become an important risk factor for diarrheal illness. It will be important to have additional treatment options for patients who are not able to tolerate ritonavir-lopinavir.

1833

DIAGNOSIS OF NEUROLOGICAL TOXOPLASMOSIS IN URINE IN PERSONS LIVING WITH HIV

Hannah Steinberg[1], Andrea Diestra[2], Cusi Ferradas[2], Maritza Calderón[2], Catherine Apaza[2], Marilly Donayre Urquizo[3], Melanie Ayachi López[4], Viviana Pinedo Cancino[3], Lastenia Ruiz[3], Cesar Ramal[4], Paul Russo[5], Natalie Bowman[6], Lance Liotta[5], Alessandra Luchini[5], Robert H. Gilman[7]

[1]*University of Illinois Chicago, Chicago, IL, United States,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Universidad Nacional de la Amazonía Peruana, Iquitos, Peru,* [4]*Hospital Regional de Loreto, Iquitos, Peru,* [5]*George Mason University, Manassas, VA, United States,* [6]*University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [7]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Secondary neurological infections in persons with HIV are challenging to diagnose. CT and MRIs with a clinical suspicion can guide treatment, but definitive diagnosis remains elusive for many patients. One causative pathogen is *Toxoplasma gondii (T. gondii).* Though difficult to diagnosis, it has up to 90% clinical response rate to treatment. A point of care, non-invasive diagnostic would allow for the expeditious ruling in or out of neurological toxoplasmosis. Our team has developed a diagnostic western blot with a hydrogel nanoparticle concentration step in urine for *T. gondii* antigen. To complete the assay, urine is incubated with reactive blue 221 dyed hydrogel nanoparticles. The reactive blue 221 dye semi-specifically binds to the *T.* gondii antigens in the patients' urine. The nanoparticles are then collected by centrifugation and eluted in sample buffer for gel electrophoresis and transferred to PVDF membrane. PVDF membrane is blotted using standard western blot technique for anti-GRA1 and anti-SAG1. The nanoparticle concentration step allows for the detection of as little as 7.8pg/ml of antigen. The detection of antigen in patient urine is consistent with toxoplasmosis symptoms and CT findings. The detection of antigen is most sensitive and specific in patients prior to the initiation of treatment; however it is possible to detect antigen in urine up to a week after the initiation of treatment for toxoplasmosis. Additionally, testing of urine in asymptomatic toxoplasmosis seropositive persons does not yield a positive result. It is our hope to continue this research to transition our western blot to a lateral flow assay to improve the accessibility of this diagnostic approach.

1834

HUMAN IMMUNODEFICIENCY VIRUS ASSOCIATED MULTICENTRIC CASTLEMAN'S DISEASE (MCD) WITH COEXISTING KAPOSI SARCOMA TRIGGERING POTENTIALLY FATAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Vijai Bhola[1], Steven Hatch[2], Nicole Theodoropoulos[2], Salwa Khedr[2]

[1]*University of Massachusetts Medical School, Shrewsbury, MA, United States,* [2]*University of Massachusetts Medical School, Worcester, MA, United States*

A 47 year old HIV positive male from the Central African Republic presented to a US hospital with persistent fevers, hypotension and lymphadenopathy. HIV Viral load of 123 copies/mL and CD4 count of 346, on long term HAART. He was started on broad-spectrum antibiotics. Routine radiographs and cultures were unrevealing, but fevers persisted. CT scan revealed diffuse abdominal lymphadenopathy, and splenomegaly. His ferritin level was markedly elevated at 2147 ng/mL; soluble Interleukin 2 receptor alpha (s-IL2R) level was 14,770 pg/ml (normal less than 1033 pg/ml); and an HHV8 PCR was positive at 3,300,000 copies/mL. A left inguinal lymph node biopsy revealed coexistent MCD and KS. He was diagnosed with HLH in the setting of HHV8/KS-associated MCD. He was started on etoposide, rituximab, valganciclovir, and dexamethasone; fever and hypotension resolved, with rapid clinical improvement. MCD is a rare lymphoproliferative disease characterized by hyperactive immune cells secreting multiple cytokines leading to fevers, lymphadenopathy, and organ dysfunction. It is classically seen in HIV patients who are HHV8-positive. HLH is a hyper-inflammatory syndrome characterized by uncontrolled T cell activation with subsequent cytokine storm and potentially lethal multi-organ failure. Diagnosis of HLH requires meeting at least five of eight criteria: fever; splenomegaly; cytopenias; elevated ferritin; elevated IL2R; hypertriglyceridemia; low or absent NK cell activity; and visual evidence of hemophagocytosis on biopsy (our patient met the first five criteria; a bone marrow biopsy was not performed once he met criteria). HLH triggered by HIV-MCD is quite rare. In one HIV-associated MCD cohort in Europe, only nine percent of patients developed HLH. Treatment regimens include etoposide, which halts T-cell expansion, and anti-inflammatory agents such as dexamethasone. Our case highlights the rare condition of MCD in an HIV-positive patient, the role of HHV8 in

tumorigenesis, and the possibility of simultaneous MCD and KS. We also highlight that HLH is a rare, but potentially lethal, complication of MCD, and can easily be misdiagnosed for sepsis.

1835

COUMARIN ANTIFUNGAL LEAD COMPOUNDS FROM *MILLETTIA THONNINGII* AND THEIR PREDICTED MECHANISM OF ACTION

Sylvester Kaminta[1], Daniel M. Ayine-Tora[2], Abdul-Salim Musah[3], Felix C. Mills-Robertson[4]

[1]*Monash University, Clayton, Melbourne, Austria,* [2]*University of Auckland, Auckland, New Zealand,* [3]*St. Theresa's Hospital, Nandom, Upper-west region, Ghana,* [4]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

Fungal infections including candidiasis remains a public health threat of high morbidity and mortality, affecting immunosuppressive patients such as HIV/AIDS victims despite several attempts to control its invasion. The etiological agent of candidiasis is *Candida albicans*. Currently, effective antifungal drugs that were used to treat infections due to *C. albicans* are challenged with drugs resistance. Thus, the search for new antifungal drugs that are safe and effective to replace them is warranted. Plants have shown to be a valuable source of antifungal agents. Here, we demonstrated the antifungal activity of isolated compounds from seeds of *M. thonningii*, robustic acid, thonningine-C, alpinumisoflavone and O'O-methylalpinumisoflavone against the wild *C. albicans* strain and a reference strain (ATCC18804). The agar well diffusion method was used to evaluate zones of inhibition. Thermochemical calculations were performed using Gausian 09 software suite. The predicted mechanism and bioassay results revealed that the tested fungal strains were sensitive to robustic acid, thonningine-C. Two coumarins, robustic acid and thonningine-C isolated from *M. thonningii*, show promising activity against the fungus, *C. albicans* with minimum fungicidal concentration of 1.0 and 0.5 mg/ml, respectively. Molecular modelling against the putative bio-molecular target, lanosterol 14α-demethylase (CYP51), revealed a possible binding mode for the active compounds, in which the hydroxyl group binds with a methionine backbone carboxylic group blocking access to the iron catalytic site. This binding disrupts the synthesis of several essential sterols for the survival of fungi. The findings provided useful data on the antifungal potential of *M. thonningii* compounds that can be used in drug developing for the treatment of candida infections.

1836

HISTOPLASMOSIS AND TUBERCULOSIS CONFECTION IN PEOPLE LIVING WITH HIV: A RETROSPECTIVE CASE-SERIES

Audrey Valdes, Pierre Couppie, Roxane Schaub, Romain Blaizot, Felix Djossou, Loic Epelboin, Mathieu Nacher, Denis Blanchet, Magalie Demar, Antoine Adenis

Centre Hospitalier de Cayenne, Cayenne, French Guiana

Tuberculosis and histoplasmosis coinfection is rarely suspected though case series report over 10% coinfections in people living with HIV (PLWHIV) being diagnosed for histoplasmosis. Coinfections represent a diagnostic and therapeutic challenge for clinicians with no evidence-based guidance available in PLWHIV guidelines. Thus, the objective of the study was to describe tuberculosis and histoplasmosis coinfections in PLWHIV in French Guiana. Cases of concomitant (within 3 months) culture-proven histoplasmosis and tuberculosis occurring in adult PLWHIV were identified in a retrospective cohort of PLWHIV diagnosed in Cayenne General Hospital between 01/01/1997 and 12/31/2017. Care and treatment findings were collected on a standardized form. All patients gave informed consent. Among the 14 coinfection cases identified, 8 (57%) were diagnosed simultaneously and 6 within 3 months (range:11-90 days). Lymph node (5/8) and liver (2/8) samples yielded most simultaneous diagnoses. The mean age was 40.9 ± 9.2 years. The H:F gender ratio was 2.5. Nine out of 12 patients (75.0%) declared drug addictions, half of them being crack cocaine users. Fever was reported in all cases. Cough (9/14), diffuse abdominal pain (6/14) and lymph node enlargement (6/14) were mainly reported. The median CD4 count level was 50/mm³ (Interquartile range 25%-75%: 25-79) [Range:6-253]. Hepatic cytolysis was found in 2/14 cases, hepatic cholestasis in 7/14 and an increase in LDH in 8/9 patients. An interstitial syndrome was reported in 5/10 chest X-ray performed. Within 3 months after treatment initiation no deaths and one case of lost to follow-up were observed. Similarly to previous reports, histoplasmosis and tuberculosis coinfections described herein reported a disseminated pattern in PLWHIV at the advanced stage of HIV disease. But, in comparison to other reports, prognosis was good using first line treatment for the two diseases. Clinical or biological features alone may not give clinicians relevant clues in the diagnosis of coinfections. It relies on a high index of clinical suspicion and the access to relevant diagnostic facilities for the two diseases.

1837

FREQUENCY OF NON-AIDS DEFINING EVENTS IN PATIENTS INFECTED WITH HIV IN AN OUTPATIENT CLINIC IN SANTO DOMINGO, DOMINICAN REPUBLIC

Guillermo Alexander Asmar Vargas, Mylene Gisela Asmar-Rios, Leandro Tapia, Robert Paulino-Ramirez

Institute for Tropical Medicine and Global Health, Universidad Iberoamericana, Santo Domingo, Dominican Republic

With new advances in combined antiretroviral therapy, the frequency of AIDS defining events have decreased, however, an increase in the frequency of non-AIDS defining events has been observed. The objective of this study was to establish the frequency of non-AIDS defining events in patients infected with HIV in an outpatient clinic in Santo Domingo, Dominican Republic. Patients enrolled in care in a community- based clinic were evaluated for clinical indicators of non-AIDS defining events. Socio-demographic determinants and serological data were collected from clinical files, and statistical analyses were used to assess their distribution in different key populations. A total of 500 patients were analyzed, 66 of the had described non-AIDS defining events. Cardiovascular Disease and Chronic Liver Disease accounted for 26.3% of the presented non-AIDS defining events. Hypercholesterolemia accounted for 20%, Dyslipidemia 17.5%, Chronic Kidney Disease 6.3% and Anal Dysplasia 3.8% of the cases. Male patients were the most commonly affected gender representing 55.4% cases, followed by female patients with 35.4% cases. The most affected vulnerable population were MSM with 18.5% cases, sexual workers accounted for 15.4% of cases. 91% were adherent to HAART. 82% cases had a cd4+/cd8+ proportion <1. Although most of the patients that were studied remained adherent to the antiretroviral therapy non-AIDS defining events were still observed and the proportion of CD4 + / CD8 + lymphocytes remained <1 in the majority of the cases, reflecting the immunosenescence that can be observed in the vast majority of HIV-infected patients, possibly increasing their risk of suffering a defining or non-defining event of AIDS. The vulnerable populations of MSM and sexual workers affected with higher frequency.

1838

RAPID DIAGNOSIS OF CO-INFECTION WITH INVASIVE ASPERGILLOSIS AND ACTIVE HEPATITIS B INFECTION IN A HIV INFECTED PATIENT

Rone-Chun Lin, Joseph Y. Kim

University of Illinois, COM at Peoria, Peoria, IL, United States

A 54 year-old Hispanic MSM with a history of HIV/AIDS initially diagnosed in 1995 and nadir CD4 count of 12 presented with persistent cough for one month, progressive shortness of breath, and intermittent chills for two weeks. Four months prior to his presentation, he stopped taking his antiretroviral therapy due to nausea, but has restarted them two months later. Prior to the onset of his presenting symptoms, he was given prednisone for 14 days by his primary care physician due to generalized weakness. He was later diagnosed with influenza A, and received Bactrim and later Levofloxacin for presumed bacterial bronchitis. He subsequently

spiked a fever of 102F, and was admitted for further evaluation and treatment. He was found to have a CD4 of 76 (prior CD4 was 379 four months earlier) with an undetectable HIV viral load. Chest X ray showed bilateral infiltrates, and laboratory testing showed mild transaminase level elevation. Next-generation sequencing (NGS) of cell-free DNA was performed. Within 72 hours, the result came back with detection of Aspergillus fumigatus, Hepatitis B and CMV. Sputum cultures eventually grew Aspergillus fumigatus, and subsequent confirmatory testing revealed elevated serum aspergillus antigen and beta-D-glucan levels. Hepatitis B DNA of >100 million IU/ml and CMV of 256 IU/ml were also detected. Despite previous Hepatitis B vaccination with negative Hepatitis B surface antigen and positive surface antibody titers one year earlier, he was found to have lost his Hepatitis B immunity, and demonstrated positive Hepatitis B surface antigen, core antibody and E antigen. CT scan of the chest revealed multilobar pneumonia with a four-centimeter cavitary lesion of left lower lobe. MRI of brain revealed multiple ring-enhancing lesions likely representing abscesses from Aspergillosis. LP was unremarkable. He subsequently improved with voriconazole and micafungin treatment, and was discharged home. This case illustrated the potential utility of using NGS of cell-free DNA to rapidly diagnose multiple co-infections in severely immunocompromised HIV infected patients without the need for invasive diagnostic procedures.

1839

A SYSTEMATIC REVIEW OF VIRULENCE FACTORS IN THE *LEISHMANIA* GENUS

Osaru Omoruna[1], Avinash N. Mukkala[1], Ruwandi Kariyawasam[2], Eric Shao[1], Priyanka Challa[1], Michael A. Klowak[1], Shareese Clarke[1], Jamie Sookhoo[1], Dylan Kain[1], Tianna Chong-Kit[1], Olamide Egbewumi[1], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada*

Parasite-determined factors play a complementary role in the pathogenesis of leishmaniasis, a disease caused by protozoans of the genus *Leishmania* with diverse and species-specific clinical manifestations. Virulence factors (VFs), or pathogen moieties facilitating disease, can potentiate host cell damage by Leishmania species via increased expression, host cell invasion, stress tolerance, and modulation of the host immune system. Due to large eukaryotic genomes in Leishmania species, there is a wide array of VFs which contribute to different aspects of pathogenesis. Here we conduct a comprehensive, systematized review of the literature around VFs in *Leishmania* spp. and construct a complete picture of parasite-determined contributors to the pathogenesis of various clinical forms of leishmaniasis. PubMed (NCBI), MEDLINE (OVID), EMBASE (OVID), Web of Science, and LILACS (VHL) were searched from inception to July 2018 using combinations of the search terms "virulence factor*", "*Leishmania*", and "Leishmaniasis*", while accounting for unique database syntax. Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. For the systematic review, we will include primarily molecular and mechanistic pathogenesis studies in various model systems, observational studies, review studies, cohort studies, as well as clinical trials. Of 2620 articles remaining after title and abstract screening, some major VFs identified in the Leishmania genus are: heat shock proteins (HSP23, HSP70), cysteine peptidases (CPB), mannose phosphate isomerases (MPI), metalloproteases (GP63), and elongation factors (EF1-alpha), among many others. Data will be grouped and summarized by species, geographic region of endemicity, and VFs. This systematic compilation of mechanistic VF data will add to the large body of work in molecular pathogenesis of kinetoplastids and enhance our understanding of species and regional variations in Leishmania pathogenesis.

1840

NEW *LEISHMANIA* SPECIES AND ITS POTENTIAL NEW VECTOR, RESPONSIBLE FOR CUTANEOUS LEISHMANIASIS IN SOUTHEASTERN GHANA

Godwin Kwakye-Nuako[1], Mba-Tihssommah Mosore[2], Priscilla Ankamaa Opare[1], Michelle Bates[3], Rod James Dillon[3], Mary E. Wilson[4], Paul A. Bates[5]

[1]*University of Cape Coast, Department of Biomedical Sciences, School of Allied Health Sciences, College of Health and Allied Sciences, Cape Coast, Ghana,* [2]*Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana,* [3]*Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, United Kingdom,* [4]*University of Iowa, Departments of Internal Medicine, Microbiology and Immunology, and the Veterans' Affairs Medical Center, Iowa City, IA, United States,* [5]*Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lnacaster, United Kingdom*

An outbreak of human cutaneous leishmaniasis (CL) was observed in Ho District in the Volta Region of South-eastern Ghana in 1999, which was we proved to be leishmaniasis and isolated the causative protozoan from patients with skin ulcers. The study aimed at identifying the *Leishmania* species responsible for CL and their potential vectors. Aspirates from patients with cutaneous ulcers were cultured *in vitro* in *Leishmania* growth medium (Sloppy Evan's and M199). Isolates of the "yet-to-be-named" *Leishmania* species were amplified, and genomic DNA was extracted. Sequence analysis of RPL23, ITS1, RNAPolII genes were performed with reference genomes. The Ghanaian *Leishmania* isolate clade closely with the pathogens *L. orientalis*, *L. martiniguensis*, *L. marcopodum*, belong to *Leishmania (Mundinia) enriettii* complex on the evolutionary tree. The phylogenetic analysis of Ghanaian isolates and other available sequences revealed it's a newly species. To incriminate the vector transmitting the Ghanaian isolates, two potential vectors, *Lutzomyia longipalpis* (sand fly) and *Culicoides sonorensis* (biting midge) were infected with Ghana *Leishmania* isolate and maintained for more than 10 days. Representative vectors were dissected daily to check infectivity. Heavy infections were characterised in both vectors at the blood meal stage, until the blood meals were digested. Infections in *Lu. longipalpis* decreased to 0, 3 days post-bloodmeal. Infections in the *C. soronensis* were retained beyond 10 days to ≈80%, colonising the midgut and stomodeal valve. A newly identified human pathogenic *Leishmania* species responsible for CL in Ghana was able to heavily infected *C. sonorensis*, colonizing the midgut and stomodeal valve up to 10 days and beyond. Although most *Leishmania* spp. causing human disease are transmitted by a sand fly vector, this result leads us to hypothesize that midges could be vectors of this new *Leishmania* species in Ghana. Further proof is required to demonstrate successful transmission of the parasite by *C. sonorensis* to mammalian host.

1841

ACCURACY OF DIAGNOSTICS IN TEGUMENTARY LEISHMANIASIS: A SYSTEMATIC REVIEW

Sonia Igboanugo[1], Melissa S. Phuong[1], Rachel Lau[2], Robert Chris[1], Eric Shao[1], Ruwandi Kariyawasam[3], Hira Raheel[1], Sharmistha Mishra[4], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Public Health Ontario, Toronto, ON, Canada,* [3]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [4]*Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, ON, Canada*

Tegumentary leishmaniasis (TL) is characterized by cutaneous and mucocutaneous ulcerative skin lesions, caused by Leishmania parasites, that can potentially disfigure the midface. The clinical presentation of TL is similar to that of epidemiologically overlapping fungal and mycobacterial infections, thereby necessitating confirmatory diagnostics to inform appropriate treatment. Laboratory diagnostic techniques for TL include

the leishmanin skin test; microscopic identification of amastigotes from skin aspirates, biopsies and scrapings; culture; and molecular assays. We aim to determine optimal methods to accurately and efficiently diagnose TL to improve diagnostic stewardship. We searched five databases from inception to July 16, 2018 including Ovid MEDLINE, Embase, LILACS, Cochrane Library and Scopus with the following search terms: ("cut* leish*" OR "muc* leish*" OR "teg* leish*") AND (diagnosis OR diagnostic accuracy OR sensitivity OR specificity OR stard OR test*) AND NOT (viscer*). All systematic reviews, diagnostic trials and observational studies were included. Titles, abstracts and full-texts are systematically doubled screened by two reviewers with a tertiary arbitrator. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Quality Assessment of Diagnostic Accuracy Studies (QUADAS) will be employed. 6745 papers were identified from the five databases and 1278 papers remained for abstract evaluation (3391 removed) after title screening, where non-human, non-TL, non-diagnostic and case report articles were excluded. Abstract and full-text screening will be conducted. Data will be extracted from full-texts and assessed using QUADAS for selection and information bias. Heterogeneity of the studies will be determined and meta-analysis performed as appropriate. TL cannot be distinguished from competing infectious etiologies clinically, thus necessitating confirmatory diagnostics. A knowledge synthesis of accurate diagnostic assays can provide insight into the optimal approach for TL confirmation and subsequently guide therapy.

1842

ETHNOPHARMACEUTICALS FOR THE TREATMENT OF OLD WORLD CUTANEOUS LEISHMANIASIS: A SYSTEMATIC REVIEW OF TOPICAL APPLICATION OF TUMERIC

Priyanka Challa[1], Michael A. Klowak[1], Ruwandi Kariyawasam[2], Emma Hagopian[1], Eric Shao[1], Jason Kwan[1], Hira Raheel[1], Tianna Chong - Kit[1], Swana Kopalakrishnan[1], Anjola Ogunsina[1], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada*

Toxicity, expense, and accessibility limit treatment success in Old World Cutaneous Leishmaniasis (OWCL), a neglected parasitic disease caused by members of the genus *Leishmania*found in the Middle East, Mediterranean basin, Arabian Peninsula, Africa as well as the Indian Subcontinent. Better drugs are urgently needed, however, drug discovery is hindered by limited funding given geographic restriction of highly endemic OWCL to LMICs. Plant-based compounds with potential anti-leishmanial effects found in and around local endemic communities present an opportunity to overcome the aforementioned therapeutic challenges, and many such interventions are supported by anecdotal evidence of efficacy. We aim to synthesize existing evidence around available ethnopharmaceuticals to promote drug discovery for the prevention and treatment of OWCL. PubMed (NCBI), Medline (OVID), Embase (OVID), Web of Science (BioSIS) and LILACS (VHL) were searched for from inception to July 26, 2018 using combinations of the search terms "cutaneous leishmaniasis" and "ethnopharmaceuticals". Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. 3057 PubMed, 2818 Medline, 4200 Embase, 3183 Web of Science and 490 LILACS articles were retrieved for title and abstract screening; after duplicate removal, 5492 remained. 550 abstracts met inclusion criteria for full-text review, of which, 241 (43.80%) abstracts pertained to Old World species, and 113 (21%) were specific to *L. donovani*. Curcuma spp. "Turmeric" was identified in 4 articles (0.7%) to date. Synthesizing the current evidence surrounding ethnopharmaceuticals for the treatment of OWCL may contribute to drug discovery pipelines and potentially lead to novel therapeutics in a field that has not seen any new drug development for over half a century, especially in the context of turmeric.

1843

ETHNOPHARMACEUTICALS FOR THE TREATMENT OF NEW WORLD CUTANEOUS LEISHMANIASIS: A SYSTEMATIC REVIEW OF TOPICAL APPLICATION OF PEPPER AND ALLIUM

Anjola Ogunsina[1], Ruwandi Kariyawasam[2], Olamide Egbewumi[1], Sonia Igboanugo[1], Shveta Bhasker[1], Shareese Clarke[1], Paul Dunn[1], Avinash N. Mukkala[1], David Harris[1], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada*

New World Cutaneous Leishmaniasis (NWCL) is a neglected parasitic disease caused by members of the genus *Leishmania* primarily identified in Central and South America. Better drugs are urgently needed given the toxicity, expense and accessibility limits of first-line treatment options. Plant-based compounds with potential anti-leishmanial effects found in and around local endemic communities, particularly in and around the Amazon basin, present an opportunity to overcome the aforementioned therapeutic challenges, and many such interventions are supported by anecdotal evidence of efficacy. We aim to synthesize existing evidence around available ethnopharmaceuticals to promote drug discovery for the prevention and treatment of NWCL. PubMed (NCBI), Medline (OVID), Embase (OVID), Web of Science (BioSIS) and LILACS (VHL) were searched for from inception to July 26, 2018 using combinations of the search terms "cutaneous leishmaniasis" and "ethnopharmaceuticals". Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. For the systematic review, we included molecular, mechanistic, and observational studies, case reports, case series, cohort studies, as well as clinical trials reporting therapeutic outcomes, if possible using the GRADE approach. A total of 13667 abstracts were retrieved, after which 7566 duplicates were removed. Of the remaining abstracts, 550 abstracts were included in the full text review, of which 176 (32%) abstracts highlighted New World species; 116 (66.0%), 33 (18.7%), and 27 (15.3%) abstracts pertained to *L. amazonensis*, members of *Viannia* subgenus, and other New World species, respectively. Of all the abstracts included in the full text review, 25 (4.5%) and 6 (1.1%) were identified for *Piper* spp. "Pepper" and *Allium spp.* "Garlic", respectively. Synthesizing the current evidence surrounding ethnopharmaceuticals for the treatment of NWCL may contribute to drug discovery pipelines and potentially lead to novel therapeutics, particularly those targeting the *Viannia* complex, where patients often develop more severe clinical manifestations.

1844

AN UPDATE ON THE ROLE OF WOUND CARE IN THE MANAGEMENT OF OLD WORLD CUTANEOUS LEISHMANIASIS

David Harris[1], Ruwandi Kariyawasam[2], Avinash N. Mukkala[1], Christian Lecce[1], Evan Belsky[1], Andrea K. Boggild[1]

[1]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada*

Old world cutaneous leishmaniasis (OWCL) typically presents as one or several chronic, infiltrative lesions on exposed parts of the body, and is treated pharmacologically to accelerate cure, reduce scarring, and to prevent parasite dissemination or relapse. Limited data support the role of local wound care for the management of OWCL, though the scope of such benefit and to which patient populations wound care should be applied remains undetermined due to the absence of synthesized data on the subject. We aim to synthesize the literature around the role of wound care in the management of OWCL to inform treatment guidelines and evidence-based therapeutic strategies. Medline (Ovid), Embase (Ovid), and PubMed (NCBI) were searched from inception to February 2019 without language restriction using combinations of the search terms "leishmania*" and "wound care". The GRADE approach will be used to assess quality of studies reporting specific wound care interventions. 626 articles were identified with the initial search. After screening titles and

abstracts, 226 articles were selected for final review. 50 publications were specific for OWCL, 5 publications discussed wound care in both OWCL, and New World Cutaneous Leishmaniasis, and 58 publications were review articles. Study characteristics including number of participants, wound care strategy/ intervention (debridement and removal of crusts, occlusive dressings, cream/ointment containing silver, washing, topical antimicrobials), outcomes (cure, time to reepithelilization, induration reduction, scar formation-quality and cosmesis, safety/tolereability, costs, feasibility/accessibility), study location, and species identification, will be extracted from all eligible studies and analyzed. We will systematically map the literature and synthesize the current state of knowledge and topical wound-oriented management practices in OWCL in order to inform optimal adjunctive clinical approaches and guidelines. We will also identify knowledge gaps and potential prospective research questions to fill them.

1845

A SYSTEMATIC REVIEW OF WOUND CARE IN THE MANAGEMENT OF NEW WORLD CUTANEOUS LEISHMANIASIS

Ruwandi Kariyawasam[1], David Harris[2], Christian Lecce[2], Avinash N. Mukkala[2], Evan Belsky[2], Andrea K. Boggild[2]

[1]*Institute of Medical Sciences, Department of Medicine, University of Toronto, Toronto, ON, Canada,* [2]*Tropical Disease Unit, Toronto General Hospital and University of Toronto, Toronto, ON, Canada*

New world cutaneous leishmaniasis (NWCL) typically presents as one or several chronic, infiltrative lesions on exposed parts of the body, and is treated pharmacologically to accelerate cure, reduce scarring, and to prevent parasite dissemination or relapse. Limited data support the role of local wound care for the management of NWCL, though the scope of such benefit and to which patient populations wound care should be applied remains undetermined due to the absence of synthesized data on the subject. We aim to synthesize the literature around the role of wound care in the management of NWCL to inform treatment guidelines and evidence-based therapeutic strategies. Medline (Ovid), Embase (Ovid), and PubMed (NCBI) were searched from inception to February 2019 without language restriction using combinations of the search terms "leishmania*" and "wound care". The GRADE approach will be used to assess quality of studies reporting specific wound care interventions. 626 articles were identified with the initial search. After screening titles and abstracts, 226 articles were selected for final review. 113 publications were specific for NWCL, 5 publications discussed wound care in both NWCL and Old World Cutaneous Leishmaniasis, and 58 publications were review articles. Study characteristics including number of participants, wound care strategy/ intervention (debridement and removal of crusts, occlusive dressings, cream/ointment containing silver, washing, topical antimicrobials), outcomes (cure, time to reepithelilization, induration reduction, scar formation-quality and cosmesis, safety/tolereability, costs, feasibility/accessibility), study location, and species identification, will be extracted from all eligible studies and analyzed. We will systematically map the literature and synthesize the current state of knowledge and topical wound-oriented management practices in NWCL in order to inform optimal adjunctive clinical approaches and guidelines. We will also identify knowledge gaps and potential prospective research questions to fill them.

1846

PHENOTYPIC CHARACTERIZATION OF TRYPANOSOMES CELLS TREATED WITH TETRACYCLIC IRIDOID, ML F52 SUPPRESSION OF *FLAGELLA* ATTACHMENT PROTEINS

Georgina I. Djameh[1], Thelma Tetteh[1], Takuhiro Uto[2], Frederick Ayertey[3], Michael Amoa-Bosompem[4], Faustus I. Azerigyik[1], Kofi D. Kwofei[4], Tomoe Ohta[2], Irene Ayi[1], Shiro Iwanaga[4], Nobuo Ohta[4], Yukihiro Shoyama[2], Mitsuko Ohashi[4]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Nagasaki International University, Nagasaki, Japan,* [3]*Centre for Plant Medicine Research, Mampong-Akuapem, Ghana,* [4]*Tokyo Medical and Dental University, Tokyo, Japan*

Despite the recent advances in drug research, finding a safe, effective, and easy to use chemotherapy for Human African Trypanosomiasis (HAT) remains a challenging task. This condition underlines the urgent necessity for the development of new drugs for the treatment of HAT. We previously identified the anti-trypanosome activities of three novel tetracyclic iridoids; ML-2-3, Molucidin and ML-F52, isolated from *Morinda lucida* with IC_{50} values of 3.75μM, 1.27μM and 0.43μM, respectively. Immunohistochemistry (IHC) study showed that the compounds significantly suppressed the expression of PFR-2, which proceeded to the events of cell cycle alteration and apoptosis induction. Scanning Electron Microscopy revealed the severe phenotype of the flagella detached from the body of the parasite. Here we present a phenotypic characterization of ML-F52 treated trypanosomes in detail with analyzing the expression levels of Flagellum Attachment Zone (FAZ) filament proteins, Coiled-coil 2-domain containing protein (CC2D) and Flagella Attachment Zone protein 1 (FAZ-1) by IHC and Western blot assays. Immunohistochemistry study showed that ML-F52 significantly suppressed the expression of CC2D after 12 hours of post treatment whilst FAZ-1 did not show any significant suppression. After 24 hours of post treatment, cell length and FAZ length decreased with the emergence of cell containing detached flagella as compared to the control. Also, ML-F52 caused multinucleated phenotype and an increase in number of cells with only one or no visible kinetoplast. Western blot assay showed that CC2D expression was reduced more than approximately 60% by 12 hours post treatment and approximately 80% by 24 hours post treatment. Our findings suggested that ML-F52 might significantly inhibit the development and function of the flagellum.

1847

VISCERAL LEISHMANIASIS ELISA TESTING: EVALUATION OF SERIAL SERUM SAMPLES REVEALS AN UNANTICIPATED FINDING

Naomi E. Aronson[1], Nancy Koles[1], Saule Nurmukhambetova[1], Rupal Mody[2], Edgie Mark Co[3], Dutchabong Shaw[1], Robert DeFraites[1], Ines Lakhal-Naouar[1]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*William Beaumont Army Medical Center, El Paso, TX, United States,* [3]*Walter Reed National Military Medical Center, Bethesda, MD, United States*

Serologic testing for visceral leishmaniasis (VL) includes ELISA and immunochromatographic testing (ICT) methods. Generally antibody responses are reported to wane with time. 200 Iraq-deployed, healthy US servicemembers were enrolled in an asymptomatic VL surveillance study 2015-17 and ELISA testing of current, pre and post deployment sera was conducted. Their prior banked samples were requested from the Department of Defense Serum Repository; timepoints included entry to military service (accession), before deployment and upon return from Iraq. Serologic testing included a soluble *Leishmania* antigen-based ELISA using 1:400 sera dilution with positive ELISA results confirmed using a *Leishmania* Western Blot (WB, LDBios, France). Additionally, post Iraq and enrollment sera were tested with rk39 ICT (Kalazar Detect, Inbios WA). Enrollment sera from seven subjects tested positive on ELISA (3.5%) with one WB confirmed; there were no reactive rK39 serologies. Post-deployment sera tested ELISA positive in 55 subjects (27.5%), 48%

confirmed by positive WB. One subject post-deployment had a positive rK39 serology. Accession sera tested 147/200 ELISA positive (73.5%) and 63/200 (31.5%) pre-deployment sera were positive. An exploratory study of 10 persons who never traveled to Iraq or Afghanistan but had received influenza vaccination within past 3 months found 30% were seropositive on *Leishmania* ELISA testing. As expected, post-deployment serologic anti-*Leishmania* responses were identified in more subjects than currently. However, 73.5% and 31.5% ELISA positive results at accession and prior to Iraq deployment respectively were unexpected. Both groups received influenza vaccination proximate to blood draw and this may have impacted our serologic results.

1848

IN VITRO ANTI-LEISHMANIAL ACTIVITY OF UNRIPE LIME OIL AGAINST *LEISHMANIA MAJOR*

Elvis Suatey Lomotey[1], Godwin Kwakye-Nuako[2], Christian Kweku Adokoh[2], Joan Amoanab[2]

[1]*Noguchi Memorial Institute for Medical Research, Legon-Accra, Ghana,* [2]*University of Cape Coast, Cape Coast, Ghana*

The paradigm shift from chemotherapy to natural products to treat various diseases caused by bacteria, fungi, and protozoa, such as Leishmaniasis has led to the exploration of potential products from plant species, extracts and their main active compounds. This study sought to determine the anti-*Leishmania* activity of unripe lime oil extract on the promastigotes of *L. major*. Employing cell viability assay and trypan blue exclusion method, this research has analyzed and evaluated the *in vitro* inhibitory activity of unripe essential lime oil extract from *Citrus aurantifolia* of the Rutaceae family and its major active constituents, germacrene, against the growth and viability of *L. major.* The unripe lime oil extract showed significant activity against promastigote form of *L. major*, with IC_{50} of 2.76 μg/ml compared to amphotericin B with IC_{50} of 2.45 μg/ml after 72 hours. However, after 6 hours, the IC_{50} of the essential oil was 0.05 μg/ml indicating a substantial activity of the essential oil. Significant morphological alterations and disrupted membrane were observed in the promastigotes. The activity against promastigotes of *L. major* hints unripe lime oil extract as a potential new therapeutic agent against cutaneous leishmaniasis.

1849

USE OF IMAGE PROCESSING FOR A MHEALTH BASED APPROACH TO SCREEN CUTANEOUS LEISHMANIASIS LESIONS IN REMOTE AREAS

Hermali Silva[1], Shahirah Shaik[2], Kalaivani Chellappan[2], **Nadira D. Karunaweera**[1]

[1]*Faculty of Medicine, University of Colombo, Colombo* [8]*, Sri Lanka,* [2]*Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia*

mHealth infrastructure excites the healthcare industry in establishing remote screening and monitoring that is expected to meet the demands and needs of public health sector in developing countries. Leishmaniasis is an epidemic challenge affecting mostly the poor populations living in remote areas of over 80 countries across Asia, East Africa, South America, and the Mediterranean region. Among different clinical types of leishmaniasis, cutaneous leishmaniasis has higher potential to be successfully treated if patients can be identified by effective screening and monitoring. Clinical observation of skin lesions is a commonly used approach in cutaneous leishmaniasis diagnosis where facilities and expertise for confirmatory testing is not available. In the past, images captured from mobile cameras have been used for further processing in establishing the ulcer grading but the results are yet to meet the clinical decision making requirements. The researchers are in a desperate need to establish a more prominent remote image capturing approach due to the mobility limitation and medical practitioners availability in most of the prevalent areas. Establishing a prominent remote imaging solution in producing clinical applicable images will assist in more a more reliable decision making. In this study image processing technique was adapted to process the mobile captured JPEG images. The images were resized to 256x256 pixel resolution and grayscale conversion was done to standardize the variation of images. The standardized images were enhanced by using contrast stretching algorithm to identify the boundary of the lesion to be cropped. The cropped images were further processed to cluster between poor respondents and positive respondents to treatment with intralesional sodium stiboguconate injections through thresholding and contour analysis. Both the techniques resulted in a 50% accuracy in differentiating between poor respondents and respondents. In conclusion the mhealth approach assisted by imaging techniques may provide an added advantage to the remote leishmaniasis screening and treatment process.

1850

MONITORING EFFICACY OF NIFURTIMOX IN CHILDREN WITH CHAGAS DISEASE: RESULTS OF ELISA F-29 OBTAINED IN A PHASE 3 TRIAL (CHICO)

Ulrike Grossman[1], Luis Castro[2], Juan Dib[3], Jimy Pinto Rocha[4], Teresa Ramirez[5], Guillermo Moscatelli[6], Erya Huang[7], Olivia Ding[8], Jaime Altcheh[6], on behalf of the CHICO Study Group

[1]*Bayer AG, Berlin, Germany,* [2]*Centro de Atencion e Investigacion Medica S.A, Yopal, Colombia,* [3]*Centro de Investigacion – Fundacion Hospital Universidad del Norte, Soledad – Baranquilla, Colombia,* [4]*Fundación CEADES – Plataforma de Chagas, Cochabamba, Plurinational State of Bolivia,* [5]*Centro de Enfermedad de Chagas y Patologias Regionale, Santiago del Estero, Argentina,* [6]*Hospital de Ninos Ricardo Gutierrez, Buenos Aires, Argentina,* [7]*Bayer US LLC, Whippany, NJ, United States,* [8]*Bayer Healthcare Co. Ltd, Beijing, China* [9]

A challenge in the treatment of Chagas disease, which is caused by the parasite *Trypanosoma cruzi*, is the establishment of feasible cure criteria. Currently, immunological and parasitological methods have been reported with applicability for post-treatment cure assessment. For conventional serology methods, one of the most relevant limitations is the long time required for conversion of serological responses to negative. In previous studies, *T. cruzi* flagellar calcium-binding protein F-29 was proposed as an early marker of cure in Chagas disease. In a multicenter, multinational, randomized, double-blind phase 3 clinical trial using a historical control, F-29 was analyzed to evaluate the treatment response to nifurtimox in pediatric patients with Chagas disease after 1 year post-treatment follow-up. A total of 330 children aged 0 days to 17 years were assigned at a 2:1 ratio to 60-day (n=219) or 30-day (n=111) treatment with nifurtimox. Serum samples collected before (baseline) and at 7, 30, 60, 240, and 420 days after the start of nifurtimox treatment were analyzed for F-29 in a central laboratory using an enzyme-linked immunosorbent assay (ELISA). At baseline, 214 children (64.85%) tested positive for F-29, whereas in 116 children (35.15%) no F-29 was detected. However, all children showed positive test results measured by conventional ELISA serology or direct observation of *T. cruzi* by concentration test at baseline. The proportion of children with negative F-29 at baseline was similar in both treatment groups, and the majority remained negative at subsequent evaluation time points. In children who tested positive for F-29 at baseline, 66 (30.84%) showed negative responses at 1 year after the end of nifurtimox treatment. Correlations between F-29 and other serological (conventional ELISA) or molecular (quantitative polymerase chain reaction) test results were also evaluated, taking into account the different geographical regions. The response to nifurtimox treatment as evaluated by F-29 is comparable to previous reports for benznidazole in pediatric patients with Chagas disease.

1851

DISEASE AWARENESS, CLINICAL FEATURES AND TREATMENT OUTCOME ASSOCIATED WITH CUTANEOUS LEISHMANIASIS IN ANURADHAPURA, SRI LANKA

Hasna F. Riyal[1], Nilakshi T. Samaranayake[1], Deepani Munidasa[2], Nadira D. Karunaweera[1]

[1]*Faculty of Medicine, University of Colombo, Colombo 08, Sri Lanka,* [2]*Teaching Hospital, Anuradhapura, Anuradhapura, Sri Lanka*

Cutaneous Leishmaniasis (CL) is endemic in Sri Lanka with a high incidence in North-Central Province. Efforts were made to study the patients' awareness on the disease & their compliance for treatment given at a tertiary care hospital. 116 clinically-suspect CL patients were included in the study & their clinical data collected. Disease awareness was evaluated, Patients were followed up over an year to record their status of treatment progress & outcome. 102 patients(87.93%) were parasitologically-confirmed for CL with 73 males(71.56%). 42(41.18%) were between 25-40 years & 31(30.39%) were between 41-55 years. Upper limb was the commonest(59.80%) site of lesion, while lower limbs were affected in 23(22.55%). Fifty(49.02%) of them had nodules, 37(36.27%) had ulcers among which 10 had secondary infections. 66(64.70%) of the lesions were smaller than 1.5cm. A notable number of patients(n=43 ;42.16%) tended to seek medical care after lengthy delay i.e. about 3 months after appearance of lesion.Only a small number(n=16 ; 15.69%) of patients had adequate knowledge on CL while the majority of 59(57.84%) patients were totally unaware of the disease. These patients had no idea about sandfly, the prevention methods to get rid from the bites or how important it is to get the lesion cured. Majority(n=61; 59.80%) of the patients successfully completed their course of treatment while 29(28.43%) had discontinued therapy on their own and the rest of the patients continued with the prescribed treatment. Difficulty in attending weekly clinics with lengthy waiting periods were the common complaints the patients had. Patients feeling better as per their own perception after a few doses of medications was another reason for pre-mature termination of treatment. Delay in seeking treatment after the lesion onset & the lack of disease awareness among CL patients are causes for concern. Premature discontinuation of treatment, points towards the need for better patient education and more-patient friendly therapy. A national framework is required to educate the community on the importance of early detection and continuation of treatment for better containment of CL.

1852

NEW PEDIATRIC FORMULATION ALLOWS INDIVIDUALIZED DOSING OF ORAL NIFURTIMOX FOR TREATMENT OF CHILDREN WITH CHAGAS DISEASE

Heino H. Stass[1], Ethel C. Feleder[2], Gustavo Yerino[2], Facundo Garcia-Bournisen[3], Boris Weimann[4], Jaime Altcheh[3]

[1]*Bayer AG, Wuppertal, Germany,* [2]*Pharmacokinetic Unit FP Clinical Pharma SRL, Buenos Aires, Argentina,* [3]*Servicio Parasitologia – Chagas, Hospital de Niños R. Gutierrez, Buenos Aires, Argentina,* [4]*Chrestos Concept GmbH & Co. KG, Essen, Germany*

Nifurtimox (NFX) is one of only two approved treatments for Chagas disease (CD). Pediatric use of NFX requires body weight-adjusted dosing. However, the only NFX formulation currently available (i.e. 120 mg) hinders accurate pediatric dosing, particularly for small children. We performed biopharmaceutical investigations based on pharmacokinetic (PK) evaluations in 3 groups of adult CD patients using the available 120 mg tablet and a new formulation (i.e. 30 mg): 1) adults (n=24) were fed a high-fat, high-calorie meal 30 minutes before receiving a single 120 mg tablet or 4×30 mg tablets; 2) fed adults (n=12) were administered 4×30 mg NFX either as tablets or as an aqueous slurry; 3) adults (n=35) received 4×30 mg tablets in fed or fasting (≥10 hours) states. Washout between treatments was of ≥5 days in each crossover cohort. Blood was sampled over 24 hours post-dose for PK analysis in all studies. In all cohorts, most patients were female (66.7%, 100%, 89%, respectively), and mean age was 32.8, 32.4, and 33.9 years, respectively. Bioavailability was unaffected by formulation: area under the curve from baseline to last measurable NFX concentration ($AUC_{0\text{-tlast}}$) ratio: single 120 mg tablet vs 4×30 mg tablets, 1.05 (90% CI 0.99-1.11); 4×30 mg slurry vs tablets, 0.93 (0.84-1.03). Maximum plasma concentrations (C_{max}) were similar for the two tablets (ratio 1.02 [0.89-1.16]), but slightly lower for the slurry vs tablets (0.76 [0.69-0.85]). Food increased NFX exposure by about 70%: fed vs fasting $AUC_{0\text{-tlast}}$ ratio 1.72 (1.54-1.92); C_{max} ratio 1.68 (1.50-1.87). Only mild-to-moderate NFX adverse events (headache, nausea, abdominal pain, vomiting) were observed, in a minority of patients. Together, these findings help define clear dosing instructions for NFX treatment of young patients with CD in the outpatient setting. The ability to dose in increments from 15 mg to 120 mg, with a slurry option, facilitates reliable, personalized, body weight-based dosing of NFX. Efficacy of the pediatric formulation is under evaluation in an ongoing randomized phase 3 trial in pediatric CD patients (NCT02625974). Interim data from this trial will be presented at the meeting.

1853

EVALUATING NEW REGIMENS FOR THE TREATMENT OF CHRONIC CHAGAS DISEASE: THE BENDITA TRIAL

Fabiana Barreira da Silva Rocha, Bethania Blum, Sergio Estani

Drugs for Neglected Diseases initiative, Rio de Janeiro, Brazil

The current treatment for Chagas disease (CD) has a long duration, and safety and tolerability concerns. Benznidazole (BZN), a nitro-heterocyclic drug, is administered twice daily at a dose of 5 mg/kg/day, for 60 days in adults. Data from recently trials suggest the potential for new therapeutic approaches to improve response, tolerability and reduce resistance. Bendita, a double-blind, randomized, placebo-controlled, phase 2 trial, conducted in Bolivia from 2016 to 2018, assessed efficacy and safety of different regimens of BZN as monotherapy, or in combination with E1224, a broad-spectrum antifungal triazole, in reducing and clearing parasitaemia in adults with chronic indeterminate CD. Primary efficacy endpoint was parasitological response by serial qualitative PCR at end of treatment and sustained until 6 months. 210 participants were randomized to seven groups: BZN 300mg daily for a) 8 weeks, b) 4 weeks or c) 2 weeks); d) BZN 150mg daily for 4 weeks; e) BZN 150mg daily for 4 weeks in combination with E1224 300mg weekly; f) BZN 300mg weekly for 8 weeks in combination with E1224 300mg weekly; g) matching placebos. 202 patients completed the study. Primary efficacy analysis (intention-to-treat population) showed 89.3% of sustained clearance in parasitaemia at 6 months in BZN 300mg 8 weeks and 4 weeks; 82.8% in BZN 300mg 2 weeks; 83.3% in BZN 150mg 4 weeks; 85.2% in BZN 150mg 4 weeks in combination with E1224 and, 82.8% in BZN 300mg weekly in combination with E1224, in comparison to 3.3% in the placebo. Six patients (20%) interrupted treatment in BZN 300mg 8 weeks; 1 (3.3%) in 4 weeks; none in 2 weeks; 1 (3.3%) in BZN 150mg 4 weeks; 3 (10%) in BZN 150mg 4 weeks in combination with E1224 and 4 (13.3%) in BZN 300mg weekly in combination with E1224. Most adverse events were mild to moderate, 6 patients presented serious events (SAE): 2 (6.7%) in the BZN 300mg 8 weeks; 1 (3.3%) in both 300mg 4 weeks and in BZN 150mg 4 weeks in combination with E1224; 2 (6.7%) in BZN 300mg weekly in combination with E1224. Shorter treatment with BZN sustained parasitological response, was well tolerated and may have potential as new treatment for CD.

1854

DEVELOPING TOOLS FOR THE COLLECTION AND EVALUATION OF COSTS OF HUMAN AFRICA TRYPANOSOMIASIS INTERVENTION PROGRAMS

Xia Wang-Steverding[1], Marina Antillon[2], Alex P. Shaw[3], Ron Crump[1], Ching-I Huang[1], Fabrizio Tediosi[2], Jason Madan[1], Paul Bessell[4], Kat S. Rock[1]

[1]*University of Warwick, Coventry, United Kingdom,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Division of Infection and*

Pathway Medicine, The University of Edinburgh and AP Consultants, Andover, Edinburgh, United Kingdom, [4]Epi Interventions, Glasgow, United Kingdom

Gambiense human African trypanosomiasis (gHAT) is an infectious, vector-borne, parasitic disease of humans. HAT is a neglected tropical disease, and whilst targeted for elimination of transmission, there are limited data and analyses on costs of complete intervention programmes. Consequently there are relatively few economic evaluations assessing the cost-effectiveness of strategies for controlling gHAT. In order to determine and measure the costs of gHAT elimination strategies in endemic countries, the HAT Modelling and Economic Predictions for Policy (HAT MEPP) team developed the Cost Collection Tool (CCT) to collect economic data, which could be used to inform decision-making around strategies for gHAT elimination. Based on a literature review and expert knowledge the CCT was designed to collect country- and focus-specific information on unit and total costs associated with gHAT control programmes. Cost data collected include costs of diagnosis, treatment, and vector control. Additionally, the costs of running the programme itself and sensitisation activities were considered. The CCT is then completed by national gHAT control programmes before analysis of the data by the health economic team. Consequently, the CCT can contribute to the tailored allocation of scarce health resources for specific HAT *foci* to support attainment of local elimination targets and consequently the global elimination goals set by WHO.

1855

EFFORTS AGAINST GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS IN CHAD: A MATHEMATICAL AND ECONOMIC MODELING STUDY OF END-GAME INTERVENTIONS

Marina Antillon[1], Xia Wang-Steverding[2], Ron E. Crump[2], Ching-I Huang[2], Rian Snijders[3], Mallaye Peka[4], Severine Mbainda[4], Kat S. Rock[2], Fabrizio Tediosi[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Warwick University, Warwick, United Kingdom, [3]Institute of Tropical Medicine, Antwerp, Belgium, [4]Ministry of Health, N'Djaména, Chad*

The incidence of human African trypanosomiasis caused by the gambiense strain of *Trypanosoma brucei* (gHAT) has been on the decline since the 1990's, and it is now a disease identified for elimination of transmission by 2030. gHAT is transmitted by the tsetse fly and the disease is typically fatal if untreated. Among the locations where gHAT remains a concern is Mandoul, Chad. This is an insightful focus for the present case-study on the cost-effectiveness of interventions as the transmission dynamics have been well-characterized and elimination is within reach. No vaccine against gHAT exists and the side-effect profile of treatments precludes mass drug administration strategies, therefore prevention activities rely on identifying and treating infected individuals and complementary vector control. The approval of a novel, multiple-dose oral treatment – fexinidazole –provides an improvement over drugs that require parenteral in-hospital administration, and represents a potential game-changer for gHAT elimination. In our analysis we use a transmission dynamic model fit to epidemiological data from Mandoul to evaluate the cost-effectiveness of combinations of active screening, "enhanced" passive surveillance (expanding the number of health posts capable of screening for gHAT), and vector control activities employing tsetse targets. Our primary outcome is disability-adjusted life-years (DALYs) and costs are denominated in 2018 US$. Although the novel drug fexinidazole will cut inpatient and drug costs, interventions that include vector control provide good value-for-money (at less than $1000/DALY averted) and increase the probability of reaching the elimination target. Active screening activities present the largest cost drivers, so interventions that include vector control would allow safe scale-back of these activities, thus minimizing costs once low prevalence yields few identified cases during surveillance activities.

1856

ANTI-LEISHMANIAL ACTIVITIES OF COMPUTER-DESIGNED PROTEIN DISULFIDE ISOMERASE INHIBITORS

Susie Pham[1], Peter Sedillo[1], Noureddine Ben Khalaf[2], Valeria Pittala[3], Ivy Hurwitz[1]

[1]*University of New Mexico, Albuquerque, NM, United States, [2]Arabian Gulf University, Manama, Bahrain, [3]Univerity of Catania, Catania, Italy*

Leishmaniasis is a neglected tropical disease that affects 12 million people across 98 countries worldwide, with an annual incidence rate between 700,000 to 1.2 million. No vaccines are available, and current standards of treatment are inconvenient and toxic. Furthermore, there is evidence of drug resistant strains of *Leishmania spp*. Therefore, there is a demand for new treatment options. Protein disulfide isomerase plays a central role in the folding of newly synthesized proteins with further molecular chaperone and anti-chaperone activities. *Leishmania major* PDI (*Lm*PDI) is expressed and secreted by both promastigote and amastigote stages of *L. majo*r. In previous studies, deletion of *lmpdi* genes render resulting parasites non-virulent in animal models, suggesting a putative role for *Lm*PDI in parasite pathogenicity. These results further led to the hypothesis that *Lm*PDI may be a suitable target for anti-leishmania chemotherapy. A series of *Lm*PDI inhibitors, VP13, was designed utilizing a computer-aided approach. Fragment-based virtual screening allowed for better understanding of the inhibitors' modes of action on *Lm*PDI. In this work, the anti-leishmanial activities of the VP13 series of PDI inhibitors were investigated. In these assays, the cytotoxicities of the VP13 compounds were tested against *L. major* promastigotes and monocyte-derived macrophages (MDM). Specifically, VP13/74, VP13/83, VP13/98, and VP13/103 at 40uM effectively inhibited growth of *L. major* without significant MDM cytotoxicity. In preliminary experiments, the abilities of the four compounds to clear infection were tested by exposing 40uM of each compound to *L. major*-infected MDM. Following 48-hrs of treatment, there were significant decreases in the numbers of parasites per cell when compared to controls. The ability of the four VP13 compounds to clear *L. major* infections in a dose dependent manner are being investigated. Further, the ability of this class of *Lm*PDI inhibitors to minimize metacyclic *L. major* infection of MDM are in progress.

1857

DETERMINATION OF A MURINE MODEL TO EVALUATE NEW THERAPIES IN THE CHRONIC PHASE OF *TRYPANOSOMA CRUZI* H1 INFECTION ACCORDING TO ITS CARDIAC ELECTRICAL FUNCTION

Bárbara Carolina Arias Argáez[1], Xenia López Blanco[1], Landy Pech Pisté[1], Paulina Haro[2], Eric Dumonteil[3], Miguel Rosado Vallado[1]

[1]*Laboratorio de Parasitología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, Mérida, Mexico, [2]CONACYT-Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, Mérida, Mexico, [3]Department of Tropical Medicine, School of Public Health and Tropical Medicine, and Vector-Borne and Infectious Disease Research Center, Tulane University, New Orleans, LA, United States*

Chagas disease (CD), presents two clinical phases. In the acute phase, patients present a high parasitemia with nonspecific febrile illness. The disease progresses to a long-lasting indeterminate chronic phase that is characterized by low parasitemia and lack of any clinical, radiographic, or electrocardiographic abnormalities. Then, about 30% of patients progress to a heart pathology, which is the most serious consequence of CD. To evaluate effective therapies for CD, there is a need for an animal model that can display symptoms of cardiac pathology analogous to those found in humans. Thus, we aimed to define the murine model that better characterize the chronic phase of infection. Since during the course of CD, electrocardiographic alterations are usually the first clinical evidence of disease progression, we evaluated the development of cardiomyopathy through electrocardiographic studies in order to define the early and late chronic phase of infection. Thirteen BALB/c mice and thirteen ICR

mice were infected with 500 blood form trypomastigotes of *T. cruzi* H1. Electrocardiographic studies were performed every 35 days until 210 days post-infection (dpi) using a non-invasive equipment for conscious mice (ECGenie electrocardiograph). A total of 20-25 signals were recorded and analyzed using e-MOUSE software. ICR mice presented electrical alterations before the end of the acute phase (50 dpi); therefore, it was not possible to identify the early chronic phase. On the other hand, BALB/c mice had not only high survival (100%) in comparison with ICR mice (54.5%), but also had electrocardiographic abnormalities only at chronic phase. Cardiac abnormalities found include decreased heart rate and longer RR, QTc, QRS, ST, PR and PQ intervals. We conclude that at 70 dpi BALB/c mice were in the early chronic phase, since no parasites and electrical abnormalities were registered. While the onset of the late chronic phase starting at 105 dpi, as the first electrical abnormalities were recorded. Thus, the BALB/c strain is an appropriate murine model to evaluate therapeutic strategies in the different stages of the chronic phase of *T. cruzi* H1 infection.

1858

BURDEN OF *ANGIOSTRONGYLUS CANTONENSIS* LARVAE IN JUVENILE *PARMARION MARTENSI*

William L. Gosnell, Randi Rollins, Kenton Kramer, Jourdan Posner, Robert Cowie

University of Hawaíi at Manoa, Honolulu, HI, United States

Angiostrongylus cantonensis, the rat lungworm, is a parasitic nematode that is transmitted between rats and mollusks (slugs or snails). Humans and other animals such as dogs may become infected by ingestion of *A. cantonensis* infected slugs or snails. Human angiostrongyliasis presents as an eosinophilic meningitis/meningoencephalitis and has been reported globally. Documented cases of human angiostrongyliasis in Hawaíi and other Pacific islands have been reported since the early 1960's but over the past 10 years there appears to have been an increase in cases for Hawaíi as a whole but particularly for the Big Island of Hawaíi and Maui County. Epidemiological studies have shown that human angiostrongyliasis is commonly acquired via individual and cultural food practices of purposefully consuming raw or partially cooked snails. However, for the majority of recent cases in Hawaíi the route of infection with *A. cantonensis* has remained unknown, although assumed to have been the result of inadvertent ingestion of *A. cantonensis* infected slugs or snails. Among the different species of mollusks in Hawaíi that participate in this parasite's lifecycle one recent invasive species, *Parmarion martensi*, sometimes referred to as a semi-slug, has been reported to have a particularly high prevalence of natural infection with *A. cantonesis*. These findings are important, since Hawaíi and other regions of the world are experiencing conditions that are portentous for the rapid dispersal of this zoonosis and the likely spread and increased abundance of the invasive *P. martensi* and other well-known intermediate molluscan hosts of *A. cantonensis*. Therefore, because of the importance attributed to gastropods in the transmission of *A. cantonensis*, studies of the parasite-host interaction have been conducted as part of a strategy to develop measures to control snails and slugs and consequently lessen the risk of human angiostrongyliasis in Hawaíi.

1859

IDENTIFYING THE ROLE OF THE DIFFERENT RESERVOIR HOSTS OF ZOONOTIC SCHISTOSOMIASIS IN WEST AFRICA

Elsa Leger[1], Stefano Catalano[1], Anna M. Borlase[1], Cheikh B. Fall[2], Samba D. Diop[3], Bonnie L. Webster[4], David Rollinson[4], Nicolas D. Diouf[5], Khalilou Bâ[6], Mariama Sene[5], Joanne P. Webster[1]

[1]*Royal Veterinary College, Hatfield, United Kingdom,* [2]*University Cheikh Anta Diop, Dakar, Senegal,* [3]*University of Thies, Bambey, Senegal,* [4]*Natural History Museum, London, United Kingdom,* [5]*University Gaston Berger, Saint-Louis, Senegal,* [6]*CBGP, Institut de Recherche pour le Developpement, Dakar, Senegal*

Schistosomiasis is a neglected tropical disease caused by *Schistosoma* parasitic worms, which inflicts a significant burden on human and animal populations, particularly across sub-Saharan Africa. Anthropogenic land-use changes affect the distribution and availability of suitable definitive and intermediate hosts, increasing opportunities for hybridization between human and animal schistosomes with subsequent zoonotic transmission. This can have a substantial impact on the dynamics and distribution of schistosomiasis, with further challenges and constraints for effective control. Our aim was to elucidate the role of different definitive hosts as reservoirs of zoonotic *Schistosoma* single species and hybrids in a region of northern Senegal subject to important anthropogenic change. Extensive and systematic parasite sampling from human, livestock, and rodent definitive hosts, combined with snail intermediate hosts, were performed over three years across key transmission sites in northern Senegal. Multi-locus molecular analyses of all *Schistosoma* isolates, followed by Maximum Likelihood (ML) and Bayesian Inference (BI), were used to infer phylogenetic and transmission dynamics between the circulating zoonotic *Schistosoma* species/hybrids and their hosts. Molecular analyses confirmed the presence of widespread viable hybridization within and between *Schistosoma* species of humans and animals. Phylogenetic analyses indicated shared transmission of zoonotic *Schistosoma* species and hybrids between humans and animals (both wild and domestic), providing unique insights into the role of different host species in maintaining transmission. Our study emphasizes the need for a One Health multi-host framework for schistosomiasis control in both people and animals living in high zoonotic transmission zones of sub-Saharan Africa.

1860

LOW RISK PERCEPTION AROUND HANDLING OF LIVING AND DEAD ANIMALS POSES BARRIERS TO ZOONOTIC DISEASE PREVENTION AND PREPAREDNESS IN COTE D'IVOIRE

Danielle Naugle[1], Natalie Tibbels[1], Abdul Dosso[2], William Benié[2], Walter Kra[3], Corinne Fordham[1], Mieko McKay[2], Valère Konan[4], Jeanne Brou[5], Jocelyne Nebre[5], Adaman Kouadio[4], Zandra Andre[6], Diarra Kamara[2], Stella Babalola[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Johns Hopkins University, Abidjan, Côte D'Ivoire,* [3]*Alassane Ouattara University, Bouaké, Côte D'Ivoire,* [4]*Department of Veterinarian Services Ministry of Animal Resources and Fisheries, Abidjan, Côte D'Ivoire,* [5]*National Institute of Public Hygiene, Abidjan, Côte D'Ivoire,* [6]*U.S. Agency for International Development, Abidjan, Côte D'Ivoire*

Partner-supported government preparedness efforts during the 2014 Ebola outbreak helped prevent the incursion of the epidemic into Cote d'Ivoire (CdI); however, gaps remain in knowledge of transmission and appropriate prevention measures to limit the spread of zoonotic diseases. Public health actors in CdI have identified five priority zoonotic disease groups - rabies, mycobacteria, bacterial and parasitic diseases, viral hemorrhagic fevers, and respiratory zoonoses - to target for risk communication and social and behavior change (SBC) interventions. We conducted a qualitative study to understand the determinants of risk and prevention behaviors related to the five priority zoonoses across four sites in CdI through 32 focus groups, 32 individual interviews, 20 observations and 20 community maps with members of the general population and handlers of livestock and poultry. The interviews were recorded, transcribed, coded and analyzed. The data suggest that low knowledge of zoonoses and low perceived risk influence the limited adoption of hygienic or other protective measures by the Ivorian population. There is a general lack of understanding of animal to human disease transmission due to generations of co-habitation with animals, feelings of fondness towards animals, and a lack of first-hand experience with the transmission of zoonoses. Animal handlers and the general public perceive little risk in handling animals and potentially contaminated meat. This results in many risky behaviors, including direct human contact with sick or dead animals, the handling of animal and by-

products without protection, failure to sterilize hands, utensils or surfaces, and the consumption of sick animals. In addition, risk perception, as in the case of Ebola, appears short-lived; once the crisis passed, so did the perception of risk and the practice of related prevention behaviors like hand-washing. SBC programs must raise awareness of zoonotic risks to promote more favorable prevention practices. A key challenge will be creating awareness of the potential for risk and the need for sustained prevention in times where there is no ongoing crisis.

1861

CONCEPTUALIZING CHICKFLOWS IN MAPUTO, MOZAMBIQUE: HIGH-RISK BEHAVIORS AND PATHWAYS FOR CHILDHOOD EXPOSURE TO CHICKEN FECES

Frederica Lamar, Matthew C. Freeman, Karen Levy

Emory University, Atlanta, GA, United States

The risk of exposure to animal feces is unquantified yet likely contributes largely to the global burden of diarrheal disease in children. Studies assessing the impact of water, sanitation, and hygiene (WASH)- related exposures have focused predominantly on infrastructure and associated behaviors along the traditional F-diagram, with less emphasis on exposure to animal sourced pathogens. Poultry is a major reservoir for *Campylobacter* and non-typhoidal *Salmonella* (NTS), two of the top four global causes of the global burden of diarrhea. Poultry production in sub-Saharan Africa is encouraged for economic gain and nutrition, yet the risks associated with zoonotic pathogens may offset the benefits. To understand opportunities for childhood exposure from production to market to consumption in Maputo, Mozambique, we performed formative research to understand and map the chicken value chain - the ChickFlows approach. We conducted key informant interviews to identify potential high- risk behaviors and pathways that expose children to chicken feces. Our preliminary assessment included direct and indirect interactions between children and chickens/chicken products, WASH indicators, biosecurity measures, and waste management. We identified three high-risk settings: 1) live, informal markets, 2) small- scale, backyard farms, and 3) households. Children may also be exposed to chicken feces via the application of chicken litter to produce as fertilizer. Biosecurity measures were minimal to nonexistent across all settings. Adherence to disinfection periods between broiler flocks, antibiotic schedules, and microbial standards was variable and not regulated or enforced. Improved understanding of this complex network of high-risk behaviors and pathways can provide guidance on which locations within and upstream of the household should be prioritized to mitigate exposures. Collaboration with the agriculture sector and assessing risks within the formal and informal food systems may suggest innovative WASH interventions that could limit exposure to zoonotic infection.

1862

PATTERNS AND RISK FACTORS FOR ANTIBIOTIC RESISTANCE AMONG COAGULASE-POSITIVE *STAPHYLOCOCCUS* (CPS) ISOLATED FROM DOGS AND CATS THAT RESIDE WITH A PATIENT RECENTLY DIAGNOSED WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* SKIN OR SOFT-TISSUE INFECTION

Cusi Ferradas[1], Caitlin Cotter[2], Jonathan Shahbazian[2], Sally Ann Iverson[2], Patrick Baron[2], Ana Misic[3], Amy M. Brazil[2], Irving Nachamkin[3], Jacqueline M. Ferguson[2], Ebbing Lautenbach[3], Daniel O. Morris[4], Andrés G. Lescano[1], Meghan F. Davis[2]

[1]*School of Public Health and Administration, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States,* [3]*School of Medicine, University of Pennsylvania, Philadelphia, PA, United States,* [4]*School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, United States*

Companion animals may be important in the maintenance of methicillin-resistant (MR) and multidrug resistant (MDR) staphylococci in the household. Although risk factors for carriage of staphylococci in veterinary hospital settings have been evaluated, community-acquired staphylococci have not received the same attention. This study is part of a nested sub-study to a randomized controlled trial on methicillin-resistant *S. aureus* (MRSA) decolonization treatment. The objective of this study was to examine the patterns of and risk factors for antibiotic resistance among *S. aureus* (*n*=30), *S. pseudintermedius (n*=24) and other species of coagulase-positive *Staphylococcus* (CPS) *(n*=56) isolated from dogs and cats whose owners were recently diagnosed with MRSA skin or soft-tissue infection. A single isolate per pet was selected based on most likely MRSA phenotype and tested for antibiotic susceptibility against 11 drugs using Kirby Bauer disk diffusion. We used binomial logistic regression to identify risk factors for antibiotic resistance and evaluated the potential for bacterial species to modify associations between putative risk factors and antibiotic resistance outcomes. More than 50% of the *S. pseudintermedius* and more than 20% of other CPS isolates were sensitive to all classes of antibiotics tested, while only 4% of *S. aureus* isolates were susceptible to all classes of antibiotics tested. In addition, more than 8% of. *S. aureus* and more than 10% of CPS isolates were resistant to five or more classes of antibiotics. In a bivariate analysis, the association between a number of factors including: neuter status, use of topical antibiotics, use of clindamycin by the owner or the pet, contact with animals outside the house, and evidence of unwanted pests in the household and antibiotic resistance was modified by bacterial species. In conclusion, *S. aureus* isolates were more likely to be MDR compared to *S. pseudintermedius*. Furthermore, the results suggest that drivers of antibiotic resistance in household staphylococci may vary by bacterial species, which could have implications for intervention strategies.

1863

CHICKEN OWNERSHIP IS NOT ASSOCIATED WITH *CAMPYLOBACTER* INFECTION OR ANEMIA AMONG CHILDREN 6 TO 59 MONTHS OLD IN THE GREATER ACCRA REGION, GHANA

Nathalie J. Lambrecht[1], Dave Bridges[1], Bright Adu[2], Mark L. Wilson[1], Andrew D. Jones[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Ghana*

Household chicken ownership in low-income countries may increase exposure of young children to enteric pathogens such as *Campylobacter*. In these settings, anemia may develop from such enteric infections through inflammatory mechanisms. In this study, we aimed to determine the association between household chicken ownership and anemia among young children in Ghana and evaluate the potential role of inflammation due to bacterial infection in mediating this association. We randomly sampled 484 children aged 6 to 59 months from 18 communities in the Greater Accra Region. Surveys assessed household ownership of livestock species and household sociodemographic characteristics. Blood samples were analyzed for hemoglobin, serum ferritin (SF), serum transferrin receptor (sTfR), α-1-acid glycoprotein (AGP), and C-reactive protein (CRP). Among a subsample of 264 children, DNA extracted from stool samples was analyzed for *Campylobacter* spp. using the cadF gene by quantitative polymerase chain reaction. Among this subsample, 45.8% of children were anemic (Hb<11.0g/dL) and 17.1% were positive for *Campylobacter* infection (cadF>10^3 copies/gram of stool). In multiple regression models adjusting for child age and sex, household sociodemographic characteristics, and village cluster, household chicken ownership was associated neither with *Campylobacter* infection (OR (95% CI): 1.2 (0.6, 2.6)) nor anemia (0.8 (0.5, 1.3)) in children. In adjusted models, *Campylobacter* infection was marginally associated (p<0.1) with iron deficiency (high sTfR: 1.9 (0.9, 3.8); low SF: 1.9 (0.9, 3.9)) but was not associated with anemia (1.1 (0.6, 1.9). Elevated AGP (AGP>1g/L), indicative of chronic inflammation, was higher among children with *Campylobacter* infection (51.2% vs. 35.2%, p<0.05). In conclusion, we did not find evidence to suggest that *Campylobacter* infection in children

is associated with household chicken ownership. Although *Campylobacter* infection was not associated with child anemia, infection may contribute to iron deficiency among children due to inflammation.

1864

CHARACTERIZING ANTIBIOTIC RESISTOMES IN HUMANS AND DOMESTIC ANIMALS FROM RURAL AND URBAN BANGLADESH

Jenna Swarthout[1], Erica R. Fuhrmeister[2], Angela R. Harris[3], Emily S. Gurley[4], Syed M. Satter[5], Alexandria B. Boehm[6], Amy J. Pickering[1]

[1]*Tufts University, Medford, MA, United States,* [2]*University of California Berkeley, Berkeley, CA, United States,* [3]*North Carolina State University, Raleigh, NC, United States,* [4]*Johns Hopkins University, Baltimore, MD, United States,* [5]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh,* [6]*Stanford University, Stanford, CA, United States*

Antibiotic resistance is a global public health concern, and animals are known reservoirs of resistant bacteria. Antibiotic use as prophylaxis and growth promotion is common in animal husbandry, and resistance development in animal gut microbiomes and exchange with humans has been shown in high-income countries and intensive farming environments. Data on community-acquired drug-resistant infections in low- and middle-income countries, however, are sparse. Widespread antibiotic misuse and insufficient water treatment, sanitation and hygiene practices exacerbate the risk of environmental contamination with resistant bacteria, particularly in urban areas where humans often cohabitate with domestic animals. Our primary objective is to assess whether humans and animals share resistance profiles in Bangladesh and whether there is a stronger correlation between resistomes in urban compared to rural settings. We collected human, goat and chicken fecal samples from one rural and one urban community in Bangladesh to create four fecal composites (pooling five individual fecal samples) for each species within each community. We completed long-read sequencing of all composites with the Oxford Nanopore MinION. Sequence data from composites of each sample type, defined by species and community, were pooled for further analysis. Minimap2 was used to align reads to antibiotic resistance gene (ARG) sequences in the ResFinder database, and Centrifuge was used to assign taxonomic classifications, referencing NCBI's RefSeq database. The sequencing yielded, on average, 9.9 million reads per sample type, with an average length of 1734 bp. Preliminary results show relative ARG abundance (normalized by Gbp) is greater in urban compared to rural samples, across all fecal hosts. Findings will be presented on ARGs circulating among humans and animals in Bangladesh, and long reads will enable identification of pathogenic bacteria carrying particular ARGs. Our findings will contribute to understanding the potential for zoonotic exchange of ARGs, which is necessary to inform antibiotic stewardship strategies in medical and veterinary practices.

1865

DETERMINING THE PRESENCE OF AN ANIMAL RESERVOIR FOR GAMBIAN HAT VIA MATHEMATICAL MODELLING

Ron E. Crump[1], Ching-I Huang[1], Erick M. Miaka[2], Matt J. Keeling[1], Kat S. Rock[1]

[1]*The University of Warwick, Coventry, United Kingdom,* [2]*Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of the Congo*

Gambiense human African trypanosomiasis (gHAT) is a disease of humans caused by the parasite *Trypanosoma brucei gambiense* which is transmitted via tsetse. Unlike the closely-related zoonotic disease Rhodesiense HAT, which has a transmission cycle including humans and animals, gHAT is often considered to be anthroponotic despite evidence that animals can be infected. The existence, and importance of an animal reservoir for gHAT is unclear. In this study we explore the transmission dynamics of gHAT in humans, using models with and without an animal reservoir. The models are fitted to human case incidence data recorded at the health zone level across the Democratic Republic of Congo (DRC). Although the data are only on human cases of gHAT, the observed patterns over time may be better explained if an animal reservoir exists. In addition, the animal-specific reproduction numbers for gHAT at the health zone level are calculated and these allow us to postulate about the ability of any animal reservoir to sustain transmission independently of human hosts. Additionally, the impact of presence or absence of such reservoir on future infection dynamics is examined. A number of intervention strategies are considered, with future projections being made for both models. The use of vector control, via tiny targets, will be particularly valuable where an animal reservoir exists as this intervention impacts upon transmission in both human and animal populations.

1866

LAND USE AND HUMAN BEHAVIORAL RISK FACTORS FOR ZOONOTIC DISEASE EXPOSURE IN LAIKIPIA COUNTY, KENYA

Elizabeth Ashby[1], Joseph Kamau[2], James Hassell[3], Dawn Zimmerman[3], Jennifer Yu[3], Lindsey Shields[4], Suzan Murray[3]

[1]*George Mason University, Fairfax, VA, United States,* [2]*Institute of Primate Research, Nairobi, Kenya,* [3]*Smithsonian Institution, Global Health Program, Washington, DC, United States,* [4]*PATH, Washington, DC, United States*

A majority of emerging infectious diseases (EIDs) are zoonotic, highlighting human-animal interactions as a driver of spillover. Laikipia County, Kenya was selected as an area of focus in PREDICT, a global disease surveillance project, due to its human-wildlife-livestock interface. Questionnaires that assessed high-risk behaviors (animal interactions, food safety) were distributed to 327 participants among five communities in Laikipia. Behavioral trends were assessed in R via chi square analysis and LASSO regression. Communities were classified by land use as: Pastoralist (collectively owned group ranches), Commercial Ranching (CR; privately owned land), or Wildlife Conservancy (WC; herding within a private wildlife conservancy). Pastoralist communities expressed the most frequent human-animal interactions and high-risk food and water practices; a majority reported eating sick animals (98%, 55%) and sharing drinking water sources with animals (90%, 100%). The CR community was less likely to report collecting dead animals and sharing a water source when compared to pastoralists ($p < 0.01$). The two WC communities were least likely to report food-safety risk, such as eating sick animals (2%, 0%), but water sharing with animals varied (43%, 11%). LASSO regression revealed that proper management of human waste was a protective factor (OR 0.84) and ill household members were a risk factor (OR 16.68) to participant reports of illness, indicating risk for pastoral communities. Risk is most influenced by human behavior among pastoralists, and higher wildlife densities in WC communities. Livestock-wildlife land sharing is unavoidable as resource availability decreases in a changing climate, and protective human behaviors may reduce spillover. This study addresses an information gap in land use and behavioral EID risk. Effective community intervention should emphasize training for local health workers, who are liaisons between researchers and at-risk communities. Further studies should include qualitative research to develop tailored intervention strategies and assess reasons for behavior variation in land use systems. .

1867

ONE HEALTH SURVEILLANCE FOR BAT-BORNE VIRUSES AT CAVE TOURISM DESTINATIONS IN SOUTHEAST ASIA

Heather S. Davies[1], Alexis C. Garretson[2], Kathryn Hogan[1], A. Alonso Aguirre[1], Michael von Fricken[1]

[1]*George Mason University, Fairfax, VA, United States,* [2]*Brigham Young University, Provo, UT, United States*

Recreational cave tourism may pose risk of zoonotic disease transmission. Tourists to the Python Cave in Uganda contracted Marburg Haemorrhagic Fever; bats subsequently tested positive for high-identity viruses. Cave tourism is popular across Southeast Asia, a promoted activity for the 100+ million visitors to the region where, also, cave-roosting bats are deemed to be reservoirs for coronaviruses that may have been the indirect

source of SARS. While most human-bat contact events do not result in emerging infectious disease spillover, characterizing risk factors at caves with intensive human activity can inform conservation management approaches. This research proposes a cave surveillance network for monitoring bat health and virus shedding. Bat-virus-location associations were abstracted from publications (1997-2019). Spatial range data for cave-roosting bats present in the Philippines, Malaysia, Indonesia, Thailand, Cambodia, Laos, and Vietnam were used to identify areas of high diversity of potential host species. Representative show caves were selected from these areas for possible inclusion in a sentinel monitoring network, with coverage across the region. Disease emergence risk factors were compiled for sentinel caves, including visitation data, roosting ecology, population size, and proximity of bat-virus detection among present species. Surveillance planning considerations were identified, including target virus species by location, use of screening detection methods for longitudinal sampling programs, data sharing and validation measures. Viruses have been detected in nearly 200 chiropteran species in 40 genera across Southeast Asia. Location-specific risk profiles, along with longitudinal monitoring, can support One Health management to protect bat and human health, as well as sustainable cave tourism.

1868

LEPROSY CHEMOPROPHYLAXIS OF HOUSEHOLD CONTACTS: A SURVEY OF CANADIAN INFECTIOUS DISEASE AND TROPICAL MEDICINE SPECIALISTS

Carl Boodman[1], Jay Keystone[2]

[1]*University of British Columbia, Vancouver, BC, Canada,* [2]*University of Toronto, Toronto, ON, Canada*

Leprosy is uncommon in Canada. However, immigration from leprosy endemic areas, notably India, Vietnam and the Philippines, has introduced leprosy to a Canadian context where health care practitioners have little to no knowledge of the disease. While post-exposure chemoprophylaxis (PEP) has been shown to decrease leprosy transmission, no Canadian guidelines exist to advise clinical decision-making about leprosy PEP. We characterized the practice patterns of Canadian infectious disease and tropical medicine specialists regarding the use of PEP for household contacts via an online anonymous survey. Descriptive statistics and Exact Multinomial Tests were performed. Survey response rate was 47% (20 of 43). 35% responded that PEP is needed for household contacts. 45% responded that PEP is not needed for household contacts. 25% did not know whether PEP is needed (multinomial test $P = 0.785$). 25% responded that PEP should be given to all household contacts. 62.5% responded that PEP should only be given to contacts of multibacillary cases and 25% responded that PEP should only be given to contacts that are genetically-related to the index case. For specialists who prescribe PEP, 57.14% use rifampicin, ofloxacin (levofloxacin), minocycline, 14.29% prescribe Single Dose Rifampicin, 28.57% prescribe multiple doses of rifampicin, 14.29% prescribe another unknown regimen (multinomial test $P = 0.106$). 68.42% recommend yearly screening of household contacts, while 31.58% do not (multinomial test $P = 0.167$). A striking lack of consensus defines the practices patterns of Canadian infectious disease and tropical medicine specialists regarding PEP of leprosy household contacts.

1869

MODELING THE IMPACT OF THE USE OF INFLUENZA VACCINE IN CHILDREN UNDER FIVE YEARS OF AGE ON THE CUMULATIVE CASE COUNT OF INFLUENZA IN MALI

Nancy Ortiz[1], Adama Mamby Keita[2], Flanon Coulibaly[2], Uma Onwuchekwa[2], Samba O. Sow[2], Arthur L. Reingold[1], Milagritos Tapia[3]

[1]*University of California Berkeley, Berkeley, CA, United States,* [2]*Centre pour le Développement des Vaccins-Mali, Bamako, Mali,* [3]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States*

Children under five years of age are at high risk of influenza, experiencing a disproportionate burden of influenza illness and are also at risk for severe influenza-related complications. In Mali, one of the poorest countries in the world, half of the population is under fifteen years of age. Rates of respiratory illness among children are highest in Africa, while influenza vaccination in most of Africa is rare. Understanding influenza seasonality is important for the timing of vaccination. In Mali, influenza transmission occurs in September/October and February annually. We used an SEIR compartmental model and developed ordinary differential equations to model the seasonal peaks of influenza activity in Mali to model the impact of introducing influenza vaccination in children on cumulative case count of influenza among children residing with other vulnerable populations at risk for severe influenza-related outcomes (i.e. pregnant, postpartum women and infants). We found that even nominal influenza vaccine coverage (10%) reduced the total epidemic size considerably, to nearly three-fourths of the number of cases observed in the same population without vaccination. Our analyses also demonstrated that the timing of vaccination efforts played an important role in the final epidemic size, with implementation of vaccination after the first case of influenza had appeared resulting in larger projected epidemic sizes than if vaccination had been implemented four weeks before influenza activity began. Vaccinating in late August and third week of December led to the largest projected reductions in total case counts. Our modeling analysis demonstrated that even with minimal vaccination of children, influenza vaccination cut the number of cases dramatically and that projected cumulative case counts increased with each week of delay of vaccination.

1870

SYNTHESIS AND ANTIMYCOBACTERIAL EVALUATION OF D-CYCLOSERINE ANALOGUES

Shoneeze Simone Renga, Vinayak Singh, Kelly Chibale

University of Cape Town, Cape Town, South Africa

Tuberculosis (TB) continues to be the leading cause of death from a single infectious agent worldwide and the rapid emergence of multidrug-resistant and extremely drug-resistant underpin the urgent need for novel, safe and efficacious drugs. D-Cycloserine (DCS) is an oral bacteriostatic anti-tubercular drug used for the treatment of drug-resistant TB. The antimycobacterial mechanism of action of DCS is two-fold. DCS interferes with bacterial cell-wall synthesis by competitively inhibiting the L-alanine racemase and D-alanine:D-alanine ligase enzymes. Additionally, it also inhibits other pyridoxal-phosphate dependent enzymes. Despite attractive properties, DCS displays significant toxicity at effective dosages. However, a synthetic analogue of DCS, terizidone, has been shown to have improved safety margins. The present study utilizes DCS and terizidone as a starting point for the development of a small library of antimycobacterial compounds. The structure-activity relationship is investigated in order to identify new antitubercular agents potentially able to circumvent antitubercular drug-resistance using a pro-drug approach. Herein we report the synthesis of a series of 15 monomers of DCS-based analogues of terizidone, and two hybrid analogues of DCS. These were evaluated for their antimycobacterial activity against the *Mtb* H37Rv strain. Further work is in progress to establish the mechanism of resistance/action of DCS analogues.

1871

MULTIPLEXED DETECTION OF PATHOGENS IN RESPIRATORY ILLNESS IN LATIN AMERICA

Julia S. Ampuero[1], Ivette Lorenzana[2], Doris Gomez[3], Margarita Ochoa-Diaz[3], Ana Arango[4], Nicolas Aguayo[5], Marina Gonzalez[6], Yelin Roca[7], Victor Ocaña[8], Edward Chavez[9], Kimberly Garcia[2], Crystyan Siles[10], Maria Silva[1]

[1]*U.S. Naval Medical Research Unit No. 6 (NAMRU-6), Lima, Peru,* [2]*Universidad Nacional Autónoma de Honduras-UNAH, Tegucigalpa, Honduras,* [3]*Doctorado en Medicina Tropical, Grupo UNIMOL, Universidad de Cartagena, Cartagena, Colombia,* [4]*Grupo Inmunovirologia, Universidad de Antioquia, Medellin, Colombia,* [5]*NGO Rayos de Sol, Asuncion, Paraguay,* [6]*Laboratorio de Salud Pública, Secretaría de Salud del Meta, Villavicencio, Colombia,* [7]*Centro de Enfermedades Tropicales, CENETROP, Santa Cruz, Plurinational State of Bolivia,* [8]*Centro de Salud Pachitea. Ministerio de Salud, Piura, Peru,* [9]*Centro Médico Militar,* [32]*a Brigada de Infantería, Trujillo, Peru,* [10]*U.S. Naval Medical Research Unit No. 6 (NAMRU-6), Iquitos, Peru*

The availability and use of multiplexed diagnostic devices now allows for a much more comprehensive understanding of pathogens associated with disease. This devices, particularly those that detect bacterial and viral agents, are of high importance to guide treatment and prevent improper use of antibiotics. The objective of this study was to characterize the infectious causes of acute respiratory disease using a multiplex PCR system. Subjects invited to participate in the study were those who visited health care centers in Bolivia, Colombia, Honduras, Paraguay, and Peru. The inclusion criteria for participants was reporting illness for ≤5 days, fever (≥ 38°C oral, tympanic, or rectal; ≥ 37.5°C axillary) or history of fever within 24 hours of arrival to the clinic and either one or more of the following symptoms: sore throat, cough, or runny nose. Upon obtaining an informed consent, clinical and epidemiological data and a nasopharyngeal swab sample were collected. All samples were processed using the Biofire® FilmArray® respiratory panel (17 viruses, 3 bacteria) using FilmArray 2.0® equipment (BiofireDX, USA). Between July 2016 and March 2019 a total of 2,562 participants were enrolled. An infectious respiratory agent was detected in 68.9% of the samples (1766/2562), and 15.3% out of these presented with co-detection of agents (270/1766). Viruses were more frequently detected (1748/1766) than bacteria (35/1766). The top five agents identified and the average age ± SD, were: 26.6% human rhinovirus/enterovirus (HRV/ENT; 682/2060, 15.0 ± 17.2), 11% influenza A (H1N1)pdm09 (228/2060, 20.7 ± 19.4), 10% influenza B (212/2060), 8% respiratory syncytial virus (174/2060, 5.7 ± 12.8) and 7% human metapneumovirus (142/2060, 14.0 ± 21.7). Importantly, the most frequently detected pathogen (HRV/ENT) causes a self-limiting disease where treatment focuses on mitigating symptoms and bacterial infections were rare. Continued use of multiplexed diagnostic devices are warranted to better guide therapeutic treatment and for broader surveillance efforts.

1872

STREPTOCOCCUS PNEUMONIAE COLONIZATION OF THE NASOPHARYNX IN MOTHER-INFANT PAIRS: A CROSS-SECTIONAL STUDY

Dennis Gyasi Konadu[1], Kaali Seyram[1], Darby Jack[2], Abena Konadu Yawson[1], Louisa F. Iddrisu[1], Zuwera Yidana[1], Farrid Boadu[1], Felix Boakye Oppong[1], Dennis Adu-Gyasi[1], Steven Chillrud[3], David K. Dosoo[1], Patrick Kinney[4], Kwaku Poku Asante[1]

[1]*Kintampo Health Research Centre, Kintampo, Ghana,* [2]*Columbia University Mailman School of Public Health, New York, NY, United States,* [3]*Lamont-Doherty Earth Observatory at Columbia University, Palisades, NY, United States,* [4]*Boston University School of Public Health, Boston, MA, United States*

Bacterial pathogens including *Streptococcus pneumoniae* frequently colonize the nasopharynx and anterior nasal cavity in adults and children and such colonizations increase the risk of Acute Lower Respiratory Infections in children. We set up this study to determine the prevalence of *S. pneumonia*e carriage and antibiotic susceptibility using oxacillin to predict susceptibility to β-lactams in the nasopharynx of mother-infant pairs in the middle-belt of Ghana. We adopted a cross-sectional study design nested into the Ghana Randomized Air Pollution and Household Survey (GRAPHS) in the Kintampo North Municipality and Kintampo South District. The GRAPHS study sort to evaluate the effect of improved cook stove on pregnancy outcome and infant health. Demographic data of mother and infants (>2years) were collected. Nasopharyngeal swabs were obtained from 123 mother-infants pairs and cultured on blood agar plate containing gentamicin to suppress growth of other commensals. Pneumococcal isolates were identified and analyzed for antimicrobial susceptibility testing using the Kirby Bauer disc diffusion method. Prevalence of pneumococcal carriage was 23.6% (29/123) and 70.7% (87/123) among mothers and infants respectively. Maternal pneumococcal carriage was significantly associated with that of infant using chi square test (*P*-value= 0.036). Forty-two (37.2%) out of 113 *Streptococcus pneumoniae* isolates were resistant to oxacillin (Oxacillin zone ≥20 mm). Prevalence of pneumococcal carriage was high among infants in the municipality compared to their mothers. The high level of oxacillin resistance should be of concern and monitored carefully since this predict resistance to β-lactams. Further studies in molecular techniques need to be carried out to elucidate the association of maternal carriage to that of infants.

1873

ETIOLOGY OF ACUTE RESPIRATORY INFECTIONS IN CHILDREN UNDER FIVE YEARS OLD. RESULTS FROM AN ACTIVE COMMUNITY SURVEILLANCE AND PASSIVE HOSPITAL SURVEILLANCE IN LIMA, PERU

Yeny O. Tinoco[1], Candice Romero[1], Felices Vidal[1], Giselle Soto[1], Maria Silva[1], Danielle Iuliano[2], Andrea J. McCoy[1]

[1]*Naval Medical Research Unit-6, Callao, Peru,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Acute respiratory infections (ARI) constitutes a major cause of morbidity and mortality in children. Different pathogens were described to cause ARI however their contribution and role as etiological agents are not well understood. We aimed to describe the etiology of ARI among children < 5 years in two settings: (1) Severe acute respiratory illness (SARI) in a hospital setting and (2) influenza-like illness (ILI) from a community active surveillance, both in a similar geographic area and time period. To perform the testing we used Taqman® Array Cards, which detect 30 respiratory pathogens. From February 2014—March 2015, we sampled 258 nasopharyngeal & oropharyngeal (OP) swabs from SARI children. SARI was defined as fever ≥38 C with cough & disease onset in last 10 days prior to hospitalization. Simultaneously, we collected 150 OP swabs from children with ILI in community cohort; ILI was defined as sudden onset of fever ≥38 C, with cough and/or rhinorrhea and/ or nasal congestion. Of the SARI-patients, 95% (244) had at least one agent detected. Respiratory Syncytial Virus was the most common (33%, 13) followed by *Streptococcus pneumonia* ([STPN], 15%, 6) and Human metapneumovirus (13%, 5). Among ILI patients, 91% (136) had at least one agent detected. Among those with a detected, pathogen, 31% (12) were *Moraxella catarrhalis* (MOCA) and 13% (5) for each Influenza A&B, *Haemophilus influenza* (Hib) and STPN. Identification of only one pathogen was not common among SARI patients (16%, 39) or ILI patients (29%, 39); however, there were statistically significantly more single detections among ILI patients compared with SARI patients (p= 0.003). Hib, MOCA and STPN were the most common co-detections (46% -62%) found among ILI and SARI children. Importantly, we found that *Legionella* species, Human coronavirus NL63 and 229E, *Bordetella pertussis*, *Pseudomonas aeruginosa*, Influenza C, Group A streptococcus and *Pneumocystis jiroveci* were present only in SARI hospitalized children. Our results demonstrate that more than 90% of pathogens could be identified in OP swabs and pathogens detected were different between community and hospitalized settings.

1874

EVALUATING COMMUNITY KNOWLEDGE OF TUBERCULOSIS AND ISONIAZID PREVENTATIVE THERAPY IN RURAL SOUTH AFRICA

Carlo Foppiano Palacios[1], Tejaswi Kompala[2], Anthony Moll[3], Laurie Andrews[4], Sheela Shenoi[4]

[1]*University of Maryland Medical Center, Baltimore, MD, United States,* [2]*University of California San Francisco, San Francisco, CA, United States,* [3]*Church of Scotland Hospital, Tugela Ferry, South Africa,* [4]*Yale University School of Medicine, New Haven, CT, United States*

Isoniazid preventive therapy (IPT) is an efficacious strategy to reduce TB incidence among HIV infected patients, though implementation has been suboptimal. The World Health Organization (WHO) recently expanded the categories of individuals who would benefit from IPT. We sought to understand community members' knowledge of TB, HIV, and IPT to inform future implementation efforts. In rural South Africa, individuals were interviewed anonymously at community events as part of a larger HIV and TB screening project. Four different domains of TB knowledge were evaluated: causes, transmission, treatment and prevention of TB, and IPT. An overall TB knowledge assessment was composed of the sum of the TB causes, transmission, and treatment and prevention scores. Among 104 respondents, the mean age was 35±9.3 years, 65% were female, and 26% completed secondary school. Overall, respondents had poor knowledge about the causes (mean 61±27%) and transmission (mean 46±21%), and good knowledge of the treatment and prevention (mean 88±18%) of TB. Respondents scored lowest on TB transmission, significantly lower than TB causes ($p=0.003$) and treatment and prevention ($p<0.0001$). Years of formal education was significantly associated with total TB knowledge ($p<0.0001$). Specifically, increased education was associated with improved knowledge of TB causes ($p<0.0001$) and transmission ($p<0.0001$), but not with knowledge of treatment and prevention of TB ($p=0.19$) or of IPT ($p=0.82$). Greater knowledge of IPT was associated with higher scores of total knowledge of TB ($p=0.01$) and specifically TB treatment and prevention ($p<0.0001$), with wanting to take care of family ($p<0.0001$), and wanting to keep their family healthy from TB ($p<0.0001$). Community members in rural South Africa had poor TB knowledge, particularly about transmission of TB. Formal education was associated with knowledge of TB. Knowledge of IPT was associated with the motivation to care for family. To implement the new WHO TB prevention guidelines, resource limited settings will require improved public health and individual-level education about tuberculosis.

1875

IMPROVED LATENT TUBERCULOSIS THERAPY COMPLETION RATES IN REFUGEE PATIENTS THROUGH USE OF A CLINICAL PHARMACIST

Kimberly L. Carter[1], Joseph Garland[2]

[1]*University of Pennsylvania, Philadelphia, PA, United States,* [2]*Alpert Medical School, Brown University, RI, United States*

On average 700 refugees resettle in Philadelphia annually and approximately 30% have LTBI upon arrival. In 2012, a pharmacist-run LTBI clinic was established at Penn Center for Primary Care (PCPC) in attempts to improve LTBI completion rates among newly arriving refugees. Prior to 2012, LTBI completion rates at PCPC were less than 20%. A structured model was developed to efficiently track patients and ensure successful completion occurred. All patients diagnosed with LTBI during the study period were referred to the clinical pharmacist for treatment initiation and follow-up. At a minimum, the pharmacist met with each patient at treatment initiation, 5-week follow-up, and treatment completion. Monthly follow-up was arranged for patients with adherence issues. The pharmacist provided extensive counseling and monitored adherence through pill counts and refill dates. Interventions made by the pharmacist were recorded. Patients who successfully completed their treatment according to CDC standards were provided with a Completion of Therapy Certificate. Completion reports were forwarded to the Philadelphia Department of Health for tracking and statistical purposes. Between 2012 and 2017, 150 (21.8%) of 687 refugee patients screened were diagnosed with LTBI and 132 patients were referred to the pharmacist-run LTBI clinic to initiate treatment. Of those referred, 94% successfully completed LTBI treatment within the designated period and 40% required an intervention from the pharmacist to remain adherent. Interestingly, refugees from Africa tended to need more interventions compared to refugees from other countries. LTBI completion rates more than tripled after implementation of a pharmacist-run LTBI clinic. This successful model indicates that incorporating clinical pharmacists into interdisciplinary healthcare teams can enhance medication adherence and completion rates within refugee populations, leading to improved public health outcomes.

1876

INFLUENZA AND OTHER RESPIRATORY PATHOGENS AMONG HOSPITALIZED CHILDREN IN PUBLIC HOSPITALS IN PERU

Candice Romero[1], Giselle Soto[1], Isabel Bazan[2], Wilma Casanova[3], Hugo Rodriguez[3], Roger Hernandez[4], Yeny Tinoco[1], Andrea Mc Coy[1]

[1]*U.S. Naval Medical Research Unit - 6, Lima, Peru,* [2]*U.S. Naval Medical Research Unit - 6, Iquitos - Loreto, Peru,* [3]*Universidad Nacional de la Amazonia Peruana, Iquitos- Loreto, Peru,* [4]*Hospital Nacional Cayetano Heredia, Lima, Peru*

Acute Lower Respiratory Infections (ALRI) are a major cause of morbidity and mortality in young children. Influenza is an important etiology (from 9% to 17%) of Severe Acute Respiratory Infection (SARI) among hospitalized children while respiratory syncytial virus (RSV) is also responsible for substantial global morbidity and mortality. Estimates of the burden of respiratory virus associated hospitalization from low-middle income country are limited and most SARI cases in public hospitals are diagnosed syndromically. From August 2017 to July 2018 we conducted a passive surveillance of SARI among children under 5 years in 3 public hospitals in Peru. SARI case definition followed that of WHO. A nasopharyngeal swab was collected and tested by FilmArray Respiratory Panel, which detects 20 pathogens (17 virus and 3 bacteria). We enrolled 489 SARI hospitalized children and detected at least one pathogen in 84% (411/489) of the sample tested. We identified RSV in 34% (165/489), Human Rhinovirus/Enterovirus in 28% (135/489), Human Metapneumovirus in 13% (65/489), Influenza in 8% (37/489), Parainfluenza virus in 7% (33/489), Coronavirus in 6% (28/489), *Bordetella pertusis* in 2% (8/489) and Adenovirus in 1% (4/489). From April to June 2018, we observed an increase detection of RSV with an average of 72% of positivity. These results provide a description of the etiology of SARI among children less than 5 years. Collectively, the data generated from continued analysis may help to guide clinical judgment of physician in public hospitals and plan mitigation strategies and vaccination policies.

1877

TOWARD A NANOTECHNOLOGY-BASED RAPID DIAGNOSTIC TEST FOR TUBERCULOSIS SCREENING IN LOW-RESOURCE SETTINGS

Ruben Magni, Marissa Howard, Sara Sharif, Sameen Yusuf, Lance Liotta, Alessandra Luchini

George Mason University, Manassas, VA, United States

While Tuberculosis (TB) affects more than 10 million people worldwide, nearly 40% of the patients are never diagnosed or treated. TB drug resistance is also a severe world-wide issue negatively affecting treatment success rate and increasing the spread of the disease. We propose a novel nanotechnology-based integrated urine collection and rapid test platform for TB screening, which can offer high sensitivity and specificity and can be used directly in the field. We employ hydrogel nanoparticles which can selectively sequester and preserve target protein analytes from bio-fluids and enhance assay sensitivity > 100-fold. The individual donating urine for the test simply urinates in a nanoparticle-loaded collapsible collection cup, then dumps out the urine. TB derived molecules, immediately

sequestered by the nanoparticles, can be visualized by plugging a one-step self-working disposable lateral-flow immunoassay in the collection device. Alternatively, the collection device can be collapsed, sealed, stored at room temperature indefinitely or mailed to a laboratory facility for further investigation of TB markers via mass spectrometry. Detection of low-abundance TB antigens was evaluated on a set of 150 urine culture and PCR positive after nanoparticle processing and mass spectrometry analysis. Among the >100 identified proteins, we found markers of active disease as well as potential drug targets (LppX_LprAFG lipoprotein, Alanine racemase, thymidylate synthase, PadR-like family transcriptional regulator, leucyl aminopeptidase, integral membrane indolylacetylinositol arabinosyltransferase EmbB) and proteins associated with drug resistance mechanism (ABS transporter ATP-binding protein, Taurine ABC transporter permease protein, TetR family transcriptional regulator). Further sample testing and validation of the TB molecules identified will be performed in order to identify the panel of most promising markers. This information will be used to develop multiplexed lateral flow assays that can be conducted in the field, in underdeveloped global regions, for tuberculosis screening and patient management.

1878

EVALUATING THE RELATIONSHIP BETWEEN INTRODUCTION OF ACELLULAR PERTUSSIS VACCINE AND WHOOPING COUGH REEMERGENCE IN THE UNITED STATES

Jeegan U. Parikh, Miguel Reina, Ricardo Izurieta

College of Public Health, University of South Florida, Tampa, FL, United States

The incidence of *Bordetella Pertussis,* a highly contagious organism, has increased since 1996 in the United States with cyclical outbreaks in 2010, 2012 and 2014. This increased incidence coincides with the switch from the whole-cell pertussis vaccine (DTwP) to acellular pertussis vaccine (DTaP), which was partially implemented in 1992 and completely implemented in 1997. In addition, the epidemiological profile of cases has also changed in recent years, with increased incidence being observed in adolescents and vaccinated children. Pertussis cases from 1922 to 2016 was obtained from "*Nationally Notifiable Disease Surveillance System*", surveillance reports, CDC & from the WHO vaccination coverage data. A trend analysis was conducted to assess whether correlation between reported incidence and changes in vaccine policy and their implementation. There has been an increase in the number of cases due to *B. Pertussis* with changing epidemiological pattern of the disease. The number of Pertussis cases increased from 4,083 in 1992 to 17,972 in 2016 with cyclical outbreaks of 27,550 cases in 2010, 48,277 cases in 2012, 28,639 cases in 2013 and 32,971 cases in 2014.The incidence in age group of 7-10 and 11-19 increased from 1.95 and 1.99 per 100,000 cases to 14.84 and 16.31 per 100,000 cases. It is impossible to establish causation due to study limitations; however there seems to exist a temporal association between incidence and policy change/implementation. Waning immunity, which is more pronounced in DTaP (0.018 yr^{-1}) as compare to DTwP (3 x 10^{-5} yr^{-1}) and would induce susceptibility to pertussis among adolescents, selective pressure of non-vaccine bacterial strains or a combination of both factors, could explain the observed results. Further research is needed to study the immunological responses of those affected to explain disease re-emergence. Implications of pertussis vaccine policy should be considered.

1879

DEVELOPMENT OF SUSTAINABLE WATER INFRASTRUCTURE FOR SCHISTOSOMIASIS CONTROL IN ETHIOPIA

Meseret Desalegn[1], Feleke Zewge[1], Muluwork Maru[1], Laura Braun[2], Michael R. Templeton[2]

[1]*Addis Ababa University, Addis Ababa, Ethiopia,* [2]*Imperial College London, London, United Kingdom*

Schistosomiasis is a neglected tropical parasitic disease caused by several species of the genus *Schistosoma* worms affecting over 200 million people in 78 countries worldwide, with 90% of the burden currently concentrated in Africa. Many people in developing countries, including Ethiopia, do not have access to piped water and are forced to rely on untreated surface water sources for drinking, washing, and recreational activities. The repeated contact with water infested with cercariae, the human infective stage, hence increases the risk of contamination. As an additional intervention to the commonly practiced preventive and deworming chemotherapy using the drug praziquantel, provision of treated water for schistosomiasis control is considered under this study. Slow sand filtration is a sustainable option for rural water treatment because it is relatively low cost and simple to operate. This study aims to establish specific design and operational parameters for the use of slow sand filters to eliminate cerecariae from water. The filter setup consists of PVC pipes (diameter of 15.4 cm) with an endcap, plastic hose with diameter of 6 mm for water outlet, plastic diffuser and locally available river sand. The parameters chosen for optimization are grain size and depth of sand. Hence, three filters were filled with a drainage layer (15cm of gravel size 2-11.2 mm), and then a 60 cm sand layer of mixed grain size sand: 0.15-0.85 mm (filter 1), 0.425-0.6 mm sand (filter 2) and 0.15-0.425 mm sand (filter 3). Finally, a diffuser was placed 5 cm above the sand layer. Infected snails were collected from nearby endemic areas (Lake Ziway, Ethiopia) and kept in the lab under suitable condition to produce enough cercariae. Results obtained show that filters with sand depth of 60 cm reduce the number of cercariae more than 95 %. As filter depth increases, the efficiency of cercaria removal also increases. Therefore, we conclude that providing treated water with slow sand filters for various uses for rural communities can be a feasible option to control schistosomiasis.

1880

EFFECTIVITY OF WASH/MALARIA EDUCATIONAL COMMUNITY-BASED INTERVENTION IN REDUCING ANEMIA AMONG PRESCHOOL CHILDREN FROM BENGO, ANGOLA

Claudia Fançony, Ania Soares, **Miguel Brito**

CISA - Health Research Centre in Angola, Caxito, Angola

Exclusive therapeutic approaches promptly clear infections, however, in heavy contaminated environments high reinfections and incidence rates may occur limiting the sustainability of these approaches. Integrating therapeutic and preventive WASH/malaria educational strategies can simultaneously treat infections and reduce disease transmission, resulting in reduced anemia. This study, nested into a major research project investigating the efficacy of educational approaches in reducing anemia, was conducted in 2015 /2016 in CISA´s study area, located Bengo - Angola, and included 312 children randomized to the WASH/Malaria arm. Demographic, socio-economic, Water, Sanitation, Hygiene and Malaria parental practices, and parasitological and biochemical data were collected at baseline and at 12-month follow up. These moments were intercalated with 3 monthly domiciliary educational visits aiming at increasing the health literacy of caretakers and collect indicators of changed/improved behavior. T-student, McNemar Test and Chi-2 tests were used to determine variations on the primary and secondary outcomes. 202 children have completed the study. Despite that the mean hemoglobin increased levels and total anemia prevalence reduction were not statistically significant (from 11.2g/dL to 11.4g/dL, p=0.21 and from 38.6 to 33.2%, p= 0.21 respectively), a 8.4% reduction in the Iron Deficiency Anemia prevalence was observed (p=0.01). A significant increase in the prevalence of infections was observed, mainly *P. falciparum* (1.5 to 6.4%), *G.lamblia* (9.2 to 20.6%) and *A. lumbricoides* (6.1 to 26.7%). 50.5% of children's hands and 43.4% of the household's observations scored 5-6 points for the cleaning state of the nails and for the cleaning state of latrines, respectively. 81.2% of the households scored 0-1 point regarding having water in the latrine to wash hands, 53.3%, 60.1% and 78.5% were observed to have garbage, loose domestic animals or puddles in the home surroundings. Neither the reduction of IDA or the increased infection prevalence were found to be statistically associated with the educational process indicators collected here.

1881

IMPACT OF WATER, SANITATION AND HYGIENE ON COMMUNITY-LEVEL INTESTINAL PARASITES IN ETHIOPIA: THE GESHIYARO PROJECT

Anna E. Phillips[1], Kalkidan Mekete[2], Alison Ower[1], Ebba Abate[2], Julia Dunn[1], Heven Sime[2], Gemechu Tadesse[2], Roy Malcolm Anderson[1]

[1]*Imperial College, London, United Kingdom,* [2]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia*

Improving water, sanitation, and hygiene (WaSH) is increasingly seen as a key component of a sustainable soil-transmitted helminth (STH) and schistosomiasis control strategy due to their lifecycles. Current STH and schistosomiasis control strategies rely primarily on preventive chemotherapy, which is effective at killing adult worms in the human body but does not prevent re-infection after treatment. Few large-scale studies have provided WaSH facilities and quantified the relationship between WaSH, treatment and the subsequent impact on transmission of these intestinal parasites. The Geshiyaro project will test the feasibility of interrupting transmission of STH and schistosomiasis in the Wolayita zone of south-western Ethiopia. The monitoring model is quasi-experimental with three arms of interventions (i) expanded community-wide MDA plus WaSH and behaviour change communication (ii) expanded community-wide MDA only and (iii) annual school-based MDA (National STH/SCH control program). In October and November 2018, a population census was conducted collecting household and community WaSH data where individuals and households were registered using biometric fingerprint and barcoded identification cards. Scores were then constructed reflecting exposure to both STH and schistosomiasis, according to adequacy of water access, sanitation facilities and hygiene. Under the assumption that WaSH will reduce parasite transmission, the impact of WaSH was evaluated through epidemiological assessments to assess whether people with better access to WaSH were less likely to be infected. Baseline parasitological mapping was conducted in December 2018 across all age groups (pre-school-aged children (SAC), SAC, adolescents, and adults) and individual results correlated with WaSH data using the biometric and ID cards. Overall, WaSH and parasitology data were available for 11,086 individuals across 32 communities. Our findings demonstrated that improving WaSH may reduce transmission of these parasites. However, different elements of WaSH interventions appear to have different effects on specific parasites.

1882

CHLORINE TABLETS FOR EMERGENCY HOUSEHOLD WATER TREATMENT: QUALITATIVE ASSESSMENT AND DEVELOPMENT OF TABLET SELECTION GUIDELINES

Marlene Wolfe, Brittany Mitro, Mateo Galeano, Mustafa Sikder, Karin Gallandat, Daniele Lantagne

Tufts University, Medford, MA, United States

Chlorine tablets are commonly distributed in emergencies. However, confirmed use ranges widely (from 7-87%), raising concerns about effectiveness. Currently, there is no process for selecting a technically and socially acceptable tablet. We conducted a study in two phases: 1) a qualitative assessment of barriers and facilitators of tablet distribution, and 2) development and field trialing of guidelines to select tablets for distribution. In phase 1, we completed key informant interviews (KIIs) on tablet distribution in emergencies and chlorine taste and odor acceptance and rejection, and a literature review on taste and odor. In phase 2, we convened a working group to develop a process for selecting a context-appropriate chlorine tablet(s) which was then field tested in Cox's Bazar, Bangladesh. In phase 1, we found: 1) chlorine tablets are regarded as an effective and appropriate intervention in emergencies, 2) dosing confusion and taste and odor rejection are perceived as the main problems limiting effectiveness, and 3) the primary solutions suggested were social and behavioral. In phase 2, a guidance document was developed including sections on: 1) gathering key information, and 2) undertaking tablet selection. Tools are provided for assessing: water quality parameters, chlorine demand, and taste and odor acceptance. After information is gathered, tablets that maintain appropriate FCR throughout storage are pre-selected; a final choice is made after consideration of qualitative parameters such as taste and odor preferences. In a field trial in Cox's Bazar, respondents identified needing to chlorinate 10 L of water for 12 hours, and taste and odor rejection was high after 1.0 mg/L FCR. A 17 mg tablet was selected, resulting in a measured FCR of 0.6-0.8 mg/L for 24 hrs. We recommend that social and behavioral scientists are integrated into tablet programming, more research is conducted on taste and odor rejection, and improved guidance is used to select and promote tablets. Our trial suggests that a structured process to select a context-appropriate tablet can alleviate confusion and direct the use of tablets that are context-appropriate.

1883

SMALL INTESTINE BACTERIAL OVERGROWTH IS ASSOCIATED WITH LINEAR GROWTH DELAY IN A LONGITUDINAL ANALYSIS OF BANGLADESHI CHILDREN

Jeffrey Donowitz[1], Zhen Pu[2], Ye Lin[2], Masud Alam[3], Mamun Kabir[3], Tahsin Ferdous[3], Ayesha Zerin[3], Uma Nayak[2], Jennie Z. Ma[2], Rashidul Haque[3], William A. Petri[2]

[1]*Virginia Commonwealth University, Richmond, VA, United States,* [2]*University of Virginia, Charlottesville, VA, United States,* [3]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh*

Introduction: Small intestine bacterial overgrowth (SIBO) is present in up to 30% of children living in low-income countries and has been associated with lower socioeconomic status, poor sanitation, enteric inflammation, and linear growth delays in cross-sectional studies. Methods: We conducted a birth cohort study to assess the effect of SIBO on growth and neurodevelopment. Healthy children were enrolled within the first 7 days of life and followed for two years. SIBO was measured via glucose-hydrogen breath test at 18, 52, 78, and 104 weeks. Socioeconomic and maternal data were collected at enrollment. Early life biomarkers previously associated with growth or neurodevelopmental delays were collected in the serum or stool at 18 weeks. Neurodevelopment was assessed at two years using a culturally adapted version of the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). Area under the hydrogen curve (AUC) was calculated and standardized for time for each glucose-hydrogen breath test and averaged for the four tests. Linear regressions with stepwise selection were performed for length-for-age Z score (LAZ) at two years, and the components of the Bayley-III (cognitive, language, and motor) as outcomes. Co-variates included average SIBO AUC, LAZ at enrollment, sex, mother's weight, maternal education, income, water treatment, improved septic, IL-4 at 18 weeks, fecal myeloperoxidase at 18 weeks, and fecal Reg 1B at 18 weeks. Results: 11.1% of children had a positive breath test (an increase in breath hydrogen > 12 ppm over their baseline) at 18 weeks. 36.4% were positive at 52 weeks, 44.3% at 78 weeks, and 34.5% at 104 weeks. SIBO AUC ranged from 3.0 to 50.0 ppm/min. A one unit increase in average SIBO AUC was associated a 0.1 SD decrease in LAZ at 2 years of age after adjusting for LAZ at enrollment, mother's weight, and maternal education. SIBO was not significantly associated with the three Bayley-III components. Conclusions: SIBO in the first two years of life is associated with linear growth delay but not neurodevelopmental outcomes in young Bangladeshi children.

1884

EVALUATING FETCHING TIME, WATER USAGE AND DIARRHEA PREVALENCE IN RURAL PIPED WATER SYSTEMS IN SOUTHERN ZAMBIA

James Winter[1], Jennifer Davis[2]

[1]*Stanford University, Palo Alto, CA, United States,* [2]*Stanford University, Stanford, CA, United States*

Evaluating fetching time and water usage in rural piped water systems in southern Zambia NGO-led efforts to install piped water systems in rural villages is accelerating in Zambia. This project measures the benefits of

piped water systems on two villages in southern Zambia and two matched control villages in the same district. The piped water systems consist of taps located in shared or private yards of households. We conducted surveys of 150 total households and measured the concentration of fecal indicator bacteria in source and stored water and the hands of respondents. We observed the size and composition of respondents' gardens as a measure of economic utilization of water and, for a subset of respondents, we used GPS tracking devices to measure their location and walking speed every 5 seconds for 16-20 hours. We have collected two rounds of data in May and September of 2018. I will collect endline data in May and June of 2019. To date, we have measured significant decreases in time spent fetching water for men, women and girls in the household and significant increases in total water use per capita. We hypothesize that extended exposure to the piped water will significantly improve garden utilization and crop diversity. We anticipate presenting information on results from GPS tracking data to estimate path-based walk time to water sources, walking v*elocit*y, and time spent in and outside the home.

1885

SANITATION, PATHOGEN EXPOSURE AND CHILD OUTCOMES IN ADDIS ABABA, ETHIOPIA

Leon Espira[1], Brook Gesesse[2], Kaleab Baye[2], Andrew Jones[1], Nancy G. Love[1], Joseph N. Eisenberg[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Addis Ababa University, Addis Ababa, Ethiopia*

The provision of clean water and access to sanitation is central to the outcomes set in 2015 by the Sustainable Development Goals (SDGs). The reason for the prominence of water and sanitation in the SDGs is that 2.4 billion people still lack access to basic water and sanitation facilities, placing them at higher risk of diarrheal diseases. Diarrheal diseases remain one of the leading causes of death for children under five years of age. However, current approaches to prevent diarrheal disease and chronic sequele focus primarily on increasing access to sanitation facilities and less on minimizing transmission of enteric or waterborne pathogens. Given that enteric pathogens have multiple and often interdependent transmission pathways, accurate measurement of pathogen loads and types is critical to fully understand the impact of sanitation access. We conducted a study in Addis Ababa, Ethiopia in which 712 households were surveyed across 12 informal settlements with infants aged 6-23 months. Along with data on demographic, socioeconomic, and sanitation variables, we collected 136 stool samples. Using droplet digital PCR (ddPCR) we screened the stool for 16 pathogens and conducted a genomic analysis of the stool samples to determine microbiome composition. The results of this study will enable us to quantify the direct and indirect effects of sanitation by identifying thresholds at which diarrheal risk and stool pathogen profiles change. This will provide a mechanistic understanding of how sanitation interventions work, enabling the delivery of more effective solutions.

1886

ANALYSIS OF ENVIRONMENTAL PATTERNS AND LEPROSY IN MINAS GERAIS, BRAZIL USING SPATIAL AND TEMPORAL STATISTICS

Shaiana Oliveira-Streiff[1], Uriel Kitron[2], José A. Ferreira[3], Maria A. de Faria Grossi[3], Adauto C. Pugedo[4], Maria do Carmo R. de Miranda[4], Jessica K. Fairley[5]

[1]*Emory Rollins School of Public Health, Atlanta, GA, United States,* [2]*Emory University, Atlanta, GA, United States,* [3]*Faculdade da Saude e Ecologia Humana, Vespasiano, Brazil,* [4]*Secretaria de Estado da Saúde de Minas Gerais, Belo Horizonte, Brazil,* [5]*Emory University School of Medicine, Atlanta, GA, United States*

Brazil has the second highest number of new leprosy cases reported annually with the state of Minas Gerais (MG) having pockets of highly endemic leprosy. Transmission remains only partially understood, and in addition to a respiratory route, transmission may also be related to environmental conditions. Potentially viable *Mycobacterium leprae* has been found in water, soil, and armadillos. To investigate the role of the environment on transmission of leprosy, we identified spatial clusters of cases and likely associations between leprosy incidence and environmental predictors, specifically, (1) elevation, (2) normalized difference vegetation index (NDVI), (3) temperature, and (4) precipitation through a cross-sectional study using the Brazilian Notifiable Disease Surveillance System (SINAN) data from 853 municipalities in MG from 2009 to 2013. Multivariable Poisson regression models were used to estimate the rate ratio (or incidence density ratio (IDR)) to compare incidence across municipalities. We then used spatial statistics (global autocorrelation, local indicator of spatial autocorrelation [LISA], Getis Ord Gi(d)*) to analyze clustering of leprosy cases and incidence. Overall incidence decreased from 8.76 per 100,000 in 2009 to 5.04/100,000 in 2013 with the average municipality leprosy incidence at 7.11 per 100,000 annually. The local autocorrelation analysis identified 51 high-high clusters of leprosy incidence in the northeast and west of Minas Gerais. Temperature was positively correlated with leprosy incidence (IDR=1.41, [CI: 1.37, 1.46)], p= <0.0001), while precipitation (IDR=0.96, [CI: 0.94, 0.99], p= <0.0033), NDVI (IDR= 0.94, [CI:0.92, 0.96], p= <0.0001), and elevation (IDR=0.53, [CI:0.52, 0.55], p= <0.0001) were negatively associated with leprosy incidence. The associations between leprosy and environmental predictors, especially higher temperatures, indicate that the role of the environment and geographical conditions need to be considered in the context of disease transmission and viability of *M. leprae* in the environment, especially in the era of global warming and climate change.

1887

BEHAVIORAL AND ENVIRONMENTAL RISK FACTORS ASSOCIATED WITH NEONATAL SEPSIS IN UGANDAN HEALTHCARE FACILITIES

Habib Yakubu[1], Richard Mugambe[2], Constance Bwire[2], Joanne McGriff[1], Christine Moe[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Makerere University, Kampala, Uganda*

Neonate exposure to pathogens in the healthcare facility (HCF) environment can cause healthcare associated infections including sepsis. Sepsis is responsible for 15%-20% of neonatal deaths globally and about 30%-40% occur at the time of birth. Low resource settings are disproportionally affected by the burden of neonatal deaths. In Uganda, newborn deaths constitute 38% of all infant mortality, and an estimated 31% of newborn deaths are attributed to sepsis. However, little evidence exists on behavioral and environmental risk factors for sepsis. The objective of this study is to assess environmental and behavioral risk factors associated with neonatal sepsis in labor & delivery (LD) and post-natal (PN) wards. Two public HCFs in Kampala, Uganda with contrasting water, sanitation and hygiene (WASH) conditions and infrastructure were purposively selected and classified as "good WASH" and "poor WASH" HCF. Structured and unstructured observations were conducted to understand what surfaces and equipment neonates come into contact with and the frequency of contact to inform environmental sampling and identify potential pathogen transmission pathways. An observation checklist was developed based on literature and unstructured observations within each HCF. Structured observations were subsequently conducted using the checklist for 3 hours each in the morning, afternoon and evening for 2 weeks by trained enumerators. In the PN wards, bedsheets (26%) and walls (21%) were the most frequently touched surfaces in the good WASH HCF. In the poor WASH HCF, the most frequently touched surfaces were mobile phones (10%) and delivery kits (9%). In the LD wards, fetal scopes (16%) and bedrails (13%) were the most frequently touched in the good WASH HCF whilst sink faucets (7%) and bed linings (6%) were the most frequently touched in the poor WASH HCF. This information was used to target environmental sampling to determine the pathways of exposure that may pose the greatest risk to neonates. Analysis of the sampling results in conjunction with the behaviors observed will be useful in planning evidence-based interventions in HCFs to reduce pathogen transmission.

1888

INVESTIGATING EXPOSURE TO HEAVY METALS AS A POTENTIAL ETIOLOGY OF MESOAMERICAN NEPHROPATHY, AN UNEXPLAINED EPIDEMIC KIDNEY DISEASE IN LATIN AMERICA

Rebecca S. Fischer[1], Wayne Sanderson[2], Max Costa[3], Kristy O. Murray[4]

[1]*Texas A&M University Health Science Center, College Station, TX, United States,* [2]*University of Kentucky, Lexington, KY, United States,* [3]*New York University, Environmental Medicine, Biochemistry and Molecular Pharmacology, Tuxedo, NY, United States,* [4]*Baylor College of Medicine, Tropical Medicine and Human Immunobiology, Houston, TX, United States*

Mesoamerican Nephropathy (MeN) is a medical mystery affecting working-age adults in rural agricultural communities throughout the Pacific lowlands of Central America. Premature renal mortality there tops 50,000. MeN presents as an aggressive interstitial nephritis, sometimes initiated by acute kidney injury with systemic inflammation and anemia. Clinical and pathologic evidence suggests a toxic, inflammatory etiology, Several heavy metals are nephrotoxic, causing interstitial nephritis as well as disrupted immune and inflammatory processes. To measure exposure to metals and consider metal toxicity in the genesis of MeN, toenail samples were collected from 18 agriculture workers with acute MeN (median age 26.9 yrs), detected through surveillance for AKI and CKD in Nicaragua, and 36 healthy controls (median age 29.5 yrs) without history of renal impairment, recruited at health screenings from the same population. Clinical data was recorded, and nails were aseptically collected 3-6 months later. Zinc, Aluminum, Iron, Copper, Manganese. Selenium, Nickel, Cobalt, Mercury, Vanadium, Chromium, Arsenic, Lead, Cadmium, and Uranium were measured by ICPMS. Nickel (Ni) was the only exposure common to all cases but only 58% of controls ($p=0.001$), and levels were higher in cases (1.55mg/kg, 0.17-42.65; $p=0.003$). Higher levels of Ni correlated ($p<0.05$) positively with serum creatinine, leukocytosis, and neutrophilia, and inversely with hemoglobin/hematocrit and glomerular filtration rate. Clinically, Ni-exposed individuals mirrored what is observed in MeN. Clinical events in Ni toxicity can mirror what is observed during acute MeN: Ni accumulates in kidneys, causing interstitial nephritis with focal inflammatory damage at the corticomedullary junction, and leukocytosis, and anemia are common. Little is known about geogenic or anthropogenic sources of nickel and other metals posing hazards to human and animal health in this and other tropical regions. Human exposure and environmental sources should be thoroughly explored to assess its role in MeN, whether through low-level chronic exposure or acute exposure to toxic levels.

1889

ENVIRONMENTAL CONTAMINATION WITH INTESTINAL PARASITES IN THE SOUTHERN USA

Christine C. Blackburn[1], Macey Lively[1], Catherine Flowers[2], Nicholas L. Herrera[3], Maria Jose Villar Mondragon[3], Rojelio Mejia[3]

[1]*Texas A and M, College Station, TX, United States,* [2]*Center for Rural Enterprise and Environmental Justice, Montgomery, AL, United States,* [3]*Baylor College of Medicine, Houston, TX, United States*

A growing body of evidence shows that intestinal parasites are present in the United States. Communities within the United States that lack proper infrastructure, sanitation, and health access are at risk for parasite infection. The goals of this study were to determine the prevalence of environmental contamination of intestinal parasites in the United States. Soil samples were collected from Louisiana, Mississippi, Alabama, South Carolina, and Texas. Communities were selected based on median household income, rural status, population size, the percentage of residents living in poverty, and having poor sanitation. One hundred environmental samples were collected from each study location for a total of 500 samples. Each sample contained approximately 50 grams of soil or 2 liters of wastewater, which were collected from private residences (60%) and public parks (40%). Both soil and wastewater were processed by a novel concentration and DNA extraction methods using bead beating. Multi-parallel quantitative real-time PCR (qPCR) was performed for *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Taenia solium, Toxocara canis/cati, Trichuris trichiura, Blastocystis* species*, Cryptosporidium* species*, Entamoeba histolytica,* and *Giardia lamblia*. Preliminary results of all five states were 25% soil/water samples were positive for *Blastocystis* species, *Entamoeba histolytica* (2.9%), *Giardia lamblia* (2.9%), and *Toxocara canis* or *cati* (7.4%). In Mississippi, there were 30% positive for *Blastocystis* species*, Toxocara cati* (20%), and *Giardia lamblia* (20%). In Alabama, there were 36.8% positive for *Blastocystis* species*, Toxocara cati* and *canis* (2.6% and 5.2%, total 7.9%), and *Entamoeba histolytica* (5.3%). These environmental studies align with previous human serology and stool findings and provide additional evidence that parasites contaminate environmental samples throughout the Southern United States. Future work will include mapping and modeling the qPCR data to location and environmental conditions to provide remote sensing analysis of predicted parasite exposure in the USA.

1890

USEFULNESS OF LATERAL FLOW ASSAYS FOR RAPID ON-SITE TESTING FOR DETECTION OF BACTERIAL, VIRAL AND TOXIN AGENTS IN ENVIRONMENTAL SAMPLES

Kodumudi S. Venkateswaran, Thomas O'Brien, Neeraja Venkateswaran

Tetracore Inc., Rockville, MD, United States

Environmental samples suspected to have biological agents with potential of harming human, animal and plant health need to be detected quickly to take timely countermeasures to reduce the deleterious effects. Such biohazardous agents can be bacteria, virus or toxins. Various methods using specific immunoreagents are used for detection of harmful biothreat agents or their components directly from the suspected environmental samples. Molecular methods for testing for nucleic acids specific to the agents are also being used for very sensitive detection of various bacterial and viral biothreat agents. Lateral flow immunoassay (LFA) using the principle of immunochromatography is a simple, low cost and rapid method for onsite testing of biothreat agents. Singleplex LFA developed for detection *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, ricin, abrin, staphylococcal enterotoxin B, botulinum neurotoxins and orthopox virus. These LFAs were evaluated to measure detection limit, cross reactivity and interference by environmental sample matrices. Multiplex assays for either four bacterial agents or four toxin agents were also tested and evaluated for their performance. Multiplex assay needs only fraction of sample needed compared to the volume needed for singleplex assays. LFA results can be seen visually without the need for any instruments. However, an optional field portable LFA reader has also been developed for objective visualization of the LFA result and to digitize data for easy storage and effective sharing of the results. Data will be presented summarizing all the testing done. Performance evaluation of the singleplex and multiplex assays were done using well defined inclusivity, exclusivity, near neighbor, informational and environmental organism panels for each biological agent. Assessment of LFAs were performed by statistical analysis and calculation of sensitivity, specificity and accuracy of visual results and reader results. Our study showed LFA tests as reliable tool for rapid, field portable detection of various biothreat agents.

1891

APACT TRIAL: MULTICENTER THERAPEUTIC EFFICACY ASSESSMENT OF PYRONARIDINE-ARTESUNATE (PYRAMAX®) AND NEW DRUG COMBINATIONS WITH ATOVAQUONE-PROGUANIL FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CAMBODIA

Mariusz Wojnarski[1], Chanthap Lon[1], Somethy Sok[2], Darapiseth Sea[1], Krisada Jongsakul[1], Michele Spring[1], Kimberly A. Edgel[3], Nillawan Buathong[1], Sabaithip Sriwichai[1], Soklyda Chann[4], Chandara Sok[4], Nichapat Uthaimonkol[1], Tyler Warkentien[3], Catherine Berjohn[3], Panita Gosi[1], Nonlawat Boonyalai[1], Piyaporn Saingam[1], Chaiyaporn Chaisatit[1], Pattaraporn Vanachayangkul[1], Bertha Nyagaya-Wojnarski[1], Kittijarankon Phontham[1], Worachet Kuntawunginn[1], Jessica Lin[5], Shannon Takala-Harrison[6], Dennis Faix[3], Prom Satharath[2], Jurgen Venitz[7], Pascal Ringwald[8], Rekol Huy[9], Dysoley Lek[9], Philip Smith[1], John S. Brooks[3], Nicholas J. Martin[1], Mark Fukuda[1], Norman Waters[1]

[1]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*Ministry of National Defense, Department of Health, Phnom Penh, Cambodia,* [3]*Naval Medical Research Unit 2, Phnom Penh, Cambodia,* [4]*Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia,* [5]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [6]*University of Maryland School of Medicine, Baltimore, MD, United States,* [7]*Virginia Commonwealth University, Richmond, VA, United States,* [8]*World Health Organization, Geneva, Switzerland,* [9]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*

Multi-drug resistance (MDR) threatens malaria control in Southeast Asia, underscoring the need for identification of more effective drug combinations for *Plasmodium falciparum (P.f)*. The APACT trial was launched in October 2018 in response to the growing threat that malaria may become untreatable by available drug combinations. The main objective is to assess the therapeutic efficacy of the latest approved artemisinin combination therapy, artesunate-pyronaridine (ASPY), and novel multi-drug combinations, atovaquone-proguanil+ASPY (AP+ASPY) and atovaquone-proguanil+artesunate-mefloquine (AP+ASMQ) for *P.f* malaria. The study is being conducted at 2 sites in Cambodia, where study drugs are administered by directly observed therapy, with active follow-up for up to 8 weeks, after a minimum of 3 days inpatient treatment. Volunteers are randomized, open-label, to one of three treatment arms, ASPY, AP+ASPY, and AP+ASMQ. The target sample size is 252. Among the first 24 volunteers who completed 6-8 weeks follow up to date, we have observed 100% adequate clinical parasitological response rate against *P.f* malaria among all 3 treatment arms. Four volunteers (17%) had *Plasmodium vivax* recurrence consistent with the high rate of suspected liver stage *P.vivax* co-infections in this area. Observed transient mild liver enzyme elevations are consistent with prior reports for the study drugs and none of the volunteers met Hy's Law criteria for liver toxicity. No serious adverse events were reported for the drug combinations thus far. Tolerability was high with 100% volunteers reporting that combination treatments were simple to take. Most volunteers (86%) reported that taking multiple tablets was acceptable and 76% were comfortable with taking the same drug regimen the next time they have malaria, while 19% preferred a shorter treatment course. Information on the efficacy, safety and tolerability, and community perceptions about the new drug combinations tested in this trial will be forthcoming, in our effort to respond to the growing threat in Southeast Asia of multidrug resistant malaria that may become untreatable with standard drug regimens.

1892

PYRONARIDINE-ARTESUNATE (PYRAMAX®) FOR THE TREATMENT OF *PLASMODIUM VIVAX* AND DIHYDROARTEMISININ-PIPERAQUINE RESISTANT FALCIPARUM MALARIA IN DAK NONG PROVINCE IN THE HIGHLANDS OF VIETNAM

Nguyen D. Manh[1], Marina Chavchich[2], Nguyen N. San[3], Huynh H. Quang[4], Nguyen V. Thanh[1], Nguyen T. Van[1], Geoffrey W. Birrell[2], Kimberly A. Edgel[5], Nicholas W. Martin[5], Michael D. Edstein[2]

[1]*Military Institute of Preventive Medicine, Hanoi, Vietnam,* [2]*Australian Defence Force Malaria and Infectious Disease Institute, Brisbane, Australia,* [3]*Hanoi Medical University, Hanoi, Vietnam,* [4]*Institute of Malariology, Parasitology and Entomology, Quy Nhon, Vietnam,* [5]*U.S. Naval Medical Research Unit - 2, Singapore, Singapore*

Dihydroartemisinin-piperaquine (DHA-PPQ), Vietnam's first-line treatment drug for uncomplicated *Plasmodium falciparum,* is failing in Binh Phuoc province at an alarming rate of >50%. To contain the spread of DHA-PPQ resistance from south into central Vietnam, there is an urgent need to assess the efficacy of other artemisinin combination therapies. In the present study, we evaluated the therapeutic efficacy of pyronaridine-artesunate (Pyramax®) in treating uncomplicated *P. falciparum* and *P. vivax* malaria in Cu Jut and Dak Mil districts of Dak Nong province. In this open label study, a total of 93 patients have been recruited so far. Of these, 36 participants (39%) were infected with *P. falciparum,* 56 (60%) with *P. vivax* and 1 (1%) had a mixed *P. falciparum/P. vivax* infection. All patients received a 3-day course of Pyramax® with a 42-day follow-up period. Patients with vivax malaria or a mixed infection were treated with primaquine (0.25 mg/kg daily for 14 days). The study is ongoing with a sample size target of 50 evaluable cases for both falciparum and vivax malaria. Pyramax® rapidly cleared vivax malaria within 24 hours of starting treatment in 93% (52/56) of participants, with only 2% (1 in 48 evaluable patients) having a recurrence of malaria. However, of the 35 patients with *P. falciparum,* who completed Pyramax® treatment, 40% (14/35) had parasites present on day 3. Recrudescent parasites were detected in 2 participants on days 31 and 39 after starting treatment. Notably, 94% (33/35) of clinical isolates carried C580Y mutation in the *Kelch 13* gene, implicated in artemisinin resistance. Furthermore, 63% (22/35) of the isolates had multiple copies of plasmepsins 2-3 and 86% (30/35) of parasites had mutation E415G in the *Exo415* gene, both are molecular markers for PPQ resistance. *In vitro* evaluation of *P. falciparum* isolates revealed reduced susceptibility to DHA and PPQ by ring stage survival and PPQ survival assays in 3 out of 5 isolates tested so far, including that from a participant who experienced a recrudescence. The role of Pyramax® for treatment of vivax and artemisinin-piperaquine resistant falciparum malaria in Vietnam will be discussed.

1893

EMERGENCE OF ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM* WITH *KELCH13* C580Y MUTATIONS IN PAPUA NEW GUINEA

Olivo Miotto[1], Makoto Sekihara[2], Shin-Ichiro Tachibana[2], Masato Yamauchi[2], Mie Ikeda[2], Toshiyuki Mori[2], Makoto Hirai[2], Richard D. Pearson[3], Roberto Amato[4], Sonia Morgado Gonçalves[4], Rintis Noviyanti[5], Jutta Marfurt[6], Sarah Auburn[6], Ric Price[6], Ivo Mueller[7], Alyssa Barry[7], Moses Laman[8], Livingstone Tavul[8], Manuel Hetzel[9], Pascal Ringwald[10], Jun Ohashi[11], Francis Hombhanje[12], Dominic P. Kwiatkowski[4], Toshihiro Mita[2]

[1]*MORU - University of Oxford, Bangkok, Thailand,* [2]*Juntendo University Faculty of Medicine, Tokyo, Japan,* [3]*University of Oxford, Oxford, United Kingdom,* [4]*Wellcome Sanger Institute, Hinxton, United Kingdom,* [5]*Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [6]*Menzies School of Health Research, Darwin, Australia,* [7]*Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia,* [8]*Papua New Guinea Institute of Medical Research, Port Moresby, Papua New Guinea,* [9]*Swiss TPH,*

Basel, Switzerland, [10]*World Health Organization, Geneva, Switzerland,* [11]*University of Tokyo, Tokyo, Japan,* [12]*Divine Word University, Madang, Papua New Guinea*

The rapid and aggressive spread of artemisinin-resistant *Plasmodium falciparum* carrying the *kelch13* C580Y mutation is a growing threat to malaria elimination in Southeast Asia, but there is no evidence of their spread to other regions. We conducted cross-sectional surveys in 2016 and 2017 at two clinics in Wewak, Papua New Guinea (PNG) where, among 239 genotyped clinical samples, we identified three infections caused by C580Y mutants. Ring-stage survival assays (RSA) for artemisinin showed that these mutants exhibited the highest survival rate (6.8%) among the parasites surveyed. Analyses of *kelch13* flanking regions by microsatellite markers did not suggest a common origin of mutants in PNG and Cambodia. Comparative analyses based on deep sequencing data from 389 clinical samples from PNG, Papua Indonesia and Western Cambodia supported an independent origin of the Wewak C580Y mutation, showing that the mutants possess several distinctive genetic features. Identity by descent (IBD) showed that multiple portions of the mutants' genomes share a common origin with parasites found in Papua Indonesia. Within these shared haplotypes, a number of alleles differentiate Wewak C580Y mutants from other PNG samples, including several within genes previously associated with drug resistance, such as *mdr1*, *ferredoxin*, *atg18* and *pnp*. These findings suggest that *P. falciparum* lineages are spreading across New Guinea, gradually acquiring a complex ensemble of variants, including *kelch13* C580Y, which may affect their drug sensitivity. This worrying development reinforces the need for increased genetic surveillance of the evolving parasite populations on the island, to contain the spread of resistance.

1894

A LONG-LASTING PROTECTION OF CHEMOPROPHYLAXIS IMPLANT AGAINST MALARIAL INFECTION

Hongxing Wang[1], Shuanghong Liang[1], Yinzhou Fan[1], Zhenping Huang[2], Xiaoyi Huang[1], Siting Zhao[3], Li Qin[2], Xiaoping Chen[3]

[1]*Bluelight Pharmatech. Co. LTD, Guangzhou, China,* [2]*CAS Lamvac Biotech Co., Guangzhou, China,* [3]*Guangzhou GIBH CAS, Guangzhou, China*

Liver stage *Plasmodium* control is essential to decrease new malaria infection and to prevent relapsing malaria. One of the approaches is to parenterally administer an implant to provide a slow and long-acting release of antimalarial agent for causal prophylaxis. In the recent study, we have created a series of formulations for intramuscular or subcutaneous depot of decoquinate (DQ) by using hot melt extrusion (HME) or co-solvency methods. Compared to classic co-solvency method using synthetic polymers typical for controlled drug release, the implants prepared by the HME technique at doses of DQ 50-200 mg/kg provide much better efficacy in preventing *Plasmodium* infection in mice. A single intramuscular injection of an optimized, HME made, cholesterol-based composition, provides a DQ release at a slow rate and maintains a sufficient level of the desired drug active in mice for preventing *Plasmodium* infection for up to 4 months with a single inoculation of *Plasmodium* parasite sporozoites (50,000) by tail vein. To reflect real-world endemic exposure, repeated inoculations of the same number of parasites (sporozoites) were given each time at an interval of each month to mice with initial one time intramuscular injection of the implant. Surprisingly in this case, the mice remained free of the disease for as long as 7 months. The implants made by HME also demonstrated far better protection for the same period of time than the simple physical mixing of the same formulation components, indicating that the mechanic process and the validated parameters for the HME play an important role in making desired implants. Our innovative, safe and controlled-released intramuscular implants utilize naturally available materials that are easily metabolized or degraded or excreted by animals or humans. The implant in the aqueous suspension (saline) for intramuscular placement results in limited pain or distress and the surgical removal rarely is needed. Furthermore, the HME process is environmentally clean, and the product quality and the massive production can also be quarantined. More studies using primates will be needed to further evaluate the implants.

1895

TRIPLE ARTEMISININ COMBINATION THERAPIES: A NEW PARADIGM FOR THE TREATMENT OF MALARIA?

Chanaki Amaratunga[1], Mehul Dhorda[1], Rob van der Pluijm[1], Joel Tarning[1], Ricardo Aguas[1], Maciej F. Boni[2], Phaik Yeong Cheah[1], Paulina Tindana[3], Freek de Haan[4], Wouter Boon[4], Ellen H. Moors[4], Katherine Plewes[1], Rupam Tripura[1], Nick P. Day[1], Nick J. White[1], Arjen M. Dondorp[1]

[1]*Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [2]*Center for Infectious Disease Dynamics, Department of Biology, Pennsylvania State University, University Park, PA, United States,* [3]*School of Public Health, College of Health Sciences, University of Ghana, Accra, Ghana,* [4]*Innovation Studies, Copernicus Institute of Sustainable Development, Utrecht University, Utrecht, Netherlands*

Artemisinin Combination Therapies (ACT) are first-line treatments for malaria. The Tracking Resistance to Artemisinin Collaboration (TRAC) mapped the spread of artemisinin resistance in 10 countries in Asia (7) and Africa (3) and described artemisinin resistance in Southeast Asia (SEA). In Cambodia, Thailand, and Vietnam, artemisinin resistance is compounded by partner drug resistance resulting in dihydroartemisinin-piperaquine (DHA-PPQ) treatment failure rates of >60%. The TRACII study conducted in 8 countries in Asia (7) and Africa (1) explored the concept of combining an ACT with a third antimalarial drug and assessed efficacy, safety and tolerability of two Triple ACT (TACT): DHA-PPQ+mefloquine (DHA-PPQ+MQ) and artemether-lumefantrine+amodiaquine (AL+AQ). Both TACT were safe, well tolerated and highly efficacious against ACT-resistant parasites in SEA. TACT could become standard treatment for malaria worldwide as part of strategies to prevent or delay emergence of drug resistant malaria in regions outside of SEA. A new project titled Development of Triple Artemisinin Combination Therapies (DeTACT) will take a multifaceted approach to assess potential benefits and disadvantages of deploying TACT as first-line antimalarial treatments. In a randomized, controlled, non-inferiority trial, we will compare safety, tolerability and efficacy of dose-optimised artesunate-PPQ+MQ and AL+AQ TACT in blistered co-packages versus ACT+placebo in 13 countries in Asia (5) and Africa (8). The potential of TACT to delay emergence and spread of antimalarial resistance and cost-effectiveness of deploying TACT will be assessed through mathematical modelling. We will address ethical issues such as balancing individual and community disadvantages versus public benefits of introducing TACT in regions where ACT are still efficacious. Market and demand related issues involved in development, implementation and deployment of TACT will be studied to guide future introduction in the global marketplace and communicated to stakeholders via a strategic engagement plan. Development of the project and progress to date will be presented.

1896

EFFECT OF ARTEMISININ ON THE SEXUAL CONVERSION OF *PLASMODIUM FALCIPARUM*

Harvie P. Portugaliza[1], Anna Rosanas-Urgell[2], Alfred Cortés[1]

[1]*ISGlobal - University of Barcelona, Barcelona, Spain,* [2]*Institute of Tropical Medicine, Antwerp, Belgium*

Artemisinin-based combination therapies (ACTs) remain the frontline treatment for malaria. However, ACTs show limited efficacy against the transmissible sexual forms of the parasite, termed gametocytes, and some reports suggest that drug treatment increases sexual conversion rates. Here we combined *in vitro* and field-based approaches to determine whether artemisinin affects the production of *Plasmodium falciparum* sexual stages. We collected serial blood samples from 32 Vietnamese patients with uncomplicated falciparum malaria enrolled in an artemisinin clinical

trial, and quantified by RT-qPCR the relative transcript levels of the sexual commitment marker *pfap2-g* and other early gametocyte markers every 12h from day 0 to 3. We observed activation of *pfap2-g* (>2 fold) after the first dose in almost 50% of the patients, which was associated with subsequent bloodstream release of mature gametocytes (*pfs25* transcripts peak) on day 7 or 14 (p=0.026). Additionally, fast-clearing infections were associated with *pfap2-g* induction (OR 8.9; 95%CI 1.3-63; p=0.029). We also tested the effect of artemisinin on *pfap2-g* transcript levels and gametocyte production in culture-adapted parasites. For a robust and accurate measurement of sexual conversion, we first generated reporter lines using the CRISPR/Cas9 system and optimized the determination of sexual conversion rates by flow cytometry and immunofluorescence assays. We found that exposure to sublethal concentrations of artemisinin at the trophozoite stage induces sexual conversion and enhances gametocytemia. This was observed under basal conditions, but not under metabolic conditions that stimulate sexual conversion (choline depletion). Altogether, our results show that artemisinin can stimulate gametocyte production. The *in vitro* observation that this can occur at sublethal concentrations implies that substandard drugs, underdosing, and noncompliance with the ACT regimen pose risks to malaria control efforts. We also show that *pfap2-g* can serve as an early marker to test the immediate effect of drugs on gametocyte production both *in vitro* and in epidemiological studies.

1897

TRANSMISSION-BLOCKING EFFECTS OF PRIMAQUINE AND METHYLENE BLUE SUGGEST *PLASMODIUM FALCIPARUM* GAMETOCYTE STERILIZATION RATHER THAN EFFECTS ON SEX RATIO

John Bradley[1], Harouna Soumare[2], Michelle Roh[3], Michael Delves[1], Chris Drakeley[1], Thomas S. Churcher[4], Alassane Dicko[2], Roly Gosling[3], Teun Bousema[5]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*University of Science, Techniques and Technologies, Bamako, Mali,* [3]*University of California, San Francisco, CA, United States,* [4]*Imperial College, London, United Kingdom,* [5]*Radboud University, Nijmegen, Netherlands*

Artemisinins clear asexual parasites but have limited efficacy against gametocytes and do not fully prevent transmission. Primaquine (PQ) and methylene blue (MB) are potent gametocytocidal drugs which reduce transmission. Their effects on transmission may occur before reducing gametocyte density, but it is not clear why. Preferential clearance of male or female gametocytes, resulting in a non-viable sex ratio, is a possible explanation; an alternative is a gametocyte sterilizing effect. We examine these hypotheses using a mathematical model of transmission and data from a clinical trial. The trial in Mali compared sulfadoxine-pyrimethamine plus amodiaquine (SP/AQ) and dihydroartemisinin-piperaquine (DP) as non-gametocytocidal drugs with SP/AQ plus a single dose of 0·25 mg/kg PQ (SP/AQ-PQ), and DP plus 15 mg/kg MB per day for 3 days (DP-MB) in asymptomatic microscopy positive gametocyte carriers. Infectivity was assessed by membrane feeding assay on days 2 and 7. Density of male and female gametocytes was determined by quantitative reverse transcription polymerase chain reaction. On day 2, male and female densities were similar in those on PQ or MB compared to those who took only non-gametocidal drugs. However, despite considerable overlap of gametocyte densities, infectivity after DP-MB (0%) and SP/AQ-PQ (5%) was lower than those on non-gametocytocidal drugs (61%). A model showed strong evidence of lower infectivity at a given male and female density for those on PQ or MB (p<0.001). On day 7, infectivity after DP-MB (0%) and SP/AQ-PQ (0%) was again lower than for those on non-gametocytocidal drugs (53%). Although gametocyte densities were lower, a model suggested that infectivity on PQ or MB was lower than for those on non-gametocital drugs, controlling for density (p=0.044). We have confirmed that PQ and MB reduce infectivity before reducing gametocyte density. Our results show this is due to a sterilizing effect on gametocytes, rather than an effect on sex ratio. The major implication is that studies examining transmission blocking effects of drugs and potentially vaccines require mosquito feeding assays to measure infectivity.

1898

THE ROLE OF IP-10 AND CXCR3 SIGNALING IN ZIKA VIRUS PROSTATE CELL INFECTION

Jennifer L.S. Clinton, Linda L. Tran, Megan B. Vogt, David R. Rowley, Jason T. Kimata, Rebecca R. Rico-Hesse

Baylor College of Medicine, Houston, TX, United States

Zika virus (ZIKV) is a *Flavivirus* recently classified as a sexually-transmitted infection. Infectious ZIKV has been detected in semen up to 69 days after infection, suggesting ZIKV may persist in the male urogenital tract. Flaviviruses can develop persistent infections by inducing specific cytokines and hijacking the interferon (IFN) pathway. However, little is known about which persistence mechanisms mediate long-term sexual transmission. Previously, we demonstrated that ZIKV infects prostate stromal mesenchymal stem cells (MSCs) and immortalized prostate epithelial cells, and that ZIKV preferentially replicates in MSCs. To understand the mechanisms of this differential replication, we measured induction of 41 cytokines during ZIKV infection in three prostate cell types by multiplex immunoassay. To determine which cytokines might augment replication, we treated prostate cells with individual upregulated cytokines before or after infection and measured their effects by qRT-PCR. Our results show that three ZIKV isolates elicit distinct cytokine profiles in infected MSCs and epithelial cells, with an increase in IFNs and IFN-related proteins (IP-10). We focused on the effects of IP-10 due to its association with severe infection with other sexually transmitted viruses. IP-10 treatment of prostate epithelial cells three hours before or 24 hours after ZIKV infection significantly reduced levels of viral RNA compared to untreated infected cells, without altering viability. While IP-10 inhibits replication in epithelial cells, it only slows viral replication kinetics in MSCs. We see similar effects on viral replication when prostate cells are pretreated with an agonist for the IP-10 receptor, CXCR3. This suggests that ZIKV preferentially replicates in prostate stem cells, where it may evade the suppressive effects of IP-10/CXCR3 signaling. IP-10 has been associated with promoting (HIV, Herpes) or protecting (Coronavirus, EBV) against viral infection in different cell types, but the mechanism of action of CXCR3 has not been elucidated. This could lead to treatment options for ZIKV infection, to block sexual transmission.

1899

A POINT MUTATION BETWEEN ASIAN AND AFRICAN LINEAGE ZIKA VIRUSES AUGMENTS MOSQUITO INFECTIVITY

Emily Gallichotte[1], Reyes Murrieta[1], Eric Bellis[1], Thomas Friedrich[2], Matthew Aliota[3], Gregory Ebel[1]

[1]*Colorado State University, Fort Collins, CO, United States,* [2]*University of Wisconsin, Madison, WI, United States,* [3]*University of Minnesota, St Paul, MN, United States*

Zika virus (ZIKV) was isolated in 1947 where it caused only rare infections in humans. After it was introduced to Brazil in 2015, it caused a massive outbreak with over one million infections. It is unclear if changes to the virus lead to the increased incidence of infection. Phylogenetic analyses found that ZIKV contains two lineages, African and Asian. Within Asian-lineage ZIKVs, a serine-to-asparagine substitution emerged at residue 139 of the viral polyprotein. This substitution sits within the pr domain of the prM protein, which covers the fusion-loop of the virion and prevents fusion upon viral egress. Cellular furin cleaves pr from other structural proteins, and upon virus release, cleaved pr dissociates from M. Using a Puerto Rican Asian-lineage ZIKV infectious clone, we generated a reversion mutant (N139S) to test the role of both lineage amino acids at this position. We found that the N139S mutation does not alter furin cleavage or virus maturity. The mutant virus has similar thermostabilities as the parental Asian-lineage virus at multiple temperatures, and replicates similarly in mammalian cells. In the *Aedes aegypti* cell line Aag2, we found that the mutant virus replicates to higher titers than the wild-type virus. Additionally, the N139S mutant is more infectious in *Ae. aegypti* mosquitoes, with higher infection and dissemination rates, and higher viral RNA levels, compared to the wild-type virus. These results are consistent

with multiple studies showing African-lineage ZIKVs are more infectious in mosquitoes compared with Asian-lineage viruses. These results suggest the recent emergence and maintenance of asparagine at position 139 in Asian-lineage ZIKVs was not a result of selection for improved fitness in mosquitoes. Therefore, the substitution may result in a fitness advantage within the mammalian host, or alter the virus-vector interaction through another mechanism that results in enhanced fitness. Additional experimentation in mosquito cells and directly in mosquitoes will test differences in virus binding, entry, interactions with host-receptors, and kinetics of infection to more fully elucidate the role of N139S.

1900

STRUCTURAL PROTEINS (PRME) DICTATE SEXUAL TRANSMISSION POTENTIAL OF ZIKV AND SPONV IN AN *IN VITRO* EPIDIDYMAL EPITHELIAL CELL MODEL

Erin M. McDonald, Aaron C. Brault

Centers for Disease Control and Prevention, Fort Collins, CO, United States

The Spondweni serogroup of flaviviruses (*Flaviviridae*, *Flavivirus*) is comprised of Spondweni virus (SPONV) and Zika virus (ZIKV), which are mosquito-borne viruses capable of eliciting human disease. Following the emergence of ZIKV in the Americas, sexual transmission in humans has been documented. Furthermore, ZIKV can be transmitted *in utero*, leading to ZIKV congenital syndrome. While SPONV is not known to elicit similar congenital pathogenic effects or to be transmitted sexually and has been limited to an African geographic distribution, it was recently identified in mosquitoes in Haiti. In C57bl/6 mice treated with monoclonal antibody to Ifnar1, SPONV can infect the placenta and fetal tissues, including the fetal head, but it does not cause fetal resorption or microcephaly. In interferon α/β and –γ receptor knockout (AG129) mice, ZIKV replication in epididymal epithelial cells in the male reproductive tract (MRT) has been correlated with ZIKV sexual transmission potential. Although SPONV infects the AG129 MRT, including epididymal epithelial cells, it is not found in high frequency in ejaculates from these mice. To determine if viral structural elements mediate sexual transmission potential differences observed between ZIKV and SPONV, the prME genes from Spondweni SAAr94 were cloned into a cDNA clone of ZIKV. In a murine epididymal epithelial cell line, neither SPONV nor the SPONVprME/ZIKV chimeric virus demonstrated viral growth. In contrast, ZIKV was able to infect and replicate in these cells. The sexual transmission potential and *in utero* transmission potential of SPONV prME chimeric virus will be further assessed in a sensitive immunodeficient (AG129) murine model.

1901

AFRICAN-LINEAGE ZIKA VIRUS CAUSES PLACENTAL PATHOLOGY IN PREGNANT RHESUS MACAQUES

Chelsea M. Crooks[1], Anna S. Jaeger[2], Andrea M. Weiler[3], Sierra L. Rybarczyk[3], Mason I. Bliss[3], Elizabeth A. Brown[1], Heather A. Simmons[3], Jennifer M. Hayes[3], Andres Mejia[3], Keisuke Yamamoto[4], Phoenix Shepherd[4], Megan E. Murphy[5], Thaddeus G. Golos[5], Amber Possell[3], Kara Weaver[3], Terry K. Morgan[6], Dawn M. Dudley[4], Nancy Schultz-Darken[3], Eric Peterson[3], David H. O'Connor[4], Matthew T. Aliota[2], Thomas C. Friedrich[1]

[1]*Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, United States,* [2]*Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, United States,* [3]*Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, United States,* [4]*Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, United States,* [5]*Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, United States,* [6]*Departments of Pathology and Obstetrics and Gynecology, Oregon Health Sciences University, Portland, OR, United States*

Did Zika virus (ZIKV) recently acquire the ability to cause congenital Zika syndrome (CZS)? Data from vertical transmission studies in mice suggest that ZIKVs of both African and Asian lineages have the capacity to cause placental damage and fetal harm. We used an established macaque model of ZIKV, which more closely mimics the reproductive physiology of humans, to further evaluate the pathogenic potential of African-lineage Zika viruses. Three pregnant Indian rhesus macaques were subcutaneously inoculated with 10^4 PFU of ZIKV DAK AR 41524, a low-passage ZIKV isolate from Senegal, during mid-first trimester (approximately gestation day 45; full term is 165±10 days). ZIKV infection was monitored via plasma viremia and fetal growth by weekly ultrasounds. The first pregnancy progressed to term without adverse events and no gross fetal abnormalities were noted at delivery at gestation day 155. A comprehensive set of ~50 maternal and fetal tissue samples were collected at delivery. Sensitive QRT-PCR detected no ZIKV RNA in fetal tissues, but vRNA was detected in a variety of maternal-fetal interface tissues, with particularly high levels found in fetal membranes and the chorionic plate. Histopathological analysis showed acute inflammation in both maternal and fetal tissues, consistent with viral infection—in 7 of 16 placental cotyledons there were microscopic acute, non-hemorrhagic, placental infarctions with neutrophils and histiocytes. Data collected thus far support the hypothesis that ZIKV of both African and Asian lineage pose a threat to women and their fetuses. The other two pregnancies are in progress and we expect a full data set later this summer.

1902

IMPACT OF ZIKA VIRUS EVOLUTION ON MOSQUITO TRANSMISSION DURING THE EPIDEMIC IN THE AMERICAS

Chantal B. Vogels[1], Glenn Oliveira[2], Sharada Saraf[2], Carlos Ontiveros[2], Rimjhim Agarwal[2], Raphaëlle Klitting[2], Joseph R. Fauver[1], Anderson F. Brito[1], Emma Allen[1], James Weger-Lucarelli[3], Gregory D. Ebel[4], Kristian G. Andersen[2], Nathan D. Grubaugh[1]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*The Scripps Research Institute, La Jolla, CA, United States,* [3]*Virginia Polytechnic Institute and State University, Blacksburg, VA, United States,* [4]*Colorado State University, Fort Collins, CO, United States*

The rapid and often unpredictable emergence of mosquito-borne viruses such as Zika, chikungunya, and dengue presents serious challenges for public health. Mosquito-borne RNA viruses lack proofreading ability, replicate rapidly, and form large within-host populations, resulting in high evolutionary rates during transmission. Consequently, these viruses have an incredible adaptive potential which allows for continuous emergence into new territories. Previous studies have (1) shown that virus evolutionary rates increase during rapidly expanding epidemics and (2) linked adaptive potential of viruses to few specific mutations; yet, functional evolution of viruses has not been comprehensively studied during an epidemic. Here we present the first thorough investigation of how Zika virus evolved during the rapid spreading epidemic in the Americas. From our preliminary data, we demonstrate that Zika viruses isolated from Cambodia (pre-epidemic), Brazil, Panama, Honduras, and Puerto Rico exhibit different levels of competitive fitness *in vitro*, leading us to hypothesize that Zika virus evolution during its spread within the Americas may alter transmission fitness in mosquitoes (*in vivo*). To test this hypothesis, we constructed a phylogenetic tree using our previously sequenced and available Zika virus genomes and identified 13 clade-defining amino acid changes that appeared during the epidemic. Next, we engineered these mutations in a Zika virus cDNA clone derived from a 2015 Brazilian isolate, and will use these viruses to infect *Aedes aegypti* mosquitoes and evaluate the impact on transmission. We will use outcomes from *in vivo* assays to inform vectorial capacity models which allows us to assess the impact of specific mutations on Zika virus transmission potential. Our study system provides a framework for evaluating functional evolution of viruses during rapidly evolving outbreaks and to investigate if emergence was facilitated by recent virus evolution.

1903

CHARACTERIZING THE IMMUNE RESPONSE TO ZIKA VIRUS USING EPITOPE MAPPING: REPORTER VIRUS PARTICLES AND ANTI-ZIKV ANTIBODIES

Edgar Davidson[1], Chuck Whitbeck[1], Anu Thomas[1], Aubrey L. Bryan[1], Tabb Sullivan[1], Lewis J. Stafford[1], Ross Chambers[1], Michael G. Rossmann[2], James E. Crowe Jr.[3], Benjamin J. Doranz[1]

[1]*Integral Molecular, Inc., Philadelphia, PA, United States,* [2]*Department of Biological Sciences, Purdue University, West Lafayette, IN, United States,* [3]*Departments of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN, United States*

We have characterized the immune response to ZIKV infection and vaccines by epitope mapping over 70 anti-ZIKV MAbs at amino acid-resolution, using a comprehensive ZIKV prM/E library of 672 single alanine mutants expressed in human cells. Published studies described epitopes of MAbs isolated from a Brazilian patient, including a highly neutralizing MAb protective in animal models of ZIKV fetal disease. The epitope locations suggested that the some MAbs act by binding across adjacent protein E dimers, preventing the rearrangements necessary for ZIKV infectivity. The epitope maps obtained also reveal which epitopes are specific for ZIKV or are common to DENV, information that can be used to create better vaccines and therapeutics. We have also identified mutations that increased ZIKV RVP budding, and which may be applicable to the design and production of anti-ZIKV vaccines, by screening each individual ZIKV library prM/E variant for ZIKV particle budding and infectivity. To provide critical reagents, we have isolated anti-ZIKV MAbs for ZIKV-specific immunodetection, diagnostic applications, and ZIKV neutralization studies. MAbs were isolated after immunization with DNA and sub-viral particles, and phage library panning with ZIKV reporter virus particles (RVPs) that we have developed. RVPs are capable of one round of infectivity, with luminescent or fluorescent readout. We isolated 48 conformational MAbs specific to prM/E from ZIKV, but not DENV, including a MAb that potently neutralized ZIKV RVPs (IC_{50} 45 ng/ml) and for which mapping identified a quaternary epitope spanning adjacent E proteins. We have also used ZIKV RVPs to identify new cellular receptors and attachment factors that enable ZIKV entry. ZIKV RVPs were tested on our Membrane Proteome Array (MPA), comprising 5,300 unique human membrane proteins individually expressed in live human cells. Known receptors and attachment factors were identified (validating the approach), as well as a number of membrane proteins not previously known to enable ZIKV entry. These newly identified proteins help explain viral tropism and pathogenesis, and may be useful as therapeutic targets.

1904

CD8+ LYMPHOCYTES MODULATE ZIKA VIRUS DYNAMICS AND TISSUE DISSEMINATION AND ORCHESTRATE ANTIVIRAL IMMUNITY

Blake Schouest[1], Marissa Fahlberg[1], Elizabeth A. Scheef[1], Matthew J. Ward[2], Kyra Headrick[2], Dawn M. Szeltner[1], Robert V. Blair[1], Margaret H. Gilbert[1], Lara A. Doyle-Meyers[1], Victoria W. Danner[1], Dawn M. Wesson[2], Antonito T. Panganiban[1], Nicholas J. Maness[1]

[1]*Tulane National Primate Research Center, Covington, LA, United States,* [2]*Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

CD8+ lymphocytes are critically important in the protection and control of viral infections, but their roles in acute Zika virus (ZIKV) infection remain incompletely explored. Nonhuman primate models of ZIKV have been valuable in understanding immune mechanisms of ZIKV control due to similarities in immune function to humans and due to their susceptibility to infection with primary human isolates of the virus. Here, we use CD8+ lymphocyte depletion to dissect immune responses and viral dynamics in adult male rhesus and cynomolgus macaques infected with ZIKV. CD8 depletion delayed serum viremia and dysregulated patterns of innate immune responses in the blood, demonstrated by a complete lack of neutrophil recruitment and a striking absence of monocyte-driven transcriptional changes in type-I interferon and other key immune genes. Depletion also modulated patterns of monocyte expansion and activation. CD8-depleted macaques showed evidence of compensatory adaptive immune responses, with elevated Th1 activity and persistence of neutralizing antibodies beyond the clearance of serum viremia. The absence of CD8+ lymphocytes increased viral burdens in lymphatic tissues, semen, and cerebrospinal fluid, and neural lesions were also evident in both CD8-depleted rhesus macaques. Together, these data support a role for CD8+ lymphocytes in the control of ZIKV dissemination and in maintaining immune regulation during acute infection of nonhuman primates.

1905

A LOCALIZED SANITARY SURVEY AS A PROXY FOR FECAL CONTAMINATION IN LOW-INCOME URBAN MAPUTO, MOZAMBIQUE

Drew Capone[1], David Berendes[2], David Holcomb[3], Jackie Knee[1], Joe Brown[1]

[1]*Georgia Institute of Technology, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Sanitary surveys are increasingly used in low- and middle-income countries to assess water, sanitation, and hygiene conditions, but few data exist comparing such surveys with direct measures of environmental fecal contamination. We conducted a cross-sectional survey of sanitary conditions in low-income neighborhoods of Maputo, Mozambique to assess the association between compound level (household clusters) sanitary conditions and *E. coli* counts in soils and on surfaces. We adapted the World Bank's Urban Sanitation Status Index to implement a sanitary survey tool specifically for compounds: a Localized Sanitation Status Index (LSSI) ranging from 0 (poor sanitary conditions) to 1 (better sanitary conditions) calculated from 20 variables that characterized local sanitary conditions. We measured the variation in the LSSI with environmental *E. coli* counts in soil (nine locations per compound) and surface swabs (seven locations per compound) in 80 compounds to assess reliability. We detected *E. coli* in 74% of soil samples at an average concentration of 4.10 $\log_{10}$ CFU *E. coli* per gram of dry soil and detected *E. coli* at 5.4% of swab locations. The presence of chickens, soil moisture, sun exposure, intra-compound location, and the compound's wealth index were all significantly associated with *E. coli* counts in soil. No covariates were significantly associated with the detection of *E. coli* on compound surfaces in univariable or multivariable Poisson regression. Multivariable linear regression indicated that a ten-percentage point increase in LSSI (e.g. 0.55 to 0.65) was associated with 0.05 (95% CI: 0.00, 0.10) $\log_{10}$ fewer *E. coli* per dry gram in compound soil and a 2% decrease (95% CI: 0%, 4%) in the risk of detection of any *E. coli* from compound soil. Findings suggest the LSSI can be a useful proxy for environmental fecal contamination as measured by *E. coli* counts in this low-income urban setting. However, the expected reduction in *E. coli* counts from substantial improvements in the LSSI may not reflect a meaningful difference in compound level exposure risk to feces-associated enteric pathogens.

1906

EXPLORING THE POTENTIAL RELATIONSHIP BETWEEN FECAL EXPOSURE PATHWAYS AND SYMPTOMATIC AND ASYMPTOMATIC ENTERIC INFECTIONS IN CHILDREN IN AN URBAN ENVIRONMENT IN VELLORE, INDIA

Yuke Wang[1], Sydney Hubbard[1], Gagandeep Kang[2], Suraja Raj[1], Habib Yakubu[1], Arun Karthikeyan[2], Senthil Kumar[2], Venkata R. Mohan[2], Christine Moe[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Christian Medical College of Vellore, India, Vellore, India*

Rapid urbanization has led to a sanitation crisis in many low- and middle-income countries (LMIC). In March 2014, SaniPath in collaboration with

the Christian Medical College (CMC) of Vellore, India conducted an exposure assessment in Old Town, a dense, urban unplanned settlement in Vellore. A total of 191 samples were collected from open drains, drinking water, public latrines, soil, raw produce, bathing water, child handrinse, and toy feeding spoon rinse and analyzed for *E. coli*. From March 2010 - February 2012, the MAL-ED study, a multi-site project examining enteric and growth outcomes enrolled a birth cohort of 190 children in Old Town. Multiple stool samples were collected from each child over two years of follow up and tested for bacterial and viral pathogens. Symptomatic illness was recorded. Each child in the MAL-ED study was linked with the closest environmental samples from the SaniPath study. Spatial variables, like the distance to the closest open defecation site, were generated. Generalized linear models were used with the bacterial infection rate, viral infection rate, and symptomatic illness rate as outcomes and environmental fecal contamination from different pathways and spatial variables as covariates. *E. coli* concentration from the closest public latrine and the distance to the closest open defecation site were significant predictors of bacterial infection rate in children. The sum of the open drain lengths within a 100-meter radius of the child, as well as the sum of street lengths within a 100-meter radius of the child, were significant predictors of viral infections in children. The *E. coli* concentration of the closest piped water was the only significant predictor of symptomatic illness in children. These results highlight the need for safe excreta management in dense, urban settings to prevent bacterial infections, while contaminated drinking water seems to be a major driver of symptomatic illness in this population. Human congestion, as proxied by summative surrounding street lengths and open drains, is a key risk factor for viral infection.

1907

TRACKING TRANSMISSION SOURCES OF DIARRHEA: AN INVESTIGATION ON DIARRHEAGENIC *ESCHERICHIA COLI* IN URBAN HOUSEHOLDS OF BANGLADESH

Zenat Zebin Hossain[1], Rokaia Sultana[1], Anowara Begum[1], Peter Kjær Jensen[2]

[1]*University of Dhaka, Dhaka, Bangladesh,* [2]*University of Copenhagen, Copenhagen, Denmark*

It is already recognized that poor hygiene practices may trigger the rapid distribution of pathogens, particularly waterborne diarrheagenic bacteria, in overpopulated urban settings. Prevalence of pathogenic *E. coli* contamination in diarrhea case household environments of low-income urban area in Bangladesh has not been explained before. This study collected diarrhea patients' rectal swabs and in-house environmental samples from case households in Arichpur, Dhaka city, Bangladesh for four months period to reveal in-house environmental hotspots for diarrheagenic *E. coli* transmission and their involvement in diarrhea. The environmental samples include: swabs from four frequently touched surfaces (water vessel used for drinking water, cutting knife or "Boti"- a floor based knife, latrine doorknob, and food plate), drinking water and food which were collected from a total of 34 low-income households with reporting diarrhea patients. Total 245 DNA samples were examined for virulence genes characteristic of five major diarrheagenic *E. coli* pathotypes (ETEC, EHEC, EIEC, EPEC, EAEC) by PCR. The isolated *E. coli* strains were analyzed for virulence typing.Results showed that genomic presence of diarrheagenic *E. coli* was detected in 36% (89 of 245) of all samples. The frequency rate of virulent genes of *E. coli* from the rectal swab, household swabs, food, water samples were 28% (10/36), 44% (63/144), 12% (4/34), 10% (3/31) respectively in PCR analysis. Six out of 36 rectal swab samples and associated household swabs, water, and food samples showed the presence of similar *E. coli* pathotypic genes, and among the different sample types, drinking vessel surface contamination was highest. One EAEC strain was commonly found in both clinical and latrine door knob swab. Kitchen tools and utensils appeared to play a role in the circulation of diarrheagenic *E. coli* within the household. This study data suggests the existence of high-risk hotspots, particularly surfaces directly related to the human food chain for diarrheagenic *E. coli* contamination within low-income case household environment in Bangladesh.

1908

WATER, SANITATION, AND ANIMAL-SPECIFIC RISK FACTORS FOR MODERATE-TO-SEVERE DIARRHEA IN YOUNG CHILDREN IN THE VACCINE IMPACT ON DIARRHEA IN AFRICA (VIDA) STUDY—THE GAMBIA, KENYA, AND MALI, 2015-2018

David Berendes[1], Kirsten Fagerli[1], Sunkyung Kim[1], Dilruba Nasrin[2], Helen Powell[2], Irene Kasumba[2], Sharon Tennant[2], Anna Roose[2], M. Jahangir Hossain[3], Joquina Chiquita M. Jones[3], Syed MA Zaman[3], Richard Omore[4], Ben Ochieng[4], Jennifer Verani[5], Marc-Alain Widdowson[5], Samba Sow[2], Dramane Malle[6], Sanogo Doh[6], Eric Mintz[1], Karen Kotloff[2]

[1]*Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Medical Research Council Unit, The Gambia, Bakau, Gambia,* [4]*Kenya Medical Research Institute, Kisumu, Kenya,* [5]*Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya,* [6]*Center for Vaccine Development-Mali, Bamako, Mali*

Environmental exposure to contaminated water, unsafe sanitation, and animals contributes to child diarrhea. We examined associations between these risk factors and moderate-to-severe diarrhea (MSD) in children &lt5 years old in the Vaccine Impact on Diarrhea in Africa (VIDA) case-control study in The Gambia, Kenya, and Mali. We enrolled cases seeking care for MSD at health facilities; age-, gender-, time- and community-matched controls were enrolled at home. Site-specific conditional logistic regression models, adjusted *a priori* for mother's education, evaluated associations between household drinking water source, sanitation type, animal ownership, and MSD. Preliminary risk factor proportions in cases and controls and matched Odds Ratios with 95% confidence intervals are reported. From 2015-2018, sites enrolled 4,841 cases and 6,214 matched controls. Compared to controls, cases had higher odds of using an unimproved main drinking water source recently in all sites (Gambia: 19% v. 14%, OR: 1.76 [1.39-2.23]; Mali: 10% v. 8%, OR: 1.38 [1.09-1.75]; Kenya: 29% v. 25%, OR: 1.22 [1.03-1.46]). Associations between levels of the Joint Monitoring Program sanitation ladder and MSD varied by site. Compared to at least basic sanitation, cases had lower odds of using an unimproved facility in The Gambia (54% v. 67%, OR: 0.56 [0.48-0.65]). In Kenya, cases had higher odds of using a shared sanitation facility (33% v. 29%, OR: 1.42 [1.13-1.77]) but lower odds of using no facility (3.6% v. 7.9%, OR: 0.48 [0.33-0.70]). In The Gambia, cases were more likely to own ruminants (87% v. 85%, OR: 1.26 [1.01-1.56]) and fowl (84% v. 81%, OR: 1.32 [1.10-1.59]). In Kenya, the reverse association was noted (ruminants: 73% v. 80%, OR: 0.66 [0.56-0.78]); fowl: 90% v. 94%, OR: 0.57 [0.44-0.74]). No associations between sanitation or animal ownership and MSD were observed in Mali. Results suggest that use of unimproved water was a consistent risk factor for MSD, but contributions of other environmental exposure pathways were site-specific. Further analyses will explore variation in etiologic agents associated with MSD with the coverage and types of waste containment by site.

1909

AN INDIVIDUAL PARTICIPANT DATA META-ANALYSIS ON THE ASSOCIATION BETWEEN ENVIRONMENTAL FECAL CONTAMINATION AND CHILD HEALTH

Frederick G. Goddard[1], Amy J. Pickering[2], Ayse Ercumen[3], Joe Brown[4], Howard H. Chang[1], Thomas F. Clasen[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Tufts University, Boston, MA, United States,* [3]*North Carolina State University, Raleigh, NC, United States,* [4]*Georgia Institute of Technology, Atlanta, GA, United States*

Water, sanitation and hygiene (WaSH) interventions have historically sought to improve child health by interrupting fecal-oral transmission of enteric pathogens. However, recently published evaluations of large WaSH trials have shown mixed results on diarrhea and growth, raising questions about the extent to which WaSH interventions reduce fecal contamination

in the environment. To design more effective interventions, there is a need to better understand the associations between fecal contamination and child health and how those differ along various transmission pathways. We systematically identified studies that quantified household-level fecal indicator bacteria concentrations in environmental samples or fly densities, and individual-level reported diarrhea or growth for children <5 years in low- and middle-income settings. We pooled raw individual participant data from eligible studies and used multilevel generalized mixed effects models to estimate the odds ratio for diarrhea and the change in height-for-age Z (HAZ) scores associated with a 1-log increase in fecal contamination along individual transmission pathways. We obtained data from 16 eligible studies, totaling over 42,000 data points on reported diarrhea and over 20,000 growth measures, linked to four transmission pathways: drinking water, child hands, fly density and fomites (sentinel toys). We found significant increases in odds of diarrhea with an increase in fecal contamination in drinking water and on hands, but not on fomites or kitchen fly densities. Children exposed to higher levels of fecal contamination in water had reduced HAZ scores, but we did not find this effect for other pathways. Our findings indicate that fecal contamination in the domestic environment is associated with child diarrhea and growth. Interventions that failed to yield health benefits may not have sufficiently interrupted fecal-oral transmission. Our mixed findings by pathway suggest that fecal contamination along proximal pathways (water and hands) is more strongly associated with child health than contamination along distal pathways in the broader domestic environment.

1910

ENVIRONMENTAL PATHOGEN SURVEILLANCE OF WASTEWATER: TIME-VARYING VIRAL SHEDDING INTENSITY IN THE 2013 SILENT POLIO OUTBREAK IN ISRAEL

Andrew F. Brouwer, Joseph N. Eisenberg, James S. Koopman, Lester M. Shulman, Marisa C. Eisenberg

University of Michigan, Ann Arbor, MI, United States

The power of environmental pathogen surveillance was apparent in the 2013 polio outbreak in Israel when scientists used it to detect circulation despite the absence of any cases of human paralysis. Polio researchers have promoted the use of environmental surveillance to enhance traditional epidemiologic disease surveillance; it is now an important tool used in the global effort to eradicated polio. Beyond its use in detection, quantitative, longitudinal environmental surveillance has the potential to describe the epidemiology of waterborne outbreaks for a wide variety of pathogens. To do this, we need to characterize the dynamics of pathogen shedding, including both the fraction of people likely to be shedding as function of time since exposure and varying pathogen shedding intensity over that time period. Here, we analyzed data from 665 quantitative environmental surveillance samples for 3 strains of polio from 7 sentinel sites in southern Israel during the 2013 silent epidemic using a transmission modeling framework we developed to incorporate environmental surveillance data. We found that the data are consistent with a shedding duration (29 days (95% CI 27-31 days)) closer to what experts have predicted for fully susceptible individuals than to what might be expected for individuals who have had multiple doses of inactivated polio vaccine, as was believed to be the case for this population. We characterized our modeled infection stages as having high or low shedding rates and found that the outbreak dynamics were consistent with person-to-person pathogen transmission occurring only during high shedding periods. Our work highlights the need for realistic models of time-varying pathogen shedding to understand how to convert the observed environmental surveillance signals into public health metrics. With this understanding, environmental surveillance has the potential to transform our analysis of waterborne pathogen epidemiology and, in turn, help inform control strategies.

1911

ASSOCIATION BETWEEN GASTROINTESTINAL DISEASE IN CHILDREN AND WASTEWATER AGRICULTURAL IRRIGATION IN VALLE DEL MEZQUITAL, MÉXICO

Eunice Elizabeth Félix-Arellano[1], Sandra Leticia Rodriguez-Dozal[1], Jesse Contreras[2], Rafael Meza[2], Joseph N.S. Eisenberg[2], Horacio Riojas-Rodriguez[1]

[1]*National Institute of Public Health, Cuernavaca, Mexico,* [2]*University of Michigan, Ann Arbor, MI, United States*

Wastewater reuse for agriculture has intensified over the years partly due to increases in urbanization. In the Valle del Mezquital, Hidalgo, wastewater from Mexico City is used for agricultural irrigation. Human and animal pathogens are continuously high in wastewater, potentially putting farmers and their families at risk. The Atotonilco Wastewater Treatment Plant began partially treating wastewater in May 2017, providing treated water to some irrigation districts of the Mezquital. We compared the risk of gastrointestinal diseases in children under five living in three areas of the Mezquital: zone A where crops are irrigated with treated wastewater after the treatment plant began operation, zone B where crops are irrigated with untreated wastewater, and zone C where crops are irrigated with well water. We conducted a longitudinal study following 880 children under five with three household visits: recruitment (November 2016-April 2017), first follow-up (May-July 2017), and second follow-up (August-November 2017). We administered questionnaires to the primary caregiver about diarrheal disease in children under five, use of water in the household, hygiene practices, and participation in agriculture. We estimated the odds of presenting with diarrhea using multivariate logistic regression models with fixed and random effects. Children under five living in zones that irrigated crops with wastewater (zones A and B) had 2.0 times the odds of diarrheal disease compared to zone C (CI 95% 1.27 - 3.1). Compared to Zone C, Zone B had 2.3 times the odds of diarrheal disease (95% CI 1.41 - 3.84), followed by zone A (OR = 1.72 times, 95% CI 1.04 - 2.86), after adjusting for age, sex, presence of chickens, and access to sewerage. The odds of diarrhea in children under five increased in the presence of chickens (OR = 1.74, 95% CI 1.18 - 2.57) and decreased with access to sewerage (OR = 0.50, 95% CI 0.27 - 0.93). Our results suggest that exposure to wastewater used for irrigation, with or without treatment, contributes to the presence of diarrheal disease in children under five years of Valle del Mezquital.

1912

EXPLORING THE EFFECTS OF NEXT-GENERATION NETS ON HIGHLY PYRETHROID-RESISTANT *ANOPHELES COLUZZII* MOSQUITOES USING BENCHTOP BEHAVIOR ASSAYS

Natalie Lissenden, Jeff Jones, Hilary Ranson, Philip J. McCall

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

In Burkina Faso, malaria is increasing despite high coverage of WHO recommended vector control tools - a phenomenon that could be partly due to surging pyrethroid-resistance in the vector population. Insecticide-resistant populations can be controlled using insecticide synergists, such as piperonyl butoxide (PBO). We used novel benchtop video assays to investigate the physiological and behavioural impacts of exposure to pyrethroid-PBO nets of a highly pyrethroid-resistant *An. coluzzii* population in South-West Burkina Faso. Adult females reared from larvae were exposed to test netting (baited with a human host) in filmed assays for 3 (Video Cone Tests) or 20 (Baited Box Test) minutes, and immediate and delayed mortality was recorded. Composite video analysis and scan sampling were used to examine mosquito responses. During exposure to PermaNet 3.0 roof (i.e. pyrethroid-PBO net) in the Baited Box Test, flight activity declined rapidly until knockdown began at 180 seconds. In Video Cone Tests, flight declined after 60 seconds accompanied by an increase in resting. In both assays 24hr mortality was 100% following exposure to PermaNet 3.0 roof. Following exposure to PermaNet 3.0 sides (i.e. pyrethroid-only net) 24hr mortality was 0% in Baited Box and 2.13% in Video Cone tests - this increased to 9.68% and 6.38% after 7 days,

respectively. Mosquito flight behaviour on exposure to PermaNet 3.0 sides was comparable to untreated controls. Composite video analysis revealed that contact with the pyrethroid-PBO net was greatly reduced in comparison with untreated controls, but this brief exposure is enough to rapidly knock down and kill highly pyrethroid-resistant vectors. The implications of these results for the design of next-generation bed nets for targeting insecticide resistant mosquitoes are considered.

1913

PYRIPROXYFEN REDUCES FECUNDITY IN *ANOPHELES ARABIENSIS*: A NEW POTENTIAL TOOL FOR MALARIA CONTROL IN ETHIOPIA

Solomon Kibret[1], Delenasaw Yewhalaw[2], Guofa Zhou[1], Guiyun Yan[1]

[1]*University of California Irvine, Irvine, CA, United States,* [2]*Tropical and Infectious Diseases Research Center, Jimma University, Jimma, Ethiopia*

Insecticide resistance poses a major threat to current malaria control campaigns. Insecticides with novel modes of action are therefore needed to improve malaria control. Pyriproxyfen (PPF), a conventional juvenile mosquitocide, has a unique mode of action that also sterilizes adult mosquitoes upon direct contact. However, the application of PPF on larval vs adult mosquitoes has not been well studied. This study investigated the rate of fecundity of *Anopheles arabiensis* after PPF exposure to determine the potential of PPF-induced sterilization in malaria mosquitoes control. Larvae of *An. arabiensis* were collected from field and two treatment regimens were tested. First, we placed 60 larvae each in 12 washbasins (used as larval breeding habitat) half-filled with water treated with 0.0001% of PPF in the malariasphere and evaluated the rate of larval development and mortality. Second, we released adult *An. arabiensis* into the malariasphere with a wall dusted with PPF and evaluated autodissemination of the PPF by mosquitoes from the wall to breeding habitats in the malariasphere. Fecundity was measured as the number of viable eggs laid by the mosquitoes after blood feeding. The results showed that 92% *An. arabiensis* larvae placed in breeding habitats treated with PPF died either at their larval or pupal stage compared to only 15% of the larvae that died in the control breeding habitats (with no PPF treatment). The average number of eggs laid by *An. arabiensis* resting on PPF dusted walls was 32.4 eggs/batch compared to 189.6 eggs/batch in controls without the PPF. None of the eggs laid by mosquitoes subjected to PPF dusted walls were developed to larvae when placed in breeding habitats while 102 eggs/batch in the control developed to larvae and 72% of them pupated. Our study found out that PPF autodessemination is effective in controlling *An. arabiensis* mosquitoes, the major malaria vector in Ethiopia.

1914

ENTOMOLOGICAL EVALUATION OF INDOOR RESIDUAL SPRAYING (PYRIMIPHOS-METHYL) ON MALARIA TRANSMISSION IN DIEBOUGOU DISTRICT, SOUTHWEST BURKINA FASO

Dieudonne Diloma Soma[1], Jacques Edou Gnambani[1], Georges Anicet Ouedraogo[2], Alphonsine Koffi[3], Cedric Pennetier[4], Roch K. Dabire[1], Nicolas Moiroux[4]

[1]*Institut de Recherche en Sciences de la Sante, Bobo-Dioulasso, Burkina Faso,* [2]*Université Nazi Boni, Bobo-Dioulasso, Burkina Faso,* [3]*Institut Pierre Richet, Bouake, Côte D'Ivoire,* [4]*MIVEGEC, IRD, CNRS, University Montpellier, Montpellier, France*

The rapid spread of insecticide resistance in malaria vectors and the possible recent increase in malaria cases in Africa require to develop and evaluate new vector strategies able to manage resistance. The combination of two insecticides targeting different time of the life cycle of the *Anopheles* vector is one possibility. A randomized controlled trial was performed with the objective to evaluate in communities, the effect of indoor residual spraying (IRS) with pyrimiphos-methyl (PM) in combination with Long-Lasting Insecticidal Nets (LLINs) on malaria transmission and the vectors diversity, resistance and behaviour. The study took place in 13 villages (5 sprayed and 8 control) between November 2017 to June 2018. We performed 4 surveys of hourly mosquito collection following the implementation of IRS using the human-landing collection technique from 17:00 to 09:00 in each village. Malaria vectors species, *Plasmodium* infection, blood-meal source, *kdr-west*, *kdr-east* and *ace1* target-site mutations were searched by molecular technique. Residual activity of PM was monitored with susceptible *"Kisumu"* and wild strains using the WHO cone wall bioassay technique. The residual efficacy of the PM ranged 88-100% for 7 months on mud and cement walls against both susceptible and wild strains of *Anopheles gambiae s.l.* Average densities of *Anopheles* sp were 0.69 bites per human per night in the sprayed villages, significantly lower than in the control villages (3.2 $b.h^{-1}.n^{-1}$; RR= 0.38 ; 95%CI[0.15-0.94]). The majority of biting activities of *Anopheles* occurred between 02:00 to 3:00h and 06:00 to 7:00h in the control villages and sprayed villages respectively. Overall, entomological inoculation rate was lower in the sprayed villages (0.14 infective bites per human per night) compared to the control villages (0.84 $ib.h^{-1}.n^{-1}$). PM insecticide IRS allowed to drastically reduce malaria transmission in our trial. PM should be considered as a potential good complementary tool to LLINs in high transmission areas of Burkina Faso but in association with surveillance of malaria vector behaviour.

1915

FEEDING AND RESTING BEHAVIOR OF *ANOPHELES GAMBIAE* S.L. IN AREAS GETTING INDOOR RESIDUAL SPRAYING FOR MALARIA VECTOR CONTROL AND AREAS NOT SPRAYED IN NORTHERN GHANA

Sylvester Coleman[1], Samuel K. Dadzie[2], Yemane Yihdego[1], Frank Gyamfi[1], Lena Kolyada[1], Dereje Dengela[3], Aklilu Seyoum[3], Jon Eric Tongren[4], Sixte Zigirumugabe[5], Dominic Dery[5], Kristen George[6], Jennifer Armistead[6], Maxwell Appawu[2], Kingsley Badu[7], Kwasi Obiri-Danso[7], Daniel Boakye[2], Daniel Szumlas[8]

[1]*U.S. President's Malaria Initiative Vectorlink Project, Accra, Ghana,* [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana,* [3]*U.S. President's Malaria Initiative Vectorlink Project, Abt Associates Inc., Bethesda, MD, United States,* [4]*U.S. President's Malaria Initiative, Malaria Branch, U.S. Centers for Disease Control and Prevention, Accra, Ghana,* [5]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Accra, Ghana,* [6]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Washington, DC, United States,* [7]*Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [8]*Armed Forces Pest Management Board, Silver Spring, MD, United States*

Since 2008, Ghana has implemented indoor residual spraying (IRS) in the Northern region, where a 48% decrease in parasite prevalence was observed from 2011 to 2016 along with significant reductions in entomological indices of malaria transmission. However, preference of *Anopheles gambiae* s.l. for outdoor biting has also been observed. To better understand how mosquito outdoor behavior relates to the dynamics of malaria transmission, we investigated the feeding and resting activity of *An. gambiae* s.l. in selected sprayed and unsprayed rural areas of Northern Ghana, during the rainy and dry seasons of 2017 and 2018. We collected mosquitoes monthly using prokopack aspiration and used enzyme-linked immunosorbent assay to measure human blood index (HBI). *An. gambiae* s.l. (93%) was the predominant species, primarily collected from places outside of sleeping rooms in both sprayed (94.9%) and unsprayed (82.7%) areas (P<0.001). Of those mosquitoes collected outside sleeping rooms, most were caught in animal shelters; 89% in sprayed and 77% in unsprayed areas (P<0.001). The indoor resting density was 0.17 and 0.95 mosquitoes per room per day for sprayed and unsprayed houses respectively (p<0.05). *An. gambiae* s.l. was further identified by polymerase chain reaction (PCR) as *An. gambiae* (81%), *An. coluzzii* (18 %) and *An. arabiensis* (1%). The HBI was lower in the sprayed areas (82%) compared to the unsprayed areas (94%) (p=0.001). Mortality of mosquitoes collected alive from non-sleeping shelters and held for 24hrs was 55% and 13% in IRS and unsprayed areas, respectively (p<0.05); indicating that even some of the mosquitoes resting outdoors have been

exposed to the insecticides on sprayed surfaces or nets indoors. Lower HBI of *An. gambiae* s.l. in sprayed compared to unsprayed areas may indicate a slight shift towards zoophagy or higher mortality of human blood fed mosquitoes in sprayed areas, possibly due to IRS. *An. gambiae* s.l. is still predominantly anthropophilic. These findings suggest that further interventions, in addition to IRS, may be required to target outdoor resting mosquitoes and achieve further reductions in malaria transmission.

1916

THE IMPORTANCE OF COPY NUMBER VARIATION IN METABOLIC INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE*

Lizzie Bridget Tchongwe[1], Eric Lucas[2], Martin Donnelly[2]

[1]*Malawi Liverpool Wellcome Trust, Blantyre, Malawi,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Insecticide based interventions have significantly contributed to the reduction of malaria, one of the leading causes of death in sub-Saharan Africa. However, insecticide resistance is a threat to this success due to intensified use of the available insecticides. Target site and metabolic resistance are the key mechanism that mosquitoes use to evade the insecticide. Genetic variations in the *Anopheles gambiae* genome are important in increased resistance as evidenced in target site mutations. However, genetic variations such as copy number variations (CNVs) have been poorly studied and their role poorly understood in metabolic resistance. The aim of this study was to identify metabolic resistance genes that exhibit copy number variation and their significance in metabolic resistance. Pyrethroid phenotyped samples from Malawi (59) and Kenya (354) were screened for the presence of a specific *Cyp6aa1* duplication known to exist in East Africa (*Cyp6aa1*-Dup1) with conventional PCR and *Cyp6aa1* duplications general with qPCR. CNV discovery was done using the *Anopheles gambiae* 1000 genomes phase 3 data from Uganda and Tanzania, focusing on known metabolic resistance genes; *Cyp6aa1*, *Gstes* and *Cyp9k1*. A high frequency of *Cyp6aa1*-Dup1 was found in both dead and alive phenotypes. A total of 177 (43 %) individuals had the duplication, 137 (33 %) did not have a duplication and 99 (24 %) were dropouts. The *Cyp6aa1*-Dup1 duplication was significantly associated with the resistance phenotypes. The CNV discovery using the *Anopheles gambiae* 1000 genomes phase 3 data found high CNV frequencies in the candidate genes screened. *Cyp6aa1*-Dup1, so far only known to exist as a duplication, was found to exist as a triplication in the Tanzania data, and a new duplication was found on *Gste2* in two individuals from Uganda. These results have shown an importance of gene duplication in pyrethroid resistance, as well as high frequency of CNVs in the three metabolic resistance genes. More work should be done to functionally validate *Cyp6aa1* in *Anopheles gambiae* and understand its role in insecticide resistance and as a diagnostic marker in metabolic insecticide resistance.

1917

USE OF GRAVID OVIPOSITION STICKY (GOS) TRAP AND DENGUE NON-STRUCTURAL 1 (NS1) ANTIGEN TEST FOR EARLY SURVEILLANCE OF DENGUE AMONG *AEDES* MOSQUITOES TO REDUCE DENGUE OUTBREAK

Jonathan Wee Kent Liew, Sivaneswari Selvarajoo, Wing Tan, Indra Vythilingam

University of Malaya, Kuala Lumpur, Malaysia

Dengue is a global disease, transmitted by *Aedes* mosquitoes. The current dengue vector surveillance/control in many nations, including Malaysia are riddled with flaws, being delayed, reactive and non-relevant. These are likely reasons for the struggle in controlling dengue epidemics. Although novel control techniques (eg *Wolbachia* to control *Aedes* populations) have great potential, these are still under trial. Urgent and effective strategies for vector surveillance/control are required pending their results. Previous studies showed that gravid oviposition sticky (GOS) traps with use of the dengue NS1 test is able to detect dengue in trapped adult *Aedes*. Subsequently, dengue cases were found to occur 1 week after detection of infected mosquitoes, with a peak lag of 2 – 3 weeks. Evidently, this method is a cheap (less than USD 0.60 per trap) and effective way for early dengue surveillance. The next phase is to determine if this early and pro-active method of surveillance is able to reduce dengue cases. Thus, a cluster, randomized controlled trial of 18 months has been initiated since October 2018 in Selangor, Malaysia. Eight apartments were randomly assigned into intervention (3686 residential units) and control (4203 residential units) arms. The primary outcome concerns the reduction of dengue cases and dengue outbreak period in the intervention arm. GOS traps were set at the intervention apartments to collect *Aedes* weekly, following which dengue NS1 antigen were detected in these mosquitoes. When a positive mosquito was detected, the communities were alerted and advised to execute vector search-and-destroy and protective measures. As of March 2019, 9 dengue NS1-positive *Aedes* pools have been found in separate weeks in the intervention arm. The trial is ongoing, and the number of dengue cases will be obtained from the District Health Office, for further analysis to achieve the primary outcome with preliminary results available in September 2019. We foresee this intervention to reduce dengue cases in the study arm and be feasibly implemented as part of the dengue control program.

1918

APPLICATION OF SPATIAL ANALYSIS METHODS TO IDENTIFY AND EXPLAIN INSECTICIDE RESISTANT CLUSTERS OF *AEDES AEGYPTI* MOSQUITOES IN FLORIDA

Stephanie J. Mundis

University of Florida, Gainesville, FL, United States

Insecticide resistance can lead to vector control failure, disease resurgence, and wasted time and resources spent applying ineffective treatments. While insecticides are widely used in Florida, monitoring resistance status can be costly and challenging, meaning resistance may go undetected. This study examined the resistance status of *Aedes aegypti*, using genetic data from the V1016I and F1534C *loci* from populations sampled at 59 sites across 18 counties in Florida. The objectives of this study were to identify statistically significant clusters of resistant and susceptible genotypes and test for associations between landscape factors and resistance frequencies. We employed a SaTScan analysis and a linear regression model selection framework. We found urban areas along the western coast of the state had high frequencies of the resistant genotype, though locations with higher than expected frequencies of susceptible genotypes were also identified along both coasts. The clusters of susceptible mosquito populations likely represent "refuges," which could be important in re-establishing susceptible populations. The linear regression models indicated that landscape characteristics including distance from agricultural land cover and the enhanced vegetation index had statistically significant associations with the outcome of insecticide resistance. These results indicate that spatial factors could be used to predict and potentially manage insecticide resistance in the field.

1919

WIDESPREAD ANTIBIOTIC USE AMONG SUSPECTED ENTERIC FEVER CASES IN NEPAL, BANGLADESH AND PAKISTAN

Krista Vaidya[1], Kristen Aiemjoy[2], Farah N. Qamar[3], Samir K. Saha[4], Caitlin Barkume[5], Denise Garrett[5], Stephan P. Luby[6], Jason R. Andrews[6]

[1]*Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Nepal,* [2]*Stanford University, San Francisco, CA, United States,* [3]*Aga Khan University, Karachi, Pakistan,* [4]*Dhaka Shishu (Children) Hospital, Dhaka, Bangladesh,* [5]*Sabin Vaccine Institute, Washington, DC, United States,* [6]*Stanford University, Stanford, CA, United States*

Enteric fever, caused by *Salmonella Typhi* and *Paratyphi*, is a major cause of morbidity globally. Antibiotic use prior to seeking care can reduce the sensitivity of blood culture for enteric fever, with implications for both clinical care and surveillance. Moreover, easy access and indiscriminate use of antibiotics prior to formal care is likely a major contributor

to the emerging threat of antimicrobial resistance. Antibiotic use is typically measured via self or caregiver report and may be vulnerable to measurement error. The Surveillance of Enteric Fever in Asia Project is a prospective study of enteric fever incidence in Nepal, Bangladesh and Pakistan. Among a subset of SEAP participants, we measured *in vitro* inhibition of growth of pan-susceptible bacteria (*Kocuria rhizophila*) as an assay for antibiotic presence in urine and inquired about antibiotic use prior to seeking care at a study hospital. All participants received a blood culture. We calculated the sensitivity and specificity of reported antibiotic use against a reference standard of antibiotics detected in the urine using binomial logit models. We enrolled 2,916 patients with suspected enteric fever over two years, from 2016 to 2018. Antibiotics were detected in 256/1000 (26.5%) of urine samples collected in Nepal, 337/910 (37.0%) of samples in Bangladesh, and 468/882 (53.1%) of samples in Pakistan. The sensitivity and specificity of reported antibiotic use, compared with the urine assay, were 79% and 80% in Bangladesh and Nepal; and 58% and 67% in Pakistan. Individuals who were culture positive for enteric fever were more likely to have antibiotics detected in the urine (RR 1.3; 95%CI 1.17, 1.48), likely because antibiotic use is an indicator of disease severity. The widespread pre-hospital antibiotic use documented in this study has implications for clinical care, enteric fever surveillance and public health. Relying on patient or caregiver-reported antibiotic use may not be sufficiently valid. High levels of pre-hospital antibiotic use may reduce the sensitivity of blood culture and thus underestimate the true burden of enteric fever.

1920

INTEGRATING TRADITIONAL MICROBIOLOGY WITH CUTTING-EDGE METAGENOMICS TO ADVANCE PATHOGEN DETECTION AND ELUCIDATE MICROBIOME SIGNATURES OF *E. COLI* INFECTION

Karen Levy[1], Angela Pena-Gonzalez[2], Maria J. Soto-Girón[2], Shanon Smith[1], Jeticia Sistrunk[1], Lorena Montero[3], Maritza Paez[3], Estefanía Ortega[3], Janet K. Hatt[4], William Cevallos[5], Gabriel Trueba[3], Konstantinos T. Konstantinidis[4]

[1]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [2]*School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, United States,* [3]*Instituto de Microbiologia, Universidad San Francisco de Quito, Quito, Ecuador,* [4]*School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA, United States,* [5]*Centro de Biomedicina, Universidad Central del Ecuador, Quito, Ecuador*

Escherichia coli infectious diarrhea is an important contributor to child mortality worldwide. While important advances have been made in characterizing the virulence mechanisms of pathogenic *E. coli*, understanding of the compositional and functional alterations in the intestinal microbiome during active infections is still limited. For example, it remains unknown how alterations in the gut microbiome vary by pathotypes with different virulence mechanisms, and whether these metagenomic signatures can be used for diagnostic purposes. Here, we present an integrative methodology that combined culture and PCR techniques with metagenomics and epidemiology to identify cases of diarrhea where *E. coli* was most likely the causative disease agent and to evaluate specific signatures in the disease-state gut microbiome that distinguish between DAEC, ETEC and EPEC pathotypes. Application of this methodology to Ecuadorian children with diarrhea showed that ~50% of the cases where an *E. coli* pathotype was detected through culture/PCR were likely not caused by *E. coli*, based on low relative abundance, level of clonality and/or virulence gene content within the metagenome. Those diarrhea samples where *E. coli* was most likely the causative agent had higher abundance of the pathogenic isolate compared to controls or commensal *E. coli*, high prevalence of virulence factors and/or enterotoxins, including the metagenomic detection of the pathotype-specific marker gene, and a reduced *E. coli* intra-population diversity compared with control samples. Our results also revealed at least four species that discriminated DAEC from ETEC infections. At the functional level, no major differences were detected. DAEC infections were accompanied by co-elution of high amounts of human DNA and conferred significant shifts in the composition of the gut microbiome relative to controls without diarrhea or ETEC infections. Our results demonstrate that metagenomic approaches can provide a high resolution approach for the detection of the etiologic agent of diarrhea and a better understanding of the disturbance of the gut microbiome caused by enteric infections.

1921

HOST GENOME-WIDE ASSOCIATION STUDY OF *SHIGELLA*-ASSOCIATED DIARRHEA IN A BIRTH COHORT OF BANGLADESHI INFANTS

Dylan Duchen[1], Rashidul Haque[2], Genevieve Wojcik[3], Laura Chen[1], Poonum Korpe[1], Beth Kirkpatrick[4], William A. Petri[5], Priya Duggal[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Stanford University, Stanford, CA, United States,* [4]*University of Vermont, Burlington, VT, United States,* [5]*University of Virgina, Charlottesville, VA, United States*

Shigella is a leading cause of moderate-to-severe diarrhea in African and South Asian children and the causative agent of shigellosis and dysentery. Associated with 80 - 165 million cases of diarrhea and up to 600,000 deaths annually, exposure to shigella is ubiquitous in many regions while colonization or infection is heterogenous. To characterize the host-genetic susceptibility to shigella-associated diarrhea, we performed two independent genome-wide association studies (GWAS) including 589 Bangladeshi infants, 429 from the PROVIDE birth cohort and 160 infants from a Cryptosporidium-focused study birth cohort in Dhaka, Bangladesh. We classified children as ever having shigella associated diarrhea or not in the first 13 months of life. A qPCR Ct distribution of the ipaH gene, carried by all four shigella species and enteroinvasive E. coli, identified a total of 143 infants with a shigella-associated diarrheal event and 446 infants with no evidence of shigella-associated diarrhea within their first 13 months of life. Host GWAS's were performed using the Illumina Infinium 5 Multiethnic Global Array and analyzed under an additive genetic model. A joint analysis (imputed variants n=6,547,362) identified *loci* of interest on chromosomes 11 (rs582240, within the *KRT18P59* pseudogene, average MAF=29.4%, $p=8.37x10^{-8}$) and 8 (rs12550437, within the lincRNA *RP11-115J16.1*, average MAF=38.1%, $p=1.69x10^{-7}$). This study suggests host genetic factors may influence the response to shigella colonization and pathogen-associated diarrhea. Additional replication and further research on the function of these genes and their association with shigellosis and other pathogen-associated diarrheal diseases is warranted.

1922

EPIDEMIOLOGY OF *SHIGELLA* INFECTIONS AND DIARRHEA IN THE FIRST TWO YEARS OF LIFE USING CULTURE-INDEPENDENT DIAGNOSTICS IN THE MAL-ED STUDY

Najeeha Iqbal[1], Elizabeth T. Rogawski McQuade[2], Arjumand Rizvi[1], Fariha Shaheen[1], Furqan Kabir[1], James A. Platts-Mills[2], Fatima Aziz[1], Adil Kalam[1], Shahida Qureshi[1], Jie Liu[2], Aldo A. Lima[3], Gagandeep Kang[4], Amidou Samie[5], Rashidul Haque[6], Estomih R. Mduma[7], Margaret N. Kosek[2], Jose Paulo Leite[8], Ladaporn Bodhidatta[9], Nicola Page[10], Ireen Kiwelu[11], Tahmeed Ahmed[6], Eric R. Houpt[2], Zulfiqar Bhutta[1]

[1]*Aga Khan University, Karachi, Pakistan,* [2]*University of Virginia, Charlottesville, VA, United States,* [3]*Federal University of Ceara, Fortaleza, Brazil,* [4]*Christian Medical College, Vellore, Vellore, India,* [5]*University of Venda, Thohoyandou, South Africa,* [6]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [7]*Haydom Global Health Institute, Haydom, United Republic of Tanzania,* [8]*Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil,* [9]*Armed Forces Research Institute of*

Medical Sciences (AFRIMS), Bangkok, Thailand, [10]National Institute for Communicable Diseases, Johannesburg, South Africa, [11]Kilimanjaro Clinical Research Institute, Moshi, United Republic of Tanzania

The use of culture-independent diagnostics has revealed a larger burden of *Shigella* diarrhea and *Shigella*-associated linear growth decrements among children in low-resource settings than previously recognized. We further characterized the epidemiology, risk factors, and seasonality of *Shigella* in the first two years of life in the multisite longitudinal birth cohort, MAL-ED. We tested 41,405 diarrheal and monthly non-diarrheal stools for *Shigella* by quantitative PCR and culture. To identify risk factors, model seasonality, and assess culture positivity, we used mixed effects log-binomial regression with a random effect for individuals. The prevalence of *Shigella* varied from 4.9%-17.8% in non-diarrheal stools across the 8 sites, and the incidence of *Shigella*-attributable diarrhea was 22.9 cases (95% CI: 21.3, 24.6) per 100 child-years (range 2.2-68.4 across sites). Among approximately half of *Shigella*-attributable diarrheal stools that could be typed to *S. flexneri* or *S. sonnei*, 60.6% were *S. flexneri* and 44.7% were *S. sonnei*. The sensitivity and specificity of culture compared to qPCR were 6.6% and 99.8%, respectively. Sensitivity increased to 17.1% in the subset of *Shigella*-attributable diarrheal stools and to 27.8% in *Shigella*-attributable dysentery. PCR-positive stools from younger children were less likely to be culture positive. Older age (RR: 1.73, 95% CI: 1.68, 1.78 per 6-month increase in age), unimproved sanitation (RR: 1.19, 95% CI: 1.06, 1.35), low maternal education (<10 years, RR: 1.18, 95% CI: 1.07, 1.30), less than 3 months of exclusive breastfeeding (RR: 1.12, 95% CI: 1.02, 1.22), and malnutrition (RR: 0.92, 95% CI: 0.89, 0.96 per unit increase in weight-for-age z-score) were risk factors for *Shigella* infection. *Shigella* was highly seasonal in some sites, such as a peak in December/January in Tanzania and a peak in March/April in the South Asian sites. The burden of *Shigella* varied widely across sites, but uniformly increased through the second year of life. Culture missed most clinically relevant cases of severe diarrhea and dysentery, signaling the critical inadequacy of culture and necessity of molecular methods.

1923

IDENTIFICATION OF HOUSEHOLD RESERVOIRS AND TRANSMISSION PATHWAYS ASSOCIATED WITH *SHIGELLA FLEXNERI* DIARRHEA AMONG CHILDREN FROM THE MIRZAPUR, BANGLADESH SITE OF THE GLOBAL ENTERIC MULTICENTER STUDY

Kurt Z. Long[1], AGS Faruque[2], Inong R. Gunanti[3], Johanna Sanchez[3], James P. Nataro[4], Dilruba Nasrin[5], Myron Levine[6], Karen Kotloff[7]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh,* [3]*Faculty of Medicine and Biomedical Sciences, University of Queensland, Brisbane, Australia,* [4]*Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA, United States,* [5]*Center for Vaccine Development, Baltimore, MD, United States,* [6]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States,* [7]*Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD, United States*

Rural communities in Bangladesh have some of the highest rates of shigellosis reported among young children. Identification of environmental reservoirs and transmission pathways for *Shigella flexneri* in these communities can aid in the development of more effective intervention strategies to reduce this burden. Bayesian path analyses were used to model the effect of household pathogen transmission pathways on the risk of *Shigella* infections among children enrolled in the Bangladeshi component of the Global Enteric Multicenter Study (GEMS). Models were developed to test whether such potential pathogen reservoirs as water source and treatment, sanitation facilities and dirt flooring were associated directly with greater *Shigella* infections or had indirect effects mediated by hygiene behaviors. There were 430 isolates of *S. flexneri* identified in stools collected from the 3,859 children enrolled in Mirzapur, Bangladesh. The disposal of a child's feces in a latrine or toilet was significantly associated with a higher risk of *Shigella* diarrhea compared to disposal in the bush while having a concrete or tile floor was associated with a reduced risk. A further positive association of feces disposal in a latrine was indirectly associated with a higher risk when the latrine was unimproved. Children from households with unimproved sanitation facilities had a significantly lower risk of diarrhea when their caretakers washed hands before cooking while no effect of handwashing was found in households with improved sanitation. Deep tube wells were positively associated with *Shigella* diarrhea directly and indirectly through unimproved sanitation but this indirect association was reduced when handwashing before cooking was reported. Water treatment also had an indirect positive association through unimproved sanitation but this indirect association was again reduced with reported handwashing before cooking. These results suggest that handling and disposal of feces can lead to greater transmission of *Shigella* directly and indirectly through water sources but that specific handwashing behaviors can block such transmission.

1924

DEVELOPMENT OF A MULTIPLEXED *SHIGELLA*-SPECIFIC BACTERICIDAL ASSAY

Hailey Petersen Weerts, Akamol E. Suvarnapunya, Robert W. Kaminski

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Shigella is a leading cause of diarrhea-associated global morbidity and mortality in children under the age of 5. Developing a *Shigella* vaccine has been complicated because there are multiple serotypes of *Shigella,* and previous infection provides protection from subsequent infection only in a serotype-specific manner. Current epidemiological data suggest that a quadrivalent vaccine will be required to protect against 80% of global disease caused by *Shigella*. A multivalent vaccine will require assessment of immune responses directed to multiple *Shigella* serotypes and, as the target population for vaccination is children under 5, sample volume will be a limiting factor. Multiplexed assays will therefore play an essential role in assessment of immune responses in vaccine field trials. A multiplexed *Shigella*-specific bactericidal assay (SBA) is under development to allow for high-throughput analysis of functional antibody responses while also conserving sample volumes. To develop this multiplexed SBA, *S. flexneri* 2a, *S. flexneri* 3a, and *S. sonnei* strains were transformed with plasmids containing GFPuv, iRFP670, or mRuby2, and frozen bacterial stocks prepared. Bactericidal assays were conducted with modifications to previously published protocols by incubating all three transformed *Shigella* strains, monoclonal or polyclonal antibodies, and exogenous baby rabbit complement in 96 well plates. Plates were subsequently incubated at 37°C for two hours, transferred onto PVDF filter bottom 96 well plates and incubated overnight to allow for bacterial growth. Fluorescent colonies were detected and enumerated using a CTL Immunospot S6 Analyzer. Monoclonal antibodies were used to develop appropriate parameters, controls, and bacterial growth conditions. This multiplexed SBA allows for the simultaneous measurement of bactericidal activity against three *Shigella* serotypes, ultimately conserving time, reagents, and sample. As the field moves towards evaluating multivalent *Shigella* vaccines in target populations, multiplexed assays will become increasingly necessary to evaluate immune responses induced after immunization.

1925

SIGNIFICANCE OF DIARRHEAL DISEASES TO UNDER-FIVE MORTALITY AND DIAGNOSTIC VALUE OF RECTAL SWABS IN CHILDREN WITH FATAL DIARRHEAL DISEASES IN SUB-SAHARAN AFRICA

Portia Mutevedzi, Richard Chawana, Shabir Madhi

RMPRU, Johannesburg, South Africa

Diarrheal disease is the second leading under-five mortality cause worldwide. To achieve the World Health Organisation (WHO) sustainable development goal for child mortality of at most 25 deaths per 1000 live births by 2030, it is imperative to implement targeted and contextually

effective morbidity and mortality prevention strategies as well as ensure early disease specific diagnosis. Our study aimed to quantify under-five mortality attributable to diarrheal disease and assess the diagnostic value of rectal swabs collected as part of the Child Health and Mortality Prevention Surveillance (CHAMPS). CHAMPS network procedures involved minimally invasive tissue sampling (MITS) within 24-36 hours of death in children aged 1-59 months, between 18 January 2017 and 1 June 2018 in Soweto, South Africa (one of the 7 CHAMPS sites). Microbiological culture and/or molecular tests were performed on lung, blood, cerebrospinal fluid, nasopharyngeal swab and stool samples. The causes of death (CoD) were determined for each case by a multi-disciplinary panel of 12-15 specialists. A total of 365 deaths in children aged 1-59 months were evaluated; 221 (60.5%) were males, majority 248 (67.9%) were neonates whilst 44 (12.1%) were aged 1-5 years. Diarrheal related deaths accounted for 13% of child deaths; 14 (29.1%) classified as immediate, 14 (29.1%) morbid and 20 (41.6%) underlying respectively. Twenty-two (6%) of the total deaths presented with diarrhea for a median of 4 days (IQR: 2-7days); 2 of whom had persistent diarrhea as underlying CoD. Of the 248 neonatal deaths, 4 (1.6%) had listeriosis in the causal chain. E.coli was detected in stool samples from 5 (10.4%) children whose immediate or underlying CoD was classified as acute gastroenteritis whilst in one death classified as dysentery both E.coli and Shigella were isolated. Rotavirus A was detected in 3 children whose underlying CoD was considered to be diarrheal related. Our study highlights significant under-five preventable mortality attributable to diarrheal disease which may be successfully diagnosed through rectal swabs.

1926

WUCHERERIA BANCROFTI CIRCULATING FILARIAL ANTIGEN EXHIBITS DIFFERENT LECTIN-BINDING SPECIFICITY AND GREATER PROTEASE RESISTANCE COMPARED TO OTHER FILARIAL GLYCOPROTEINS

Marla Hertz[1], Amy Rush[2], Philip Budge[2]

[1]*Washington University in St. Louis, St. Louis, MO, United States,* [2]*Washington University in St. Louis, St Louis, MO, United States*

The Global Program for the Elimination of Lymphatic Filaraiasis relies on rapid diagnostic tests (RDTs) for LF that detect a ~200-250 kDa circulating *W. bancrofti* antigen via a carbohydrate epitope that is recognized by the monoclonal antibody AD12. This epitope is present on many glycoproteins of other filarial nematodes, but little is known about its structure and function. Nor is it clear why the *W. bancrofti* circulating filarial antigen (Wb-CFA) is persistently present in infected individuals, while antigens containing this epitope are not detected in individuals with brugian filariasis and are only detected in a subset of patients with loiasis. We sought to improve our understanding of this epitope and the differences between Wb-CFA and other filarial antigens decorated with it. Binding to a lectin array showed that AD12 epitope-containing proteins from *B. malayi* are captured by many lectins, while the Wb-CFA was not well captured, suggesting that a variety of other glycans are present on epitope-containing *Brugia* glycoproteins, but not on the Wb-CFA. Mass spectrometry analysis of *B. malayi* AD12-epitope containing proteins identified many of the same antigens present in cross-reactive loiasis sera. We found that the AD12 epitope is not removed from Wb-CFA or other filarial antigens by PNGase F digestion, nor by several other commercially available glycosidases. Wb-CFA was also more resistant to several proteases than AD12 glycoproteins of *B. malayi*, including trypsin, pronase, lys-C and O-sialoglycoprotein endopeptidase. Preliminary observations indicate that treatment of bancroftian filariasis releases additional AD12 glycoproteins of lower molecular weight that Wb-CFA; these transiently released *Wuchereria* antigens are also more susceptible to protease digestion that the high molecular weight Wb-CFA. The inherent stability of the Wb CFA may explain how it is consistently detected in sera while other AD12-eptiope containing proteins are more readily lost.

1927

POTENTIAL OF CYTOSOLIC AND ENDOSOMAL PRR-AGONISTS IN IMPROVING VACCINATION EFFICACY AGAINST THE FILARIAL NEMATODE *LITOMOSOIDES SIGMODONTIS*

Johanna F. Scheunemann, Frederic Risch, Julia J. Reichwald, Alexandra Ehrens, Marianne Koschel, Achim Hoerauf, Christoph Coch, Beatrix Schumak, Marc P. Hübner

University Hospital Bonn, Bonn, Germany

Current efforts to eliminate human filarial infections are hampered by the lack of protective vaccination approaches. Here we investigated the potential of cytosolic vs. endosomal PRR-ligands to boost the efficacy of anti-filarial vaccination. *In vitro* studies with human peripheral blood mononuclear cells revealed that RNA and DNA of the rodent filarial nematode *Litomosoides sigmodontis* but not human RNA or DNA trigger the release of pro-inflammatory cytokines. Cytosolic RNA delivery in human PBMC induced a strong IFNα release, while endosomal delivery led to TNF and IL-1β secretion. This correlated with monocyte activation as indicated by significantly increased expression of MHC class II and CD80. In order to test *in vivo* whether different nucleic acid receptor agonists improve vaccination efficacy using irradiated *L. sigmodontis* L3 larvae, BALB/c mice received 3 subcutaneous injections in 2-week intervals with irradiated L3 larvae in combination with different agonists prior to a challenge infection via the natural mite vector. The vaccination approach using irradiated L3 larvae alone reduces the adult worm burden by 80%. First results indicate that subcutaneous injection of 3pRNA (RIG-I agonist), naked poly (I:C) (TLR3 ligand) or 2'3'cGAMP (STING agonist) within 4h induced the recruitment of immune cells, in particular neutrophils, which were previously shown to mediate protection against invading L3 larvae. Further, 2'3'cGAMP triggered upregulation of co-stimulatory molecule CD86 on myeloid cells. For all agonists this was accompanied by elevated local CXCL-10 mRNA levels (skin), but minor changes in systemic CXCL-10 levels. Ongoing experiments address the impact of a combined vaccination with irradiated L3 larvae with different cytosolic and endosomal PRR ligands on the adult worm recovery, microfilariae release, generation of *L. sigmodontis*-specific antibodies, systemic cytokine and chemokine levels and immune cell activation. We hypothesize that an activation of nucleic acid receptors boosts the elicited immune response and triggers protective immune responses against invading L3 larvae.

1928

LYMPHATIC FILARIASIS ELIMINATION IN SAMOA: EVALUATING THE USE OF MOLECULAR XENOMONITORING AS A SURVEILLANCE TOOL

Brady McPherson[1], Sarah Sheridan[2], Kei Owada[3], Take Naseri[4], Robert Thomsen[4], Tautala Mauala[5], Helen Mayfield[1], Lisa Rigby[6], Silvia Ciocchetta[3], Julia Maguire[1], Nils Pilotte[7], Andrew M. Gonzalez[7], Steven A. Williams[7], Katherine Gass[8], Patricia M. Graves[9], Colleen L. Lau[1]

[1]*Australian National University, Canberra, Australia,* [2]*University of New South Wales, Sydney, Australia,* [3]*University of Queensland, Brisbane, Australia,* [4]*Samoa Ministry of Health, Apia, Samoa,* [5]*Samoa Red Cross, Apia, Samoa,* [6]*Queensland Institute of Medical Research, Brisbane, Australia,* [7]*Smith College, Northampton, MA, United States,* [8]*Task Force for Global Health, Atlanta, GA, United States,* [9]*James Cook University, Cairns, Australia*

The World Health Organization recently recommended triple drug mass drug administration (ivermectin, diethylcarbamazine, albendazole: IDA) in areas with slow progress towards lymphatic filariasis (LF) elimination. The 2018 'Surveillance and Monitoring to Eliminate Lymphatic Filariasis and Scabies from Samoa' project consisted of human and mosquito surveys to identify the best indicators for determining when LF transmission has been interrupted by IDA. To assess the value of molecular xenomonitoring (MX) as a surveillance tool, we investigated village-level associations between the presence of PCR-positive mosquitoes for *Wuchereria bancrofti* DNA (PCR+) and circulating filarial antigen (Ag) in humans. Mosquitoes were

collected from 28 randomly selected villages using BG Sentinel traps, and sorted into 4 categories: *Aedes polynesiensis* (the reported main vector in Samoa), *Ae. (Finlaya) spp*, other *Aedes spp*, and *Culex spp*. Pools of ≤25 mosquitoes of each category were tested using PCR. Analyses were conducted for each category and for 'all species combined'. Of 475 pools tested (29% *Ae. polynesiensis*, 7% *Ae. (Finlaya) spp,* 22% other *Aedes spp,* 42% *Culex spp*; total 8506 mosquitoes), 18.1% were PCR+. Pools with the highest positivity rate were other *Aedes spp* (29.5%) and *Ae. polynesiensis* (24.3%). Infection prevalence (maximum likelihood estimate using Poolscreen 2) was 1.1% (95% CI 0.9-1.4%) for 'all species', 2.0% for other *Aedes spp,* and 1.5% for *Ae. polynesiensis*. The 'all species' category proved most sensitive for detecting villages with Ag-positive humans, with PCR+ pools identified in 81.8% (18/22) of these villages, compared to 50.0% sensitivity with *Ae. polynesiensis*. Presence of PCR+ *Ae. polynesiensis* pools provided the best predictor of villages with Ag-positive people with a positive predictive value (PPV) of 78.6% (11/14) followed by the 'all species' category with a PPV of 78.3% (18/23). Our study provides promising evidence to support the value of MX in post-MDA surveillance, even if there is no entomological capacity to sort mosquitoes by species. Further work in 2019 will explore the use of MX to assess the impact of IDA.

1929

DIAGNOSTIC PERFORMANCE OF ELISA, RAPID DIAGNOSTIC TESTS AND MULTIPLEX BEAD ASSAY TO DETECT ONCHOCERCIASIS OV-16 IGG4 ANTIBODY REACTIVITY USING SAMPLES FROM A FORMERLY ENDEMIC AREA

Marisa Hast[1], Oscar de Leon[2], Circe McDonald[1], Renata Mendizabal de Cabrera[2], Alison Golden[3], Paul Cantey[1], Vitaliano Cama[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Universidad del Valle de Guatemala, Guatemala City, Guatemala,* [3]*PATH, Seattle, WA, United States*

The World Health Organization has published guidelines for endemic countries to verify the elimination of onchocerciasis transmission. The optimal strategy for post-elimination surveillance remains unclear, but an accurate serologic assay will be needed. We compared the relative performance of diagnostic assays to detect onchocerciasis OV-16 IgG4 antibodies in a formerly endemic area: a multiplex bead assay (MBA), an SD BIOLINE rapid diagnostic test (RDT), and an alkaline phosphatase (AP) enzyme-linked immunosorbent assay (ELISA) developed in Guatemala. Using sera and dried blood spots (DBS) collected in 2014 from 209 participants in a post-endemic focus in Guatemala, we compared overall and positive agreement for five OV-16 detection methods: AP ELISA, RDT on performed on stored sera and read at 20 minutes (wet), RDT read the next day (dry), and an MBA on both sera and DBS. We calculated MBA cutoffs using receiver operating characteristic (ROC) analyses on a panel of 86 known positive and 454 negative samples. To calculate positive agreement, sera MBA and the wet RDT were the references where applicable. The MBA was positive in 60.3% of sera samples and 43.8% of DBS samples, with 79.4% overall agreement and 68.3% positive agreement. The wet and dry RDTs were positive in 24.6% and 25.9% of samples, respectively, with 96.9% overall and 95.7% positive agreement. The AP ELISA was positive in only 7.2% of samples. Overall and positive agreement between the sera MBA and dry RDT were 63.3% and 40.7%, respectively, and were 46.6% and 11.4% between the sera MBA and AP ELISA. Of five methods for measuring OV-16 IgG4 serology for onchocerciasis, MBA using sera had the highest positivity, followed by MBA using DBS, dry RDT, wet RDT and AP ELISA. These results suggest that MBA assays may be the most sensitive diagnostic tool for post-elimination surveillance. The potential to incorporate MBA OV-16 detection into integrated disease serosurveillance efforts should be evaluated further. Additional research is needed to investigate the causes of disparities between OV-16 detection methods and to further understand the performance of the MBA assay.

1930

ENTOMOLOGICAL SURVEILLANCE GUIDED PARASITOLOGICAL SURVEILLANCE, AN EFFECTIVE POST-ELIMINATION STRATEGY TO CLEAR LAST FEW LYMPHATIC FILARIASIS CASES IN SRI LANKA

Indeewarie Eranga Gunaratna[1], Dammika de Mel[1], Manjula W. Punchihewa[2], Isuri C. Wijethunga[3], Tharanga D. Dassanayake[1], Lakmini K. Liyanage[1], Wimal J. Migelhewa[2], Sameera R. Meegahapalage[1], Devika Mendis[1]

[1]*Anti Filariasis Campaign, Colombo* [05]*, Sri Lanka,* [2]*Regional Anti-Filariasis Unit, Galle, Sri Lanka,* [3]*Office of Medical Officer of Health, Balapitiya, Sri Lanka*

Microfilaria rate in Sri Lanka was less than 0.5% since 1981. This was further reduced to 0.05% with five rounds of Mass Drug Administration (MDA) in all eight endemic districts during 2002-2006 and three rounds in the highest risk Galle district during 2014-2016. Sri Lanka was certified as a country eliminated filariasis as a public health problem in 2016. Anti Filariasis Campaign (AFC) introduced a new post-elimination strategy aiming to reach total elimination of filariasis by 2021; entomological surveillance guided parasitological surveillance with house to house night blood film (NBF) screening in 2016 to detect reservoir infection who contribute to continuous transmission. To assess the effectiveness of the new strategy, it was piloted in two high risk health administrative areas; Balapitiya and Habaraduwa in Galle district in 2018. High risk localities within selected areas to carryout NBF were identified by positivity of gravid traps. Total of 672 pools (25 mosquitoes per pool) were obtained from 336 traps were subjected to Real Time Polymerase Chain Reaction. Maximum likelihood filarial DNA rate was 0.64%, CI 0.46-0.85 (trap positivity rate 22.2%, pool positivity rate 33.3%). All trap locations were mapped and areas to be screened around positive trap sites were marked for human survey. NBF screening programme was organized along with community mobilization (advocacy, house to house messaging, school programmes, community awareness through media) within 200m radius of positive trap sites. Thick blood smears were prepared for microfilaria detection by microscopy. Total of 51 patients were identified from 31,370 people screened (44% males) from all households within selected areas. Microfilaria rate was 0.16%. Majority (88%) of patients were males (microfilaria rate is 0.32%) and 78% were >50 years of age (microfilaria rates 0.29%). Results suggest that screening should focus on males and older people. This strategy can be used as an end game strategy with higher coverage to plan out focused high risk screening to detect hidden cases and effective treatment and recommend for countries in post-elimination phase to stop transmission.

1931

TESTING A METHOD OF SAMPLING FOR ENTOMOLOGICAL DETERMINATION OF TRANSMISSION OF *WUCHERIA BANCROFTI* TO INFORM LYMPHATIC FILIARIASIS (LF) TREATMENT STRATEGY IN URBAN SETTINGS.

Rogers Nditanchou[1], Ruth Dixon[2], Benjamin Koudou[3], Dung Pam[4], Sunday Isiyaku[5], Christian Nwosu[5], Safiya Sanda[5], Elena Schmidt[2], David Molyneux[6]

[1]*Sightsavers, Yaounde, Cameroon,* [2]*Sightsavers, Haywards Heath, United Kingdom,* [3]*Centre Swisse de Recherches Scientifiques en Cote d'Ivoire, Abidjan, Côte D'Ivoire,* [4]*Department of Zoology, University of Jos, Jos, Nigeria,* [5]*Sightsavers, Kaduna, Nigeria,* [6]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

There is an on-going debate about scale-up of LF treatment to include urban areas. Determining transmission is more complex in these settings; entomological methodologies with no clear approach. Within Kaduna and Minna cities in Nigeria, we selected 3 communities with pre-disposing risk factors and LGA level TAS data indicating transmission. After mapping local breeding sites we selected 41 households in proximity to the risk factors for 5 gravid traps (GT), 15 exit traps (ET) and 21 pyrethrum spray catch sites (PSC). We collected mosquitos over 5 months corresponding

to high transmission season using a combination of entomologists and community based researchers. Mosquitos were counted, speciated and abdominal state determined on the day of collection. We targeted 10,000 mosquitos per city inclusive of 1500 *Anopheles* in order for transmission to be determined based on presence of the L3 infective stage of *Wuchereria bancrofti.* In 5 427 trapping events, we collected 36,880 mosquitos (2818 *Anopheles)*. 92% were *Culex* species. PSC were the most likely to yield *Anopheles* with a mean of 1 per trapping event compared to 0.5 for ET and 0.1 for GT. 77% of *Anopheles* came from ETs with successful *Anopheles* catches occurring later in the season. Community researchers were influential in ensuring collections were acceptable and were able to conduct collections independently. 14.5% of trapping events were impacted by householder actions, weather conditions or trap malfunction. ETs were the most cost effective way to catch *Anopheles* (6.4$ per trapping event and 12.8$ per *Anopheles* caught). Community acceptability of the methods of collection was high. Sample size for mosquitos was met though *Anopheles* catch was insufficient in one city. Findings of transmission are hard to apply as cities themselves are not implementation units and collection coverage was not city-wide. Methods need adapting to maximise *Anopheles* catch: we propose planning 250 GT and 3724 ET events in similar settings and weighting trapping events later in the rainy season. Evaluation units should be analogous with implementation units/ the units at which treatment decisions are made.

1932

DEVELOPMENT AND EVALUATION OF SCFV (SINGLE-CHAIN VARIABLE FRAGMENT) ANTIBODIES AGAINST RWB-SXP1 AND ITS IMPLICATION IN THE DIAGNOSIS OF FILARIAL SXP-1

Kaliraj Perumal, Kamatchi R, Mahalakshmi N, Prince R. Prabhu, Meenakshisundaram S

Anna University, Chennai, India

Lymphatic filariasis is one among the globally challenging neglected tropical disease. In the wake of MDA efforts to block transmission of infection in endemic areas, there is a growing need for more efficacious diagnostic tools. Hence, we have already developed various antigen/ antibody based immunodiagnostic methods using a parasite-mf-surface protein *Wb*SXP-1 identified from *Wuchereria bancrofti*. A rapid field applicable immunoassay kit for diagnosis/surveillance is currently available for screening. To augment this effort, we have also developed a functional single-chain fragment variable (scFv) antibody specific for Wb-SXP-1 antigen as an alternative to the production of complete antibodies using hybridomas. The variable heavy chain (VH) and the variable light chain (kappa) (Vκ) genes were amplified from the mouse hybridoma cell line and were linked together with a flexible linker by overlap extension PCR. The ScFv construct (Vκ-Linker-VH) was expressed and purified by IMAC. Immunoblotting and sandwich ELISA were used to analyze the antigen binding affinity of purified scFv, which also recognized recombinant and native Wb-SXP-1 antigen in microfilariae-positive patient sera. To further enhance the efficiency of the scFv against rWb-SXP1, a procedure was developed to create diverse libraries of scFv based on a single DNA framework with all the requisites for an *in vitro* protein synthesis and ribosomal display technique. Sandwich ELISA technique was devised to detect the Kd value between WbSXP1 and native scFv (His tagged). Screening by ribosome display, shows Kd value for WbSXP1 to be 10 fold lesser than the Kd value of native scFv. Reactivity with clinical samples showed significant augment ($p<0.0001$) in reactivity to MF samples with evolved scFv in comparison to wild-type scFv. This evolutionary method coupled with ribosome display has facilitated us to improve the reactivity of the scFv without diminishing the specificity.

1933

THE GLOBAL PREVALENCE OF *CYP2D6* HAPLOTYPE VARIATION AND PREDICTIONS FOR PRIMAQUINE EFFECTIVENESS IN MADAGASCAR

Estee Y. Cramer[1], Rajeev Mehlotra[1], Ernest Chan[1], Jacquelaine Bartlett[1], Rosalind Howes[2], Daniel Tisch[1], Andrea Gaedigk[3], Arsene Ratsimbasoa[4], Scott Williams[1], Peter Zimmerman[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Big Data Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [3]*University of Missouri, Kansas City, MO, United States,* [4]*National Malaria Control Programme of Madagascar, Ministry of Health, Antananarivo, Madagascar*

Plasmodium vivax is treated with primaquine (PQ) to prevent relapsing malaria. PQ must be activated during phase 1 metabolism by CYP2D6 in order to be effective. CYP2D6 has four phenotypic classifications of metabolic activity. Poor (PM) and intermediate (IM) metabolizers appear to exhibit compromised PQ-bioactivating capacity, rendering the medication not or less effective. There are currently no guidelines regarding PQ administration for patients with PM or IM status. The study population includes 2504 samples from the 1000 Genomes Project and 66 samples from Madagascar. Genome-wide haplotype data was generated by whole genome sequence (1000G) or the Illumina MEGAv1.0 SNP array (Madagascar) to perform a principle component analysis (PCA). In addition, *CYP2D6* genomic data (5kb upstream and downstream, 36 SNPs) was also analyzed for PCA. Because the haplotypes are phased in this dataset, nonfunctional (e.g. *CYP2D6*4*) and decreased function haplotypes (e.g. **9, *10, *29, *41*) can be determined. Results from the genome-wide PCA demonstrate that the genetic variation in the Malagasy population draws from both African and East-Asian population origins, consistent with migration patterns into Madagascar. The frequency of the *CYP2D6*4* allele (13.25%) is higher in the Malagasy population than in the East-Asian population (0.4%) and the African population (12%). Frequencies of *CYP2D6*9* (0%), **10* (61%), *29* (6.3%), and **41* (4.8%) alleles in the Malagasy population are in between those seen in African (0.15%, 22%, 21%, 3.6%) and East-Asian (0%, 84%, 0%, 7.5%) populations. Our results highlight the prevalence of nonfunctional and decreased activity alleles in Madagascar, which puts the population at risk of less effective or ineffective PQ treatment. Additionally, our PCA results are consistent with the migration patterns of African and East-Asian populations into Madagascar. To reach *Plasmodium vivax* elimination goals, additional guidelines regarding PQ administration should include *CYP2D6* haplotype information.

1934

FUNCTIONAL IMPLICATIONS OF *PLASMODIUM*-CONSERVED ESSENTIAL GENES IN *P. VIVAX* MALARIA TRANSMISSION BIOLOGY

Jenna Oberstaller, Justin Nicholas, John H. Adams

University of South Florida, Tampa, FL, United States

Genome-wide screens of the most-deadly malaria parasite *Plasmodium falciparum* and the primary rodent-malaria model *Plasmodium berghei* have identified genes essential for parasite survival during asexual blood-stage development. These breakthrough studies provide revelatory insights into prioritizing parasite pathways and processes for new therapeutic interventions. Since most essential genes are conserved among all *Plasmodium spp*., the new functional annotations can be applied to *Plasmodium vivax*, which is much more difficult to study due to lack of continuous culture systems. Though *P. vivax* and *P. falciparum* are both human-infective malaria parasites, they are as far-removed evolutionarily from each other as they are from the rodent-infective *P. berghei*, and it may be the 'dispensable' and 'essential' roles for these genes that are more likely to vary among these species than the gene conservation. While some of the unique *P. vivax* clinical characteristics make it a major hurdle to elimination efforts and will be important metabolic functions to target, we expect that most high-value core metabolic functions will be conserved as

~80% of *P. vivax* genes have an ortholog in *P. falciparum* and *P. berghei*. In this study, we use data from the essentiality screens, coupled with recent transcriptome analyses of *P. vivax* sporozoites and *in vivo* transcriptomic data from longitudinal infection studies in macaques of the vivax-like *P. cynomolgi*, to explore the relationship between blood-stage essentiality and multi-stage function in *P. vivax*. Furthermore, we explore the potential functional implications of *Plasmodium*-conserved essential genes in *P. vivax* sporozoite activation and hepatocyte infectivity to evaluate multi-stage essential genes. Streamlined parasite genomes suggest that every gene is essential in some condition or developmental stage; here we apply an integrated approach to predict multi-stage essential genes to prioritize as targets for intervention.

1935

EXAMINING QUININE MECHANISM OF ACTION AND RESISTANCE USING A NOVEL *PLASMODIUM FALCIPARUM* GENETIC CROSS IN HUMANIZED MICE

Mariko Kanai[1], Leila S. Ross[1], Tomas Yeo[1], Melanie J. Shears[2], Abhai Tripathi[2], Sachel Mok[1], Photini Sinnis[2], David A. Fidock[1]

[1]*Columbia University Irving Medical Center, New York, NY, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Quinine (QN) is used for the treatment of severe *Plasmodium falciparum* malaria; however, it has a complex mechanism of action (MOA) and resistance that remains unresolved. Previous studies suggest a multifactorial mechanism, as polymorphisms in *P. falciparum* chloroquine resistance transporter (*pfcrt*) and *P. falciparum* multidrug resistance-1 (*pfmdr1*) and possibly *pfut* (HECT E3 ubiquitin-protein ligase) partially associate with cytostatic (growth inhibitory) QN resistance (QN^R), as defined by 72-hour drug susceptibility assays (IC_{50}). None of these genes have been validated as the primary QN^R driver and at least one other unidentified mediator is thought to exist. To interrogate the QN mechanism, we have used FRG-NOD human liver-chimeric mice to perform a genetic cross between Cam3.II (QN^R; $pfcrt^{Dd2}$ $pfmdr1^{184F}$) and NF54 (QN-sensitive (QN^S); $pfcrt^{WT}$ $pfmdr1^{WT}$). Microsatellite genotyping with 12 markers showed that of the 163 cloned progeny, at least 64 were genetically distinct recombinants. Preliminary IC_{50} studies with 20 progeny tested at lower QN concentrations indicate that the primary driver of resistance to cytostatic QN activity is neither *pfcrt* nor *pfmdr1*, as two $pfcrt^{Dd2}$ $pfmdr1^{184F}$ progeny had QN sensitivity similar to the QN^S NF54. Similarly, in a QN lethal dose (LD_{50}) assay (N=10) where parasites were exposed to high QN concentrations for 48 hours, washed, and analyzed 24 hours later, variations in LD_{50} values within the $pfcrt^{WT}$ $pfmdr1^{WT}$ genotype also indicate that *pfcrt* and *pfmdr1* do not fully explain resistance to cytocidal (cell killing) QN action at clinically relevant concentrations. Different sensitivities were also observed at the cytocidal vs. cytostatic QN level, suggesting that distinct mechanisms may be involved. We have obtained whole-genome sequences of the independent recombinant progeny and parents, and are proceeding with quantitative trait locus analysis to identify QN resistance mediators. This study will help identify novel surveillance markers and inform new targets for drug development.

1936

WHOLE-GENOME ANALYSIS OF *PLASMODIUM FALCIPARUM* TO UNDERSTAND CLINICAL IMMUNITY TO MALARIA

Zalak Shah[1], Alexis Boleda[2], Kara Moser[1], Matthew Adams[1], Andrea Buchwald[3], Karl Seydel[4], Don Mathanga[5], David Serre[1], Miriam K. Laufer[1], Michael Cummings[2], Joana C. Silva[1], Shannon Takala-Harrison[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Maryland College Park, College Park, MD, United States,* [3]*University of Colorado School of Public Health, Aurora, CO, United States,* [4]*Michigan State University, East Lansing, MI, United States,* [5]*University of Malawi College of Medicine, Blantyre, Malawi*

After repeated *Plasmodium falciparum* infections, individuals in high-transmission areas acquire clinical immunity to malaria. However, the mechanisms important in determining clinical immunity are not entirely known. Here we take a whole-genome approach to identify genes that may be involved in acquisition of clinical immunity to malaria by sequencing and analyzing parasite genomes collected from infected individuals followed over time as part of a longitudinal study in Malawi. We compared parasite genomes from individuals with varying levels of clinical immunity, defined using an individual's age and number of symptomatic infections per year. We also examined pairs of parasites collected at different time points from the same individuals, hypothesizing that these parasites will be more different from each other than expected by chance, at *loci* important for clinical immunity. Using F_{ST} as a measure of genetic differentiation, we identified several SNPs, including SNPs in vaccine candidate antigens such as AMA1, LSAP2 and RESA, that are significantly genetically differentiated between individuals with varying levels of clinical immunity. Analysis of infections from the same individuals showed that there is lower identity-by-descent between parasites from the same individual compared to parasites from different individuals, highlighting the role of allele-specific immunity. We also found that several vaccine candidate antigens, such as SERA5, MSP6, AMA1, etc., differ more than expected by chance in parasites from the same individual compared to parasites from different individuals. SNPs in genes such as *ama1*, *rifin*, and *clag2,* as well as several genes encoding proteins of unknown function, were identified by both analyses, lending further support for their involvement in the development of immunity. We will analyze *in silico* predicted T/B-cell epitope regions in *loci* these genes to understand which regions of these proteins might be immunologically important. Identifying and further analyzing these genomic regions will provide insights into mechanisms involved in acquired immunity and antigenic escape.

1937

THE MIRAGE PROJECT - MALARIA INFECTIOUS RESERVOIR AND GENOMICS, IN SEARCH OF ELUSIVE MALARIA PARASITES IN THE DRY SEASON

Antoine Claessens[1], Benoit Aliaga[1], Sukai Ceesay[2], Sarah Tarr[3], David Conway[3], Teun Bousema[4], Umberto D'Alessandro[2]

[1]*University of Montpellier, Montpellier, France,* [2]*MRC-Gambia, Banjul, Gambia,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*RadboudUMC, Nijmegen, Netherlands*

Malaria in The Gambia is seasonal, with virtually all cases occurring during or just after the four months wet season. As there is very little transmission during the dry season, the reservoir for *Plasmodium falciparum* parasites is thought to be within asymptomatic chronic infections. How the parasite population can switch from a typically high parasitaemia virulent phenotype in the wet season to a 'dormant-like' state in the dry season is not understood. To address this question, at the start of the dry season, we established a cohort with 60 *P. falciparum*-positive asymptomatic Gambian participants who were bled monthly for 6 months. 45% were still carriers at the end of the dry season. We now have a unique dataset of parasite and human serum samples from the same individuals over a 6-month period to investigate how *P. falciparum* establishes a chronic infection. In collaboration with MalariaGEN, parasite genomes are being sequenced to investigate the genetic diversity in the wet and dry seasons. With the Conway lab at LSHTM, we analyse how the parasite senses its environment and adapt to it using (1) Parasite Multiplication Rate assays, and (2) RNA-Seq, to compare parasite transcriptomes across monthly timepoints. Our novel protocol can sequence a whole transcriptome from as few as 1000 parasites. Ongoing data analysis reveals a specific 'transcriptomal signature' of an isolate within its host, stable over time. To the best of our knowledge, this is the first characterization of monthly parasite transcriptomes derived from the same ongoing infection. This

study design, which has the potential to answer a multitude of biological questions, will greatly advance our understanding of the parasite, host and vector interactions, with direct implications for malaria eradication.

1938

SPATIAL ANALYSIS OF PARASITE POPULATION GENOMICS DURING MALARIA ELIMINATION EFFORTS IN EASTERN MYANMAR

Xue Li[1], Grace A. Arya[1], Ann Reyes[1], Aung Myint Thu[2], Gilles Delmas[2], Daniel M. Parker[3], Khin Maung Lwin[2], Kanlaya Sriprawat[2], François Nosten[4], Tim Anderson[1]

[1]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [2]*Shoklo Malaria Research Unit, Mae Sot, Thailand,* [3]*University of California, Irvine, CA, United States,* [4]*University of Oxford, Oxford, United Kingdom*

Early diagnosis and effective antimalarial treatment, combined with mass treatment of "hotspot" villages has proven to be extremely effective in reducing malaria transmission in Kayin State in Eastern Myanmar, an area of multidrug resistance. We describe how genomic surveillance of malaria parasite samples collected during this elimination program can be used to monitor the spread of resistant alleles, to examine patterns of parasite transmission, and to inform control efforts. We prepared DNA from > 3000 blood spots from *Plasmodium falciparum* infected patients collected from > 300 malaria posts in a region of Kayin state spanning ~200km from the northernmost to southernmost village during malaria elimination efforts between 2016 and 2018. We optimized selective whole genome amplification (sWGA) protocols for amplifying low levels of malaria DNA from contaminating human DNA. We are able to use sWGA to recover sufficient malaria DNA for genome sequencing from samples with >24.8 pg malaria DNA as measured by qPCR (approximately one third of the samples) and we have now successfully sequenced >1000 parasite genomes. We are using this dataset, with precise spatial and temporal information for each sample, to examine the spread of resistance alleles under intense selection, to estimate spatial patterns of relatedness (and parasite transmission) using identity-by-descent relationships and landscape features, and to trace mutational changes in clonal lineages of inbred parasites.

1939

SPATIAL-GENETIC ANALYSIS OF *PLASMODIUM FALCIPARUM* IN THE DEMOCRATIC REPUBLIC OF THE CONGO THROUGH MOLECULAR INVERSION PROBES

Robert Verity[1], Ozkan Aydemir[2], Nicholas F. Brazeau[3], Oliver J. Watson[1], Nicholas J. Hathaway[4], Melchior K. Mwandagalirwa[5], Patrick K. Marsh[2], Travis Fulton[3], Madeline Denton[3], Andrew Morgan[3], Jonathan Parr[3], Philip J. Rosenthal[6], Patrick Tumwebaze[7], Julie Gutman[8], William Moss[9], Modest Mulenga[10], Anita Ghansah[11], Benidicta Menseh[11], Antoinette K. Tshefu[12], Azra C. Ghani[1], Steven R. Meshnick[3], Jonathan J. Juliano[3], Jeffrey A. Bailey[2]

[1]*Imperial College London, London, United Kingdom,* [2]*Brown, Providence, RI, United States,* [3]*University of North Carolina, Raleigh-Durham, NC, United States,* [4]*University of Massachusetts, Worcester, MA, United States,* [5]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo,* [6]*University of California San Francisco, San Francisco, CA, United States,* [7]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [8]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [9]*John Hopkins, Baltimore, MD, United States,* [10]*Tropical Disease Research Centre, Ndola, Zambia,* [11]*Noguchi Memorial Institute for Medical Research, University of Ghana, Noguchi, Ghana,* [12]*Kinshasa School of Public Health, Hôpital General Provincial de Reference de Kinshasa, Kinshasa, Democratic Republic of the Congo*

The Democratic Republic of the Congo (DRC) is estimated to harbor 20% of the global malaria burden. Historically, malaria control efforts have been hampered by the evolution of antimalarial resistance, which is reflected in complex spatial patterns of molecular markers of drug resistance, such as pfcrt and pfdhps. The fine-scale genetic structure that this imposes on the parasite population is currently poorly understood. Using two panels of molecular inversion probes (MIPs), one targeting 1793 SNPs across the genome and one targeting known drug resistance mutations, we carried out the largest nationally representative study of *Plasmodium falciparum* genetic population structure in Africa to date. We used 1951 geo-referenced samples from the 2013 Demographic and Health Survey (DHS) and 435 samples from surrounding countries. PCA showed that parasites in the DRC link traditional East African and West African parasite populations with a Northwest to Southeast cline, consistent with traditional isolation by distance. Subsequent principal components showed population differentiation from both known drug resistance alleles and novel genes not previously characterized. Embedded within these relationships, the high-density SNP genotyping allowed for assessment of highly related pairs of parasites that occurred over distances beyond the expected geographic decay of relatedness. These patterns suggest migration events and gene sharing occurring between Kinshasa and outlying regions in the country. This study provides the first data on fine-scale genetic structure of parasites at a national scale in Africa, and provides a baseline that can be used to study how implementation programs impact parasite populations in the region. The newly implemented MIP platform represents a highly scalable and cost-effective means of providing genome-wide genetic data, without the costs of whole genome sequencing, on large numbers of samples needed for large population surveys. The highly flexible nature of the platform allows it to be rapidly scaled in terms of targets and samples leading it to be applicable across malaria endemic countries.

1940

MALARIA CHEMOPREVENTION WITH MONTHLY TREATMENT WITH DIHYDROARTEMISININ PIPERAQUINE FOR THE POST DISCHARGE MANAGEMENT OF SEVERE ANAEMIA IN CHILDREN AGED LESS THAN FIVE YEARS IN UGANDA AND KENYA: A 3 YEAR, MULTI-CENTER, TWO ARM RANDOMIZED PLACEBO CONTROLLED SUPERIORITY TRIAL

Titus K. Kwambai[1], Aggrey Dhabangi[2], Richard Idro[2], Robert Opoka[2], Simon Kariuki[1], Victoria Watson[3], Nickline Ashitiba[1], Kephas Otieno[1], Aaron M. Samuels[4], Meghna Desai[4], Chandy C. John[5], Bjarne Robberstad[6], Michael Boele van Hensbroek[7], Duolao Wang[3], Kamija Phiri[8], Feiko O. ter Kuile[3]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Makerere University College of Health Sciences, Kampala, Uganda,* [3]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [4]*Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [5]*Ryan White Center for Pediatric Infectious Disease and Global Health, Indiana University School of Medicine, Indianapolis, IN, United States,* [6]*Centre for International Health, University of Bergen, Bergen, Norway,* [7]*Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands,* [8]*School of Public Health and Family Medicine, College of Medicine, University of Malawi, Blantyre, Malawi*

Children hospitalised with severe-anaemia in malaria-endemic areas are at high risk of readmission or death within 6 months post discharge. No strategy specifically addresses this post-discharge period. We conducted a multi-centre, two-arm, placebo-controlled, randomized trial in nine hospitals in Kenya and Uganda to determine if 3 months of post-discharge malaria chemoprevention (PMC) with monthly 3-day treatment courses of dihydroartemisinin-piperaquine (DP) reduced the rate of all-cause readmissions and deaths by 6 months post discharge (primary outcome) compared to a single 3-day treatment course with artemether-lumefantrine provided at discharge (control). Between May 2016 and November 2018, children aged &lt5 years with admission haemoglobin of &lt5g/dL received standard in-hospital care for severe-anaemia and a standard 3-day course with artemether-lumefantrine at discharge. They were randomized two weeks later to receive DP or placebo-DP at 2, 6 and 10 weeks and followed until week 26 inclusive using passive case-detection. Conditional risk set modelling for repeated events (Prentice-Williams-Peterson total-time) were used to obtain hazard ratios

(HR). Overall, 1049 children were randomized (PMC=524, control=525). Between 2-26 weeks post-discharge, there were 189 primary outcome events in the PMC arm and 324 in the placebo arm (HR=0.64 (95% CI 0.53-0.78). The HR during the PMC-intervention period (2-14 weeks) was HR=0.36 (0.27-0.47) and HR=1.17 (0.88-1.55) during the extended follow-up period (14-26 weeks). The median time to the first event was delayed from 47 to 111 days. In areas with intense malaria transmission, three months of malaria chemoprevention in children recently admitted with severe anaemia results in major reductions in all-cause readmissions or death post-discharge.

1941

MATERNAL AND CHILD MALARIA CHEMOPREVENTION TO ENHANCE CHILD DEVELOPMENT: A DOUBLE-BLINDED RANDOMIZED CONTROLLED TRIAL

Paul Bangirana[1], Andrea L. Conroy[2], Robert O. Opoka[1], Margaret Semrud-Clikeman[3], Maria Kroupina[3], Michael Georgieff[3], Grant M. Dorsey[4], Moses R. Kamya[1], Diane Havlir[4], Chandy C. John[2]

[1]*Makerere University, Kampala, Uganda,* [2]*Indiana University, Indianapolis, IN, United States,* [3]*University of Minnesota, Minneapolis, MN, United States,* [4]*University of California San Francisco, San Francisco, CA, United States*

Malaria in pregnancy is associated with adverse birth outcomes like premature birth and low birth weight which often affect neurodevelopment in the developing fetus and child. In addition, childhood severe malaria is associated with 1 in 4 children having long-term cognitive impairment. Prevention of malaria in pregnancy and in young children may thus prevent poor neurodevelopmental outcomes in children in malaria endemic areas. We present neurodevelopmental outcomes of children at age 36 months following chemoprevention in mothers and the children. Three hundred pregnant HIV-uninfected women were randomized at 12-20 weeks of gestation to malaria chemoprevention with either 3 doses of sulfadoxine-pyrimethamine (SP), or 3 doses of dihydroartemisinin-piperaquine (DP), or monthly DP. After birth, the children were randomized to receive DP chemoprevention monthly or every 3 months from 2 to 24 months age. Neurodevelopmental testing was performed using the Bayley Scales of Infant and Toddler Development third edition and parental completion of the Child Behavior Checklist. Monthly DP was associated with a lower risk of maternal and placental malaria compared to SP or 3 doses of DP during pregnancy and with a lower risk of malaria compared to DP every 3 months during infancy. Placental malarial and malaria in pregnancy were associated with poorer language development (mean difference 1.28, 95% CI: 0.12 to 2.45, p=0.03 and mean difference 2.21, 95% CI: 0.72 to 3.70, p=0.004 respectively). Malaria in the first 12 months was associated with poorer motor and language development (mean difference 5.82, 95% CI: 0.38 to 11.26, p=0.04 and mean difference 3.60, 95% CI: 1.24 to 5.96, p=0.003 respectively) at 36 months. Pathway analysis is being conducted to assess whether malaria chemoprevention mediates protection against adverse neurodevelopmental and behavioral outcomes. Placental malaria, malaria in pregnancy in the mother and malaria in the first 12 months of life in the child are associated with worse child neurodevelopmental outcomes at 36 months.

1942

INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE CONFERS NON-MALARIAL EFFECT ON BIRTHWEIGHT: RESULTS FROM A MEDIATION ANALYSIS

Michelle Roh[1], M. Maria Glymour[1], Stephen Shiboski[1], Roly Gosling[1], Anne L'Ianziva[2], Abel Kakuru[3], Richard Kajubi[3], Meghna Desai[4], Julie Gutman[4], Feiko ter Kuile[5], Moses R. Kamya[6], Grant Dorsey[1], R. Matthew Chico[7]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*Centers for Disease Control and Prevention (CDC), Kisumu, Kenya,* [3]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [4]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, US Centers for Diseases Control and Prevention, Atlanta, GA, United States,* [5]*Liverpool School of Tropical Medicine, London, United Kingdom,* [6]*School of Medicine, Makerere University College of Health Sciences, Kampala, Uganda,* [7]*Faculty of Infectious and Tropical Disease, London School of Hygiene & Tropical Medicine, London, United Kingdom*

The World Health Organization recommends intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) in areas of high to moderate malaria transmission to prevent consequences of malaria infection. Parasite resistance to SP has led researchers to evaluate IPTp with dihydroartemisinin-piperaquine (DP) as an alternative to SP. Three IPTp trials in East Africa have shown DP to be more effective than SP in preventing malaria infection, but not superior at improving newborn birthweight. We conducted mediation analysis using individual-level data from 1,612 HIV-uninfected women enrolled in IPTp trials in Uganda (n=2) and Kenya (n=1) to decompose the non-malarial and antimalarial effects of SP versus DP on birthweight. Treatment was defined as random assignment to SP or DP; the mediator was placental malaria at delivery; the outcomes were birthweight and low birthweight (LBW; <2.5 kg). We accounted for confounders (maternal age, education, household wealth, and gravidity) and interaction by gravidity. Meta-analyses were used to obtain pooled estimates of the total, non-malarial, and antimalarial effects of SP vs DP. Meta-analyses showed SP increased birthweight 73 g [95% CI: 30, 115] compared to DP. This effect was attributed mainly to SP's non-malarial activity (non-malarial effect vs DP=104 g [95% CI: 53, 155]). In contrast, the more potent antimalarial properties of DP produced a modest 34 g [95% CI: -8, 77] increase in birthweight compared to SP via antimalarial mechanisms. DP's antimalarial effect was larger and statistically significant in Uganda, where malaria transmission was higher (74 g [95% CI: 12, 137]). Multigravidae benefited more from the non-malarial effects of SP on birthweight compared to primigravidae (103 g [95% CI: 51, 154] vs 46 g [95% CI: -40, 131]), though differences were seen mainly in Ugandan data. Similar results were observed with LBW. SP appears to have potent, non-malarial effects on birthweight and LBW, independent of its antimalarial activity. Future research should evaluate combining SP with DP for IPTp to prevent both malarial and non-malarial causes of poor pregnancy outcomes.

1943

THE DURATION OF PROTECTION FROM AZITHROMYCIN AGAINST MALARIA, PNEUMONIA AND GASTROENTERITIS WHEN GIVEN ALONGSIDE SEASONAL MALARIA CHEMOPREVENTION: SECONDARY ANALYSIS OF DATA FROM A CLINICAL TRIAL IN HOUNDÉ, BURKINA FASO AND BOUGOUNI, MALI

Matt Cairns[1], **Mphatso Phiri**[2], Issaka Zongo[3], Issaka Sagara[4], Irene Kuepfer[1], Frederic Nikiema[3], Modibo Diarra[4], Amadou Barry[4], Amadou Tapily[4], Paul Milligan[1], Jean Bosco Ouédraogo[3], Daniel Chandramohan[1], Alassane Dicko[4], Brian Greenwood[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Malawi-Liverpool-Wellcome Trust Clinical Research Programme, London School of Hygiene & Tropical Medicine, Blantyre, Malawi,* [3]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,* [4]*Malaria Research and Training Center, University of Science, Techniques, and Technologies of Bamako, Bamako, Mali*

Mass administration of azithromycin (AZ) is being considered as a strategy to reduce mortality in young children in sub-Saharan Africa, but the potential mechanism by which AZ reduces child mortality is not well characterised. To better understand the nature and extent of protection provided by AZ against common causes of childhood illness, we explored the changing profile of protection against clinical malaria, clinical pneumonia and gastroenteritis by time since administration, using data from a household-randomized, placebo-controlled trial of azithromycin given alongside seasonal malaria chemoprevention (SMC) with sulphadoxine-pyrimethamine plus amodiaquine in Burkina Faso and

Mali. In this study, a cohort of approximately 20,000 children received AZ or placebo with SMC on four occasions during the peak malaria transmission season each year from 2014 and 2016. Poisson regression with a gamma-distributed random effect was used to obtain the incidence rate ratio comparing children given SMC + AZ versus children given SMC + placebo in fixed time strata post-treatment, accounting for the household-randomized design and within-individual clustering of malaria, pneumonia and gastroenteritis episodes. The likelihood ratio test was used to assess evidence for the time-treatment group interaction. The additional protection of SMC + AZ against malaria, relative to SMC + placebo was confined entirely to the first two weeks after administration (Protective efficacy 24.2% (95% CI: 17.8%, 30.1%) P<0.001; suggesting either improved cure of existing infections, or a short-lived contribution to post-treatment prophylaxis. There was evidence of higher initial protection against pneumonia and gastroenteritis, and some evidence of protection beyond the first two weeks post-treatment. We will discuss the possible implications for the mechanism of AZ protection, including the role of curative versus prophylactic effects, and the role of reinfection rates for different pathogens in determining the overall benefit of treatment.

1944

THE EFFECTIVENESS OF SEASONAL MALARIA CHEMOPREVENTION (SMC) IN THE OPERATIONAL PROGRAMMING CONTEXT OF GUINEA

Donal Bisanzio[1], Aissata Fofana[2], Timothée Guilavogui[3], Eugene Kaman Lama[4], Elizabeth Fitch[5], Adam Preston[6], Mamadou Aliou Baldé[2], Jean-Luc Taton[1], Lamine Bangoura[7], Richard Reithinger[1]

[1]*RTI International, Washington, DC, United States,* [2]*PMI StopPalu+ Project, RTI International, Conakry, Guinea,* [3]*National Directorate of Disease Control, Ministry of Health, Conakry, Guinea,* [4]*National Malaria Control Program, Conakry, Guinea,* [5]*RTI International, RTP, NC, United States,* [6]*RTI International, Fort Collins, CO, United States,* [7]*President's Malaria Initiative, US Agency for International Development, Conakry, Guinea*

Seasonal malaria chemoprevention (SMC) is one of the main interventions recommended by WHO to prevent and reduce malaria cases in children under five years of age living in high transmission areas. While the efficacy of SMC in randomized controlled clinical research trials is well established, reports on its epidemiological impact in an operational programming context is more limited. Since 2015, thanks to international support, Guinea's National Malaria Control Program (NMCP) has implemented SMC targeting children ages 3-59 months (CU5) in districts with high malaria incidence. SMC-targeted districts have increased from 8 in 2015 to 13 in 2018. Since 2018, the PMI-funded StopPalu+ project supported the NMCP in implementing SMC in 8 of the countries' 38 districts. We report on the epidemiological impact of the SMC implementation in a context of scaled-up malaria intervention programming, including enhanced case detection and case management, long-lasting insecticidal nets and disease surveillance. Using trend analysis and generalized linear modelling, we compared the trends of malaria test positivity rate (MTPR) in CU5 among SMC and non-SMC covered districts from 2014 (i.e. one year prior to SMC being implemented) to 2018 using data from the routine malaria information system; data included health facility and community-level data. Additionally, we compared MTPR in CU5 to individuals above 5 years of age in SMC districts. Results show that a greater reduction in MTPR in CU5 in SMC districts than in non-SMC districts between 2014 and 2018, with the mean MTPR of CU5 decreasing from 35.1% to 29.8% (15.3% reduction) in SMC districts and from 29.8% to 27.1% (6.8 % reduction) in non-SMC districts ($p<0.05$). Moreover, in SMC districts, the reduction in MTPR in CU5 was 1.4-fold higher than in individuals aged 5 years and above ($p<0.05$). The reduction in MPTR did not show strong heterogeneity among the SMC districts. Our results confirm that—even in an operational programming context—adding SMC to the comprehensive package of malaria interventions yields an epidemiological impact and results in greater reduction in MTPR in CU5.

1945

OPTIMIZING DELIVERY OF SEASONAL MALARIA CHEMOPREVENTION (SMC) FOR CHILDREN UNDER FIVE YEARS OF AGE: VERY HIGH COVERAGE CONSISTENTLY ACHIEVED THROUGH DOOR-TO-DOOR CAMPAIGNS IN BURKINA FASO

Issaka Zongo[1], Jean Bosco Ouédraogo[1], Yacouba Sawadogo[2], Sham Lal[3], Matt Cairns[3], Paul Snell[3], Johanna Stenstrom Johansson[4], Diego Moroso[5], Paul J. Milligan[3]

[1]*Institut de Recherche en Sciences de la Santé (IRSS)/Institut des Sciences et Techniques (INSTech), Bobo Dioulasso, Burkina Faso,* [2]*Programme Nationale Lutte Contre le Paludisme, Burkian Faso, Ouagadougou, Burkina Faso,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Malaria Consortium, Ougadougou, Burkina Faso,* [5]*Malaria Consortium, Kampala, Uganda*

According to WHO estimates Burkina Faso consistently ranks among the small group of countries contributing the vast majority of the world's malaria deaths each year. To address this public health emergency, SMC was introduced in 2014 and scaled up in subsequent years so that by 2018 SMC was implemented in 65 of the 70 districts in the country, over 4 months during and immediately after the rainy season. A survey in 2016 by ACCESS-SMC showed very high levels of uptake, 99% of eligible children received at least one SMC treatment, and 91% received 4 treatments. That survey was undertaken at the end of the season and relied on caregiver recall for children who did not have an SMC record card or whose card was not completed. To verify the validity of estimating SMC coverage at the end of the season, in 2017 we conducted surveys immediately after each monthly cycle, to compare coverage estimates with retrospective estimates made for the same month in the final survey. 1050 children eligible for SMC were surveyed after the July campaign, and 1567, 1692 and 1776 after the Aug, Sep and Oct campaigns. SMC record cards were available for inspection in 77%, 85%, 79% and 88% of children in each month. The coverage in Jul, Aug and Oct was 95%, 96% and 95%. This compares with retrospective estimates for the same months of 96%, 97% and 96%, obtained in the final survey. The close agreement indicates that coverage can be reliably assessed through a single survey at the end of the season. Coverage in the final month was 96%. 90.5% of children received SMC 4 times. Adherence was high, out of the children who received SMC, the percentage reported to have received all 3 daily doses was 98.2%, 99.5%, 99.5% and 99.6% each month. Out of the children who received a blister pack, the first dose was directly observed each month in 96.8%, 99.2%, 99.1% and 98.5%. Out of those who slept in the household the night before the survey, 95% of children and 92% of all household members slept under an LLIN. SMC is a life-saving intervention. We have shown that very high coverage of SMC can be consistently achieved. This should encourage other countries to investigate barriers to full coverage and take steps to overcome them.

1946

THE EFFECTIVENESS OF REPELLENT DELIVERED THROUGH VILLAGE HEALTH VOLUNTEERS ON MALARIA INCIDENCE IN SOUTHEAST MYANMAR: A STEPPED-WEDGE CLUSTER-RANDOMIZED CONTROLLED TRIAL

Paul Agius[1], Win Han Oo[2], Naanki Pasricha[1], Katherine O'Flaherty[1], Kyaw Zayar Aung[2], Aung Thi[3], Myat Mon Thein[2], Nyi Nyi Zaw[2], Htin Kyaw Thu[2], Wai Yan Min Htay[2], Aung Paing Soe[2], Nicole Romero[1], Zahra Razook[4], Alyssa Barry[4], Angela Devine[5], Julie Simpson[6], Brendan S. Crabb[1], James G. Beeson[1], Julia Cutts[1], **Freya J. Fowkes**[1]

[1]*Burnet Institute, Melbourne, Australia,* [2]*Burnet Institute, Yangon, Myanmar,* [3]*Myanmar Ministry of Health and Sports, Nay Pyi Taw, Myanmar,*

[4]*Walter and Eliza Hall Institute, Melbourne, Australia,* [5]*Menzies School of Health Research, Darwin, Australia,* [6]*University of Melbourne, Melbourne, Australia*

In February 2019 the World Health Organization released the guidelines for malaria vector control. Personal repellents have the potential to provide individual protection against mosquito bites, however their deployment in large-scale public health campaigns is not recommended due to the lack of high quality evidence of their public health value. In the Great Mekong Subregion malaria services are typically provided by village health volunteers to ensure access to malaria services in hard to reach populations. In order to provide 'real-world' evidence for the effectiveness of repellent distributed by village health volunteers on *Plasmodium* spp. infection (detected by rapid diagnostic test [RDT] and PCR) we performed a stepped-wedge cluster randomized trial 116 villages in South-east Myanmar over 15 months . *Plasmodium* spp. incidence was low over the duration of the study (0.16%, RDT; 3.2% PCR). We observed a significant decline in *Plasmodium* spp. infection detected by RDT across the study period, independent of the repellent intervention and seasonal variations in incidence which is most likely due to the increased access to malaria services provided by the village health volunteers. However, this decline was not observed for PCR-detectable infection which fluctuated over time. *Plasmodium* spp. detected by both RDT and PCR significantly reduced once villages transitioned into repellent distribution. There was significant heterogeneity observed in the nature of the effect of repellent distribution between villages and the protective effect was greatest for *P. falciparum*, with no protection observed against *P. vivax*. The observed reduction in *P. falciparum* infection incidence after repellent distribution, suggests that the incorporation of repellent into existing malaria services may be an effective strategy in combating malaria in Myanmar and the Greater Mekong Subregion more broadly.

1947

PHASE 1 EVALUATION OF A LIVE ATTENUATED VACCINE FOR THE PREVENTION OF ZIKA

Anna P. Durbin[1], Kristen K. Pierce[2], Beth D. Kirkpatrick[2], Rachel Blankenheim[1], Jennifer Han[1], Patricia Lutton[2], Xi Fang[1], Radmila Pavlovic[1], Marya Carmolli[2], Connor Klopfer[2], Stephen S. Whitehead[3]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*University of Vermont, Burlington, VT, United States,* [3]*National Institutes of Health, Bethesda, MD, United States*

Zika virus (ZIKV) is a mosquito-borne flavivirus that was first isolated from a sentinel macaque in the Zika forest of Uganda in 1947. It caused sporadic human infection throughout Africa and Asia until the first large outbreak on the island of Yap in Micronesia in 2007. The second large outbreak occurred in French Polynesia in 2013. Beginning in early 2015, Brazil experienced a very large outbreak of Zika which rapidly spread throughout the country as well as neighboring countries in Latin America. By the end of 2015, an association between ZIKV infection in pregnancy and microcephaly was established. Due to the devasting effects of congenital Zika syndrome (CZS), a safe and effective Zika vaccine is needed. Scientists at the U.S. NIH have developed the live attenuated Zika vaccine, rZIKV/DEN4Δ30), using a similar approach as that which resulted in the development of the live attenuated dengue vaccine TV003 currently in Phase 3 clinical evaluation. The eventual goal of this program is to develop a pentavalent dengue/Zika vaccine. rZIKV/DEN4Δ30 was developed by substituting the prM and E proteins of ZIKV Paraiba/2015 into the background of the DENV-4 vaccine candidate rDEN4Δ30. The vaccine was evaluated in a Phase 1 clinical trial conducted at Johns Hopkins University and the University of Vermont. Twenty-eight subjects were enrolled (20 received vaccine; 8 received placebo). Subjects received a single subcutaneous dose of vaccine. They were evaluated in the clinic approximately every other day for the first 3 weeks of the trial. At each visit, subjects were evaluated by a clinician and safety laboratory studies were performed. Blood, urine, semen, and cervico-vaginal secretions were collected and tested for the presence of vaccine virus at frequent intervals throughout the study. Blood for serology was obtained on study days 0, 28, 56, 90, 150, and 180. Complete safety, virologic, and serologic responses will be presented.

1948

SAFETY OF A PURIFIED INACTIVATED ZIKA VIRUS VACCINE (PIZV) CANDIDATE IN FLAVIVIRUS PRIMED HEALTHY ADULTS

Htay Htay Han, The ZIK-101 Study Group

[1]*Takeda Vaccines Inc., Cambridge, MA, United States*

Due to the co-circulation of Zika virus (ZIKV) and other flaviviruses (FV) in endemic regions, it is important to evaluate how preexisting immunity to other FV might impact the outcomes of ZIKV vaccines in development. We evaluated the safety and immunogenicity of a two-dose regimen of PIZV at three dose levels (2, 5 or 10 μg) in a two-stage phase 1 study, first in FV-naïve (data not shown) and second in FV-primed healthy adults. This study was conducted at multiple sites in the US and Puerto Rico. The FV-primed healthy adults aged 18-49 years (N=146) were enrolled and randomized into four groups (1:1:1:1) to receive either two administrations of placebo or one of three PIZV dose levels with 28 days interval. Study participants recorded solicited adverse events (AEs) for 7 days, and unsolicited AEs for 28 days after each dose. Serious adverse events (SAEs) were recorded throughout the study period. Safety laboratory parameters were measured at baseline and 7 days post vaccination. Neutralizing antibodies were measured by a plaque reduction neutralizing test (PRNT) before vaccination and 28 days after each dose. Here we report the cumulative safety data from an interim analysis. Out of 146 participants enrolled, 136 received two doses and completed the 28 day post-dose 1 visit and the 7 day post-dose 2 visit, and 99 completed the 28 day post-dose 2 visit. Reported solicited local reactions were higher in PIZV groups compared with placebo. Pain was the most frequently reported reaction, but mostly mild in intensity across all PIZV groups. Reported solicited systemic AEs in PIZV groups were comparable to placebo recipients, generally mild to moderate in intensity across the groups with no apparent increase with increasing dosage. Unsolicited AEs were reported with similar frequencies in all groups; two unrelated SAEs were reported. There were no changes of concern in safety lab parameters across all vaccine groups. Similar safety profiles were observed in all PIZV groups. In summary, the PIZV vaccine was well tolerated with an acceptable safety profile at all dose levels in FV-primed healthy adults. Immunogenicity analyses are being reported later.

1949

ASSOCIATION BETWEEN ZIKA VIRUS MICROCEPHALY IN THE NEWBORN WITH THE RS3775291 VARIANT AT TOLL-LIKE RECEPTOR 3 AND RS1799964 VARIANT AT TNFA GENES

Amélia R. Ribeiro[1], Camilla N. Santos[1], Danielle R. Ribeiro[1], Juliana A. Cardoso[1], Rodrigo A. Cazzaniga[1], Lucas S. Magalhães[1], Mércia S. de Souza[1], Adriana B. Fonseca[1], Ana J. Bispo[1], Roseane L. Porto[1], Cliomar A. dos Santos[1], Ângela M. da Silva[1], Mauro M. Teixeira[2], Roque P. de Almeida[1]

[1]*Federal University of Sergipe, Aracaju, Sergipe State, Brazil,* [2]*Federal University of Minas Gerais, Belo Horizonte, Minas Gerais State, Brazil*

Congenital Zika syndrome (CZS) is a cluster of malformation and the mechanisms that lead to it are still unclear. Seventy women who gave birth to babies with CZS from August 2015 to March 2017, seventy children with CZS, attended to the pediatric service of the University Hospital in Sergipe, Brazil, and twenty-three fathers were recruited to participate in this study. We performed clinical evaluation to confirm the neurological damage and serological tests to exclude other causes that might induce neurological injury, such as microcephaly. According to the World Health Organizations patterns, forty-two of these CZS babies were classified with severe microcephaly (more than 2 standard deviations below the mean for gestational age and sex) and seventeen with microcephaly (more than 3 standard deviations below the mean for gestational age and sex). All parents of these seventy CZS babies were ZIKV IgG positive by ELISA test. Other infections associated to microcephaly were discarded

(Cytomegalovirus, *Toxoplasma gondii*, Rubella virus and Herpes simplex virus). The control group was composed of forty-three mothers who live in the same endemic areas and their healthy babies, born during the same period of the case group. Epidemiological data were obtained by a questionnaire. Among the control group, 15 mothers were ZIKV IgG positive and 28 were negative. Whole blood sample were collected from all participants to obtain DNA, plasm and serum samples. We genotyped DNA samples using TLR3 rs3775291 and TNFα rs1799964 TaqMan® probe by qPCR. This study shows that a Single Nucleotide Polymorphism (SNP) in rs3775291 at TLR3, that trigger to type I interferons antiviral responses, in mothers infected by ZIKV during pregnancy is associated with CZS occurrence. Moreover, the T allele in SNP rs1799964 at TNFα gene in the CZS babies is associated with microcephaly severity.

1950

MAPPING THE ASSOCIATION BETWEEN ZIKA VIRUS INFECTION AND MICROCEPHALY IN BRAZIL

Oliver J. Brady[1], Simon I. Hay[2], Robert C. Reiner[2], Fatima Marinho[2]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*IHME, University of Washington, Seattle, WA, United States*

Towards the end of 2015, high rates of microcephaly were reported in Northeast Brazil following the first South American Zika virus (ZIKV) outbreak. Despite ZIKV subsequently spreading throughout Brazil, reported rates of microcephaly varied up to ten-fold in different parts of the country. While evidence from a range of laboratory-based and clinical cohort studies have proven the link between ZIKV infection in pregnancy and microcephaly, it remains unclear why microcephaly rates would show such high geographic heterogeneity. Here we merged data from multiple national databases in Brazil to derive an individual-level trimester-specific estimates of ZIKV exposure, in addition to relevant confounders, for 3.6 million pregnancies that occurred during the outbreak. Pregnancies that resulted in births with confirmed microcephaly (cases) were compared against all other births (controls) to derive population-representative estimates of risk. We estimate an absolute risk of microcephaly of 40.8 (95% CI 34.2-49.3) per 10,000 births and a relative risk of 16.8 (95% CI 3.2-369.1) given ZIKV infection in the first or second trimester of pregnancy; however, most pregnant women in Brazil during the ZIKV outbreak will have been subject to lower and more variable risk levels which we map at a high geographic resolution. Statistically significant associations of ZIKV with other birth defects were also detected, but at lower relative risks than that of microcephaly (relative risk < 1.5). This study strengthens the evidence that congenital ZIKV infection, particularly in the first two trimesters of pregnancy, is associated with microcephaly and less frequently with other birth defects. Our estimates of risk given exposure are comparable to previous clinical cohort studies, showing the utility of passive reporting data if analysis can carefully take into account its various biases and gaps. Our maps also suggest that the ZIKV epidemic was much more geographically restricted than previously thought which may imply higher risk of future ZIKV outbreaks in Brazil than previously thought.

1951

MOTOR FUNCTION AT 18 MONTHS AMONG INFANTS FROM THE PEDIATRIC OUTCOMES OF PRENATAL ZIKA EXPOSURE (POPZE) STUDY IN SOUTHERN PUERTO RICO

Luisa I. Alvarado-Domenech[1], Viviana Rosario-Villafañe[1], Nicole M. Pérez-Rodríguez[2], Irelis C. Repollet-Carrer[1], Luzeida Vargas-Lassalle[1], Vanessa Rivera-Amill[2], Mary Rodriguez-Rabassa[2]

[1]*Ponce Health Sciences University, Saint Luke's Episcopal Hospital, Ponce, Puerto Rico,* [2]*Ponce Health Sciences University, Ponce, Puerto Rico*

Zika virus (ZIKV) infection during pregnancy has been associated with adverse birth outcomes known as the Congenital Zika Syndrome (CZS), characterized by microcephaly and central nervous system abnormalities. However, microcephaly is considered the "tip of the iceberg" of this congenital infection. Consequently, children with possible congenital Zika infection born with no apparent clinical findings of CZS must be monitored over time. This study aims to describe the motor function of infants enrolled in the Pediatric Outcomes of Prenatal Zika Exposure (POPZE) cohort study. Infants born to mothers with confirmed (positive Real Time-Polymerase Chain Reaction (RT-PCR) result) or probable (positive Immunoglobulin M Enzyme-linked Immunosorbent Assay (IgM ELISA) result) ZIKV infection at a tertiary hospital in southern Puerto Rico from October 2016 to August 2017 were included. A follow-up visit was conducted at 18 months, where physical and neurologic examinations were performed. The Peabody Developmental Motor Scales (PDMS-2) was used to measure the gross motor development through the Gross Motor Quotient (GMQ) which is comprised by three specific subtests. Descriptive statistics were performed according to sociodemographic characteristics, clinical features, and the GMQ results from this visit. Additionally, comparisons between specific GMQ subtests' standard scores and their corresponding percentiles will be presented. A total of 40 infants were examined, 24 (60%) were females, and 8 (20%) were born preterm (gestational age &lt 36). One infant (3%) had moderate microcephaly at birth. According to GMQ totals, 14 (35%) infants had below average scores compared to age-specific means. Among this group, 3 infants (21%) were hypotonic, and 1 (7%) was not walking independently. Quantifying and describing the gross motor development of infants that are at risk of gross motor delay and tone abnormalities allows early referrals for developmental interventions and may mitigate adverse impacts of this congenital infection on the development of subsequent skills.

1952

ESTIMATION OF ZIKA VIRUS INFECTION RATES IN BLOOD DONORS FOLLOWING THE 2016 EPIDEMIC IN PUERTO RICO USING TWO SEROLOGICAL ASSAYS

Graham Simmons[1], Mars Stone[1], Magelda Montoya Cruz[2], Jasmine Larrick[2], Celine Cheng[1], Inder Singh[1], Honey Dave[1], Phillip Williamson[3], Eva Harris[2], Michael Busch[1]

[1]*Vitalant Research Institute, San Francisco, CA, United States,* [2]*School of Public Health, University of California Berkeley, Berkeley, CA, United States,* [3]*Creative Testing Solutions, Tempe, AZ, United States*

Zika virus (ZIKV) caused a dramatic epidemic in Puerto Rico (PR) during 2016 and 2017, with over 36,000 reported cases; however, overall infections are likely to have been much higher. Estimating the infection rate during the epidemic in this previously naïve population would help establish the likelihood of future outbreaks in PR. Therefore, we performed a serosurvey for anti-ZIKV IgG using two assays that demonstrated strong performance in terms of specificity on blinded arbovirus panel evaluations: the Biotechne ZIKV IgG NS1 ELISA and a ZIKV NS1 blocking of binding (BoB) ELISA assay. We tested six panels of 500 blood bank donor specimens collected in PR prior to the epidemic (March 2015), at the beginning (April), peak (June) and end (October) of the 2016 epidemic, and in March 2017 and April 2018. Initially using the Biotechne ZIKV IgG assay, rates of reactivity, together with mean net OD for only the reactive samples (shown in parentheses), were calculated for each sample set, yielding 0.6% (0.4) in March 2015; 4.1% (1.6) in April 2016; 9.0% (1.6) in June 2016; 17.8% (2.0) in October 2016; 23.0% (1.3) in March 2017; and 16.2% (0.7) in April 2018. Three of the panels were confirmed using the ZIKV NS1 BoB assay, with rates of reactivity for each set of: 2.9% in April 2016; 16.6% March 2017; and 17.4% in April 2018. The peak seroprevalence of 17-23% shortly after the epidemic (March 2017) is consistent with our estimate of 22% seasonal incidence in PR during the 2016 outbreak derived from the yield of ZIKV RNA-reactive blood donors and duration of the NAT detection period. Another interesting finding was the rapid waning of ZIKV Abs between 2017 and 2018, using the Biotechne assay - both in terms of percentage of reactive donations and mean OD signal in reactive samples. This waning was not observed with the NS1 BoB assay, although overall peak seropositivity was somewhat lower. These findings have implications for assay selection, particularly during recurrent outbreaks.

1953

RAPID ACTIVE SEROPREVALENCE (RAS) SURVEYS PERFORMED IN RURAL GUATEMALA DEMONSTRATED A RAPIDLY CHANGING ZIKA DISEASE BURDEN IN 2015-16 AND PROVIDE A USEFUL TOOL TO MEASURE ARBOVIRUS DISEASE BURDEN IN RESOURCE-LIMITED SETTINGS

Daniel Olson[1], Molly Lamb[2], Maria Alejandra Paniagua-Avila[3], Alma Zacarias[3], Neudy C. Rojop[3], Andrea Chacon-Juarez[3], Shekema Hodge[4], Matthew Bonaparte[4], Maria Renee Lopez[5], Celia Cordon-Rosales[5], Edwin J. Asturias[1]

[1]*University of Colorado School of Medicine, Aurora, CO, United States,* [2]*Colorado School of Public Health, Aurora, CO, United States,* [3]*Fundacion para la Salud Integral de los Guatemaltecos, Los Encuentros, Guatemala,* [4]*Sanofi Pasteur, Swiftwater, PA, United States,* [5]*Universidad del Valle de Guatemala, Ciudad de Guatemala, Guatemala*

As new arbovirus vaccines approach public health deployment, rapid, cost-effective tools are needed to estimate the arbovirus disease burden in resource-limited settings. We conducted two cross-sectional Rapid Active Seroprevalence (RAS) Surveys in a dengue-endemic region of Guatemala during the 2015-16 Zika virus (ZIKV) epidemic to determine the age-based seroprevalence of ZIKV and dengue virus (DENV) infection. We used a satellite map-based 2-stage cluster randomization design to perform two cross-sectional RAS surveys over a 200km^2 area in rural southwest Guatemala. RAS Surveys 1 and 2 were completed over 4-6 weeks in Oct-Nov 2015 and Jan-Feb 2016, respectively. All enrolled children <15 years provided demographic, clinical, and epidemiologic data and a serum sample that was tested for DENV by RT-PCR (if fever was present in the preceding week). At study end, remaining samples were tested for ZIKV using microneutralization (MN) and anti-ZIKV NS1 IgG blockade-of-binding (BoB) assays; and for DENV by MN (DENV1-4) and anti-DENV NS1 IgG ELISA. Case definitions were created *a priori* to define ZIKV- and DENV-exposed individuals based on MN testing (Abstract # 2279). A total of 197 (RAS 1) and 186 (RAS 2) children had residual serum for MN, ZIKV NS1 BoB, and DENV NS1 IgG testing, and the two groups were comparable in age (mean 9.9 years) and gender (56% female). ZIKV seroprevalence by MN increased from 1/186 (<1%) to 54/184 (31%), and ZIKV seroprevalence by BoB (titer >10) increased from 20/197 (10%) to 69/184 (38%) between RAS Surveys 1 and 2. DENV seroprevalence by MN remained unchanged from 104/169 (62%) to 109/155 (70%), and DENV seroprevalence by NS1 IgG (titer >50) remained unchanged from 129/147 (88%) to 133/153 (87%) between RAS Surveys 1 and 2. DENV RT-PCR was negative in all 41 febrile cases (ZIKV PCR unavailable). For both ZIKV and DENV, seroprevalence increased with age, and there were no gender differences. These results demonstrate that Rapid Active Sampling (RAS) surveys coupled with currently available diagnostics were able to identify an ongoing ZIKV epidemic over a 3-month period in a highly dengue-endemic region of Guatemala.

1954

DUPLICATIONS, SELECTION AND INTROGRESSION DRIVE THE SPREAD OF RESISTANCE TO ORGANOPHOSPHATES IN WEST AFRICAN *ANOPHELES GAMBIAE*

Xavier Grau-Bove, Edi Constant, Eric Lucas, Dimita Pipini, Arjen van T' Hof, Martin J. Donnelly, **David Weetman**

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The organophosphate pirimiphos methyl is the most important insecticide for indoor residual spraying-based malaria control across Africa. However, organophosphate-resistant *Anopheles* have recently been identified in West Africa, an area where a mutation (G119S) in the insecticide target site, acetylcholinesterase (*Ace-1*) has also been increasing in frequency. Here, from whole genome sequence-based GWAS we establish a clear association between pirimiphos-methyl resistance and copy number of mutated *Ace1* genes in *An. coluzzii* from rice fields in southern Cote d'Ivoire. We establish repeatability of this association in multiple pirimiphos methyl populations from West Africa. Though the mutation appears to have emerged in *An. gambiae* and introgressed to *An. coluzzii*, divergent pathways of duplication have resulted in heterogeneous and homogeneous patterns of copy number variation, both under strong selection. Our results highlight the importance of CNV scanning in in GWAS studies and provide a strongly predictive marker system for monitoring operationally-crucial insecticide resistance in *An. gambiae*.

1955

LLIN EVALUATION IN UGANDA PROJECT (LLINEUP): DATA FROM TWO YEARS OF CROSS-SECTIONAL ENTOMOLOGICAL SURVEILLANCE CARRIED OUT IN 104 HEALTH SUB-DISTRICTS IN UGANDA

Amy R. Lynd[1], Samuel Gonahasa[2], Sarah G. Staedke[3], Ambrose Oruni[1], Catherine Maiteki-Sebuguzi[2], Grant Dorsey[4], Jimmy Opigo[5], Adoke Yeka[2], Agaba Katureebe[2], Mary Kyohere[2], Janet Hemingway[1], Moses R. Kamya[6], Martin J. Donnelly[1]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*University of California, San Francisco, CA, United States,* [5]*Uganda Ministry of Health, Kampala, Uganda,* [6]*Makerere University College of Health Sciences, Kampala, Uganda*

The primary tool for malaria control in Africa has been the utilization of Long-lasting insecticidal nets (LLINs). The success of these nets is currently threatened by increasing levels of resistance to pyrethroids in the principal vectors of malaria. To overcome this threat nets have been developed that incorporate the synergist, piperonyl butoxide (PBO), which inhibits cytochrome P450 enzymes, one of the main mechanisms of pyrethroid resistance, and so potentially overcoming pyrethroid resistance. However, data on the effectiveness of the PBO treated nets is incomplete. To address this shortcoming a large-scale cluster randomized trial was conducted in Uganda in 2017-2019 to evaluate the impact of long-lasting insecticidal bednets (LLINs), with and without PBO. Entomological surveys were carried out concurrently with community surveys prior to the distribution of LLINs in a national Universal Coverage Campaign and at 6 monthly intervals until 24 months post distribution. The survey was carried out in 104 health sub-districts and included the collection of mosquitoes from 10 randomly selected households from each health sub-district. Up to 50 *Anopheles gambiae s.l.* per health sub-district were investigated for specific insecticide resistance markers to obtain estimates of resistance at baseline, and at 6, 12, and 18 months post distribution. We present the results of these entomological collections over the study period and discuss the changes in frequency of insecticide resistance mutations in relation to the use of PBO-treated LLINs versus pyrethroid-only treated LLINs

1956

METOFLUTHRIN TREATED DEVICES FOR THE PREVENTION OF BITES IN URBAN ENVIRONMENTS: RESULTS OF A FIELD TRIAL IN THE YUCATAN, MEXICO

Wilbert Bibiano Marin[1], Mike Dunbar[2], Pablo Manrique Saide[1], Norma Pavia Ruz[1], Josue Villegas[1], Scott Ritchie[3], Tom Churcher[4], Oselyne Ong[5], Gonzalo Vazquez Prokopec[2], **Gregor Devine**[5]

[1]*Universidad Autonoma de Yucatan, Merida, Mexico,* [2]*Emory University, Atlanta, GA, United States,* [3]*James Cook University, Cairns, Australia,* [4]*Imperial College, London, United Kingdom,* [5]*QIMR Berghofer Medical Research Institute, Brisbane, Australia*

Volatile pyrethroids such as metofluthrin promise the fast and effective coverage of indoor spaces. They volatilise from surfaces at room temperature ensuring rapid diffusion within houses. In tandem with pronounced sub-lethal impacts on mosquito behaviour, they can be used to create "bite-free" spaces for human occupants. We deployed 10% w/w metofluthrin devices (10 x 16 cm) at a rate of 1-2 per room in >100 randomly selected households in Ticul, Mexico. A further 100 households served as untreated controls. The first season's trial ran from April-October 2018. Every three weeks, devices were replaced and Prokopack aspirators

were used to sample mosquitoes from control and treated households. At sporadic intervals, human landing catches were conducted to examine impacts on biting. Aspirated mosquitoes were identified as male, female or blood-fed. *Aedes aegypti* was the commonest species collected (n=9283). Pre-treatment, the mean number of blood-fed females remained similar between control and treatment arms (1.8 and 1.5 respectively, p=0.41). Immediately post-treatment the arms diverged. Treated houses yielded ca. 50% of the blood-fed mosquitoes collected in control houses (1.1 and 2.5 respectively, p=0.02). In terms of reductions in biting rates and potential epidemiological impacts, treated houses yielded landing counts that were 90% lower (0.7/minute) than those in control houses (5.9/minute, p<0.01). An analysis of *kdr* allele frequencies in *Ae. aegypti* from both control and treated households (1138 and 799 individuals respectively) showed that 83% carried at least one copy of the pyrethroid resistant *kdr* mutations F1534C and V1016I and 25% were homozygous for both. Data from "free-flight" chambers and experimental hut trials confirmed that the behavioural impacts of metofluthrin persist in the presence of these alleles. Semi-field trials also demonstrated that the 10% w/w metofluthrin devices did not repel mosquitoes. The burden of bites is therefore not transferred to untreated neighbours. We conclude that these formulations show promise in terms of bite protection and as outbreak response tools for Aedes-borne diseases.

1957

OPTIMIZING TRANSFLUTHRIN TREATED DEVICES FOR DETERRENCE OF MOSQUITOES FROM APPROACHING AND ENTERING PERMETHRIN TREATED TENT

David Oullo[1], James Mutunga[1], Sheila Ogoma[2], Thomas Gilbreath[3], Wes P. McCardle[1]

[1]*U.S. Army Medical Research Directorate - Africa, Kisumu, Kenya,* [2]*Clinton Health Access Initiative, Nairobi, Kenya,* [3]*U.S. Army Medical Research Institute of Infectious Diseases, Maryland, WA, United States*

Long lasting insecticidal nets and indoor residual spraying are among the widely used strategies for mosquito control. However, despite proven efficacy, distribution and usage it still possesses logistical and practical challenges in temporary shelters that do not have substrates for spraying insecticides. Therefore, these tools may not be feasible vector control interventions for refugee camps and temporary military installations in remote areas. Furthermore current strategies target endophagic and endophilic mosquitoes but not outdoor biting vectors. Since there is a need for novel strategies that are field expedient in areas with no structural substrate to spray, this study investigated the spatial protection of transfluthrin-treated devices against mosquito bites in a semi-field environment. All combinations of permethrin treated/untreated tent, transfluthrin-treated burlap strips, transfluthrin-treated Personal Insect Repellent Kits (PIRK) devices at distances of ^{0}m, ^{1}m, and ^{3}m from the tents were tested using a ^{4}x^{4} Latin Square design for five nights each, with a two night period between treatments. Starved, female An. gambiae were released inside the semi-field and collected using human landing catches simultaneously inside and outside the tent. All the treatments significantly reduced mosquito biting inside both treated and untreated tents, with an ^{85}% reduction in treated tents combined with transfluthrin devices. Further studies are warranted to estimate how prolonged exposure to doses of volatile pyrethroids might impact insecticide resistance in natural vector populations and warrant further monitoring and study.

1958

CHARACTERIZING THE IMMUNE PROFILE OF MOSQUITO LARVAE AFTER EXPOSURE TO A NOVEL ESSENTIAL OIL BASED LARVICIDE

Patrick H. Kelly[1], Ju-Lin Weng[1], Michael J. Workman[2], Ivy Hurwitz[2], Marcelo Ramalho-Ortigao[1]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*University of New Mexico, Albuquerque, NM, United States*

Synthetic and biological larvicides have been the primary components for larvae source management for many years. However, there are negative environmental issues, development of vector resistance, and substantial cost associated with the use of some of these lavicides. We have developed an environmentally friendly, yeast-encapsulated orange oil larvicide that is effective and inexpensive to produce. This essential oil-based larvicide (EBL) maintains lethality against mosquito larvae at concentrations below 50ppm. Following ingestion and release of the EBL within the larval gut, changes in the expression profiles of genes associated with innate response and epithelial regeneration ensue. These alterations are followed by epithelial damage and sclerotization in 3rd instar *Aedes* and *Anopheles* larvae. Midguts and corresponding carcasses from *Ae. aegypti* exposed to EBL for 4 and 24 hrs demonstrated >20-fold induction of the effector caspases *CASPS7* and *CASPS8*, the midgut epithelial regenerator (*Vein*), and the IMD negative regulator (*Pirk)* compared to control larvae. In addition, 10-fold and 2-fold differences in expression were observed in the inhibitor of apoptosis *IAP1* and *IAP2*, respectively. The activation of apoptotic and necrotic pathways were further assessed by confocal microscopy of EBL-exposed midguts. Larvae exposed to EBL display a pronounced systemic darkening in the cuticle, potentially due to wound healing mechanisms such as melanization that

may be related to the amount of EBL ingested. We are currently assessing the effects of EBL on other innate immune genes and those associated with autophagy, including *ATG1*, *ATG6*, and *ATG8*. The melanization effects observed following ingestion and midgut exposure to EBL are intriguing. Further investigation into this area, specifically between different species of mosquitoes, are ongoing. Our results strongly suggest that EBL effective killing is likely due to induction of apoptosis within the mosquito larva midgut. EBL's amenability for use as part of an integrated vector management makes this novel larvicide a practical approach for mosquito larval control in the future.

1959

SEMI-FIELD TRIALS OF A LOW-COST, DRIED ATTRACTIVE BAIT STATION FOR ADULT *AEDES AEGYPTI* CONTROL

Rachel Sippy[1], Valeria Sanchez[2], Froilan Heras[1], Efrain Ayala[3], Anna M. Stewart-Ibarra[4], Marco V. Neira[5], David A. Larsen[6]

[1]*SUNY Upstate Medical University and University of Florida, Machala, Ecuador,* [2]*SUNY Upstate Medical University, Machala, Ecuador,* [3]*Universidad Técnica de Machala, Machala, Ecuador,* [4]*SUNY Upstate Medical University and University of Florida, Syracuse, NY, United States,* [5]*Pontificia Universidad Católica del Ecuador, Quito, Ecuador,* [6]*Syracuse University, Syracuse, NY, United States*

Illnesses transmitted by *Aedes aegypti* (dengue, chikungunya and Zika) comprise a considerable global burden; options for transmission reduction focus on mosquito control. Current interventions are inadequate and insecticide resistance threatens the effectiveness of these options. We developed a novel mechanism to deliver ingested insecticide to *Ae. aegypti*called a dried attractive bait station (DABS) and tested its impact on *Ae. aegypti*in a series of semi-field trials. The DABS are a high-contrast 28 inch^{2}surface coated with dried sugar-boric acid solution that elicits an ingestion response from *Ae. aegypti*landing on the surface. We released 50 laboratory-reared female *Ae. aegypti*into experimental huts typical of peri-urban tropical communities in South America in six replicates. In Trial Series 1 & 2, the treatment hut contained four DABS and the control hut contained four DABS without the active ingredient. Trial Series 3 had identical DABS placement but an additional food source (100g cut apples) in each hut. Mosquitos were collected with backpack aspirators 24 (Series 1) or 48 hours (Series 2 & 3) after release to calculate mortality (proportion of dead mosquitoes). Mortality was monitored for 48 additional hours in laboratory conditions for Series 1. We used paired t-tests to compare total and 48-hour mortality. In Series 1, total mosquito mortality was 4—18% in the control hut and 54—96% in the treatment hut. There was a difference in mortality in control and treatment huts ($p=0.003$) after 48 hours and in total ($p<0.001$). In Series 2, total mosquito mortality was 2—23% in the control hut and 77—100% in the treatment hut. There was a difference in mortality in control and treatment huts ($p<0.001$). In Series 3, total mosquito mortality was 2—33% in the control hut and 68—100% in the treatment hut. There was a difference mortality in control and treatment huts ($p=0.006$). Our DABS are a promising intervention for control of *Ae. aegypti*and prevention of arboviral disease.

1960

THE IMPACT OF YEAST-ENCAPSULATED ORANGE OIL IN *AEDES AEGYPTI* OVIPOSITION PREFERENCE

Fabiane das Graças Caldeira Brant[1], Bruno Gomes[1], Camila P. Jesus[1], Michael J. Workman[2], Ivy Hurwitz[2], Mariana David[1], Fernando A. Genta[1]

[1]*Fiocruz - Oswaldo Cruz Institute, Rio de Janeiro, Brazil,* [2]*University of New Mexico, Albuquerque, NM, United States*

Aedes aegypti is the main vector for dengue, chikungunya and Zika virus in tropical regions. Mosquito control strategies for aquatic environments aim to stop larvae development by reducing breeding sites and/or the application of larvicides. The larvicide based on yeast-encapsulated orange oil (yeast-OO) is highly active against *Aedes aegypti*larvae (LD_{50}< 50 mg·L^{-1}) presenting an environment friendly alternative to chemical insecticides for mosquito control. Here, we are testing the impact of this novel larvicide in the oviposition of *Aedes aegypti* females. Oviposition assays were carried inside BugDorm-2400 Insect Rearing Tent (W75 x D75 x H115 cm) under laboratory conditions. Ovitraps (black pot + wooden plate) were loaded with 250 mL solution that vary between filtered tap water and a heterogeneous solution with yeast-OO (160 mg·L^{-1}). Two ovitraps of each treatment were placed inside a tent (one trap in each corner) with a diagonal pattern that vary across assays avoiding bias associated with ovitrap location. Mosquito females were artificially fed in human blood. In each assay, one blood fed female (3 - 4 days) was placed inside a tent to infer the amount of eggs laid during 48 h or 72 h. Number of eggs were manually counted in the wooden plate and the solution for each ovitrap. Most females were able to lay eggs in ovitraps under assay conditions providing a high proportion of valid assays (93%). All valid assays had eggs in ovitraps with water whereas only 60% of assays had positive ovitraps with yeast-OO. Moreover, the total number of eggs per ovitrap is significant different between yeast-OO and water (Wilcoxon-ranked test: W = -314, z = -3.57, P< 0.0004). Females seem to lay more eggs in ovitraps without yeast-encapsulated orange oil. This indicates a potential secondary effect for the larvicide based on orange oil position. The repelling action against *Aedes aegypti* oviposition may provide a strategy for reducing egg laying in primary breeding sites that cannot be removed by control agencies.

1961

GEOGRAPHIC INEQUALITY IN CHILDHOOD MORTALITY AND MORBIDITY DUE TO LOWER RESPIRATORY INFECTIONS IN LOW-INCOME AND MIDDLE-INCOME COUNTRIES, 2000-2017

Catherine A. Welgan, Mathew M. Baumann, QuynhAnh P. Nguyen, Brigette F. Blacker, Robert C. Reiner Jr.

Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, United States

Lower respiratory infections (LRIs) represent the second largest cause of disease burden among children under five in low-income and middle-income countries (LMICs), contributing to more than 336,000 deaths in 2017. Previous work documenting the burden of LRIs across space and time lacks either the spatial breadth or resolution presented here. This study aimed to produce annual estimates of LRI prevalence, incidence, and mortality among children under 5 in 102 LMICs from 2000 to 2017 at high spatial resolution (5-km^2). A Bayesian geostatistical model leveraging data from household surveys and a suite of environmental and socio-demographic covariates was used to generate estimates of LRI burden, which were then calibrated to results reported in the 2017 Global Burden of Diseases, Injuries, and Risk Factors Study. In 2017, the highest estimated mortality rates occurred in administrative subdivisions within South Sudan, Chad, Central African Republic, and Nigeria. Several administrative units in Laos and Guatemala also showed disproportionate LRI burden relative to regional trends. While reductions in LRI prevalence, incidence, and mortality were estimated for most countries, subnational estimates revealed substantial within-country differences in LRI burden. Despite the vaccine-preventable nature of several etiologies of LRIs, this study highlights the persistent burden of childhood morbidity and mortality due to LRIs in numerous countries. The high spatial resolution estimates produced here reveal areas of inequality in both burden and burden reduction over time, illustrating the geographic regions that should be targeted in the implementation of future LRI treatment and prevention programs.

1962

BACTERIAL BIOMARKER IDENTIFICATION FOR PEDIATRIC PNEUMONIA IN A WELL-CHARACTERIZED COHORT FROM MOZAMBIQUE

Christopher Uschnig[1], Michael F. Gillette[2], D. R. Mani[3], Karell G. Pellé[4], Stephen Schaffner[3], Clarissa Valim[5], Miguel Lanasapa[6], Sozinho Acácio[7], Lola Madrid[6], Pedro L. Alonso[6], Steven A. Carr[3], Bronwyn MacInnis[1], Quique Bassat[6], Danny A. Milner Jr[8], Dyann F. Wirth[1]

[1]*BROAD Institute of MIT and Harvard, Harvard T.H. Chan School of Public Health, Cambridge, MA, United States,* [2]*Broad Institute of MIT and Harvard, Massachusetts General Hospital, Cambridge, MA, United States,* [3]*Broad Institute of MIT and Harvard, Cambridge, MA, United States,* [4]*FIND - Because Diagnosis Matters, Geneva, Switzerland,* [5]*Boston University School of Public Health, Boston, MA, United States,* [6]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Centro de Investigação em Saúde de Manhiça (CISM), Barcelona, Spain,* [7]*Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique,* [8]*ASCP - American Society for Clinical Pathology, Chicago, IL, United States*

Differentiating the underlying etiology of acute febrile respiratory illness in children is challenging, but has critical implications, given the competing priorities of adequately treating those caused by bacterial infection (i.e. bacterial pneumonia) while thwarting the rise in antibiotic resistance. The challenge stems from the significant overlap in clinical presentation among the common causes of respiratory symptoms (viruses, bacteria, and malaria), a high frequency of mixed infections, and a lack of differential diagnostics. We sought to identify a set of proteins from peripheral blood that could reliably differentiate the main underlying etiology of acute respiratory symptoms, with an emphasis on correctly identifying bacterial pneumonia. Using samples from pediatric patients meeting the WHO case definition for clinical pneumonia (increased respiratory rate and cough or difficulty in breathing, and fever at or within 24 h of admission; n=195) in Mozambique, we used an aptamer-based high dynamic range assay (called SOMAScan) to measure up to 1279 proteins in blood, performed marker selection using a combination of machine learning approaches, and created predictive models to distinguish disease etiologies. Our predictive models met the thresholds of FIND's pediatric pneumonia diagnostic target product profile, for sensitivity (desirable ≥95%, acceptable ≥90%) and specificity (≥90% and ≥80%), in both single etiology and mixed infection samples. Gene ontology and protein pathway analysis revealed that bacterial pneumonia was strongly associated with neutrophil activity, and allowed us to derive a neutrophil blood protein signature. Neutrophil degranulation was one of the top pathways in all analyses, with recurrent degranulation markers including HP, LCN2, LTF, MPO, MMP8, SERPINA1, S100A9, SLPI, PGLYRP1 and RETN. Re-analysis of an independent RNA-sequencing study based on the same sample cohort confirmed our findings. Implemented with an appropriate technology, the markers identified here may provide the basis for a rapid diagnostic for field-based triage for antibiotic treatment of pediatric pneumonia.

1963

WHAT IS A BREATH? WORKING TOWARDS AN IMPROVED REFERENCE STANDARD FOR COUNTING RESPIRATORY RATE TO VALIDATE NEW AUTOMATED PNEUMONIA DIAGNOSTIC AIDS FOR CHILDREN UNDER FIVE

Charlotte Alice Ward, Alice Maurel, Ann-Sophie Stratil, Monica Anna de Cola, Tedila Habte, Kevin Baker

Malaria Consortium, London, United Kingdom

Acute respiratory infections (ARIs), primarily pneumonia, are the leading cause of death from infectious diseases among children under five years of age globally. In low-resource settings, advanced clinical pneumonia diagnostics, such as chest x-rays, are not readily available at the primary care level. Instead, frontline health workers observe the child's chest movements, manually count the number of breaths in 60 seconds using an ARI timer or other timer (mobile, watch) and classify the respiratory rate (RR) as fast or normal. With this method, the observed RR and classification can be inaccurate, which may result in incorrect treatment. New, automated RR diagnostic aids which may be more reliable at counting and classifying RR are becoming available. However, prior to their adoption at scale, their performance needs to be established which requires a suitable reference standard. However, the global health community acknowledges that this is currently lacking. We are aiming to address this gap by building the evidence base around the accuracy of manual video annotation of a child's chest movements. Between April and May 2019 in Southern Nations, Nationalities, and Peoples' Region, Ethiopia, a panel of 10 medical professionals will use video annotation software to annotate 51 videos from two of Malaria Consortium's previous pneumonia diagnostics studies. Three age groups will be equally represented therein (0-<2 months; 2-<12 months and 12-59 months). For each video, five randomly selected reviewers will mark breaths, uncertain breaths (very shallow breaths, incomplete cycles or breaths that are difficult to judge) and distortions (child moving, crying or holding its breath). Based on these counts, upper and lower RR counts will be calculated. Overall agreement between reviewers will be calculated using the intraclass correlation coefficient. The mean time taken to review a video will be calculated and the acceptability of the video annotation tool will be explored through a focus group discussion. Results will be available in June 2019, and these would be the subject of the presentation.

1964

INCIDENCE OF INFLUENZA AND INFLUENZA-LIKE ILLNESS IN HOUSEHOLDS OF PREGNANT WOMEN, POSTPARTUM WOMEN AND INFANTS UNDER SIX MONTHS OF AGE IN BAMAKO, MALI

Nancy Ortiz[1], Adama M. Keita[2], Boubou Tamboura[2], Flanon Coulibaly[2], Uma Onwuchekwa[2], Samba O. Sow[2], Arthur L. Reingold[1], Myron M. Levine[3], Milagritos D. Tapia[3]

[1]*University of California Berkeley, Berkeley, CA, United States,* [2]*Centre pour le Développement des Vaccins-Mali, Bamako, Mali,* [3]*Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States*

Pregnant women and infants <6 months of age are at high risk for influenza and its complications. However, there is a paucity of information on the burden of influenza illness among pregnant women and their infants, especially in Africa. To determine the incidence of influenza-like-illness (ILI) and influenza in both of these populations, we recruited a cohort of pregnant women and mother-infant pairs and followed them over time, and conducted surveillance for febrile illness. Oropharyngeal and/or nasopharyngeal swabs were tested for influenza by RT-PCR. We accrued 231.6 person-years (PYs) of observation for pregnant women, 701.6 (PYs) for postpartum women, and 720.9 (PYs) for infants <6 months months of age. We observed an incidence of 128.6 ILI episodes per 100 (PYs) and 3.0 cases of laboratory-confirmed influenza (LCI) per 100 (PYs) in pregnant women, and in postpartum women, we calculated 55.9 episodes of ILI per 100 (PYs) and 2.6 cases of LCI per 100 (PYs) Among infants, we observed an incidence of 218.7 ILI episodes per 100 (PYs) and 4.8 cases of LCI per 100 (PYs). Among pregnant and postpartum women, the incidences of ILI and LCI were highest in those <20 years. Women were 2.28 times as likely to develop ILI during pregnancy than during the postpartum period (95%, CI 1.89 - 2.75). While pregnant women had a greater relative risk of LCI than postpartum women, the difference was not statistically significant. Among infants, incidence of LCI and ILI was highest among 4 and 5 month olds. The risk of influenza in adult women was greater in households with a child <5 years of age, compared to households with no children. The risk of a case of influenza in an infant increased with increasing number of children <5 years of age residing in the household, although this difference was not statistically significant. Influenza circulated with defined seasonality and we observed bimodal annual peaks of LCI activity, with cases peaking in October and February. Maternal influenza vaccination administered prior to periods of peak influenza activity may help to prevent LCI in pregnant women, postpartum women and infants under <6 months of age in sub-Saharan Africa.

1965

VIRAL ETIOLOGY OF PNEUMONIA AMONG SEVERELY MALNOURISHED UNDER-FIVE CHILDREN: A PROSPECTIVE CASE-CONTROL STUDY IN AN URBAN HOSPITAL, BANGLADESH

Fahmida Chowdhury, ASM Sayeem Bin Shahid, Probir Kumar Ghosh, Mustafizur Rahman, Zakiul Hasan, Zubair Akhtar, S Mah-E Muneer, Lubaba Shahrin, Mohammod Jobayer Chisti

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

In Bangladesh, pneumonia has a higher mortality among malnourished children aged <5 years. Evaluating pneumonia etiology among malnourished children may help improve empiric treatment guidelines. During April 2015—December 2017, we conducted a prospective case-control study among severe acute malnourished (SAM) children aged <5 years admitted to icddr,b's Dhaka Hospital. We enrolled SAM children with clinical or radiological pneumonia as cases and SAM children without any respiratory symptom in last 10 days before admission as controls. We collected nasopharyngeal wash from both case and control and tested for respiratory syncytial virus (RSV), human metapneumovirus (HMPV), influenza viruses, human parainfluenza viruses (HPIV 1, 2, 3), rhinovirus and adenovirus by singleplex real-time RT-PCR. We enrolled 360 cases and 334 controls. For case and control median age was 8 months (IQR: 5-13) and 11 months (IQR: 6-18) (p=0.001) and 62% and 61% were male (p=0.7) respectively. Weight/age Z-score was -4.3 (SD ±0.7) for cases and -4.1 (SD ±1.1) for controls (p=0.01). Respiratory virus detection was high in cases 251 (70%) compared to controls148 (44%) (p=0.0001). The most frequently detected viruses among cases were rhinoviruses (32%) followed by RSV (13%), adenovirus (9%), HPIV3 (8%), influenza (6%) HMPV (6%). In the controls, rhinoviruses (55%) were most commonly detected followed by adenovirus (18%), HMPV (3%), RSV (2%), HPIV3 (3%), influenza (2%). RSV (OR 12.7; 95% CI: 3.7, 43.3)), influenza (OR 12.9; 95% CI: 2.8, 60.3), HPIV (5.7; 95% CI: 1.8, 18), HMPV (3.4; 95% CI: 1.9, 6) were identified as the independent viral pathogens causing pneumonia. Viral etiology of pneumonia in SAM children were mainly attributable to RSV, influenza, HPIV and HMPV. Our study findings may help in planning further studies to develop improved strategies targeting vaccines or antivirals for the prevention and treatment of pneumonia among SAM children.

1966

OBESITY IMPACT AND THE ROLE OF THE MICROBIOTA IN THE SUSCEPTIBILITY TO TUBERCULOSIS INFECTION

Sandra P. Palma Albornoz, Rômulo S. De Oliveira, Tamara S. Rodrigues, Ana Flávia Gembre, Leandra Z. Ramalho, Daniela Carlos, Vânia L. Bonato

Universidad São Paulo, São Paulo, Brazil

The prevalence of obesity has dramatically increased in recent years and is associated with inflammation and changes of the gut microbiota. Obesity is a major determinant of diabetes, and diabetes is a well-known risk factor for tuberculosis. However, few studies have examined the complex interaction between obesity and tuberculosis (TB). *Mycobacterium tuberculosis* is the causal agent of TB and is responsible for the highest number of infectious disease-related deaths in many low- and middle-income countries. We evaluated whether high-fat diet (HFD)-induced obesity can affect the outcome of TB. C57BL/6 mice were fed with either a low-fat diet (LFD) or high-fat diet (HFD) for ninety days. At 60 days, mice were infected with *M. tuberculosis* by intra-tracheal route. We observed an increase of pulmonary inflammation and susceptibility to infection in mice fed HFD (obese), with an associated augmentation in the frequency of CD4 IFN-γ^+ cells compared to LFD (lean) infected mice. We analyzed changes in intestinal barrier component and gut dysbiosis with an observed increase of Firmicutes and a reduction of Bacteroidetes in obese infected mice. We also found a lung dysbiosis characterized by significant increase of both Firmicutes and Bacteroidetes in infected obese mice compared to infected lean mice. Later, we used fecal microbiota transplantation (FMT) to evaluate the influence of the microbiota in mice with co-morbid obesity/tuberculosis. We found that infected obese mice that received antibiotic treatment before FMT showed a significant reduction of bacterial load and frequency of CD4 IFN-γ^+ cells. Furthermore, when these infected obese mice received FMT from non-infected obese mice, they recovered the bacterial load and the population of CD4 IFN-γ^+ cells. Our findings demonstrate that the obesity associated with dysbiosis is a contributing factor to reduce the resistance to *M. tuberculosis* infection. This study may help to develop novel therapeutic approaches for the treatment of tuberculosis in patients with obesity.

1967

SEVEN-YEAR OUTCOME ANALYSIS OF THE TUBERCULOSIS PROGRAM AT THE CENTRE HOSPITALIER RÉGIONAL SPÉCIALISÉ (CHRS) IN MACENTA, FOREST REGION, GUINEA-CONAKRY

Cornelia J. Staehelin[1], Valérie Schoenbaechler[2], Jean Hébélamou[3], Yakpazouo Guilavogui[3], Sosso Onivogui[3], Catrina Mugglin[2], Hansjakob Furrer[2], Esther Bavogui[3], Cécé Kolié[3], Pévé Zoumanigui[3], Ismaël Béavogui[3], David Leuenberger[3]

[1]*Bern University Hospital, Bern, Switzerland,* [2]*Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland,* [3]*Centre Hospitalier Régional Spécialisé, Macenta, Guinea*

We report population characteristics and treatment outcome of the tuberculosis program between 2010 and 2016 that included 3438 patients (1246, 36.2% female) with a median age of 35 years (interquartile range (IQR) 25-47). Most patients came from Macenta town and surroundings (45.4%). For patients from other places within Guinea, the median distance was 94 km (IQR 73-253 km, max. 608 km), and 106 (3.1%) came from neighbouring countries. Pulmonary tuberculosis was the main presenting form (n=2844, 82.7%), of which 2209 (78%) were sputum positive by microscopy and 635 (22.0%) were sputum negative. Extrapulmonary manifestations of tuberculosis (17.2% of total) were pleural (6.3 %), spinal (5.0%), lymph node (1.9%), peritoneal (1.3%), and other (2.9%). HIV was tested in 88.2% of all patients, 605 (17.6%) tested positive. Among these patients, 331 (54.7%) were already on antiretroviral treatment. Overall treatment success (cured plus treatment completed), according to WHO guidelines, was 74.5%. The main outcome was the percentage of new TB patients (n= 3050) successfully treated: 75.15% (sputum-positive patients 78.4%, all other patients 69.3%). Death occurred in 11.6%, treatment failure in 2.5%, 10.78% of patients were not evaluated or lost to follow-up. In a multivariable regression model, the following factors at treatment start were statistically significantly ($p<0.05$) associated with treatment success: age (per 10 year increase above 15 years) adjusted odds ratio (aOR) 0.87 (95% confidence interval (95% CI) 0.82-0.92); distance from clinic (per 100km further away) aOR 0.94 (0.89-0.99); and HIV status: HIV-positive on antiretroviral treatment (ART) aOR 0.32 (0.24-0.42) and HIV-positive not on ART with aOR 0.28 (0.21-0.38) versus HIV-negative patients. The following factors were not associated with treatment success: sex, year of treatment start, treatment start in years with ongoing Ebola epidemic (ie. 2014 + 2015) or unknown HIV-status. In conclusion, HIV co-infection, distance to clinic and age were factors that contributed to the lack of this TB program to reach WHO goals to achieve >90% treatment success in new patients.

1968

CLINICAL MANAGEMENT OF CHILDREN WITH MALARIA ACROSS EIGHT AFRICAN COUNTRIES: A CROSS-SECTIONAL ASSESSMENT OF NATIONALLY REPRESENTATIVE DIRECT OBSERVATION DATA

Jessica Cohen[1], Hannah Leslie[1], Indrani Saran[2], Guenther Fink[3]

[1]*Harvard School of Public Health, Boston, MA, United States,* [2]*Boston College School of Social Work, Boston, MA, United States,* [3]*Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland*

Nearly 300,000 children under age 5 die from malaria in Africa every year. Appropriate clinical management of malaria, including blood test confirmation and recommended antimalarial treatment, are crucial to preventing the progression to severe disease and death. This study estimated rates and predictors of clinically-appropriate malaria management among children under age 5 in sub-Saharan Africa. Cross-sectional analysis of direct observations of outpatient sick child visits in 12 nationally-representative facility surveys, including descriptive and multi-level logistic regression. 5,463 public and private health facilities in 8 countries in sub-Saharan Africa between 2007-2017. 22,100 children under age 5 visiting sampled health facilities for sick child care. Proportion of malaria cases receiving appropriate clinical management, defined as receiving both a blood test diagnosis and a recommended antimalarial. Of 22,100 sick children, 5,313 (24%) were diagnosed with malaria. Only 1,473 children with malaria received clinically-appropriate care (27.7%; 95% CI 25.3% to 30.1%), with 2,836 (53.4%; 50.5% to 56.2%) receiving a blood test and 3,040 (57.2%; 54.4% to 59.9%) prescribed an appropriate antimalarial. 1,135 children diagnosed with malaria (21.4%; 19.3% to 23.4%) received no antimalarial at all. Appropriate management was significantly associated with being seen at a facility with verified malaria testing equipment and treatment available (adjusted conditional marginal effect 0.20, 95% CI 0.14-0.26, p<.01). At well-stocked facilities, only 36.1% (1,340/3,715; 32.8% to 39.3%) of malaria cases received clinically-appropriate care. Appropriate malaria management was higher in the 2013-2017 surveys (1017 of 2718, 37.4%; 95% CI 33.6% to 41.2%) than in the 2007-2010 surveys (430 of 2595, 16.6%; 95% CI 14.2% to 18.9%). In conclusion, this study finds that three-quarters of children with malaria in sub-Saharan Africa received clinically inappropriate care. Renewed policy efforts to improve the clinical quality of care are urgently needed.

1969

AVIAN MALARIA: TROPICAL DEFORESTATION AND HOST SPECIFICITY

Ravinder N. Sehgal

San Francisco State University, San Francisco, CA, United States

The effects of environmental changes on parasite distributions are varied and despite potential consequences to ecosystem health, large-scale studies involving wildlife have been scarce. Here we present data of the effects of rapid deforestation on the prevalence and diversity of mosquitoes and avian blood parasites. In Cameroon, we have initiated a long-term study of mosquitoes, birds and avian malaria to determine how deforestation for the cultivation of palm oil plantations affects parasite transmission. For three years, we have collected samples from the same sites, pre- and post-deforestation. Sampling was done 4 times/year to account for seasonality. By analyzing over 2600 avian blood samples, and about 12000 mosquitoes, we find that habitat degradation leads to altered patterns of parasite prevalence and disruptions in parasite species dominance. The diversity of parasites, birds and mosquitoes changes significantly with deforestation. We also present data on how habitat may affect the evolution of lineage diversity and specialist vs. generalist strategies in avian malaria. Our work incorporates bioclimatic data to quantify differences among collection sites, and predict how microhabitat changes may affect the spread of infections. We have also initiated studies on genes involved in host pathogenicity, with the characterization of the transcriptomes of three parasites; *Plasmodium delichoni*, *P. homocircumflexum* and *Haemoproteus columbae*. We report orthologs of genes known for erythrocyte invasion, and host-specificity, including *msp-1*, *maebl*, *ama-1* and *ron-2*. We also comment on the evolutionary placement of *H. columbae* with respect to *Parahaemoproteus* and avian *Plasmodium* species. With our long-term agenda to discern the interplay between habitat, vector ecology, and genetics on the host-specificity of parasites, we emphasize that influences of land use changes on parasite prevalence are complex, and will require the detailed study of the vector ecology, and habitat effects. This multidisciplinary approach will aid in predicting how habitat changes will influence future scenarios of host-parasite interactions.

1970

LABORATORY QUALITY CONTROL SYSTEM FOR LARGE-SCALE MALARIA SEROSURVEYS: HAITI 2017

Lotus L. van den Hoogen[1], Jacquelin Présumé[2], Ithamare Romilus[2], Gina Mondélus[2], Tamara Elismé[2], Nuno Sepúlveda[1], Gillian Stresman[1], Thomas Druetz[3], Ruth A. Ashton[4], Vena Joseph[4], Thomas P. Eisele[4], Karen E. Hamre[5], Michelle A. Chang[5], Jean F. Lemoine[6], Kevin K. Tetteh[1], Jacques Boncy[2], Alexandre Existe[2], Chris Drakeley[1], **Eric Rogier**[5]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Laboratoire National de Santé Publique, Port au Prince, Haiti,* [3]*University of Montreal School of Public Health, Montreal, QC, Canada,* [4]*Tulane University School of Public Health & Tropical Medicine, New Orleans, LA, United States,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*Ministère de la Santé Publique et de la Population, Port au Prince, Haiti*

Measuring antibody responses to malaria can aid in estimating transmission in a human population. To compare outputs among separate serosurveys, standardized laboratory testing and data collection schemes are required. Here we describe the in-country application and retrospective quality control (QC) of a multiplex bead assay (MBA) to gather antibody data from large malaria surveys in Haiti. Data for IgG against 21 recombinant antigens and peptides were collected for 32,758 participant samples from three separate surveys. The total time for sample processing and IgG data collection in the Haitian national lab was 18 weeks. Titration curves for both a hyperimmune Haitian sera pool as well as the *Plasmodium falciparum* WHO reference standard were included on assay plates. Assay signal data were fit to a 5-parameter logistic regression model, and inspection of the median and interquartile range (IQR) for the y-inflection point of standard curves was used to determine assay precision within and between surveys. Median and IQRs were similar for Surveys 1 and 2 for most antigens, while the IQR for y-inflection points increased for some antigens in Survey 3. Levey-Jennings charts were created for standard curve points for selected antigens to indicate deviation in assay signal and allow a final pass/fail call for each plate. Of 387 assay plates, 13 (3.4%) had aberrant standard values and were repeated. A pass/fail call was determined for each individual sample if IgG binding to the generic glutathione-S-transferase (GST) protein was observed; only 659 (2.0%) of blood samples failed this criterion. An additional 455 (1.4%) of assay wells failed due to low bead numbers (<20/analyte). In total, 609,438 (96.6%) anti-malaria IgG data points from 32,099 persons passed all QC checks. As shown by this experience in Haiti, the MBA can be deployed with high-throughput collection of IgG data and low inter-plate variability. Systematic and efficient antibody data collection can allow for rapid assessment of population exposure and can directly inform decision-making for control and elimination programs.

1971

RISK OF ADVERSE PREGNANCY OUTCOMES IN WOMEN TREATED FOR MALARIA WITH DIHYDROARTEMISININ-PIPERAQUINE OR QUININE IN THE FIRST TRIMESTER OF PREGNANCY IN INDONESIA: A RETROSPECTIVE DATA ANALYSIS

Rukhsana Ahmed[1], Kerryn A. Moore[2], Theda Lukito[3], Andre-Marie Tchouatieu[4], Maud M. Lugand[4], Stephanie Dellicour[1], Feiko O. Ter Kuile[1], Richard N. Price[5], Julie A. Simpson[2], Jeanne R. Poespoprodjo[6]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*Center for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia,* [3]*Clinesia, Jakarta, Indonesia,* [4]*Medicines for Malaria Venture, Geneva, Switzerland,* [5]*Global and Tropical Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Australia,* [6]*Timika Research Facility and Department of Child Health, Faculty of Medicine, Universitas, Gadjah Mada, Yogjakarta, Indonesia*

The use of artemisinin for the treatment of malaria in the 1st trimester of pregnancy is restricted due to limited safety data in early pregnancy in humans. Instead, quinine (QN) which is less effective and not well tolerated is recommended for treatment of 1st trimester malaria. In 2006 Indonesia became the first country to introduce artemisinin-based combination therapy (dihydroartemisinin-piperaquine, DP) for the treatment of malaria in the 2nd and 3rd trimesters. We reviewed a decade of retrospective hospital data from Mimika district, Papua province, Indonesia to assess the risk of stillbirth and miscarriage in women treated with either DP or QN in the 1st trimester. Medical records from the emergency room, inpatient, antenatal clinic and delivery unit were linked with laboratory and pharmacy data using hospital identification number, date of birth and name. The date of delivery, date that the drug was dispensed and the estimated gestational age at delivery were used to estimate the gestational age at the time of malaria treatment during pregnancy. The relative risks of stillbirth and miscarriage between the two treatments were analysed by Cox regression. Between 2006-2017, there were 3,766 malaria episodes treated with DP or QN: 869 in the 1st trimester (751 women) of which 203 (23.4%) were with DP (162 women) and 666 (76.6%) with QN (589 women). In the 1st trimester QN group, 10 (1.7%) pregnancies ended in miscarriage and 2 (0.3%) were stillbirths compared to 3 (1.9%) and 1 (0.6%) respectively in the DP group. The hazard ratio for pregnancy loss in the DP group compared with QN alone or QN plus clindamycin was 0.99 (CI 0.31-3.15). Further analysis is being explored on the effect of potential unmeasured confounders such as disease severity. Despite pregnancy loss being rare in our data, our study adds to the growing evidence that DP in the 1st trimester does not increase significantly the risk of pregnancy loss relative to QN.

1972

WHAT HAPPENS WHEN THE SUPPORT ENDS? A COMPARISON OF PRIVATE SECTOR ANTIMALARIAL MARKET SITUATIONS IN MYANMAR

Si Thu Thein[1], Ye Kyaw Aung[1], Phone Si Hein[1], Aung Thi[2]

[1]*Population Services International Myanmar, Yangon, Myanmar,* [2]*National Malaria Control Program, Ministry of Health and Sports, Nay Pyi Taw, Myanmar*

Since 2012, Population Services International Myanmar (PSI) had run Artemisinin Monotherapy Replacement Project (AMTR) to promote quality-assured artemisinin-based combination therapy (QAACT), instead of oral artemisinin monotherapy (oral AMT), among private sector providers. PSI also promoted the use of rapid diagnostic tests (RDT) for malaria before providing any antimalarial treatment. Prompted by funding restraints in 2017, PSI had to drop several AMTR project areas. One year later in 2018, this study aimed to compare private sector antimalarial market situations between 'current' and 'stopped' AMTR areas. From each area, a representative sample of geographic clusters (wards and village tracts) were selected for this cross-sectional quantitative survey of private sector outlets. A total of 143 clusters from current AMTR areas and 110 from stopped areas were included. In each cluster, all private sector outlets with the potential of stocking, selling and distributing antimalarial medicines were screened, and outlets those did any of these activities were interviewed. A total of 9,933 private sector outlets (4,039 from current areas and 5,894 from stopped ones) were screened and 343 were eligible for interviews (164 current, 179 stopped). Availability of QAACT and RDT were higher in current areas compared to stopped ones (52.1% vs 36.3% and 28.7% vs. 7.0% respectively). Availability of oral AMT was lower in current areas than stopped ones (4.8% vs 11.6%), and its market share was 0.4% of the total in current areas but 6.5% in stopped ones. From outlet providers perspective, 43.7% of them in current areas mentioned QAACT as the most effective antimalarial medicine whereas 36.6% from stopped areas said so. Also, 27.4% of providers in current areas said they had reported malaria case data but 22.5% in stopped areas said so. Thus, stopping AMTR program had possible untoward effects on private sector antimalarial markets. Availability of QAACT and RDT were lower but that of oral AMT was higher in stopped AMTR areas compared to current ones. The knowledge of providers had also declined over a year but not as marked as stock situations.

1973

JOINT EFFORTS TO IMPROVE MALARIA CONTROL IN THREE REFUGEE CAMPS IN KIGOMA, TANZANIA: SUCCESSES, CHALLENGES AND LESSONS LEARNED

Shabani Kililwa Muller[1], Juma Ng'akola[1], Zephania Nyakiha[2], Godfrey Smart[3], Goodluck Tesha[1], Jasmine Chadewa[2], Agnes Kosia[2], Zahra Mkomwa[1], Dunstan Bishanga[4], Rita Noronha[2], Lusekelo Njoge[4], Gaudiosa Tibajiuka[4], Chonge Kitojo[5], Erik Reaves[5]

[1]*Path Tanzania, Kigoma, United Republic of Tanzania,* [2]*USAID Boresha Afya Project –Jhiego Tanzania, Kigoma, United Republic of Tanzania,* [3]*Regional Health Management Team-Kigoma, Tanzania, Kigoma, United Republic of Tanzania,* [4]*USAID Boresha Afya Project -Jhpiego Tanzania, Kigoma, United Republic of Tanzania,* [5]*President's Malaria Initiative/United States Agency for International Development, Kigoma, United Republic of Tanzania*

The majority of refugees from Democratic Republic of Congo (DRC) and Burundi arriving in Tanzania are hosted in Kigoma region, where 59.2% of all Tanzanian malaria cases are reported every year. This high proportion of malaria cases is thought to be mostly due to high prevalence rates in three refugee camps, namely Nyarugusu, Mtendeli and Nduta. This article highlights important interventions led by USAID Boresha Afya Program in collaboration with the National Malaria Control Program (NMCP) in the three refugee camps in Kigoma. USAID Boresha Afya provided mentorship and integrated supportive supervisions in the refugee camps in Kigoma region in 2017 and 2018 by following national guidelines. We also conducted meetings with the Regional and District heath management teams to identify and address challenges observed during supportive supervisions. Then, we examined malaria indicators in the District Health Information System (DHIS2) and compared descriptive data before and after the interventions. Following mentorship and supportive supervisions, clinical malaria cases in all refugee camps reduced from 4% in 2016 to 0.1% in December 2018. Malaria reporting rate is now 100% as compared to 41.7% in 2016. Malaria positivity rate has decreased from 59.8% to 54.4% in December 2018. In addition, the proportion of women receiving at least two doses of sulphadoxine-pyrimethamine at antenatal care visits increased from 66.3% in 2016 to 80.8% in December 2018. However, the burden of malaria in refugee camps remains high (54%) as compared to other places. Inaccuracy of data records and management, shortage of staff, and low coverage of long lasting insecticide-treated mosquito nets (63%) are among the major challenges. In addition, non-adherence to a number of recommended government policies and cultural differences hinder the NMCP's efforts to control malaria in refugee camps. Despite these challenges, the malaria burden can be reduced in the refugee camps if multiple interventions are implemented correctly through coordinated stakeholders' efforts.

1974

MMENTORING, A NEW APPROACH TO IMPROVE MALARIA CARE IN BURKINA FASO

Moumouni Bonkoungou[1], Ousmane Badolo[1], Youssouf Sawadogo[1], Stanislas Nebie[1], Thierry Ouedraogo[1], Yacouba Savadogo[2], William Brieger[3], Gladys Tetteh[4], Blami Dao[4]

[1]*PMI Improving Malaria Care Project, Ouagadougou, Burkina Faso,* [2]*Ministry of Health, National Malaria Control Program, Ouagadougou, Burkina Faso,* [3]*Johns Hopkins University, Baltimore, MD, United States,* [4]*Jhpiego Baltimore, Baltimore, MD, United States*

Malaria is the leading cause of consultation (43.3%), hospitalization (44.1%) and death (16.1 %) in Burkina Faso. In the Sahel Region, the case fatality proportion due to malaria is 2% compared to 0.8% for the national average. This region is most affected by malaria than others. Also, the Sahel Region is currently experiencing high levels of insecurity making movement of health teams difficult and unsafe. mMentoring is the use of mobile technology to ensure capacity building and continuing education among health staff. The process started by a workshop to develop messages, and briefing of the main actors. Each week, messages and quizzes (An automatic answer is sent to each quiz) are sent to 753 providers (nurses, midwives, medical doctors) of the 115 health centers in the Sahel Region. Each month, messages are revised by a team at national level before being sent. The messages sent were related to several key malaria prevention and control interventions, such as case definition, parasitological diagnosis, clinical case management of simple and severe cases, intermittent preventive treatment in pregnancy (IPTp), pre-referral treatment with rectal artesunate in children under 5 years, insecticide-treated bed nets. After 10 months of implementation, 64 reinforcement messages on case management and prevention guideline and 63 quizzes were sent. Proportion of correct responses to the quizzes ranged from 43% and 96%. The lowest scores related to topics on management of severe cases while the highest were related to diagnosis of malaria. The participation rate (number of respondents of the 753 targeted health workers) is on average 22% with 71% of participants from primary health facilities. Also, we notice IPT3 increased from 14.8% in the quarter 3 of 2017 to 45.6% in the same quarter of 2018 (with mMentoring). The rate of performance of rapid diagnostic tests (RDTs) rose from 67.5% to 77.8%. The case fatality rate during this quarter of 2017 was 3.3% and 1.8% in 2018. As a real platform for continuing training, it would be wise to extend this approach to other regions of the country and also to other health actors like community health workers.

1975

IMPACT OF ROTAVIRUS VACCINATION VARIES WITH DIFFERENTIAL ACCESS TO PIPED WATER: AN ANALYSIS OF CHILDHOOD CLINIC VISITS FOR DIARRHEA IN PERU, 2005-2015

Miranda J. Delahoy[1], Cesar Carcamo[2], Luís Ordoñez[3], Vanessa Vasquez[2], Benjamin Lopman[1], Thomas F. Clasen[1], Gustavo F. Gonzales[2], Kyle Steenland[1], Karen Levy[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Ministerio de Salud del Perú (Ministry of Health, Peru), Lima, Peru*

Peru has undergone several health and infrastructure developments since 2005, including increased access to piped drinking water and national introduction of oral rotavirus vaccination. We examined whether these factors were associated with the rate of clinic visits for diarrhea in children under five. We fit a negative binomial model investigating the impact of rotavirus vaccination and piped water access on diarrhea rates in the 195 Peruvian provinces from 2005-2015, considering the interaction between these factors, and controlling for long-term and seasonal (El Niño) trends. We compared the "pre-(rotavirus) vaccine" (2005-2009) and "post-vaccine" (2010-2015) eras. Annual percentages of households in each province with access to piped water were analyzed in quartiles. The 2005 childhood diarrhea rate was ~29 annual clinic visits per 100 children and decreased ~3% per year. Higher access to piped water was associated with significantly lower childhood diarrhea rates in the post-vaccine era only. We found no effect of the rotavirus vaccine in the lowest quartile of piped water access. Controlling for long-term trend, compared to the pre-vaccine era, the diarrhea rate was lower in the post-vaccine era by 7% (95% confidence interval (CI): 2-12%), 13% (95% CI: 7-19%), and 15% (95% CI: 10-20%) in the 2nd, 3rd, and 4th quartiles of piped water access, respectively. Diarrhea rates were significantly higher (6%, 95% CI: 4-8%) during moderate or strong El Niño events. Explanations for higher reductions in diarrhea rates from the pre- to post-vaccine era in provinces with better piped water access include: (1) children without piped water may be predisposed to environmental enteric dysfunction, diminishing oral vaccine impact, (2) the etiologic patterns of diarrhea cases may differ by predominant water source, and/or (3) vaccine coverage may be higher in provinces with better piped water access. Improved access to piped water and rotavirus vaccination may operate synergistically to reduce childhood clinic visits for diarrhea in Peru.

1976

SEROPREVALENCE OF ANTIBODIES AGAINST *CHLAMYDIA TRACHOMATIS* AND ENTEROPATHOGENS AND DISTANCE TO THE NEAREST WATER SOURCE AMONG YOUNG CHILDREN IN THE AMHARA REGION OF ETHIOPIA

Kristen Aiemjoy[1], Solomon Aragie[2], Dionna M. Fry[3], Zerihun Tadesse[2], E. Kelly Callahan[4], Sara Gwyn[5], Diana Martin[5], Jeremy D. Keenan[3], Benjamin F. Arnold[6]

[1]*Stanford University, Stanford, CA, United States,* [2]*The Carter Center, Addis Ababa, Ethiopia,* [3]*University of California San Francisco, San Francisco, CA, United States,* [4]*The Carter Center, Atlanta, GA, United States,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*University of California Berkeley, Berkeley, CA, United States*

Many pathogens follow water-related transmission pathways. Trachoma transmission is associated with water quantity while the transmission of enteric pathogens is associated with both water quantity and quality. We hypothesized that children living further from a water source would have higher levels of exposure to *Chlamydia trachomatis*, the bacterium that causes trachoma, and to enteric pathogens. To evaluate the relationship between proximity to water source and antibody-based measures of pathogen exposure, we used a multiplex bead assay to measure IgG antibody responses to *C. trachomatis*, *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Salmonella enterica* (LPS Groups B and D), *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* (ETEC heat labile toxin β subunit) and *Vibrio cholerae* in eluted dried blood spots collected from 2296 children ages 1-9 years in 40 communities in rural Ethiopia. Locations of water sources were recorded using GPS. Linear distances were calculated from the child's house to the nearest water source and divided into quartiles. We derived seroprevalence cutoffs using ROC curves, if available, or by fitting finite mixture models. We used targeted maximum likelihood estimation to estimate differences in seroprevalence according to distance to the nearest water source. Median distance to the nearest water source was 420 meters (IQR 236, 626). The mean seroprevalence among 1-9 year-olds was 42% for *C. trachomatis*, 27% for *S. enterica*, 70% for *E. histolytica*, 54% for *G. intestinalis*, 94.9% for *C. jejuni*, 78% for ETEC, 53% for *V. cholerae* and 94% for *C. parvum*. Children in the quartile living nearest to a water source had a 11% lower prevalence of antibodies to *S. enterica* LPS Group B and D compared to children living in the farthest quartile (p=0.0008). There were no statistically significant differences in seroprevalence for the remaining pathogens with respect to distance to the nearest water source. The absence of heterogeneity in seroprevalence in this high transmission setting may have masked any potential relationships between exposure to enteric pathogens and distance to water.

1977

UNDERSTANDING THE IMPACT OF RAINFALL ON DIARRHEA: TESTING THE CONCENTRATION-DILUTION HYPOTHESIS USING A SYSTEMATIC REVIEW AND META-ANALYSIS

Alicia N. Kraay[1], Olivia Man[1], Morgan C. Levy[2], Karen Levy[3], Joseph N. Eisenberg[1]

[1]*University of Michigan-Ann Arbor, Ann Arbor, MI, United States,* [2]*University of California-San Diego, San Diego, CA, United States,* [3]*Emory University, Atlanta, GA, United States*

Whether rainfall increases or decreases diarrhea rates is unclear based on prior literature. The concentration-dilution hypothesis suggests that the conflicting results might be explained by the background level of rain—rainfall following dry periods can flush pathogens into surface water, increasing diarrhea incidence, whereas rainfall following wet periods can dilute pathogen concentrations in surface water, thereby decreasing diarrhea incidence. In this analysis, we explore the extent to which the concentration-dilution hypothesis is supported by the scientific literature and identify sources of heterogeneity in exposure and study design to understand why the role of rainfall on diarrhea might be context-specific. We queried PubMed, Embase, Web of Science, and the Cochrane collection for articles assessing the relationship between rainfall, extreme rainfall, flood, drought, and season (rainy vs. dry) and diarrhea. A total of 73 articles met our inclusion criteria. Overall, the literature is consistent with the concentration-dilution hypothesis. In particular, extreme rainfall is associated with increased diarrhea following dry periods (IRR=1.04, 95% CI: 1.03, 1.06) but decreased diarrhea following wet periods (IRR=0.88, 95% CI: 0.82, 0.97). Bacterial diarrhea is more common during rainy seasons, providing support for a flushing mechanism, but neither all-cause nor other pathogen-specific diarrhea exhibited this association. This result suggests that concentration-dilution processes might be more relevant in areas where bacterial diarrhea predominates. Diarrhea incidence is highest immediately after a flood but decreases thereafter, with a second spike in transmission two weeks later. This pattern might be explained by an initial increase in exposure, with a delayed later peak due to secondary transmission. There was no association between overall rainfall and diarrheal disease, which might be explained by the lower quality of those studies. Future studies should use standard, clearly defined exposure variables to strengthen our understanding of the relationship between rainfall and diarrheal illnesses.

1978

EVALUATIONS OF THREE DRINKING WATER CHLORINATION INTERVENTIONS IN COX'S BAZAR REFUGEE CAMPS

Mustafa Sikder, Gabrielle String, Danielle Lantagne

Tufts University, Medford, MA, United States

Humanitarian responders provide drinking water to nearly 730,000 refugees in Cox's Bazar, Bangladesh. Multiple water chlorination interventions are implemented in the camps. The aim of this research is to evaluate the effectiveness of three different water chlorination interventions in Cox's Bazar. We applied a mixed-methods protocol and used water quality and reported acceptability to evaluate the programs. We have completed a bucket chlorination (BC) and an in-line chlorination (IC) evaluations. We will evaluate a centrally chlorinated piped network system in April 2019. In the BC intervention, staff dosed chlorine solution into users' water after collection from ringwells. We observed nine chlorination points (CP), conducted 10 KIIs, and led four FGs; enumerators surveyed 148 households. The stock solutions' chlorine varied 0.07-0.34%. Stratified by CP, the mean household free chlorine residual (FCR) varied 0.1-0.5 mg/L, with 28% having no detectable FCR. Furthermore, 73% households had <10 *E. coli* CFU/100mL. Most users believed the intervention made water safe (85%). Recommendations were to increase the concentration of stock solution, prepare solution regularly, use opaque bottles to store chlorine, and provide protective equipment to the staff. In the IC intervention, responders installed automated chlorination devices at handpumps. We observed nine CP, conducted two KIIs, and led two FGs; enumerators surveyed 180 households. At the CP, FCR varied <0.05-3.78 mg/L and *E. coli* were 2-14 CFU/100mL (median <5). Stratified by point, the mean household FCR varied 0.0-0.4 mg/L, with 63% having no detectable FCR. However, 91% of households had <10 *E. coli* CFU/100mL. Many users believed the intervention made the water safe (62%). Recommendations were to replace the devices with adjustable ones and regularly monitor household water quality to determine chlorine dosage. Despite varying modalities, both interventions reduced the *E. coli* and had high acceptance, but struggled to maintain FCR >0.2mg/L. Comparisons of the interventions will help responders make informed decision to select appropriate technology in camps.

1979

IMPACT OF LOW-COST POINT-OF-USE WATER TREATMENT TECHNOLOGIES ON ENTERIC INFECTIONS AND LINEAR GROWTH AMONG CHILDREN IN LIMPOPO, SOUTH AFRICA

Courtney L. Hill[1], Emanuel Nyathi[2], Kelly McCain[3], Joshua N. Edokpayi[2], David M. Kahler[4], Darwin J. Operario[1], James A. Smith[1], Richard L. Guerrant[1], Amidou Samie[2], Rebecca A. Dillingham[1], Pascal O. Bessong[2], Elizabeth T. Rogawski McQuade[1]

[1]*University of Virginia, Charlottesville, VA, United States,* [2]*University of Venda, Thohoyandou, South Africa,* [3]*Emory University, Atlanta, GA, United States,* [4]*Duquesne University, Pittsburgh, PA, United States*

Enteric infections early in life have been associated with poor linear growth among children in low-resource settings. Point-of-use water treatment technologies provide effective and low-cost solutions to reduce exposure to enteropathogens from drinking water, but it is unknown whether the use of these technologies translates to improvements in child growth. We conducted a community-based randomized controlled trial of two water treatment technologies to estimate their effects on child growth in Limpopo Province, South Africa. 404 households with a child under age 3 were randomized to receive a silver-impregnated ceramic water filter, a silver-impregnated ceramic disk (the MadiDrop), a safe-storage water container, or no intervention, and were followed quarterly for two years. Due to unexpectedly high silver concentrations in the treated water, the silver content of the interventions was reduced four times over follow-up. We estimated the effects of the interventions on linear and ponderal growth, enteric infections assessed by quantitative molecular diagnostics, and diarrhea prevalence. The ceramic water filters and silver-impregnated disks consistently achieved more than 1.5 and 2 log reductions, respectively, in total coliform bacteria in drinking water samples. The filters and disks were associated with small, non-significant differences in height (height-for-age z-score differences compared to no intervention: 0.11, 95% confidence interval: -0.12, 0.33 and 0.12, 95% CI: -0.11, 0.34, respectively). There were no effects of the interventions on weight, diarrhea prevalence, or enteric infections, except for a 22% lower (95% CI: 4, 36) prevalence of *Giardia* among children with the filters compared to those with no intervention. While the ceramic water filters and silver-impregnated disks were effective in treating drinking water and potentially limiting transmission of *Giardia*, their use was not sufficient to reduce enteric infections overall or improve child growth. Transformative WASH interventions that better prevent enteric infections are likely needed to improve long-term child growth outcomes.

1980

EFFICACY OF LOCALLY AVAILABLE CLEANING AGENTS AND METHODS TO REDUCE BIOFILMS ON WATER STORAGE CONTAINERS AND TAPS

Gabrielle String, Marta Domini, Patrick Mirindi, Hanaa Badr, Anthonia Oguidupe, Nabila Khandaker, Marlene Wolfe, Daniele Lantagne

Tufts University, Medford, MA, United States

Biofilm growth on surfaces in contact with drinking water can act as a microbial reservoir for *E. coli* and other opportunistic pathogens. A particular concern in water storage containers and taps is bacteria

proliferation inside biofilms on interior surfaces as they can shed and re-contaminate stored water. To our knowledge, in low-income contexts, the efficacy of locally-available cleaning agents and methods for biofilm removal from containers and taps has not been assessed. We completed two laboratory studies growing *E. coli* biofilms on the interior surfaces of 72 jerrycans and 96 taps, respectively. Jerrycans were cleaned with different agents (0.5% NaOCl; pebbles; sand; 0.5% NaOCl and pebbles; 0.5% NaOCl and sand; and, none) and frequencies (every day; every other day; once a week), via shaking. Taps were cleaned with different agents (0.5% NaOCl; recently boiled water; soapy water; and, 5% vinegar) and cleaning methods (flowing the solution through the tap; soaking the tap assembled or disassembled for 60 seconds or 5 minutes; scrubbing the tap assembled or disassemble; scrubbing and soaking the tap disassembled for 5 minutes; and, none). Biofilms were imaged by epifluorescence microscopy and *E. coli* concentrations on the surfaces were enumerated. Imaging and enumeration confirmed *E. coli* biofilm growth on all control jerrycans and taps. Images of jerrycan surfaces cleaned only with pebbles or sand had the densest biofilm growth across all surface areas. Biofilms were thickest on the bottom surfaces of all jerrycans. Images of tap surfaces cleaned with any method using soapy water had large, dense biofilm structures. Cleaning jerrycans with 0.5% NaOCl combined with abrasives inhibited biofilm development across all test combinations. The most efficacious cleaning methods for taps were soaking assembled for 5 minutes (0.5% NaOCl, boiled water), or scrubbing and soaking unassembled for 5 minutes (0.5%NaOCl, vinegar). For jerrycans storing low turbidity water, we recommend daily cleaning with any tested method and daily chlorine water treatment. For taps, we recommend soaking assembled in recently boiled water for 5 minutes.

1981

HOUSEHOLD WATER STORAGE MANAGEMENT AND ASSOCIATED DRINKING WATER QUALITY IN RURAL INDIA

Sarah L. McGuinness[1], Joanne O'Toole[1], Andrew B. Forbes[1], Kavita Patil[2], Asha Giriyan[2], Chetan A. Gaonkar[2], Fraddry D'Souza[2], S. Fiona Barker[1], Thomas B. Boving[3], Allen C. Cheng[1], Karin Leder[1]

[1]*Monash University, Melbourne, Australia,* [2]*The Energy and Resources Institute, Western Regional Centre, Goa, India,* [3]*University of Rhode Island, Kingston, RI, United States*

Intermittent community water supply, related to fluctuations in water availability and electricity, is a reality in rural India. Accordingly, household storage of drinking water is common. Fecal contamination of stored water may occur at the source, during collection or during storage. As part of a trial evaluating a community water intervention in four rural villages in India where open defecation is common, we conducted household-level surveys at six time points. Each survey collected information about water source, storage and treatment, as well as hygiene and sanitation practices. At five of these time points, we also visited a random sample of households to request stored drinking water samples for *Escherichia coli* testing, concurrently observing whether the storage vessel was covered and documenting water access practices. We conducted 12265 surveys amongst 2304 households, and collected 3296 stored water samples from 1739 households. Households frequently reported multiple sources of drinking water (mean 2.7, range 1-7). The majority of water storage vessels were covered (92%); the most common reported (79%) and observed (81%) method used to access stored water was to dip a cup or ladle into the water container. Stored water samples were frequently contaminated with *E. coli* (2277/3296, 69%), and most households contributing two or more samples (745) had intermittently (47%) or persistently (44%) contaminated drinking water. The highest levels of contamination of stored water were observed during the wet season, coincident with the highest levels of river water contamination, which was reported as a primary drinking water source. Until households can be reached with an on premises continuous water supply, the practice of household water storage will continue, coupled with opportunistic use and/or mixing of water supply sources, some of which are likely to be from unprotected sources. These data also show that improvements to the quality of water supply sources will be insufficient to prevent exposure to contaminated drinking water unless attention is given to improving household water storage, hygiene and sanitation practices.

1982

UNDERSTANDING THE REALITY OF MDA FOR TRACHOMA AMONG A MAASAI COMMUNITY IN TANZANIA: APPLICATION OF AN ANTHROPOLOGICAL FRAMEWORK FOR NTD INTERVENTION EFFECTIVENESS

Tara B. Mtuy[1], Matthew J. Burton[1], Kevin Bardosh[2], Janet Seeley[1], Upendo Mwingira[3], Jeremiah Ngondi[4], Sarah Craciunoiu[5], Shelley Lees[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*University of Washington, Seattle, WA, United States,* [3]*NTD Control Program, National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania,* [4]*RTI International, Dar es Salaam, United Republic of Tanzania,* [5]*IMA World Health, Washington, DC, United States*

Trachoma, a neglected tropical disease (NTD), is making progress in elimination, yet hard to tackle communities are often marginalized. Neglect encompasses social constructs and livelihoods of a community. Areas in Tanzania, predominantly inhabited by Maasai, remain endemic for trachoma. We assessed the effectiveness of mass drug administration (MDA) using an anthropological framework, drawing on an ethnographic study of trachoma among Maasai. The lead author conducted participant observation during MDA and used household interviews to understand perceptions and experience of MDA, decision-making and migration. Interviews were conducted in Maa and translated into English. A framework method for analysis was used. Five domains were applied. (1) *terrain of intervention*: Human movement during MDA of varied distances and time-frames included seasonal migration, domestic chores, grazing, school and family visits. Encounters with wildlife hindered MDA delivery. (2) *socio-cultural factors and community agency*: Norms to maintain pregnancy secret impeded women of stating their situation to community drug distributors (CDDs). Timing of CDD visits conflicted with attending cattle. Refusals occurred among *ilmurrani* age group and elder women. (3) *strategies and motivation of CDDs*: Maa speaking CDDs were critical to effective delivery of MDA. Maasai CDDs were motivated but challenged by distances, wildlife, and poor compensation. (4) *socio-materiality of technology*: Trust in the drug improved as side effects decreased over years of MDA. Restrictions to swallowing drug and/or water was relevant to post-partum women and *ilmurrani* age group. (5) *political governance*: Whilst perceptions of the program were positive, the community questioned government priorities. People complained of lack of information and involvement of community members. Effective delivery of MDA requires a critical understanding of livelihoods, norms and beliefs and should inform control programs to tailor approaches to delivering to Maasai communities. A critical social science perspective should be imbedded in planning and evaluation of all NTD programs.

1983

TRACHOMA REMAINS HYPERENDEMIC AFTER 10 OR MORE YEARS OF THE SAFE STRATEGY: RESULTS FROM 7 DISTRICT-LEVEL POPULATION-BASED SURVEYS IN AMHARA, ETHIOPIA

Tigist Astale[1], Eshetu Sata[1], Mulat Zerihun[1], Andrew W. Nute[2], Aisha E.P. Stewart[2], Melsew Chanyalew[3], Berhanu Melak[1], Zebene Ayele[1], Demelash Gessese[1], Gedefaw Ayenew[1], Bizuayehu Gashaw[3], Zerihun Tadesse[1], E. Kelly Callahan[2], Scott D. Nash[2]

[1]*The Carter Center, Addis Ababa, Ethiopia,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*Amhara National Regional Health Bureau, Bahir Dar, Ethiopia*

Within trachoma hyperendemic districts, current WHO guidelines recommend 5 years of the Surgery, Antibiotics, Facial Cleanliness and Environmental Improvement (SAFE) strategy for the elimination of trachoma as a public health problem. The Amhara region of Ethiopia was early to scale up with the SAFE strategy and has historically had

many districts hyperendemic for trachoma. This study describes the epidemiological trends of trachoma over a period of up to 11 years in 7 contiguous hyperendemic districts to assess the success of the SAFE strategy as currently recommended. Trachoma impact surveys were conducted in the 7 districts in 2014 after 5-6 years of SAFE, and again in 2019 after 10-11 years of SAFE. At both timepoints a multistage cluster-random sampling design was used to select a population-based sample. All individuals ages 1 year and above were examined for clinical signs of trachoma by certified graders, and conjunctival swabs were collected from children ages 1-5 years to test for Chlamydial infection. The 2014 impact surveys demonstrated that all 7 districts were highly endemic with a trachomatous inflammation-follicular (TF) prevalence among children ages 1-9 years ranging between 36.0% (95% Confidence Interval (CI): 26.3-47.0%) in Kalala district and 56.4% (95%CI: 42.1-69.6%) in Moretina Jiru. The district prevalence of trachomatous inflammation-intense (TI) ranged between 5.3% (95% CI: 2.9-9.5%) and 15.9% (95%CI: 9.5-25.6%) and Chlamydial infection ranged from 2.3% to 33.9% suggesting ongoing transmission. Based on these results, all 7 districts received an additional 5 years of SAFE and were surveyed again in 2019. Although the TF point estimate was lower in all 7 districts in 2019 compared to the earlier survey, the prevalence of TF still ranged from 11.8% (95%CI: 7.6-16.0%) to 36.1% (95%CI: 27.4-44.3%). Infection data will be available soon. These 7 districts appear to constitute a hotspot in the region, and 3-5 more years of SAFE, or more, will be required. The global program should reconsider existing guidelines for hyperendemic districts and should increase research on alternative antibiotic treatment strategies.

1984

TRACHOMA ELIMINATION ENDGAME IN UGANDA: PROGRESS, MILESTONES AND TIMELINES

Gilbert Baayenda[1], Benjamin Binagwa[2], Wangeci Thuo[3], Francis Mugume[1], Edridah Muheki[1], Jeremiah M. Ngondi[4]

[1]*Ministry of Health, Kampala, Uganda,* [2]*RTI International, Kampala, Uganda,* [3]*RTI International, Washington, DC, United States,* [4]*RTI International, Dar Es Salaam, United Republic of Tanzania*

Trachoma is endemic in Uganda where implementation of the surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) strategy started in 2006. We describe: baseline surveys; implementation of SAFE; trachoma impact surveys (TIS) and trachoma surveillance surveys (TSS); and preparation of dossier for validation of elimination. Data were collated from reports of SAFE implementation, scientific manuscripts, water sanitation and hygiene strategies (WASH), and national development plans. The trachoma elimination dossier narrative and data templates were prepared according to the World Health Organization standard operating procedures. From 2006 to 2018, atotal of 57 districts were surveyed at baseline of which 50 were eligible for the AFE components of SAFE.A total of 54,505 trichiasis surgeries were done from 2006 to 2018. Annual mass drug administration (MDA) was undertaken from 2007to 2018in 50 districts. Implementation of facial cleanliness and environmental change (F&E) was targeted to trachoma endemic districts and nationally through WASH programmes. Impact surveys showed that 47 out of 50eligible districts had achieved elimination threshold - trachomatous inflammation-follicular (TF) prevalence of <5%. Surveillance surveys showed that TF prevalence of <5% had been sustained 24 months after the TIS in 38 districts. Three districts are still eligible of for TIS and will require TSS after 24 months if TIS shows TF of <5%. Another set of nine districts require TSS to document sustained elimination of TF. Trachoma dossier preparation has started,and an action plan has been developed with submission of dossier to WHO projected for 2021. Uganda has made steady progress towards elimination of trachoma. The Uganda experience provides important lessons for other national programmes on implementation of SAFE and trachoma elimination.

1985

PHOTOGRAPHING OPERATED TRACHOMATOUS TRICHIASIS (TT) CASES DURING OUTREACH CAMPAIGNS: RESULTS OF A PILOT

Whitney Goldman[1], Assumpta Lucienne Bella[2], Clarisse Bougouma[3], Emilienne Epée[4], Martin Kabore[3], Issouf Bamba[5], Jean-Paul Djiatsa[5], Albert Kiemde[5], Phylippe Bayala[5], Marc Sepama[5], Julie Akame[4], Jules Patrick Evenga[4], Michel Hendji[4], Yannick Nkoumou[4], Carine Fokam Tagne[4], Lauren Johnson[1], Geri Kemper-Seeley[1], Katherine Nerses[1], Stephanie Parker[1], Emily Gower[6]

[1]*Helen Keller International, Washington, DC, United States,* [2]*Ministry of Health, Cameroon, Yaounde, Cameroon,* [3]*Ministry of Health, Burkina Faso, Ouagadougou, Burkina Faso,* [4]*Helen Keller International, Cameroon, Yaounde, Cameroon,* [5]*Helen Keller International, Burkina Faso, Ouagadougou, Burkina Faso,* [6]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Since January 2018 the U.S. Agency for International Development (USAID)'s Morbidity Management and Disability Prevention Project has collaborated with ministries of health in Burkina Faso and Cameroon to pilot photographing operated eyelids during trachomatous trichiasis (TT) surgery and 3-6 month post-operative follow-up exams. The photos were intended to be used as a quality assurance mechanism and to identify potential risk factors for complications. To explore the feasibility of taking high-quality photos of a high proportion of operated cases, photos were taken during routine TT surgery campaigns from Jan. 2018 through Feb. 2019 in Burkina Faso (5 campaigns) and Cameroon (4 campaigns) immediately before surgery, immediately after surgery, one day after surgery, and/or 3-6 months after surgery. Different photographers were used throughout the campaigns. A total of 939 photos were taken, analyzed, and classified. Photos classified as "usable" were those that revealed information about the quality of surgery provided. Photos were also assessed for blurriness, poor lighting, and poor angle. Across all photos, 86% (809/939) were found to be usable: 84% (179/214) in Burkina Faso and 87% (630/725) in Cameroon. Overall, 81% (243/301) of preoperative photos, 88% (260/294) of immediate postoperative photos, 89% (186/208) of Day 1 photos, and 88% (120/136) of 3-6 month photos were usable. The proportion of usable photos ranged from 74% to 95% depending on whether they were taken preoperatively, immediately after surgery, at Day 1, or 3-6 months after surgery. Among photos not classified as usable, image quality issues identified were poor angle (86%), blurriness (43%), and poor lighting (27%). The proportion of usable photos remained above 75% across multiple campaigns and photographers and in different country contexts. These results indicate that photo taking can generate a substantial portion of usable photos revealing information about the quality of surgery provided. Photos of eyelids can therefore be used as a programmatic monitoring tool to document trichiasis surgery and certain post-operative outcomes.

1986

THE GLOBAL BURDEN OF LEPROSY FROM 1980-2017

Harrison Chase Gottlich, Anum Najeem Khan, Taren Gorman, Steph Zimsen, Martina Vargas, Amanda Deen, Elizabeth Cromwell

Institute for Health Metrics and Evaluation, Seattle, WA, United States

Leprosy (Hansen's disease) is an infectious disease with the potential to cause lifelong physical disfigurement and social stigma. The World Health Organization has endorsed the elimination of leprosy as a public health problem by 2020, with an overall objective of reducing the incidence of disability due to leprosy. In this analysis, we estimate the incidence and prevalence of Stage 1 and Stage 2 leprosy from 1990 to 2017 as part of the Global Burden of Disease study. We then model the age and sex-specific distribution to estimate Disability Adjusted Life Years (DALYs). National leprosy reporting data were aggregated and modeled using the Bayesian meta-regression framework DisMod-MR 2.1 under the Global Burden of Disease Study. We present methods for age and sex

adjustment of national reporting data and summarize existing evidence on leprosy-related disability. Overall, the global estimates indicate increasing incidence and prevalence from 1990 to 2000 and decreasing incidence but stable prevalence from 2000 to 2017. We estimate 410,666 (95% Uncertainty Interval (UI): 385,100 to 439,230) prevalent cases (including treated persons with permanent disability) and 101,429 (95% UI: 94,429 to 109,758) incident cases of leprosy in 1990; 519,028 (95% UI: 487,012 to 554,426) prevalent cases and 120,932 (95% UI: 112,878 to 130,511) incident cases of leprosy in 2000; and 518,500 (UI: 487,700 to 552,500) prevalent cases and 48,500 (95% UI: 45,800 to 51,000) incident cases of leprosy in 2017. The prevalence of leprosy is highest among adults ages 50-60 years old, with the majority of total cases estimated in India, Brazil and China, accounting for an estimated 271,760 (95 % UI: 249,565 to 298,280) prevalent cases. Per capita, the burden of leprosy is highest in South Sudan, Madagascar, and Guinea with rates of DALYs per 100,000 DALYs ranging from 2.18 (95% UI: 1.49 to 3.13) to 2.94 (95% UI: 1.93 to 4.14).

1987

LEPROSY POST-EXPOSURE PROPHYLAXIS: AN OPTION TO ACCELERATE LEPROSY ELIMINATION

David J. Blok[1], Arielle Cavaliero[2], Peter Steinmann[3], Jan Hendrik Richardus[1]

[1]*Department of Public Health, Erasmus MC, University Medical Center, Rotterdam, Netherlands,* [2]*Novartis Foundation, Basel, Switzerland,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

The widespread availability of free multidrug treatment (MDT) has reduced the global leprosy prevalence considerably since the 1980s, but the annual number of newly detected leprosy cases worldwide has stagnated at around 210,000 over the past decade; with about 10% of new diagnoses being children, indicating ongoing transmission. To further curb the case detection rate, a different approach to leprosy elimination, defined as 'zero leprosy', is required. In 2015, the Leprosy Post-Exposure Prophylaxis (LPEP) program launched across eight countries, assessing feasibility and impact of a single-dose rifampicin (SDR) as prevention to eligible contact persons of newly diagnosed leprosy patients. SDR had proven to reduce the risk for these contacts to develop leprosy by over 50%. Household and other contacts were systematically traced, screened for symptoms of leprosy and administered SDR if they did not show signs of leprosy. After an initial increase in new diagnoses due to backlog, the results of LPEP showed a promising downward case detection trend. Since actual long term impact of SDR is difficult to establish during the course of a three-year program such as LPEP, we used the individual-based transmission model SIMCOLEP to predict the potential long-term impact of LPEP on global case detection rates. If LPEP were to continue after three years, our model predicts that it could reduce the case detection rate by 75% in 2030 (15 years after its start), and by over 90% in 2040. Our model further predicts that with the current tools, it would take beyond 2070 to reach a level below 10,000 new cases annually. Implementing LPEP globally would however accelerate that period towards 2057, which means advancing progress towards zero leprosy by more than a decade. LPEP would thereby prevent many new cases. In a nutshell, achieving zero leprosy will take several decades, but LPEP could significantly reduce leprosy transmission. We advise to integrate SDR routinely for contact persons of newly diagnosed patients within national health systems and leprosy programs.

1988

OPTIMIZED GENEXPERT POOLING STRATEGY FOR *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* REDUCES COST OF MOLECULAR STI SCREENING IN TWO LIMITED RESOURCE CLINICS IN ZAMBIA

Sarah Connolly[1], William Kilembe[2], Mubiana Inambao[2], Ana-Maria Visoiu[2], Tyronza Sharkey[2], Rachel Parker[1], Eric Hunter[1], Susan Allen[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Zambia–Emory HIV Research Project, Lusaka/Ndola, Zambia*

Sexually transmitted infections (STIs) such as chlamydia (CT) and gonorrhea (NG) have been shown to increase the risk of heterosexual HIV-1 transmission. In women, CT and NG are often asymptomatic and undetected by syndromic management. Molecular testing for STIs is highly sensitive, but time and cost restraints preclude implementation of these technologies in resource-limited settings. Pooling samples for testing together in GeneXpert cartridges is one strategy for reducing the cost per individual tested. This project aimed to develop a pooling strategy based on social and demographic factors associated with CT/NG prevalence rates. In a cohort of high-risk women in Zambia, including female sex workers and single mothers with children under the age of five, the prevalence of either CT and/or NG infection was 17%. Data from a 2016 cross-sectional sub-study on intra-vaginal practices was examined to identify associations between various sociodemographic factors, clinical symptoms, sexual behaviors, STI laboratory results, and CT/NG status. Logistic regression modeling was used to predict the probability of a positive CT/NG test result in the presence of the associated factors: city, age, education, long-acting reversible contraception usage, and laboratory results for bacterial vaginosis, *Trichomonas vaginalis*, and incident syphilis on the day of CT/NG testing. Signs and symptoms were not found to be associated. An easy-to-use diagnostic screening checklist was created to categorize women by probability of testing positive on the GeneXpert. An algorithm considering cost of each test and prevalence of disease determined the optimal pool size for each risk category. Pooling women with similar CT/NG predictive factors together, or testing those at highest risk individually, reduced the cost per test. Further implementation of this tool to guide presumptive treatment, in lieu of molecular testing, increases the cost saving potential. The strategies described in this study are applicable to other low-resource clinical settings seeking to bring the accuracy of molecular testing to their clients with a reduced financial burden.

1989

MISSED OPPORTUNITIES FOR VACCINATION EQUITY: TARGETING CHILDREN IN HEALTHCARE FACILITIES

Nicholas Albaugh[1], Joseph Mathew[2], S. Sitaraman[3], Choudhary Richa[3], Tomar Anjali[3], Ishumeet Bajwa[4], **Anita Shet**[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Post Graduate Institute of Medical Education Research, Chandigarh, India,* [3]*Sawai Man Singh Medical College, Jaipur, India,* [4]*Post Graduate Institute of Medical Education and Research, Chandigarh, India*

National vaccination rates frequently obscure inequities in vaccination coverage. Addressing missed vaccination opportunities for children within healthcare systems can increase immunization rates. Using the national immunization schedule as the standard, we aimed to quantify missed vaccination opportunities among hospitalized children in India and identify immunization barriers perceived by caregivers and healthcare providers. Between Nov 2018 and Mar 2019, we screened and enrolled 163 children aged 1-59 months at admission in two public hospitals in northern India. We excluded those with no record or recall of vaccines, and those with vaccine contraindications. We found 55% were under-immunized for their age. Among these 61% were <12 months old, and 3% were 'zero-dose' children (received no vaccines at all). Frequently missed vaccines were hepatitis B birth dose, DTP3 and measles vaccines. Exit interviews and chart reviews at discharge showed that 97% of under-immunized children

were 'missed opportunities' (i.e. received no catch-up vaccines or referral for vaccination services). Significant risk factors for under-immunization included higher birth order (OR 1.8), previous health facility contact (OR 1.8), and father as primary vaccination decision maker (OR 3.2). Immunization barriers noted by healthcare providers included perceived contraindications (child too ill to receive vaccines), physical barriers (vaccines unavailable in the inpatient ward despite availability proximally in the immunization clinic) and policy limitations (staff in the ward not authorized to give vaccines). Over 90% of caregivers supported receiving increased information on vaccination services, and automated reminder services post discharge. Our findings indicate that hospitalized children are more likely to be under-immunized for age compared to national populations, and represent missed vaccination opportunities despite contact at healthcare centers with immunization capacity. Interventions targeting these populations and leveraging existing immunization resources will substantially improve vaccination inequities.

1990

COMPARATIVE EFFECTIVENESS OF STRATEGIES TO IMPROVE PRACTICES OF LAY HEALTH WORKERS IN LOW- AND MIDDLE-INCOME COUNTRIES: A SYSTEMATIC REVIEW AND BAYESIAN NETWORK META-ANALYSIS

Samantha Rowe[1], Huseyin Naci[2], David Peters[3], Kathleen Holloway[4], Dennis Ross-Degnan[5], Alexander Rowe[1]

[1]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*London School of Economics, London, United Kingdom,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*Institute of Development Studies, University of Sussex, Brighton, United Kingdom,* [5]*Harvard Medical School, Boston, MA, United States*

Lay/community health workers (LHWs) in low- and middle-income countries (LMICs) often provide inadequate care. A comparison of the effectiveness of strategies to improve LHW practices would be useful for programmatic decision-making. We identified LHW studies from a systematic review on improving health worker performance in LMICs. We focused our analysis on LHW practice outcomes expressed as a percentage (e.g., % of patients treated correctly). Strategy effects were calculated as adjusted risk differences. We estimated mean differences and 95% credible intervals using Bayesian network meta-analysis (NMA) with random effects, adjusting for LHW baseline performance level. We searched 52 databases for published studies and 58 document inventories for unpublished studies up to 2016. We screened 216,477 citations and identified 22 studies that tested 17 strategies. The 22 studies included 30 comparisons: 22 strategy-versus-control and 8 head-to-head comparisons. Most studies (14/22 or 63.6%) had a high risk of bias. The 4 strategies that included the provision of drugs or equipment plus LHW training with or without other intervention components had effects ranging from 8.7 to 57.7 %-points (3 were statistically significant), but all were tested by <3 study comparisons each and thus had results with very limited generalizability. All 5 strategies tested by >3 study comparisons each (whose results were more generalizable) had effects that were relatively small (<6 %-points) and not statistically significant: training only, supervision only, training plus supervision, information and communication technology (ICT) for LHWs only, and provision of equipment plus supervision plus non-supervisory feedback plus ICT. A sensitivity NMA with an expanded definition of LHWs including unlicensed private sector providers had similar findings, except for significant effects for training only (2.8 %-points) and supervision only (13.3 %-points). Despite limitations, our NMA provides evidence that will help decision-makers select strategies to improve LHW performance in LMICs—and potentially avoid relatively ineffective strategies.

1991

EXAMINING THE FEASIBILITY OF COMMUNITY HEALTH WORKER DELIVERY OF SEVERE ACUTE MALNUTRITION TREATMENT USING AN INNOVATIVE SIMPLIFIED LOW-LITERACY PROTOCOL: RESULTS FROM NIGERIA

Olusola B. Oresanya[1], Olatunde Adesoro[1], Prudence Hamade[2], Helen Counihan[2], Patrick Gimba[3], Amina Isah[4], Kolawole Maxwell[1], Naoko Kozuki[5], Bethany Marron[5]

[1]*Malaria Consortium, Abuja, Nigeria,* [2]*Malaria Consortium, London, United Kingdom,* [3]*Nigers State MInistry of Health, Minna, Nigeria,* [4]*State Primary Health Care Development Agency, Minna, Nigeria,* [5]*International Rescue Committee, New York, NY, United States*

Pneumonia, diarrhea, and malaria are leading causes of death among under-fives worldwide. Malnutrition is an underlying cause in half of these deaths. Although integrated community case management (iCCM) is a recognized strategy for increasing access to life-saving treatment, severe acute malnutrition (SAM) is often not included, with treatment only available at outpatient therapeutic centers and delivered using complicated protocols. Using human-centered design, the International Rescue Committee developed a simplified protocol and tools for treating SAM in low-literacy settings, which Malaria Consortium adapted and piloted with community-oriented resource persons (CORPs) providing iCCM services in hard-to-reach areas in Niger State (2017-2018). We trained 67 CORPs and 20 supervisors on the protocol and tools, and 303 children who were in the dark red (9-10.25cm) or pink (10.25-11.5cm) zones on a modified mid-upper arm circumference (MUAC) strip, passed an appetite test and had no symptoms of severe disease were enrolled in the study. We used mixed methods – focus group discussions, in-depth interviews, and data on treatment duration and outcomes for 288 of the enrolled children – to evaluate the feasibility, acceptability and effectiveness of the protocol and tools. A one-sample non-inferiority test conducted against Sphere's 75% standard revealed an overall cure rate of 73.4%, which was non-inferior to the Sphere standard. Results of further analysis showed: median weeks to cure of 6.5; a non-response rate of 4.5%; a default rate of 22%; and referral rate of 14.8%. It also showed that children enrolled in the worst MUAC zone (20.1%) were 29% less likely to recover than their pink zone (79.9%) counterparts and were 50% more likely to be referred. Qualitative data indicated acceptability among CORPs and caregivers. This study demonstrates the feasibility, acceptability and effectiveness of using a simplified protocol for treating SAM via existing iCCM programs being implemented by low-literate CORPs. Community health workers are key to expanding access to SAM treatment if universal health coverage is to be achieved in Africa.

1992

THEMATIC ANALYSIS OF COMMUNICATION BETWEEN PEDIATRIC HEALTHCARE PROVIDERS AND THE SOMALI COMMUNITY

Kristin Maletsky[1], Jibril Mohamed[2], Stephanie Lauden[1]

[1]*Nationwide Children's Hospital, Columbus, OH, United States,* [2]*The Ohio State University, Columbus, OH, United States*

Healthcare providers (HPs) receive variable training in cultural sensitivity despite increasing US refugee populations. Somali refugees face challenges navigating the US healthcare system, yet little is known about how HPs understand or address existing communication barriers. We aimed to understand HPs' perceptions of clinical interactions with Somali patient families, specifically related to communication surrounding pediatric chronic diseases. A convenience sample of HPs (physicians, NPs/PAs, nurses) at an American academic pediatric hospital completed an anonymous, voluntary, online survey in 2019. Demographic data and a self-reported Likert scale (1=no confidence/understanding, 5=could not be more confident/understood) were collected and evaluated using descriptive statistics. HPs completed 3 open-ended questions on their understanding of how the Somali community defines chronic disease and their observations regarding communication and understanding

between HPs and the Somali community. Using Grounded Theory, a qualitative analysis was completed until saturation was theoretically achieved. A total of 162 HPs responded from 16 countries of origin. 45% have served abroad in a healthcare setting. HPs reported some understanding of Somali culture (avg 2.3) and slight confidence in their overall ability to communicate with Somali families (avg 2.8) though less confidence in conversations regarding chronic disease (avg 2.5). 40% reported feeling poorly understood by Somali patient families. Qualitative analysis revealed HP concerns regarding language barriers (34%, n=51), mismatch of expectations between HPs and families (17%, n=26), as well as positive interactions (18%, n=27). Interpreters were described as both integral (18%, n=27) and potentially inaccurate or variable in quality (17%, n=25). 77% (n=115) reported a lack of understanding when asked how the Somali community views chronic disease. Although many positive interactions occur, most HPs lack confidence or understanding regarding communication with our Somali families, presenting a significant opportunity for education and advocacy.

1993

PILOTING EXPANSION OF A PUBLIC SECTOR REPORTING TOOL INTO COMMUNITY-LEVEL PRIVATE SECTOR FACILITIES IN UGANDA

Dorcas Kemigisha[1], Emily A. Briskin[1], Luke Baertlein[1], Alex Ogwal[1], Carol Kyozira[2], Deepa Pindolia[1], Jimmy Opigo[2]

[1]*Clinton Health Access Initiative, Kampala, Uganda,* [2]*Ministry of Health, Kampala, Uganda*

The private sector is an important source of care for sick children in Uganda. With the aims of improving the quality of care and enhancing government awareness and follow-up of fever cases, in August 2018, a pilot group of 108 community-level private facilities in three districts of Uganda were trained to use a government-designed patient register book and to report a set of case count, diagnosis, treatment and stock indicators weekly via the public-sector SMS-based disease surveillance system, mTrac. Data from mTrac flows into the national DHIS-2, increasing oversight and accountability. District and national health authorities met quarterly to review HMIS data submitted by private facilities via mTrac, selecting those with poor reporting or case management to be supervised using a standardized checklist. An observational study was conducted over the six-month pilot, descriptively analyzing routine weekly mTrac data completeness and quality. Further, Mann-Whitney test was used to compare private sector reporting rates to those in the public sector, and linear regression was used to assess trends in private sector reporting rates over time. Results from this pilot period showed that frequency, timeliness, and quality of data reported by private facilities were similar to that of public facilities. With an average weekly reporting rate of 90% in private facilities and 95% in public facilities, the rates were similar, but statistically different ($p<0.01$), suggesting the need for refinement of reporting training and support practices for the private sector. Linear regression analysis showed no effect of time on private sector reporting rate (slope = 0.0, $p=0.62$), suggesting the reporting rate did not decline over the six-month pilot period. The results indicate that private facilities can be expected to report in HMIS via the mTrac SMS-based platform reliably, and that these results can be sustained. To ensure the scalability and sustainability of reporting by private sector facilities, the relevant policies, guidelines, budgets, and national training and support curricula must be updated to integrate the community-level private sector.

1994

EXPLORING COLLABORATIONS BETWEEN A CHILD MORTALITY SURVEILLANCE PROGRAM AND THE INFORMAL HEALTH CARE SYSTEM OF TRADITIONAL BIRTH ATTENDANTS TO IMPROVE DEATH NOTIFICATION

Saquina Cossa[1], Maria Maixenchs[2], Felismina Tamele[1], Zubaida Manhenge[1], John Blevins[3], Inacio Mandomando[1], Quique Bassat[2], Khatia Munguambe[1]

[1]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique,* [2]*ISGlobal, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain,* [3]*Emory Global Health Institute, CHAMPS Program Office, Emory University, Atlanta, GA, United States*

In low income countries like Mozambique, detailed information on deaths occurring in the community is lacking, as >50% of these deaths are not formally reported nor registered. Given that stillbirths and deceased neonates are buried shortly after death, tracing these events is challenging. The Child Health and Mortality Prevention Surveillance Program (CHAMPS) aims to track the Cause of Death (CoD) in children from <5 years old, through collection of clinical data, verbal autopsy, and minimally invasive tissue sampling (MITS). MITS consists of small samples from body tissues and fluids, collected within 24 hours of death. Through community engagement, a strategy was developed to determine how traditional birth attendants (TBA) manage deaths in the community and explore potential links of collaboration for reporting deaths that occur outside health facilities. Snowball sampling was used to select TBAs from Manhiça District (Southern Mozambique). Participatory meetings were held at the neighborhoods from October to December 2017. Field notes, reports and summaries from 18 meetings (engaging 190 TBAs) were introduced into a matrix for data analysis. TBAs explained that they are usually first to confirm stillbirths and deceased newborns in communities, are responsible for informing the traditional authorities about deaths, and in charge of preparing and burying the body in a discreet ceremony, as soon as possible after the death. Despite this limited time window (<1 day), TBAs stated that delaying the ceremony to perform MITS would be possible, provided that families are informed about the procedure's duration. All TBAs stated that they were willing to cooperate with a death notification process in Mozambique. They suggested reporting deaths through a telephone number dedicated for this purpose. Our findings suggest that TBA collaboration in death notification will be very valuable, as they have privileged knowledge about stillbirths and newborn deaths occurring in their areas.

1995

IMPACT OF TRAINING AND SUPPORTIVE INTERVENTIONS ON CASE MANAGEMENT AND REPORTING IN PRIVATE DRUG SHOPS IN TANZANIA

Emily A. Briskin[1], Abdallah Lusasi[2], Felix Lam[3], Richard Silumbe[4], Mathew Mganga[5], Rose Rutizibwa[4], Happy Ndomba[4], Deepa Pindolia[6], Elia Martin[2]

[1]*Clinton Health Access Initiative, Kampala, Uganda,* [2]*National Malaria Control Program, Dar es Salaam, United Republic of Tanzania,* [3]*Clinton Health Access Initiative, Boston, MA, United States,* [4]*Clinton Health Access Initiative, Dar es Salaam, United Republic of Tanzania,* [5]*President's Office Regional Administration and Local Government, Dodoma, United Republic of Tanzania,* [6]*Clinton Health Access Initiative, Nairobi, Kenya*

In Tanzania, Accredited Drug Dispensing Outlets (ADDOs) are an important source of care for sick children. Our study aimed to evaluate program interventions designed to improve case management provided by ADDOs for diarrhea, pneumonia, and malaria in children under five. In July 2017, a baseline survey was conducted to measure ADDO dispenser Febrile Illness Management (FIM) knowledge. This informed a training plan in October 2017, where 1467 ADDOs in 4 regions of Tanzania were trained on FIM. The training covered 1) how to assess and treat for diarrhea, pneumonia, and malaria and 2) submission of monthly surveillance data using register books and a mobile text-message-based reporting system.

A holistic package of interventions, including bimonthly SMS reminders, education SMS, performance feedback and targeted quarterly in-person supervision were implemented to reinforce FIM and reporting concepts. In December 2018, a midline survey of a representative sample of ADDOs was conducted to assess their FIM knowledge. We conducted a cross sectional pre- and post-intervention descriptive analysis, which showed that knowledge of appropriate referral for severely ill children increased from 5% at baseline to 76% at midline; knowledge of correct pneumonia and diarrhea treatment increased from 50% to 74% and 50% to 65%, respectively. Reporting rates for November and December 2018 were measured and compared before and after SMS reminders were sent, and differences in reporting rate were assessed using a test of proportions. Results showed that reporting rates increased significantly from 49% to 62% in November and 42% to 59% in December after SMS reminders were sent ($p<0.01$ for both months). The comprehensive set of interventions, including training, educational messages, and targeted supportive supervision, was shown to improve FIM knowledge. SMS reminders were shown to significantly improve reporting rates. SMS reminders should be continued and educational efforts to maintain ADDO dispenser case management knowledge can continue to be systematically modified and assessed to identify practices that maximize FIM knowledge.

1996

A DEEP SEQUENCING APPROACH TO DEFINE BENZIMIDAZOLE RESISTANCE GENE FREQUENCIES IN HUMAN HOOKWORM EGG SAMPLES FROM KPANDAI DISTRICT, GHANA

Santosh George[1], Peter Suwondo[1], Joseph Otchere[2], Lisa M. Harrison[1], Kaya Bilguvar[3], James Knight[3], Adalgisa Caccone[4], Debbie Humphries[5], Michael D. Wilson[2], Michael Cappello[1]

[1]*Yale Partnerships for Global Health, Yale School of Medicine, New Haven, CT, United States,* [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana,* [3]*Yale Center for Genome Analysis, Yale School of Medicine, New Haven, CT, United States,* [4]*Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, United States,* [5]*Department of Epidemiology of Microbial Diseases, Yale School of Public Health, Yale University, New Haven, CT, United States*

Global control of human hookworm and other STH infections relies on periodic Mass Drug Administration of benzimidazole drugs to high risk groups, regardless of infection status. Our previous studies in Central Ghana demonstrated poor hookworm treatment outcomes with albendazole. In an effort to further assess hookworm response to treatment, we conducted a cross-sectional epidemiological study of hookworm infection in Kpandai District in the Northern Region of Ghana. Participants (n=566) were recruited from 3 rural communities and assessed for STH infection using Kato Katz fecal microscopy. Hookworm-positive individuals were treated with single-dose albendazole(400mg) and re-evaluated after 11-14 days. The prevalence of hookworm was 19.6% and varied significantly by community (range 4.5-33.9%). Response to treatment was at or near WHO effectiveness standards (egg reduction rates 85-94%; cure rates 64-80%). Genomic DNA (gDNA) was extracted from hookworm eggs purified from infected subjects. Three single nucleotide polymorphisms (SNPs;F167Y, E198A and F200Y) corresponding to putative resistance markers within the coding region of the β- tubulin isotype-1 gene of *N. americanus* were characterized in 30 matched pre and post treatment samples using deep sequencing. Each SNP was amplified in two separate PCR reactions, and molecular barcodes were used to label each gDNA fragment and study subject sample. Post sequence analysis revealed 24 million reads, and further *in silico* analysis showed complete coverage of the amplified *loci*. The highest mean alternative nucleotide allele frequency at each position was 0.019 for the F167Y amplicon and 0.131 for the E198A/F200Y amplicon, suggesting minimal genomic variation. There were no samples found to harbor egg DNA with the SNP F167Y marker, while one study subject's sample contained low frequency reads associated with SNPs E198A(3.15%) and F200Y(3.13%). Efforts are underway to compare these results with data from quantitative real-time PCR analysis. This deep sequencing method provides a useful tool to monitor for emerging benzimidazole resistance at the individual and community level.

1997

COMPARISON OF WORLD HEALTH ORGANIZATION AND DEMOGRAPHIC AND HEALTH SURVEY DATA TO ESTIMATE SUB-NATIONAL DEWORMING COVERAGE IN PRE-SCHOOL CHILDREN

Nathan C. Lo[1], Ribhav Gupta[2], David G. Addiss[3], Eran Bendavid[4], Sam Heft-Neal[2], Alexei Mikhailov[5], Antonio Montresor[5], Pamela Sabina Mbabazi[5]

[1]*Stanford University School of Medicine; University of California San Francisco, Stanford; San Francisco, CA, United States,* [2]*Stanford University, Stanford, CA, United States,* [3]*Task Force for Global Health, Decatur, GA, United States,* [4]*Stanford University School of Medicine, Stanford, CA, United States,* [5]*World Health Organization, Geneva, Switzerland*

The key metric for monitoring of deworming programs against soil-transmitted helminthiasis remains WHO-reported national drug coverage. There is increasing interest in using sub-national coverage data as a tracer for equity and to measure within country differences. The Demographic and Health Survey (DHS) potentially offers a readily available source for sub-national data. This study compared deworming coverage reported to WHO and estimated by DHS in pre-school children from Burundi (2016-2017) and Myanmar (2015-2017). WHO provided routinely reported sub-national coverage data, which included the date and coverage of deworming campaigns. We used geospatial, cross-sectional DHS surveys that included mother's report of her child's deworming receipt in past 6 months. DHS survey data were matched to WHO deworming campaigns geographically (at a regional level) and temporally (aligning the survey question recall period with the WHO reporting period). We estimated mean difference in regional deworming coverage between WHO and DHS data and performed sensitivity analyses. We included eligible data on pre-school children from 13 of 18 regions in Burundi (3,585 DHS observations) and 11 of 14 regions in Myanmar (1,656 DHS observations). The reported national deworming coverage in Burundi was 79.8% to WHO and 75.5% by DHS, while in Myanmar coverage was reported as 92.9% to WHO and 47.9% by DHS. In Burundi, the absolute mean regional coverage difference was 10% (range: 1-33%) with a greater WHO coverage in 8 of 13 regions. In Myanmar, the absolute mean regional coverage difference was 45% (range: 24-62%) with greater WHO coverage in 11 of 11 regions. In sensitivity analysis, mean coverage difference was robust to assumptions regarding maternal recall period. WHO and DHS deworming coverage estimates were broadly consistent in Burundi and largely discrepant in Myanmar. These differences may be explained by quality of data reporting and country health information management practices. DHS data can complement WHO deworming coverage data as an independent check on data quality and as a tool to generate sub-national coverage and equity estimates.

1998

PREDISPOSITION AND HOUSEHOLD CLUSTERING OF SOIL-TRANSMITTED HELMINTH INFECTION EVIDENT IN MYANMAR COMMUNITIES THAT HAVE RECEIVED EXTENSIVE MASS DRUG ADMINISTRATION

Julia C. Dunn[1], Martin Walker[1], Alison A. Bettis[1], James E. Wright[1], Nay Yee Wyine[2], Aye Moe Moe Lwin[3], Nay Soe Maung[3], Roy M. Anderson[1]

[1]*Imperial College London, London, United Kingdom,* [2]*London Centre for Neglected Tropical Disease Research, London, United Kingdom,* [3]*University of Public Health, Yangon, Myanmar*

This study analyses the association between demographics, socioeconomic and water, sanitation and hygiene (WASH) factors and prevalence of each STH species in two Myanmar communities that have received repeated rounds of MDA. The study also assesses the degree of

individual and household clustering of infection using longitudinal data, including two MDA rounds, to examine evidence of predisposition and household clustering of infection. For the different STH species, including the individual as a random effect accounted for 8.2-26.3% of the variation in prevalence whereas including household as a random effect accounted for 9.2-19.2% of the variation in prevalence. For any STH and *A. lumbricoides* the best fit models included both individual and household random effects whereas for *T. trichiura* and hookworm the best fit models included only the individual random effect. Factors that were protective for *A. lumbricoides* infection were high socioeconomic status (Adjusted Odds Ratio (AOR)=0.27, 95% Confidence Interval (CI) 0.11-0.69) and being in older age groups (25-39: AOR=0.1 95% CI 0.01-1.29. 40+: AOR=0.11, 95% CI 0.01-1.37). Being male was a risk factor for hookworm (AOR=3.60, 95% CI 1.26-10.28). When accounting for individual predisposition and household clustering, treating household drinking water through chemical and boiling methods was a risk factor for *A. lumbricoides* infection (AOR=2.11, 95% CI 0.91-4.61). This analysis indicates that there are varying patterns of clustering of infection for different STH species. Individual predisposition to infection is evident for all species, but variation in infection with *A. lumbricoides* and when consolidating all species (any STH) is also partially explained by household clustering. The results suggest that, to improve the impact of MDA and possibly achieve transmission interruption, focus needs to be on men and the poorest households in the endemic communities.

1999

EPIDEMIOLOGY OF SOIL-TRANSMITTED-HELMINTHIASIS FOLLOWING TWENTY-ONE ROUNDS OF MASS DRUG ADMINISTRATION IN SEVEN DISTRICTS, BANGLADESH

Sanjaya Dhakal[1], Abdullah A. Kawsar[2], Mohammad J. Karim[2], Michael R. Diaz[1], Alexander J. Jones[1], Rubina Imtiaz[1]

[1]The Task Force for Global Health, Atlanta, GA, United States, [2]Department of Disease Control, Dhaka, Bangladesh

Following WHO guidelines for STH morbidity elimination, Bangladesh conducted 21 rounds of mass drug administration (MDA) targeting school-age children. Children Without Worms (CWW) partnered with the Bangladesh national STH control program to measure the subsequent impact in 7-districts (almost 10% of national population) through surveys using previously described methodology. This paper shares the results of the 7-district concatenated data focusing on parasite- and age-specific prevalence, intensity of morbidity, and treatment history. Integrated Community-based Survey for Program Monitoring (ICSPM), primarily based on TAS-STH were conducted in 7-districts in 2017 - 2018. The surveys, conducted 5-months after the last, and one month prior to the next MDA, collected information at the household and individual levels. Kato-Katz method was used to examine the stool samples. The overall prevalence of any STH was 12.4% per laboratory examination of stool samples (n=7,597). Sunamganj (40.4%) and Sirajganj (26.9%) districts had the highest STH prevalence while Satkhira (2.0%) and Manikganj (3.1%) had the lowest. Moderate to high intensity (MHI) infection of STH was prevalent among 2.9% of overall respondents, while Sunamganj (10.4%) and Sirajganj (7.1%) had the highest prevalence of this surrogate measure for STH morbidity. The similarity in prevalence across all age groups in most districts was unexpected, especially the adults who were not treated through the MOH-conducted MDAs. Systematic intervention resulted in reduction of STH burden in the majority of surveyed districts. However, there were a few smaller areas of high, persistent infection highlighting the need for granular data in advanced programs. STH prevalence was comparable across age groups in spite of several rounds of MDA for SAC only and warrants further investigation. Impact surveys like the ICSPM and TAS-STH, are essential to guide programs that have achieved high, consistent treatment coverage. Additional tools and methods are needed to further map disease clusters in low transmission settings.

2000

FACTORS ASSOCIATED WITH SOIL-TRANSMITTED HELMINTHS (STH) PREVALENCE AND INTENSITY OF INFECTION IN COMÉ, BENIN, WEST AFRICA: FINDINGS FROM A BASELINE PREVALENCE SURVEY OF DEWORM3 STH-ELIMINATION TRIAL

Euripide F. G. A Avokpaho[1], Parfait Houngbegnon[1], Manfred Accrombessi[1], Gilles Cottrell[2], Eloïc Atindegla[1], Fadel Tanimomon[1], Félicien Chabi[1], Innocent Togbevi[1], Firmine Viwami[1], Aurax Fernando[1], Wilfrid Batcho[3], Dorothee A. Kindé-Gazard[4], Achille Massougbodji[1], Andre Garcia[2], Sean Galagan[5], Arianna Means[6], Tim Littlewood[7], Kristjana H. Ásbjörnsdóttir[6], Adrian J. Luty[8], Moudachirou Ibikounle[9], Judd Walson[6]

[1]Institut de Recherche Clinique du Bénin, Cotonou, Benin, [2]MERIT UMR 216, Institut de Recherche pour le Développement, Paris, France, [3]Programme National de Lutte contre les Maladies Transmissibles (PNLMT), Ministry of Health, Cotonou, Benin, [4]Centre de Lutte Intégrée contre le Paludisme (CLIP), Calavi, Benin, [5]International Clinical Research Center (ICRC), University of Washington, Seattle, WA, United States, [6]Department of Global Health, University of Washington, Seattle, WA, United States, [7]The DeWorm3 Project, The Natural History Museum of London, London, United Kingdom, [8]MERIT UMR 216, IRD, Université Paris 5, Paris, France, [9]Département de Zoologie, Faculté des Sciences et Techniques, Université d'Abomey-Calavi 01BP526, Cotonou, Benin

The DeWorm3 project aims to test the feasibility of interrupting the transmission of STH using a series of cluster-randomized trials in Benin, Malawi and India, using community-wide mass drug administration. From March to April 2018, a baseline STH (Hookworm, *Ascaris* and *Trichuris*) prevalence survey was conducted in Comé, Benin using the Kato-Katz technique. We report the parasitological results and factors associated with the presence and intensity of hookworm infection using mixed effects models. In total 6,139 stool samples were screened. Hookworm was the most prevalent (3.2% of samples), with a higher prevalence in adults than school aged (SAC) and pre-school aged childrend (PSAC) (4.4% vs 2% vs 1% ; p<0.001). In positive samples, we found a median of 4.5 eggs/slide (IQR: 2-13). Males were more infected (4% vs 2.6%; p=0.002) than females. Unadjusted risk factors for hookworm infection were being SAC (OR=2.4, 95%CI:1.03;5.4) or adult (OR=6.98, 95%CI:3.2;15.1), wearing shoes (OR=4.1, 95%CI:1.1;14.4), moisture/vegetation (OR=4.4, 95%CI:2.1;8.8), highest (3rd) tertile of sand fraction (OR=2.7, 95%CI:1.3;5.3) and peri-urban environment (OR:3.8, 95%CI:2.0;7.5). Protective factors were being female (OR=0.57, 95%CI 0.4;0.8), the highest (4th and 5th) quintiles of asset index (OR=0.2, 95%CI:0.1;0.4 and OR=0.2, 95%CI:0.1;0.3), no history of deworming in the past year (OR=0.2, 95%CI:0.1;0.4), improved water (OR:0.4, 95%CI:0.2;0.6), improved sanitation (OR=0.4, 95%CI:0.3;0.7). We found a positive association between the intensity of hookworm infection and being an adult (coeff=3.2, 95%CI:2.0; 4.4), wearing shoes (coeff=2.9, 95%CI:0.5; 5.3), moisture/vegetation (coeff=3.2, 95%CI:1.8; 4.5). A negative association was found with being a female (coeff=-1.2, 95%CI:-1.9; 0.6), no history of deworming in the past year (coeff=-2.87, 95%CI:-3.7;-2.0), improved water (coeff=-2.5, 95%CI:-3.4;-1.5), improved sanitation (coeff=-1.6, 95%CI:-2.5;-0.8). The prevalence of hookworm infection in Comé was generally low, but higher in adults. This could be explained by the national deworming program in Benin that targets only SAC.

2001

IMPACT OF ONCE VERSUS TWICE PER YEAR MASS DRUG ADMINISTRATION WITH DIETHYLCARBAMAZINE PLUS ALBENDAZOLE FOR LYMPHATIC FILARIASIS ON HOOKWORM PREVALENCE AND HEMOGLOBIN LEVELS IN PAPUA NEW GUINEA (PNG)

Brooke Mancuso[1], Nelly Sanuku[2], Samson Satofan[2], Delma Beaso[2], Yao-Chieh Cheng[3], Tobias Muare[2], William Pomat[2], Andrew Majewski[4], James Kazura[5], Gary J. Weil[4], Peter U. Fischer[4], Christopher L. King[5]

[1]Tulane University School of Public Health, New Orleans, LA, United States, [2]Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea, [3]Temple University School of Medicine, Philadelphia, PA, United States, [4]Washington University St. Louis School of Medicine, St. Louis, MO, United States, [5]Center for Global Health and Disease, Case Western Reserve University School of Medicine, Cleveland, OH, United States

The use of albendazole (ALB) as part of the LF mass drug administration (MDA) program provides the added benefit of treating hookworm and other helminth infections. Its impact on anemia in areas with high hookworm prevalence is poorly understood. Between 2014-18 in East Sepik Province, PNG serial annual cross-sectional sampling for STH by Kato Katz and hemoglobin (Hemacue) testing was conducted with community-based surveys in eight sentinel sites (200-400 participant/site); four sites received MDA 1x/yr and four received MDA 2x/yr to evaluate the impact of annual vs biannual MDA on lymphatic filariasis (LF). This region has high prevalence *Necator americanus* infection with baseline rates of 96% (1x/yr) and 90% (2x/yr). Hemoglobin levels negatively correlated with intensity of infection, especially among women (r^2 = -0.27 P=0.02). Following 3 years of MDA, geometric mean egg per gram (epg) among infected persons decreased significantly, especially among the heavily infected, in both areas; 892 epg (710, 1074, 95%CI) to 391 epg (258, 525) in 1x/yr and from 1060 epg (877, 1244) to 636 epg (468, 805) in 2x/yr sites, with a corresponding decrease in the prevalence of hookworm infection to 81% (1x/yr) and 84% (2x/yr). The decrease in hookworm burden was slightly greater among participants receiving 1x/yr MDA. Prior to MDA anemia (Hb <11g/dL) rates were 45% (1x/yr) and 36% (2x/yr). Following 3 years of MDA the proportion of individuals with anemia changed little 44% (1x/yr) and 42% (2x/yr). However changes in anemia rates varied with respect to age and sex. Anemia rates increased in children <15 years of age (43% to 62% p<0.0001 in females, and 42% to 56% in males p<0.0001). Likewise, the proportion of women ≥15 years with anemia increased (36% to 44%, p=0.06). In contrast, anemia rates dropped in men ≥15 years (40% to 20% p<0.0001, 1x/yr) and (36% to 13%, p<0.0001, 2x/yr). Thus, MDA with ALB for LF decreased hookworm burden, but there was no advantage of biannual relative to annual MDA. Only adult men showed a reduction in anemia rates at year 3.

2002

RELEVANT SPATIAL SCALE FOR EVALUATION UNITS FOR ELIMINATION PROGRAMS FOR SOIL-TRANSMITTED HELMINTHS GIVEN GEOGRAPHY OF SETTLEMENTS AND HUMAN MOVEMENT

Carolin Vegvari, Robert Hardwick, James Truscott, Roy Anderson

Imperial College London, London, United Kingdom

Soil-transmitted helminths (STHs) are the most widespread neglected tropical disease (NTD) infectious agents. The current WHO goal for STHs is morbidity control or the elimination as a public health problem (EPHP). A number of programmes are underway to prove that elimination of transmission of STHs is feasible in defined settings. Current WHO guidelines on evaluating whether EPHP has been achieved are based on sampling school-age children from different ecological zones within implementation units. There are no official guidelines on evaluating the elimination of transmission. The geospatial scale across which EPHP and transmission interruption are to be evaluated is currently not well defined and will depend on, among other factors, the geographical clustering of settlements and human movement patterns. Like other neglected tropical diseases STHs have a tendency to cluster both geographically and within groups of people, typically the most socio-economically disadvantaged. Multiple studies have addressed large-scale heterogeneity in STH prevalence and infection intensity to assist country-wide scale-ups of STH programmes. Local clustering of STH has been identified in all these studies. Here we use geospatial data from three different STH elimination programmes (Tumikia in Kenya, DeWorm3 in India, Benin and Malawi and Geshyaro in Ethiopia) and spatial kernel data characterising human movement in sub-Saharan Africa to define the geospatial scales of evaluation units in which interruption of transmission can be confirmed for the three main STH species, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm. We present a generalised model that can be applied to STH elimination programmes, regardless of the details of geography and human movement patterns, to determine an adequate spatial scale for evaluation units. This information should be very useful for STH programme managers and implementers.

2003

THE DAMAGE SIGNAL IL-33 PROMOTES A PROTECTIVE IMMUNE RESPONSE TO *TOXOPLASMA GONDII* IN THE BRAIN

Katherine M. Still, Samantha J. Batista, Jeremy A. Thompson, Nikolas W. Hayes, Carleigh O'Brien, Tajie H. Harris

Center for Brain Immunology and Glia, Department of Neuroscience, University of Virginia, Charlottesville, VA, United States

Chronic infection with the parasite Toxoplasma gondii elicits robust immune cell recruitment to the brain which is critical for host survival. We observe clusters of peripherally-derived immune cells, primarily monocyte-derived macrophages and T cells, surrounding replicating parasite in brain tissue. Initial cues which instruct the recruitment and localization of these blood-derived cells is not well understood. One potential mechanism of broad relevance is the incitement of host cell damage. Here we find that IL-33, a nuclear alarmin released upon cell damage, is critical for control of T. gondii parasite burden in the brain. IL-33 is expressed by mature oligodendrocytes and astrocytes during T. gondii infection, and is released into the CSF of infected mice, but not uninfected controls. After its release, IL-33 signaling induces expression of monocyte and T cell chemoattractants and increases adhesion factor expression. We find that IL-33 signaling is required to recruit anti-parasitic immune cells to the brain, including IFN-γ+ proliferating T cells and iNOS+ monocyte-derived cells. Although IL-33 (and most alarmins) signal directly on immune cells in the lung, gut, and skin, we find the relevant responder to IL-33 in the brain during our infection to be radio-resistant, and therefore likely brain resident, using bone marrow chimera experiments. Upon conditionally deleting IL-33's receptor from individual brain cell types, we find that IL-33 signals on multiple cell types in a concerted effort to control of T. gondii infection. These results expand our knowledge of alarmin signaling in the brain, indicating a brain-centric signaling mechanism for IL-33-mediated recruitment peripheral immune cells to sites of T. gondii replication.

2004

BASOPHILS REGULATE HELMINTH-INDUCED INNATE LYMPHOID CELL RESPONSES BY MODULATING NEUROPEPTIDE RECEPTOR EXPRESSION

Juan Inclan-Rico, J.J. Ponessa, C.M. Hernandez, M.C. Siracusa

Center for Immunity and Inflammation, Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ, United States

Type 2 cytokine responses, characterized by the production of interleukin (IL)-4, 5, and 13; promote immunity to helminth parasites, but are also responsible for the immunopathology observed during chronic helminth infections. A growing body of evidence suggests that the coordinated actions of innate immune cell populations and the nervous system promote anti-helminth immunity. However, whether the immune and nervous systems communicate in order to limit type 2 inflammation and promote tissue integrity remains poorly defined. Here we demonstrate

that helminth-induced type 2 innate lymphoid cell (ILC2) responses are exaggerated in the absence of basophils, resulting in increased inflammation and diminished lung function. Further, we show that ILC2s from basophil-depleted mice express reduced levels of neuropeptide receptor that is associated with their enhanced activation. Critically, activation of neuropeptide-signaling pathways reduced infection-induced ILC2 responses, lung eosinophils and parasite clearance. Moreover, neuropeptide signaling was sufficient to reduce IL-5 and IL-13 expression by sort-purified lung ILC2s from control but not basophil-depleted mice. Remarkably, co-culture with basophils was sufficient to enhance neuropeptide receptor expression on ILC2s. Collectively, these data suggest that basophils mediate the ability of ILC2s to respond to neuron-derived signals necessary to limit inflammation and maintain tissue integrity.

2005

DEVELOPMENTAL COMPETENCE AND ANTIGEN SWITCH FREQUENCY CAN BE UNCOUPLED IN *TRYPANOSOMA BRUCEI*

Kirsty R. McWilliam[1], Alasdair Ivens[2], Liam J. Morrison[2], Monica Mugnier[3], Keith R. Matthews[2]

[1]University of Edinburgh, Edinburgh, Scotland, United Kingdom and Ludwig-Maximilians-Universität München, Munich, Germany, [2]University of Edinburgh, Edinburgh, Scotland, United Kingdom, [3]Johns Hopkins School of Public Health, Johns Hopkins University, Baltimore, MD, United States

African trypanosomes use an extreme form of antigenic variation to evade host immunity. This involves the switching of expressed variant surface glycoproteins, antigen exchange being a stochastic and parasite intrinsic process. Parasite development in the mammalian host is another feature of the infection dynamic, with trypanosomes undergoing quorum sensing-dependent differentiation between proliferative slender form and arrested, transmissible, stumpy forms within each parasitaemic wave. Longstanding experimental studies have suggested that the frequency of antigenic variation and transmissibility may be linked, antigen switching being higher in fly-transmissible, developmentally competent, parasites and lower in serially passaged lines. Here, we have directly tested this tenet of the infection dynamic by, firstly, generating lines that inducibly lose developmental capacity through RNAi mediated silencing of components of the stumpy induction signalling cascade ('inducible monomorphs'). Secondly, we have derived de novo lines that have lost the capacity for stumpy formation by serial passage ('selected monomorphs') and analysed their antigenic variation in comparison to isogenic pre-selected populations. Analysis of both inducible and selected monomorphs by in vitro flow-cytometry based VSG switch assays and VSGseq has established that antigen switch frequency does not change regardless of the method used to prevent parasite development. We conclude that changes in antigen switch frequency and developmental capacity can be uncoupled, these independently selected traits being important contributors to the parasite infection dynamic.

2006

MYND AND RNA-BINDING PROTEIN 6 (RBP6) AS MASTER REGULATORS OF *TRYPANOSOMA BRUCEI* DIFFERENTIATION AND MIGRATION IN THE TSETSE

Aitor Casas-Sanchez[1], Lara Lopez-Escobar[1], Aryana Zardkoohi-Burgos[1], Cintia Cansado-Utrilla[1], Lee R. Haines[1], Alistair Darby[2], Samuel Dean[3], Jannah Shamsani[4], Pegine Walrad[4], Alvaro Acosta-Serrano[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]University of Liverpool, Liverpool, United Kingdom, [3]University of Oxford, Oxford, United Kingdom, [4]University of York, York, United Kingdom

To complete its life cycle in the tsetse fly vector and become transmissible to the mammalian host, the parasite Trypanosoma brucei has to undergo a series of migration and differentiation events, ultimately controlled through gene expression. After the bloodstream forms differentiate into the procyclic stage in the tsetse midgut, trypanosomes colonize the fly proventriculus (PV) where they reach the epimastigote stage. These further migrate to the salivary glands to differentiate into the mammalian-infective metacyclic forms which can be transmitted to the host with the tsetse bite. To identify T. brucei genes important for life cycle progression in the tsetse, we compared the gene expression profiles of fly-transmissible PV trypanosomes with that of a strain unable to infect the salivary glands. Among the top upregulated transcripts in fly-transmissible parasites, we identified the RNA-binding protein 6 (RBP6) known to trigger differentiation when overexpressed in vitro, and a new hypothetical protein conserved across kinetoplastids containing a MYND (Myeloid, Nervy and DEAF-1) zinc finger domain. Strikingly, the overexpression of MYND protein in non-transmissible trypanosomes triggered differentiation to the epimastigote stage through a soluble factor in vitro and restored salivary gland infectivity in the tsetse. Pull-down assays revealed that MYND is part of a new protein complex formed by five hypothetical proteins with identifiable zinc finger domains. We found that such complex may interact and stabilize >150 mRNAs encoding RNA-binding, zinc finger and flagellar proteins, among others. In addition, MYND knockout cells presented severe growth and motility defects in vitro and were completely unable to colonize the PV and complete the cycle in the tsetse. On the contrary, RBP6 knockout cells showed no defects except for the inability to differentiate to epimastigotes later in the PV. We hypothesize that the sequential expression of the MYND complex and RBP6 is key to coordinate the developmental progression of T. brucei in the tsetse. Supported by the Wellcome Trust and ITN-FP7 GlycoPar.

2007

A BAR-SEQ FITNESS SCREEN OF LEISHMANIA CRISPR-CAS9 KNOCKOUT MUTANTS SHOWS THE IMPORTANCE OF MOTILITY IN COLONIZATION OF SANDFLIES

Tom Beneke[1], James Smith[1], Edward Hookway[2], Tomas Becvar[3], Jitka Myskova[3], Tereza Lestinova[3], Jovana Sadlova[3], Petr Volf[3], Richard Wheeler[4], Eva Gluenz[1]

[1]University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom, [2]Research Department of Pathology, University College London, London, United Kingdom, [3]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic, [4]Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

The number of fully sequenced Leishmania genomes increases steadily but the function of more than half of the predicted genes remains unstudied. To accelerate dissection of gene function in Leishmania spp. we developed a streamlined pipeline for generation of CRISPR-Cas9 mediated gene knockout in which each mutant carries a unique 17-nucleotide barcode, allowing loss-of-function screens and phenotyping of mutants in mixed populations. To date we have used this method to generate more than 400 null mutants in a knockout screen of the flagellar proteome. This mutant library enables a systematic analysis of the contribution of conserved and kinetoplastid-specific proteins to flagellar motility and function. Individual phenotyping uncovered diverse phenotypes including defects in motility, flagellar assembly, axonemal structure, paraflagellar rod (PFR) integrity and maintenance of cell shape. We show here how pooled fitness screens were used in vitro and in infections of the permissive sand fly vector Lutzomyia longipalpis to determine the effect of flagellar defects on parasite growth and ability to colonise the fly. Promastigotes that were aflagellate, paralysed or uncoordinated swimmers were severely diminished in the parasite population after defecation of the bloodmeal. Dissection of sand flies infected with L. mexicana lines lacking the central pair protein PF16 showed that these paralysed promastigotes did not reach the anterior regions of the fly alimentary tract. These results suggest that motility in Leishmania parasites is vital for colonization of sand flies and show the potential of this method for larger-scale fitness screens of Leishmania mutants.

2008

NANOSCALE ELUCIDATION OF THE INVASION APPARATUS OF APICOMPLEXAN PARASITES

Li-av Segev-Zarko[1], Stella Y. Sun[1], Peter D. Dahlberg[1], Daniel Pelt[2], Jian-Hua Chen[3], Michael F. Schmid[1], Jesus Galaz-Montoya[1], W. E. Moerner[1], Carolyn Larabell[4], James Sethian[2], Wah Chiu[1], John Boothroyd[1]

[1]Stanford University, Stanford, CA, United States, [2]University of California Berkeley, Berkeley, CA, United States, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, United States, [4]University of California San Francisco, San Francisco, CA, United States

The phylum Apicomplexa includes several of the most important and prevalent eukaryotic humanparasites, such as the malaria-causing Plasmodium spp. and Toxoplasma gondii that can causesevere neurological disease in the developing fetus. These intracellular parasites enter a host cellby deploying a remarkable machine at their anterior end known as the apical complex (AC), forwhich the phylum is named. At the start of invasion, the AC, including a unique spiral of tubulinbasedfibrils called the conoid, is protruded and secretion events occur from two types of distinctsecretory organelles, capsule-shaped micronemes and club shaped rhoptries. The exact meansby which these various components of the AC coordinate invasion has been largely a mysterydue, in part, to a lack of tools capable of resolving the structure of this extraordinary apparatusin its natural context. To address this need, we are developing a pipeline to image the complexand dynamic structure of the AC to high resolution in parasites devoid of any chemical fixationor staining. This pipeline starts with soft x-ray tomography of plunge frozen parasites to revealthe whole 3D shape of extracellular parasites and the organization of large subcellular organellesinside it. Next, cryo-fluorescence super resolution microscopy and cryo-electron tomography(cryo-ET) are used to precisely localize known apical proteins to the AC subcellular organellesthrough fusion with a photoactivable fluorescent protein, PAmKate, to record fluorescenceoriginating from single molecules. Finally, nano-scale resolution images of these same parasite'sAC are acquired using cryo-electron tomography (cryo-ET) and annotation of the reconstructedtomograms is performed using mixed scale convolutional neural network analysis. Aligning thedata from the three imaging techniques should provide the precise location of the protein whiledisplaying the 3D organization of all the subcellular organelles and uncovering possibleinteractions between them. To start, we have used cryo-ET generated images of unprecedentedresolution of the apical rings, a component of the AC thought to be conserved among allapicomplexan parasites whose structure and function is unknown. Our images reveal that theapical rings exhibit a highly organized structure of repeated units, allowing us to apply techniquesof subtomogram averaging to resolve its structure at nano-scale resolution. To validate ourpipeline, we have endogenously tagged SAS6-L, a protein that was previously shown to localizeat the apical tip of the conoid and are analyzing this line through the methods described above.

2009

LIPID TRANSPORT AT THE MALARIA PARASITE *PLASMODIUM FALCIPARUM* - RED BLOOD CELL INTERFACE IS FACILITATED AT MEMBRANE CONTACT SITES

Matthias Garten[1], Josh R. Beck[2], Robyn Roth[3], Tatyana Tenkova-Heuser[1], John Heuser[1], Christopher K. E. Bleck[4], Daniel E. Goldberg[3], Joshua Zimmerberg[1]

[1]National Institutes of Health/NICHD, Bethesda, MD, United States, [2]Iowa State University, Ames, IA, United States, [3]Washington University, St. Louis, MO, United States, [4]National Institutes of Health/NHLBI, Bethesda, MD, United States

The malaria parasite must obtain all nutrients across an interface: the parasitophorous vacuole (PV). Small water-soluble molecules can traverse the PV membrane via channels formed by EXP2, while larger molecules such as hemoglobin can cross via an endocytosis-like mechanism. So far, it is unknown how the parasite maintains lipid homeostasis across the PV lumen. When analyzing electron micrographs of the human malaria parasite, Plasmodium falciparum, to look for lipid transport in the PV lumen, vesicular transport was not evident. However, we found that the distance of the parasite plasma membrane (PPM) to the PV membrane (PVM) appears regulated, demonstrating regions of close membrane "apposition" (~10 nm PVM-PPM distance) and regions of "PV lumen" (20-30 nm PVM-PPM distance). We hypothesized that the exchange of lipids is facilitated in regions of close membrane apposition. To test the hypothesis and localize proteins of known function with respect to PVM-PPM distance, we developed and validated a fluorescent label for the PV lumen (PV-mRuby3) using correlative light electron microscopy. Using this PV lumen label, we found that PV lumen regions co-localize to the nutrient-permeable channel EXP2 (tagged with mNeonGreen) and anti-localize to the lipid transporter PfNCR1 (tagged with GFP). Both proteins appear in patches, but together EXP2 and PfNCR1 complement each other to populate the periphery of the parasite. The set of experiments suggests that regions of close PVM-PPM apposition are sites of lipid exchange. Functionally and structurally, this meets the definition of a membrane contact site. Conceptually, we propose a division of the PV into two distinct domains for the exchange of water soluble and lipidic material as a framework to unravel the functions of this unique organelle.

2010

EARLY IFN-G PRODUCTION BY INNATE LYMPHOID CELLS MEDIATES PROTECTION TO *CRYPTOSPORIDIUM TYZZERI*

Jodi Gullicksrud, Adam Sateriale, Julie Engiles, Christopher Hunter, Boris Striepen

University of Pennsylvania, Philadelphia, PA, United States

From a global health perspective, Cryptosporidium is one of the most important causes of severe diarrheal disease in a variety of epidemiological settings. Similar to other enteric pathogens that are restricted to the epithelium of the intestine, Cryptosporidium invades the villus epithelium, but the role of epithelial cells in pathogen resistance remains unknown. Here we use genetically modified C. tyzzeri, a natural mouse pathogen, and show that intestinal epithelial cell (IEC) derived IL-18 synergizes with IL-12 to drive innate lymphoid cell (ILC) production of IFN-g, which critically limits parasite expansion within the first few days of infection. Further analysis of the NK1.1+NKp46+ population suggests that intestinal ILC1s are a major source of early IFN-g. Transcriptional profiling of IECs from infected mice highlighted the induction of an IFN-g gene signature and specific deletion of STAT1 from IECs resulted in unrestricted parasite growth. Thus, infection with C. tyzzeri has identified ILCs as an innate source of IFN-g and established its downstream impact on ileal epithelial cells. Additionally, C. tyzzeri has provided a model system to demonstrate a critical role of IECs in the induction of protective immunity, where they employ cell extrinsic and intrinsic mechanisms to limit pathogen growth.

2011

COMPARATIVE CHEMICAL GENOMICS IN *BABESIA* SPECIES IDENTIFIES A NOVEL APICOMPLEXAN DRUG TARGET

Caroline D. Keroack, Brendan Elsworth, Jacob A. Tennessen, Cristina K. Moreira, Aditya S. Paul, Manoj T. Duraisingh

Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA, United States

Babesiosis is an emerging human zoonosis, and a well-established and widely distributed veterinary infection caused by 100+ species of Babesia parasite. This enormous diversity poses a challenge in identifying broadly effective therapeutic interventions. However, there is also a unique opportunity to leverage the power of comparative analyses in multiple Babesia spp. to explore novel conserved biology, facilitated by the existence of in vitro culture systems. The diversity of Babesia parasites, coupled with the lack of potent inhibitors necessitates the discovery of novel conserved targets. Here, we describe a comparative chemogenomics (CCG) pipeline for the identification of novel and conserved targets. CCG

relies on parallel in vitro evolution of resistance in parallel independent populations of evolutionarily-related Babesia spp. (B. bovis and B. divergens). After screening the MMV Malaria box against both species, we identified a potent anti-babesial inhibitor, MMV019266. This compound is also strongly active in Plasmodium and shows some efficacy in Toxoplasma. We were able to rapidly select for resistance to this compound in two species of Babesia in parallel, achieving 10-fold or greater resistance after six weeks of intermittent selection. After sequencing of multiple independently derived lines in the two species, we were able to identify mutations in a single conserved gene in both species. This gene encodes a predicted membrane-bound metallodependent phosphatase (putatively named phoD^{api}) which is homologous to alkaline phosphatase D in other apicomplexa. Identification of this gene target was greatly facilitated by the comparative approach of utilizing two species. In B. divergens (Bdiv_001570c) we have identified different mutations at the same amino acid residue in the two independent selections (A: C197F, B: C197W). In B. bovis (Bbov_I003300) we were able to identify a mutation (G527S) which is spatially proximal to those found in B. divergens. In both cases, the mutations were found in the phoD-like phosphatase domain, proximal to the ligand binding site. Interestingly, the gene is predicted to contain an apicoplast leader sequence, and the ortholog has been identified in the apicoplast proteome in Plasmodium falciparum, suggesting a potential role in the apicoplast. Strikingly, MMV019266 does not exhibit a delayed death phenotype in Plasmodium, killing in the first cycle. The ability to identify phoD^{api} in two species, and in multiple independent selections, validates the role this gene plays as the etiological driver of resistance to MMV019266 in Babesia spp. Together, these data show the power of CCG to identify not only novel pan-babesiacidal compounds, but also to identify novel conserved apicomplexan biology.

2012

IDENTIFICATION OF A MASTER REGULATOR OF DIFFERENTIATION IN *TOXOPLASMA*

Benjamin S. Waldman[1], Dominic Schwarz[2], Marc H. Wadsworth II[3], Jeroen P. Saeij[4], Alex K. Shalek[3], Sebastian Lourido[1]

[1]Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Whitehead Institute for Biomedical Research, Cambridge, MA, United States, [3]Institute for Medical Engineering & Science (IMES), Department of Chemistry, and Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, United States, [4]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, Davis, CA, United States

Toxoplasma gondii chronically infects a quarter of the world's population, and can cause life-threatening disease in immunocompromised patients. Acute symptoms of infection result from the rapid proliferation and lysis of host cells by Toxoplasma tachyzoites. Chronic infection is established by slower-growing bradyzoites, which form intracellular cysts resistant to current therapeutics and immune clearance. Differentiation of tachyzoites into bradyzoites can be induced in cell culture through a variety of stress conditions, which has allowed intensive examination of transcriptional differences between these two stages. While this dramatic change in lifestyle is accompanied by widespread morphological, metabolic, and transcriptional shifts, the molecular mechanisms regulating these differences have remained elusive. The inability to isolate Toxoplasma mutants displaying a complete block in differentiation has led to the view that no master regulator of this process exists. Through a combination of Cas9-mediated genetic screening and transcriptional profiling, we have identified and characterized a single transcription factor (BFD1, bradyzoite formation deficient 1) as necessary and sufficient for differentiation. Parasites lacking BFD1 fail to differentiate in cell culture in response to all induction conditions tested and fail to form brain cysts in mice, and these defects are reversed by complementation. Translation of BFD1 is stress dependent, and conditional overexpression of BFD1 induces differentiation in the absence of stress conditions, recapitulating many of the transcriptional changes associated with bradyzoite formation. Profiling the genomic binding pattern of BFD1 revealed preferential binding at transcriptional start sites, particularly within promoters of genes highly upregulated in bradyzoites. BFD1 provides a genetic switch to study and control Toxoplasma differentiation, and will inform prevention and treatment of chronic infection.

Abstract Author Index

The number(s) following author name refers to the abstract number.

A

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

B

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

C

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

D

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

E

Abstract Author Index

The number(s) following author name refers to the abstract number.

F

The number(s) following author name refers to the abstract number.

G

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

H

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

I

Abstract Author Index

The number(s) following author name refers to the abstract number.

J

K

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

L

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

M

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

N

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

O

The number(s) following author name refers to the abstract number.

P

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Q

R

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

T

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

U

Abstract Author Index

The number(s) following author name refers to the abstract number.

V

The number(s) following author name refers to the abstract number.

W

Abstract Author Index

The number(s) following author name refers to the abstract number.

X

Y

The number(s) following author name refers to the abstract number.

Z