

6164

## CHARACTERIZATION OF TICK-BORNE ENCEPHALITIS VIRUS SAMPLES FROM *IXODES* TICKS COLLECTED IN MONGOLIA

Bazartseren Boldbaatar<sup>1</sup>, Noel Cote<sup>2</sup>, Andrew G. Letizia<sup>2</sup>, Doniddemberel Altantogtokh<sup>3</sup>, Graham A. Matulis<sup>4</sup>, **Nora G. Cleary<sup>1</sup>**, Michael E. von Fricken<sup>4</sup>

<sup>1</sup>Mongolian University of Life Sciences, Ulaanbaatar, Mongolia, <sup>2</sup>Naval Medical Research Unit TWO (NAMRU-2), Sembawang, Singapore, <sup>3</sup>National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia, <sup>4</sup>University of Florida, Gainesville, FL, United States

Tick-Borne Encephalitis Virus (TBEV) can cause neurological disease in humans with a range of clinical severity including death, depending on the subtype. Far-Eastern subtype has the highest mortality rates while Siberian is more likely to cause chronic disease. Tick-Borne Encephalitis (TBE) is endemic in Mongolia including both Siberian and Far-Eastern TBEV subtypes and has been detected since the 1980s. *Ixodes persulcatus* is the main vector of TBEV in Mongolia but this flavivirus has also been found in *Dermacentor* species. Understanding the epidemiology and evolutionary dynamics of TBEV is necessary in shaping the public health response to this deadly disease in Mongolia. Thirteen hundred *Ixodes persulcatus* ticks were collected in May 2020 from Eruu, Khuder, and Mandal in Selenge using the dragging and flagging method. Tick samples were homogenized, pooled (pool sizes ranged from 20-50), and then the supernatant was inoculated into Vero cells. Upon observing cytopathic effect (CPE), two reverse transcription polymerase chain reactions (RT-PCRs) were conducted on cell supernatant; one was to detect TBEV and the second was to subtype TBEV. Lysed cell culture supernatant was processed with Next-Generation Sequencing (NGS) using Illumina technology to obtain FASTA files for analysis. TBEV was detected from these samples and identified as the Siberian subtype using PCR. Ongoing phylogenetic analysis of NGS results will analyze genomic changes of TBEV to previously published TBEV sequences in the region. Subtype analysis of TBEV and tracking the viral evolution in ticks, specifically *Ixodes persulcatus* in Selenge, is vital to understanding the risk to the local populations. Current vaccines have been developed based on the European and Far-Eastern strains and genomic analysis of wildtype TBEV can inform vaccine strategies. Given the high concentration of this tick species and previous documentation of TBE infection in humans and ticks in this province, further characterization of TBEV in Mongolia is needed.

6165

## GENOMIC INSIGHTS INTO THE *SPIROPLASMA SYMBIONT* OF *GLOSSINA FUSCIPES FUSCIPES*: IMPLICATIONS FOR TRYPANOSOME TRANSMISSION CONTROL

**Daniel J. Bruzese<sup>1</sup>**, Fabian Gstöttenmayer<sup>2</sup>, Brian L. Weiss<sup>1</sup>, Adly M.M Abd-Alla<sup>3</sup>, Serap Aksoy<sup>1</sup>

<sup>1</sup>Yale University, New Haven, CT, United States, <sup>2</sup>Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria, <sup>3</sup>Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Tsetse flies (*Glossina* spp.) are primary vectors of parasitic trypanosomes responsible for human and animal African trypanosomiasis in sub-Saharan Africa. In addition to trypanosomes, tsetse flies harbor both obligate and facultative symbionts, which play crucial roles in host physiology and vector competence. *Spiroplasma*, a bacterium found in the tsetse fly species *Glossina fuscipes fuscipes* (*Gff*), has emerged as a potential candidate for reducing trypanosome transmission in its tsetse host. Previous research demonstrates a negative correlation between *Spiroplasma* presence and trypanosome infection in *Gff* flies and has shown *Spiroplasma* to be an apt manipulator of *Gff* physiology. However, the mechanisms behind the putative *Spiroplasma*-induced trypanosome resistance remain unknown. Here, to better understand the *Spiroplasma* strain that infects *Gff* flies, we conducted comparative genomics of *Spiroplasma* collected from the colony located at the FAO/IAEA Insect Pest Control Laboratory (IPCL) in

Seibersdorf Austria and from a population located at Toloyang village in Northwestern Uganda. Leveraging Oxford Nanopore (ONT) sequencing technology, we generated closed *Spiroplasma* genomes from individual *Gff* flies. Both the colony and field assemblies had a high degree of similarity in gene content and structure, suggesting they belong to the same strain, denoted as sGff. Phylogenomic analyses placed sGff within the *Spiroplasma poulsonii* clade, which is a clade predominantly comprised of other Dipteran-infecting strains. Within the sGff genome, we found genes involved in nutrient transport showing that sGff relies on its host for many essential metabolites. We also identified numerous mobile genetic elements and putative defensive genes, including prophages, plasmids, and toxin genes, that could be responsible for the sGff-induced resistance to trypanosomes. This study enhances our understanding of the sGff strain and its potential role in modulating trypanosome transmission in its *Gff* host and highlights the efficacy of ONT sequencing to rapidly unravel the biology of symbionts.

6166

## MICROBIAL DIVERSITY OF *CULICOIDES REEVESI* FROM CHIHUAHUA, MEXICO: A METAGENOMIC ANALYSIS OF RRNA 16S

**Rodolfo Gonzalez Peña<sup>1</sup>**, David Orlando Hidalgo Martínez<sup>2</sup>, Herón Huerta<sup>3</sup>, Erick de Jesús De Luna Santillana<sup>4</sup>, Jaime Raúl Adame Gallegos<sup>5</sup>, Carlos Arturo Rodríguez Alarcón<sup>2</sup>, Stephanie Viridiana Laredo Tiscareño<sup>2</sup>, Ezequiel Rubio Tabarez<sup>2</sup>, Julián Everardo García Rejón<sup>1</sup>, Luis M. Hernández Triana<sup>6</sup>, **Javier Alfonso Garza Hernandez<sup>2</sup>**

<sup>1</sup>Universidad Autónoma de Yucatán, Mérida, Mexico, <sup>2</sup>Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Mexico, <sup>3</sup>Instituto de Diagnóstico y Referencia Epidemiológicos, Ciudad de México, Mexico, <sup>4</sup>Laboratorio Medicina de la Conservación, Centro de Biotecnología Genómica del Instituto Politécnico Nacional, Reynosa, Mexico, <sup>5</sup>Universidad Autónoma de Chihuahua, Chihuahua, Mexico, <sup>6</sup>Animal and Plant Health Agency, Virology Department, Rabies and Wildlife Zoonoses Research Group, Addlestone, United Kingdom

This study aimed to investigate the microbial diversity of bacteria in the composite microbial community associated with *Culicoides reevesi* biting midges from Buenaventura municipality in the state of Chihuahua, Mexico, using a Sanger sequencing 16S rRNA metagenomics approach. Adult females of *Culicoides reevesi* were collected by human landing catches in the rainy season of 2023 and morphologically identified. They were grouped into pools of 25 individuals from which genomic DNA (gDNA) was extracted. Sanger sequencing of 16S rRNA was performed for a total of 4 pools, and the amplicon sequencing of the V3-V4 hypervariable region was done on Illumina Mseq platform to detect bacterial communities. The bioinformatic analysis included quality assessment, taxonomic classification, and visualization. The evaluation of the microbial community involved assessing taxa abundance and diversity using Mothur and QIIME2 software included in Galaxy Tool Shed (<https://usegalaxy.eu/>). Our study presents, for the first time in México and worldwide, an in-depth analysis of the bacteriome composition in *C. reevesi*, utilizing a 16S rRNA metagenomic approach. We emphasize the prevalence of dominant bacterial phyla, particularly Proteobacteria, alongside varying abundances of Actinobacteria, Firmicutes, Acidobacteria, and Bacteroidota, with a notable occurrence of Tenericutes. We identified intriguing species of both human and animal pathogenic bacteria. Moreover, we observed the absence of unidentified bacterial sequences, alongside the presence of other bacterial groups associated with the environment or plants. This has implications for both healthcare and ecological management, potentially simplifying control measures but also posing risks if the dominant species are harmful. This research enhances our understanding of the microbiome associated with *Culicoides* species, such as *Culicoides reevesi*, underscoring the need for further investigation to fully grasp their ecological importance and impact on public health.