



"Uso de la metagenómica para el descubrimiento de arbovirus en garrapatas"

Dr. Javier Alfonso Garza Hernández Instituto de Ciencias Biomédicas Universidad Autónoma de Ciudad Juárez

Contenido

- 1. Introducción.
- 2. Metagenómica o SNG.
- 3. Los arbovirus y clasificación e importancia.
- 4. Vectores: garrapatas.
- 5. ¿Qué estamos haciendo y que encontramos?

Introducción

secuenciación nueva generación (NGS) representa un hito histórico en la investigación genética y la biología molecular. Antes de su aparición en la década del 2000, la secuenciación (tipo Sanger) de ADN era un proceso costoso, lento y laborioso limitaba la lectura a fragmentos relativamente cortos genoma.

La NGS revolucionó este panorama al introducir una capacidad de secuenciación a gran escala y alta velocidad.



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Genome Sequencing in Open Microfabricated High Density Picoliter Reactors

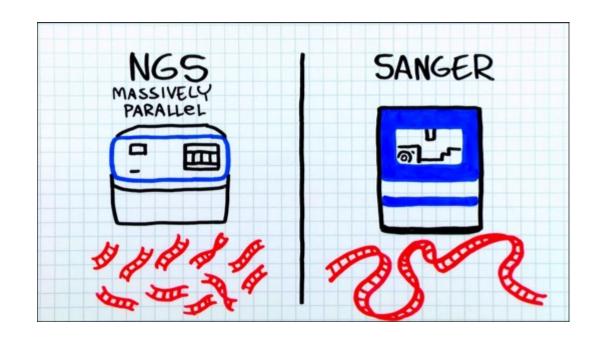
Marcel Margulies^{1,*}, Michael Egholm^{1,*}, William E. Altman¹, Said Attiya¹, Joel S. Bader¹, Lisa A. Bemben¹, Jan Berka¹, Michael S. Braverman¹, Yi-Ju Chen¹, Zhoutao Chen¹, Scott B. Dewell¹, Lei Du¹, Joseph M. Fierro¹, Xavier V. Gomes¹, Brian C. Goodwin¹, Wen He¹, Scott Helgesen¹, Chun He Ho¹, Gerard P. Irzyk¹, Szilveszter C. Jando¹, Maria L.I. Alenquer¹, Thomas P. Jarvie¹, Kshama B. Jirage¹, Jong-Bum Kim¹, James R. Knight¹, Janna R. Lanza¹, John H. Leamon¹, Steven M. Lefkowitz¹, Ming Lei¹, Jing Li¹, Kenton L. Lohman¹, Hong Lu¹, Vinod B. Makhijani¹, Keith E. McDade¹, Michael P. McKenna¹, Eugene W. Myers³, Elizabeth Nickerson¹, John R. Nobile¹, Ramona Plant¹, Bernard P. Puc¹, Michael T. Ronan¹, George T. Roth¹, Gary J. Sarkis¹, Jan Fredrik Simons¹, John W. Simpson¹, Maithreyan Srinivasan¹, Karrie R. Tartaro¹, Alexander Tomasz⁴, Kari A. Vogt¹, Greg A. Volkmer¹, Shally H. Wang¹, Yong Wang¹, Michael P. Weiner², Pengguang Yu¹, Richard F. Begley¹, and Jonathan M. Rothberg¹

1 454 Life Sciences Corp., 20 Commercial St., Branford, CT 06405, USA.

Es un sistema de secuenciación que supera en rendimiento a lo tradicional. Utiliza una placa de fibra óptica con millones de pequeños pozos y puede secuenciar grandes cantidades de ADN con alta precisión y corto tiempo. Este sistema demostró ser muy efectivo y preciso al secuenciar y ensamblar el genoma del *Mycoplasma genitalium*.

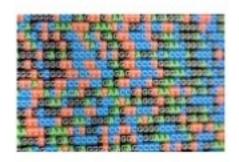
Metagenómica

La secuenciación de nueva generación (NGS-Next Generation Sequencing) es una que permite determinar tecnología secuencia de ADN y ARN con alta velocidad y a gran escala. Esto significa que puede leer miles o millones de secuencias nucleótidos simultáneamente, lo que la hace para analizar eficiente genomas muy completos y grandes cantidades de datos genéticos.

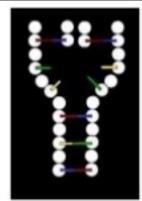


SANGERS Vs. NGS

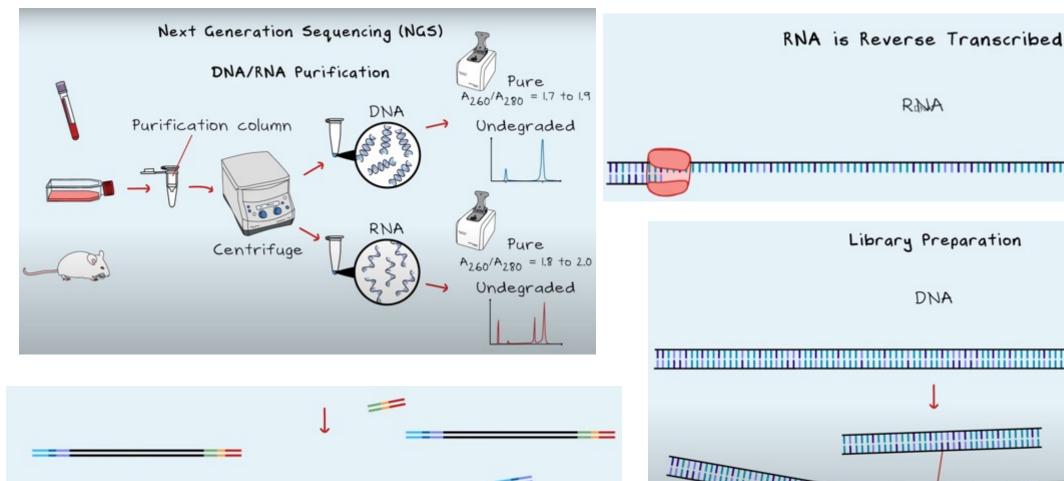
Features	Sanger	NGS	
Sequencing Samples	Clones, PCR	DNA Libraries	
Preparation Steps	Few, Sequencing reactions clean up	Many, Complex procedures	
Data Collection	Samples in plates : 96, 384	Samples on slides 1-16+	
Data	1 Read/ Sample	Thousands & Millions of Reads/ Samples.	

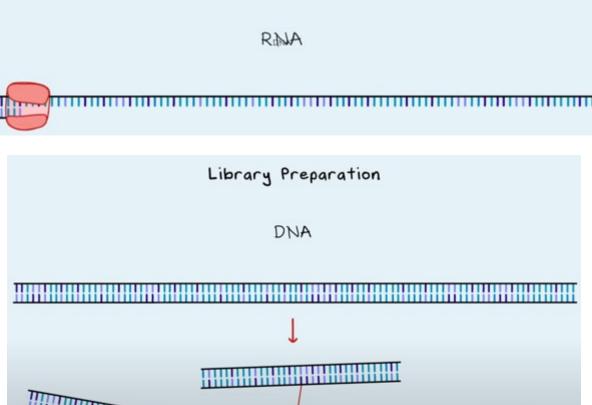


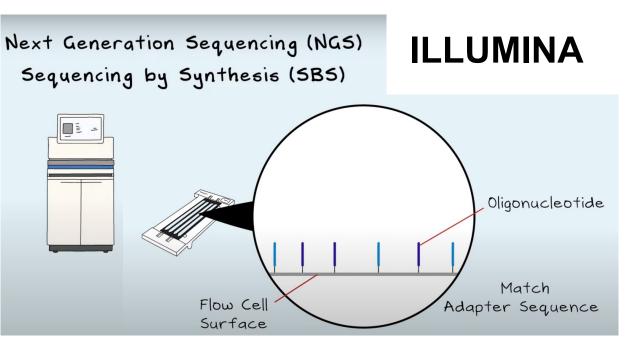


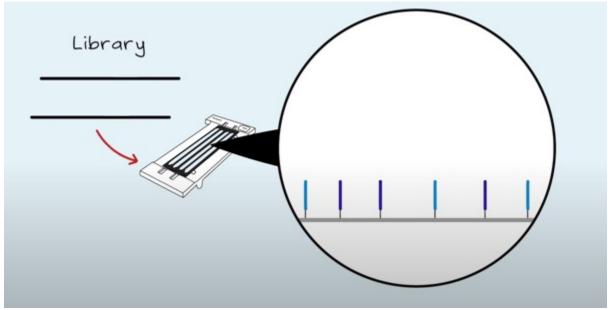


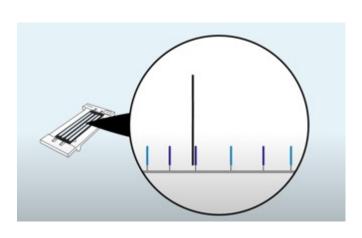
Metagenómica: ¿Cómo funciona?

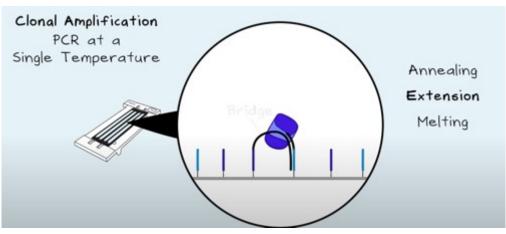


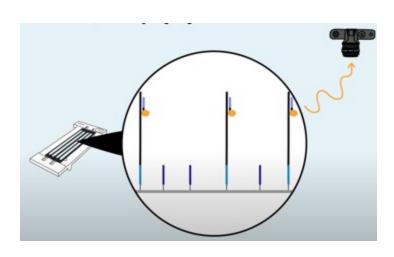












DNA

Sanger

- DNA Sequencing, individual tube format SERVICE INTERNAL **EXTERNAL** \$ 6.45 Standard sequencing rxn, and cap run, full service \$ 11.00 Standard sequencing rxn, and cap run, no redo \$ 4.30 \$ 8.35 BAC/lambda sequencing rxn, and cap run \$8.70 \$12.00 Sequencing capillary run (only) \$ 1.85 \$ 3.50

- Cluster Generation and Sequencing, NovaSeq 6000		
SERVICE	INTERNAL	EXTERNAL
100-Cycle Single Read NovaSeq 6000 SP Flow Cell Sequencing, per lane	\$ 1,855.00	\$ 2,179.00
100-Cycle Single Read NovaSeq 6000 SP Flow Cell Sequencing, per flow cell	\$ 3,272.00	\$ 3,798.00
100-Cycle Paired End NovaSeq 6000 SP Flow Cell Sequencing, per lane	\$ 2,385.00	\$ 2,785.00
100-Cycle Paired End NovaSeq 6000 SP Flow Cell Sequencing, per flow cell	\$ 4,333.00	\$ 4,999.00



NGS



La NGS ha revolucionado la forma en cómo se identifican y estudian microorganismos como bacterias, virus, hongos y otros agentes infecciosos. Algunos ejemplos de uso con patógenos incluyen:

- Diagnóstico de enfermedades infecciosas.
- Epidemiología molecular.
- Identificación de nuevos patógenos.
- Estudio de microbiomas.
- Investigación de vectores y reservorios.



Arbovirus

William C Reeves

A leading authority on the spread and control of mosquito-borne diseases

William C Reeves was only 25 years old when he and William M Harmon led the research team that isolated the western equine and St Louis encephalitis viruses from the *Culex tarsalis* mosquito.

Originally trained as an entomologist, Dr Reeves became interested in arboviruses (a term he coined for arthropod borne viruses) before going on to earn his PhD in medical entomology and parasitology in 1943 at the University of California at Berkeley, where he also earned a master's degree in epidemiology in 1949.

In a captivating series of oral history interviews in 1990, Dr Reeves said that he developed an interest in insects while growing up on a ranch in rural California. His interest was so keen that childhood friends called him "Billy Bugs Reeves."

While working for the Alameda County



Reeves (on left) created "a roadmap for understanding West Nile virus"

El término "arbovirus" fue acuñado por el virólogo estadounidense William C. Reeves en la década 1950. Se definen como un de virus grupo que transmiten a vertebrados principalmente a través de la picadura/mordedura de artrópodos, los como mosquitos, garrapatas y otros insectos.

Estos virus pertenecen a 8 diferentes familias y varios géneros. En México, han sido descritos más de 10 arbovirus que han causado problemas en salud pública.

Flavivirus Powassan virus Tick (Ixodes sp.) Orthomyxoviridae Thogotovirus Bourbon virus Tick (A. americanum) Reoviridae Coltivirus Colorado tick fever Tick (dermacentor)	Virus Family	Viral Genus	Virus		Vector Species
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Phlebovirus Sandfly Fever Sicilian virus Sandfly (phelbotomus) Phlebovirus Heartland virus Tick (A. americanum) Phlebovirus Severe fever with thrombocytopenia syndrome virus Tick (H. longicornis) Nairovirus Crimean Hemorrhagic Fever virus Tick (Hyalomma sp.) Flavivirus Dengue Virus Mosquito (Aedes sp.) Flavivirus Zika virus Mosquito (Aedes sp.) Flavivirus Yellow fever virus Mosquito (Aedes sp.) Flavivirus St. Louis Encephalitis virus Mosquito (Culex sp.) Flavivirus Japanese encephalitis virus Mosquito (Culex sp.) Flavivirus Murray Valley encephalitis virus Mosquito (Culex sp.) Flavivirus Omsk Hemorrhagic fever virus Mosquito (Culex sp.) Flavivirus Kyasanur Forest Diesease virus Tick (dermacentor) Flavivirus Powassan virus Tick-borne encephalitis virus Tick (Ixodes and Haemaphysalis sp.) Flavivirus Powassan virus Tick (Ixodes sp.) Orthomyxoviridae Thogotovirus Bourbon virus Tick (A. americanum) Reoviridae Coltivirus Vesicular Stomatitis (New Jersey) virus Sandflies (Lutz. Sp.) Mosquito (var Sp.) Mosquito (Culex sp.) Tick (Ixodes and Haemaphysalis sp.) Tick (Ixodes and Haemaphysalis sp.) Tick (Ixodes sp.) Tick (A. americanum) Tick (A. americanum) Tick (Phlebotomus Sp.) Mosquito (Aedes and Culex sp.)	Bunyaviridae			K	
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Alphavirus Chikungunya virus J Mosquito (Aedes sp.)	Togaviridae	Alphavirus	Barmah Forest Virus		Mosquito (Aedes and Culex sp.)
		Alphavirus	Chikungunya virus		Mosquito (Aedes sp.)
Alphavirus Venezuelan equine encephalitis virus Mosquito (Culex sp.)		Alphavirus	Venezuelan equine encephalitis virus	VED TO	Mosquito (Culex sp.)
Alphavirus Sindbis virus Mosquito (Culex sp.)		Alphavirus	Sindbis virus	de	Mosquito (Culex sp.)
Alphavirus Equine encephalitis virus Mosquito (Culex sp.)		Alphavirus	Equine encephalitis virus		Mosquito (Culex sp.)
Alphavirus Mayaro virus Mosquito (Haemagogus sp.)		Alphavirus	Mayaro virus		Mosquito (Haemagogus sp.)

VECTOR-BORNE AND ZOONOTIC DISEASES Volume 21, Number 11, 2021 Mary Ann Liebert, Inc. DOI: 10.1089/vbz.2021.0028

Detection of Antibodies to Lokern, Main Drain, St. Louis Encephalitis, and West Nile Viruses in Vertebrate Animals in Chihuahua, Guerrero, and Michoacán, Mexico

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Surveillance for Flaviviruses Near the Mexico-U.S. Border: Co-circulation of Dengue Virus Serotypes 1, 2, and 3 and West Nile Virus in Tamaulipas, Northern Mexico, 2014–2016

S. Viridiana Laredo-Tiscareño, 1,2,3 Javier A. Garza-Hernandez, 4 Ma Isabel Salazar, 5 Erick J. De Luna-Santillana, 6 Chandra S. Tangudu, Rosa C. Cetina-Trejo, Gloria L. Doria-Cobos, Santos Daniel Carmona-Aguirre, Julian E. García-Rejon, 2 Carlos Machain-Williams, 2 Bradley J. Blitvich, 3* and Mario Alberto Rodríguez Pérez 1

¹Laboratorio de Biomedicina Molecular, Centro de Biotecnología Genómica del Instituto Politécnico Nacional, Reynosa, Tamaulipas, México: ²Laboratorio de Arbovirología, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi," Universidad Autónoma de Yucatán, Mérida, México; Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa; *Laboratorio Entornología Médica, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Chihuahua, México: ⁶Laboratorio de Virología e Inmunovirología, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, Mexico: "Laboratorio Medicina de la Conservación, Centro de Biotecnología Genómica del Instituto Politécnico Nacional, Reynosa, Tamaulipas, México; Departamento de Epidemiología de la Cuarta Jurisdicción Sanitaria, Secretaria de Salud, Reynosa, Tamaulipas, México; ⁶Secretaria de Salud del Estado de Tamaulipas, Dirección de Epidemiología, Ciudad Victoria, Tamaulipas, México

Journal of Vector Borne Diseases

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RESEARCH ARTICLE

Ahead of Print

Host-seeking Aedes aegypti linked to dengue seropositive households at northeastern Mexico

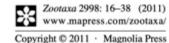
Mario A Rodriguez-Perez¹, Monsuru A Adeleke², Tanya L Russell³, Omar Olguin-Rodriguez¹, Stephanie V Laredo-Tiscareño¹, Javier A Garza-Hernandez⁴, Filiberto Reyes-Villanueva¹

- ¹ Laboratorio de Biomedicina Molecular, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamaulipas, México
- ² Public Health Entomology and Parasitology Unit, Department of Biological Sciences, Osun State University, Osogbo, Nigeria
- ³ Australian Institute of Tropical Health and Medicine, James Cook University; Cairns, Queensland, Australia
- ⁴ Laboratorio de Entomología Medica, Instituto de Ciencias Biomedicas, Universidad Autonoma de Ciudad Juarez; Cd. Juarez, Chihuahua, Mexico

Garrapatas

Grupo de artrópodos arácnidos del orden Ixodida, que se dividen en y Argasidae. A nivel Ixodidae mundial existen más de 900 especies. Son de importancia en la salud humana animal У actualmente se ha incrementado su estuio ya que han ampleado sus nichos, hospederos, distribución.





Article



The Amblyomma (Acari: Ixodida: Ixodidae) of Mexico: Identification Keys, Distribution and Hosts

CARMEN GUZMÁN-CORNEJO¹, RICHARD G. ROBBINS², ALBERTO A. GUGLIELMONE³, GRISELDA MONTIEL-PARRA⁴ & TILA MARÍA PÉREZ⁴





The Ixodes (Acari: Ixodidae) of Mexico: parasite-host and host-parasite checklists

CARMEN GUZMÁN-CORNEJO1, RICHARD G. ROBBINS2 & TILA M. PÉREZ1

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Potential Species Distribution and Richness of Ixodidae Ticks Associated with Wild Vertebrates from Michoacán, Mexico

Margarita Vargas-Sandoval^{1*}, Angel G. Priego-Santander², Alejandra Larrazábal², Carolina G. Sosa-Gutiérrez³, Blanca Lara-Chávez¹, Teresita Avila-Val¹

Email: vargasmarga@yahoo.com.mx

¹Laboratorio de Acarología, Facultad de Ciencias, Departamento de Biología Comparada, Universidad Nacional Autónoma de México. Ciudad Universitaria, Copilco, Coyoacán, México D. F., C. P. 04510. E-mail: mcgc@fciencias.unam.mx

²ISD/AFPMB, Walter Reed Army Medical Center, Washington, DC 20307-5001

Instituto Nacional de Tecnología Agropecuaria, CC 22, C.P. 2300 Rafaela, Santa Fe, Argentina

^{*}Colección Nacional de Ácaros, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México. 3er Circuito s/n Ciudad Universitaria, Copilco, Coyoacán México D. F., C.P. 04510

¹Colección Nacional de Ácaros, Instituto de Biología, Universidad Nacional Autónoma de México, 3er Circuito Exterior s/n, Ciudad Universitaria, Copilco, Coyoacán, México D.F., C.P. 04510. E-mail:cguzman@ibiologia.unam.mx

² DPMIAC/AFPMB, Walter Reed Army Medical Center, Washington, DC 20307-5001.

¹Facultad de Agrobiología "Presidente Juárez", Universidad Michoacana de San Nicolás de Hidalgo, Uruapan, México

²Centro de Investigaciones en Geografía Ambiental, UNAM, Antigua Carretera a Pátzcuaro No. 8701, ExHacienda de San José de la Huerta, Morelia, México

³Unidad de Investigación Médica de Enfermedades Infecciosas y Parasitarias, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, México, DF, México

A review of hard ticks (Acari: Ixodidae) in Colombia: The risk of tick-borne diseases

Javier Antonio Benavides-Montaño*¹©, Jesus Antonio Betancourt-Echeverri²©, Gustavo López Valencia³© and Nora Cristina Mesa-Cobo⁴©

- 1. Acarology Research Group, Parasitology Immunology and Infectious Disease Group, Animal Science Department, Faculty of Agriculture, Universidad Nacional de Colombia-Palmira, Palmira, Colombia; E-mail: abenavidesm@unal.edu.co
- 2. School of Microbiology and Bioanalysis, Faculty of Health, Industrial Universidad de Santander, Bucaramanga, Colombia; E-mail: jesantbet@yahoo.com
- 3. Asesor Científico Instituto Colombiano de Medicina Tropical, ICMT, Universidad CES, Medellín, Colombia; E-mail: glopez@ces.edu.co
- 4. Acarology Research group, Immunology and infectious disease group, Agriculture Department, Faculty of Agriculture, Universidad Nacional de Colombia-Palmira, Palmira, Colombia; E-mail: ncmesac@unal.edu.co

ABSTRACT

This paper reviews the ixodid tick species that are present in Colombia. The different databases reported 50 species of ticks, of which 29 belong to Amblyomma: A. auricularium, A. cajennense sensu lato (s.l.); for Colombia A. calcaratum, A. coelebs, A. crassum, A. dissimile, A. geayi, A. goeldii, A. humerale, A. longirostre, A. maculatum, A. tigrinum, A. triste, A. mixtum, A. multipunctum, A. naponense, A. neumanni, A. nodosum, A. oblongoguttatum, A. ovale, A. pacae, A. pecarium, A. patinoi, A. rotundatum, A. sabanerae, A. scalpturatum, A. tapirellum, A. varium, A. incisum, A. parvum; two Haemaphysalis species: H. juxtakochi, H. leporispalustris, two Rhipicephalus species: R. (Boophilus) microplus, R. sanguineus s.s., two Dermacentor species: Anocentor (D.) nitens, D. imitans, and 15 species of Ixodes: I. affinis, I. andinus, I. auritulus, I. bocatorensis, I. boliviensis, I. brunneus, I. fuscipes, I. lasallei, I. loricatus, I. luciae, I. montoyanus, I. pararicinus, I. tapirus, I. tropicalis, I. venezuelensis. Some of these species need to be validated, their presence confirmed, and their role as a risk for human and animal health studied. Therefore, a countrywide survey of Ixodidae ticks would be of great value to update the information presented in this work.

KEY WORDS: America; biodiversity; checklist; health; Ixodida; vector.

^{*} Corresponding author

Arbovirus transmitidos por garrapatas

Tick-borne viruses

M. LABUDA^{1*} and P. A. NUTTALL²

Ejemplos:

- Crimean-Congo hemorrhagic fever virus.
- Kyasanur Forest disease virus.
- Powassan virus.
- African swine fever virus.

SUMMARY

At least 38 viral species are transmitted by ticks. Virus-tick-vertebrate host relationships are highly specific and less than 10% of all tick species (Argasidae and Ixodidae) are known to play a role as vectors of arboviruses. However, a few tick species transmit several (e.g. Ixodes ricinus, Amblyomma variegatum) or many (I. uriae) tick-borne viruses. Tick-borne viruses are found in six different virus families (Asfarviridae, Reoviridae, Rhabdoviridae, Orthomyxoviridae, Bunyaviridae, Flaviviridae) and at least 9 genera. Some as yet unassigned tick-borne viruses may belong to a seventh family, the Arenaviridae. With only one exception (African swine fever virus, family Asfarviridae) all tick-borne viruses (as well as all other arboviruses) are RNA viruses. Tick-borne viruses are found in all the RNA virus families in which insect-borne members are found, with the exception of the family Togaviridae. Some tick-borne viruses pose a significant threat to the health of humans (Tick-borne encephalitis virus, Crimean-Congo haemorrhagic fever virus) or livestock (African swine fever virus, Nairobi sheep disease virus). Key challenges are to determine the molecular adaptations that allow tick-borne viruses to infect and replicate in both tick and vertebrate cells, and to identify the principal ecological determinants of tick-borne virus survival.

Key words: Arboviruses, tick-borne viruses, ticks, vectors.

¹ Institute of Zoology, Slovak Academy of Sciences, Dubravska cesta 9, 845 06 Bratislava, Slovakia

² Centre for Ecology & Hydrology, Mansfield Rd., Oxford, OX1 3SR, UK

International Committee on Taxonomy of Viruses: ICTV

Official Taxonomic Resources





Find taxa and browse the virus taxonomy



Master Species List

MSL: Spreadsheet of all current species



VMR: Virus exemplars for every species



The Master Species List: A Spreadsheet of Current Taxonomy

The most recent Master Species List (MSL) below provides access to the official, current virus taxonomy approved by the ICTV. A new MSL will be uploaded whenever a new, revised taxonom ratified by the ICTV membership. These Master Species Lists (MSL) provide the complete ICTV taxonomy as a downloadable spreadsheet. MSL files are available for each release of the virus taxonomy since 2008.

S Download Current Master Species List (MSL)

ownload archive

To download folders and files in bulk, select the checkbox next to the desired downloads and then click on the "Download archive" button to receive a .zip file of your requested files.

Name * Created Size 1.91 149 D ICTY Master Species List 2022 MSL38.v3.xlsx 09/11/2023 - 11:17 D ICTY Master Species List 2022 MSL38.v2.xlox 06/07/2023 - 14:42 1.35 MB D ICTV Master Species List 2022 MSL36.vLxlsx 04/08/2023 - 08:53 1.25 MB D ICTY Master Species List 2021 v3.xbx 11/01/2022 - 12:01 898.16 KB ICTY Master Species List 2021 v2.xlsx 06/28/2022 - 12:52 895.99 KB

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Correspondence | Published: 10 July 2023

Guidelines for public database submission of uncultivated virus genome sequences for taxonomic classification

Evelien M. Adriaenssens ⊡, Simon Roux, J. Rodney Brister, Ilene Karsch-Mizrachi, Jens H. Kuhn, Arvind Varsani, Tong Yigang, Alejandro Reyes, Cédric Lood, Elliot J. Lefkowitz, Matthew B. Sullivan, Robert A. Edwards, Peter Simmonds, Luisa Rubino, Sead Sabanadzovic, Mart Krupovic & Bas E. Dutilh

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Ticks and Tick-borne Diseases



Volume 10, Issue 5, August 2019, Pages 1028-1032

Short communication

Field samplings of *Ixodes ricinus* ticks from a tick-borne encephalitis virus micro-focus in Northern Zealand, Denmark

Andreas Petersen a b c A Maiken Worsøe Rosenstierne b, Morten Rasmussen b, Kurt Fuursted c, Henrik Vedel Nielsen c, Lee O'Brien Andersen c, René Bødker d, Anders Fomsgaard b e

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https://doi.org/10.1016/j.ttbdis.2019.05.005 7

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Abstract

In 2008–2009 a tick-borne encephalitis virus (TBEV) micro-focus was detected in Northern Zealand, Denmark. No new cases of <u>TBE</u> with an epidemiological link to Northern Zealand has been reported since. Here we undertook to investigate <u>Ixodes</u> ricinus ticks from this endemic micro-focus in 2016 and 2017. In addition to TBEV, *I.*



Virome Analysis of Amblyomma americanum, Dermacentor variabilis, and Ixodes scapularis Ticks Reveals Novel Highly Divergent Vertebrate and Invertebrate Viruses

Rafal Tokarz, Simon Hedley Williams, Stephen Sameroff, Maria Sanchez Leon, Komal Jain, W. Ian Lipkin Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York, USA

ABSTRACT

A wide range of bacterial pathogens have been identified in ticks, yet the diversity of viruses in ticks is largely unexplored. In the United States, Amblyomma americanum, Dermacentor variabilis, and Ixodes scapularis are among the principal tick species associated with pathogen transmission. We used high-throughput sequencing to characterize the viromes of these tick species and identified the presence of Powassan virus and eight novel viruses. These included the most divergent nairovirus described to date, two new clades of tick-borne phleboviruses, a mononegavirus, and viruses with similarity to plant and insect viruses. Our analysis revealed that ticks are reservoirs for a wide range of viruses and suggests that discovery and characterization of tick-borne viruses will have implications for viral taxonomy and may provide insight into tick-transmitted diseases.

Detection of multiple novel viruses in Argasidae and Ixodidae ticks in Mexico

S. Viridiana Laredo-Tiscareño¹, Javier A. Garza-Hernandez², Chandra S. Tangudu¹, Carlos A.

Rodríguez-Alarcón³, Rodolfo Gonzalez-Peña⁴, Jaime R. Adame-Gallegos⁵, Diana M. Beristain-

Ruiz³, Ignacio Netzahualcoyotl Barajas-López⁶, Alissa M. Hargett¹, Ulrike G. Munderloh⁷,

Bradley J. Blitvich^{1*}

(accepted Ticks & tick-borne diseases)

Colecta de las garrapatas

Chihuahua Michoacán Guerrero Chiapas

Figure 1B

Chiapas 1. Acacoyagua

Guerrero

1. La Unión de Isidro Montes de Oca



Chihuahua

- 1. Buenaventura
- 2. Guadalupe
- 3. Juárez



Michoacán

1. Lázaro Cárdenas













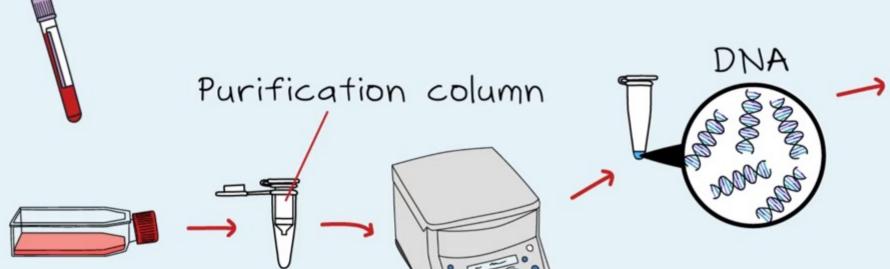






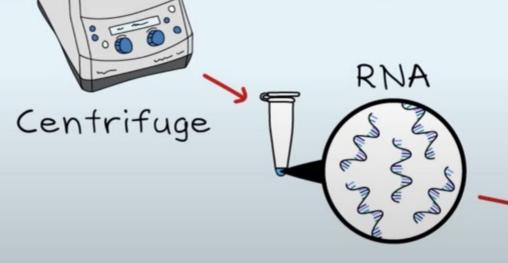
Next Generation Sequencing (NGS)

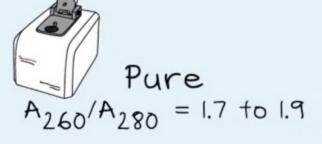
DNA/RNA Purification



Flujo metodologico:

Homogenización, Cultivo celular, asilamiento viral, precipitación glicol polietinelo, secuenciación, bioinformática.





Undegraded



Pure

 $A_{260}/A_{280} = 1.8 + 0.2.0$

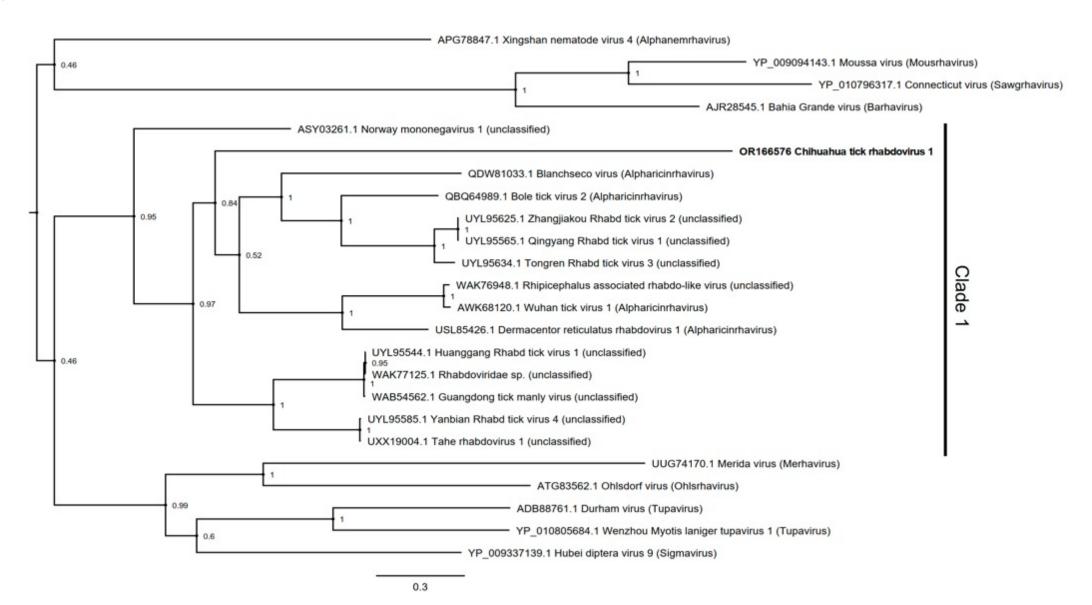
Undegraded

Resultados

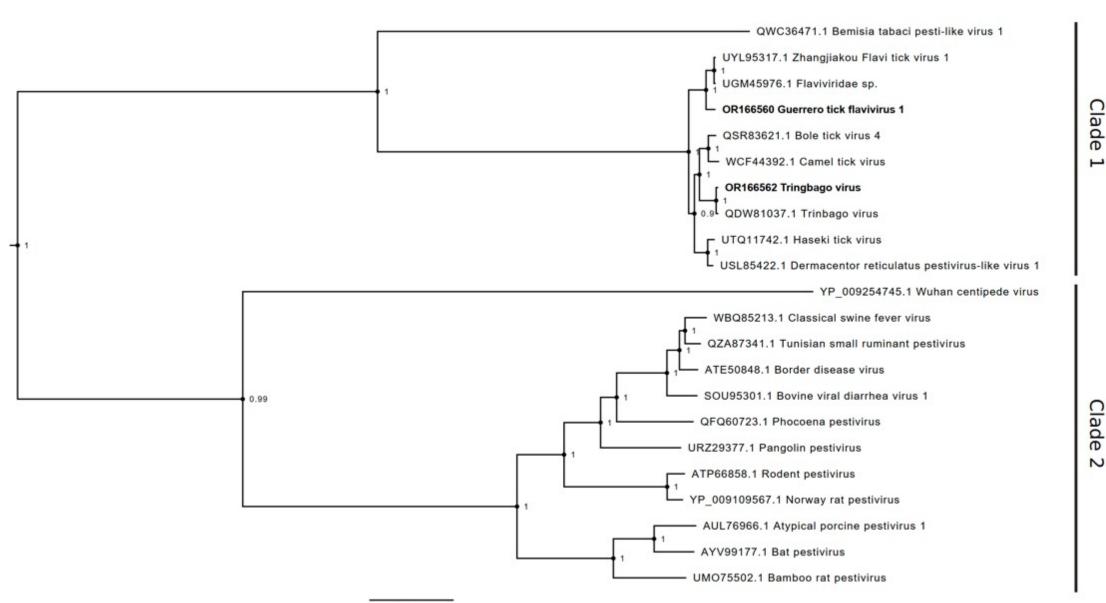
Colectamos para este estudio un total de 3131 garrapatas de animales domésticos en México entre 2019 y 2021. 5 especies de garrapatas duras y una especie de garrapata blanda.

Se detectaron siete virus nuevos y 12 virus reconocidos, en 7 familias: Totiviridae (virus que infectan protozoos y microhongos), Solemoviridae (virus que infectan plantas), Nyamiviridae (Tick-borne virus), Nodaviridae (virus que infectan animales), **Rhabdoviridae, Flaviviridae y Bunyavirales**.

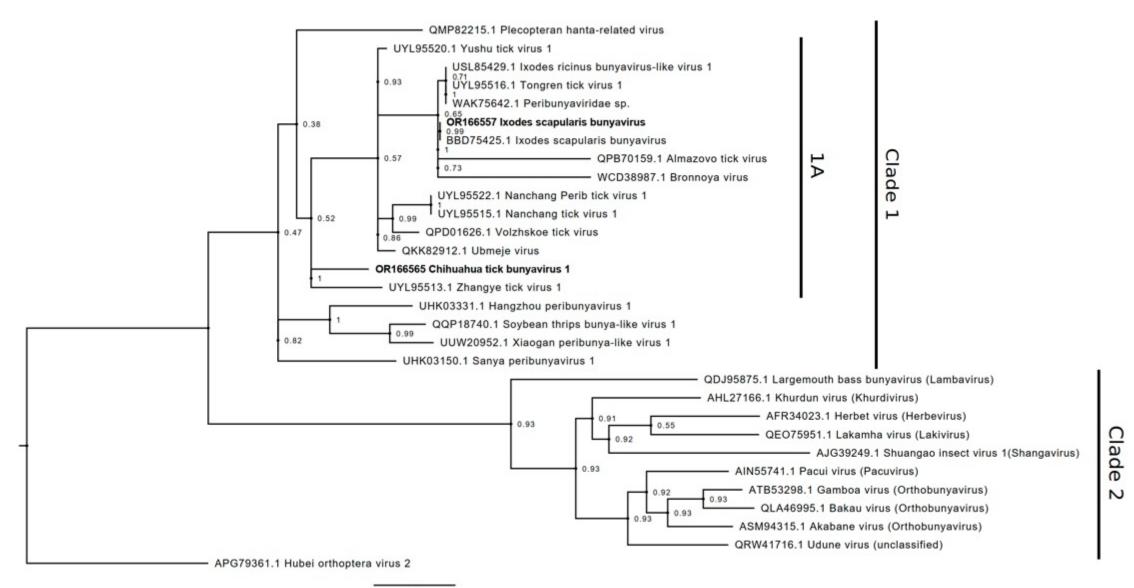
Filogenia de los nuevos <u>Rhabdoviridae</u>, Flaviviridae y Bunyavirales



Flaviviridae



Bunyavirales



En resumen, gracias a este estudio identificamos una amplia variedad de virus, tanto conocidos como nuevos, en garrapatas argásidas e ixódidas que parasitan animales domésticos en México. Este hallazgo contribuye significativamente a nuestra comprensión del viroma presente en estos parásitos, respaldando investigaciones previas que ya habían señalado la diversidad taxonómica de los virus en garrapatas.