

Presence of Arenavirus in *Mus musculus*, Chiapas, Mexico

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An outbreak of a highly lethal hemorrhagic fever, caused by an *Arenavirus* whose reservoir was *Peromyscus mexicanus*, occurred in the state of Chiapas, Mexico, in June 1967. In order to determine whether any *Arenavirus* is nowadays present in the same region of the state of Chiapas (municipalities of Ocozocoautla de Espinosa and Berriozábal), we surveyed rodents as potential *Arenavirus* reservoirs. Three species of rodents, *Mus musculus*, *Peromyscus mexicanus*, and *Rattus rattus*, previously identified as *Arenavirus* reservoirs, were registered in the study area. We detected the presence of *Arenavirus* RNA in one *Mus musculus* individual captured in an urban area. It is necessary to continue monitoring wildlife and carry out serological and molecular analyzes to investigate the incidence and future prevalence of *Arenavirus* in the area and propose specific actions for its control.

En junio de 1967, en el estado de Chiapas, ocurrió un brote de fiebre hemorrágica altamente letal, el cual se le atribuyó a un *Arenavirus* cuyo reservorio fue *Peromyscus mexicanus*. Para determinar hoy en día la posible existencia de *Arenavirus* en la misma región del estado de Chiapas (municipios de Ocozocoautla de Espinosa y Berriozábal), se evaluó a los roedores presentes en el área como posibles reservorios de *Arenavirus*. Tres especies de roedores que han sido identificados como reservorios de *Arenavirus*, *Mus musculus*, *Peromyscus mexicanus* y *Rattus rattus* se registraron en el área de estudio. Se detectó la presencia de *Arenavirus* RNA en un individuo de *M. musculus* capturado en un área urbana. Es necesario continuar monitoreando la fauna silvestre y realizar análisis serológicos y moleculares para investigar la incidencia y la prevalencia futura de *Arenavirus* en el área, así como proponer acciones específicas para su control.

Key words: Chiapas; *Mus musculus*; *Rattus rattus*; rodent; virus.

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Introduction

An outbreak of a highly lethal hemorrhagic fever occurred in the state of Chiapas in June 1967, in San Leandro ranch at 35 miles (56.3 km) southwest of Palenque ([Goldsmith and Shields 1971](#)), in the municipality of Chilón, and 96 km SW (in straight line) of the capital city, Tuxtla Gutiérrez. This hemorrhagic fever in Chiapas clinically resembled hemorrhagic fevers caused by arenaviruses from South America, and the outbreak was preceded by a large-scale deforestation and an increase in the abundance of rodents in and around houses in the epidemic area in the 3-year period before 1967 ([Goldsmith and Shields 1971](#)). Hypothetically, Ocozocoautla de Espinosa Virus (OCEV) or an *Arenavirus* phylogenetically closely related to OCEV was the etiologic agent in the hemorrhagic fever epidemic in Chiapas in 1967. At present, OCEV is the cause of a human disease that is clinically indistinct from dengue hemorrhagic fever and other severe febrile illnesses that are endemic to Chiapas ([Cajimat et al. 2012](#)). [Milazzo et al. \(2010\)](#) reported antibody against a Tacaribe serocomplex virus (*Arenavirus*) in 3 (25 %) of 12 Mexican deer mice (*Peromyscus mexicanus*) captured in the municipality of Ocozocoautla de Espinosa, and [Cajimat et al. \(2012\)](#) isolated *Arenavirus* RNA from Mexican deer mice (*P. mexicanus*) captured in the same locality. Analyses

of nucleotide and amino acid sequence data indicated that the deer mice were infected with a novel Tacaribe serocomplex virus, which was named for the first time Ocozocoautla de Espinosa Virus.

In order to confirm if the *Arenavirus* RNA is current present in Ocozocoautla de Espinosa and Berriozábal, we surveyed rodents as potential reservoirs of this RNA virus.

Materials and Methods

Study area. The study was carried out in the municipalities of Ocozocoautla de Espinosa and Berriozábal, Chiapas. Rodents were surveyed at different sampling sites (one night per locality) in Ocozocoautla de Espinosa (25-26 September 2017 and 1-2 February 2018) and Berriozábal (8-9 February, 27-28 September 2017, and 24-27 September 2018). To capture the rodents, we used 200 Sherman traps in each sampling sites, which were placed in different types of vegetation and in urban areas. Species were identified using specialized mammal guides ([Álvarez-Castañeda et al. 2017](#)). The animals were collected under the scientific collection permit number FAUT-0143 of CL (official letter No. SGPA/DGVS/002779/18), following the protocols established for capturing and handling mammals by the American Society of Mammalogists ([Sikes et al. 2016](#)).

Strict biosecurity measures were used while sampling species, including use of disposable laboratory coats, goggles, latex gloves, and masks with filters to avoid inhalation of possible pathogens and other contaminating particles. For each specimen, body measurements, weight, sex, and reproductive condition were recorded, as well as type of habitat and geographic location (Martin *et al.* 2011). Tissue samples (liver) were taken from two specimens of each species and placed in sterile 2 ml tubes with RNAlater™ (Sigma) and preserved at -20 °C. All specimens and tissues were deposited and cataloged in the Mammal Collection of El Colegio de la Frontera Sur (ECO-SC-M), San Cristóbal de Las Casas, Chiapas. The rest of specimens were released in the site in which were captured.

Detection of Arenavirus RNA. This analysis was made in the Laboratorio de Ecología Evolutiva, in the Universidad de Ciencias y Artes de Chiapas. RNA was extracted from liver samples RNAlater™ (Sigma) and preserved at -20 °C to determine the presence of *Arenavirus*. RNA extraction was carried out with the QIAamp Viral RNA Mini extraction kit (Qiagen, USA), according to manufacturer instructions.

The molecular detection of *Arenavirus* genes was carried out using the reverse transcription polymerase chain reaction (RT-PCR). The retrotranscription was performed according to manufacturer instructions by the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit for obtaining complementary DNA (cDNA). The reaction mixture was prepared with 5 µl of sample of RNA from liver tissue of 21 samples of rodents: 1 µl of Oligo (dT)₁₈ primer and 6 µl of nuclease-free water; it was mixed, briefly centrifuged and incubated at 65 °C for 5 minutes. The following components were added: 4 µl reaction buffer (5x), 1 µl ribonuclease inhibitor (RiboLock Rnase Inhibitor), 12 µl of 10mM deoxynucleotide triphosphate (dNTP's mix) and 1 µl of reverse transcriptase (RevertAid M RT). All was mixed, centrifuged briefly and incubated at 42 °C for 60 minutes and the reaction was terminated by heating at 70 °C for 5 minutes.

The detection of *Arenavirus* positive controls was carried out through the nested RT-PCR reaction with a first round of amplification where the Aren 1+ (5'-CWA TRT ANG GCC AIC CIT CIC C-3') and Aren 1- (5'-TNR WYA AYC ART TYG GIW CIR TKC C-3') primers were used, amplifying approximately 450 base pairs (bp; Castellar *et al.* 2017). The reaction had an initial denaturation time at 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, an alignment at 60 °C for 1 minute, an extension of 72 °C for 30 seconds; decreasing 0.5 °C per cycle, then 15 cycles of denaturation at 94 °C for 1 minute, 45 °C of alignment for 1 minute and an extension at 72 °C for 30 seconds and a final extension at 72 °C for 5 minutes.

In the second round, 1 µl of the amplified product was used, under the following conditions: a denaturation at 94 °C for 2 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, alignment at 45 °C for 1 minute, an extension at 72

°C for 30 seconds and a final extension phase at 72 °C for 5 minutes, using the primers Aren 2+ (5'- CAN ANY TTR TAN ARN AIR TTY TCR TAI GG-3') and Aren 2- (5'- AGY YTN KNN GCN GCI CTI AAR GC-3'), which amplify in an internal fragment of 200 bp. The mixtures for both rounds of amplification were prepared under the following conditions: 5 µl of Green GoTaq Flexi Buffer 1X, 1.5 µl of MgCl₂ (25 mM), 0.5 µl of dNTPs (10 mM), 1 µl of each primer (10 pmol), 14.9 µl of nuclease-free water and 0.15 µl of GoTaq DNA polymerase (5µ/µl) Promega® for a final volume of 24 µl.

The amplified products were visualized on a 2 % agarose gel using 3 µl of sample. In each gel was included a 1 µl line of 100 bp molecular weight marker whose total length is 1,500 bp per line; for 90 minutes at 100 V. The gel was stained with ethidium bromide (3,8-diamino-6-ethyl-5-phenylphenanthridium bromide; 10 mg/ml), taking an image with the Vision Works Software and the GelMaxImager UVP® equipment.

Results

Rodents as reservoirs of Arenavirus. A total of 26 rodent specimens were collected in Ocozocoautla de Espinosa (3 *Handleyomys rostratus*, 1 *Heteromys desmarestianus*, 2 *Mus musculus*, 5 *Peromyscus aztecus*, 15 *Peromyscus mexicanus*), and 84 in Berriozábal (7 *H. desmarestianus*, 1 *M. musculus*, 1 *Oryzomys couesi*, 21 *P. aztecus*, 1 *P. leucopus*, 44 *P. mexicanus*, 1 *Rattus rattus*, 4 *Scotinomys teguina*, 4 *Sigmodon toltecus*), from which *M. musculus* (Sudia *et al.* 1975; Bowen *et al.* 1997), *P. mexicanus* (Salazar-Bravo *et al.* 2004; Milazzo *et al.* 2010; Cajimat *et al.* 2012), and *R. rattus* (Li *et al.* 2015) have been considered natural reservoirs of *Arenavirus*.

Detection of Arenavirus RNA. Of the 20 samples analyzed (5 *P. aztecus*, 9 *P. mexicanus*, 3 *H. rostratus*, 1 *H. desmarestianus*, 2 *M. musculus*), one was positive to the presence of *Arenavirus* RNA. The positive case corresponded to a specimen of *M. musculus* (ECO-SC-M 8945) collected in February 1st, 2018 in Ocuilapa, municipality Ocozocoautla de Espinosa, Chiapas (16.8542° N, -93.4107° W), inside the kitchen of one house located in the center of the city.

Discussion

The finding of *Arenavirus* in a synanthropic rodent species (*M. musculus*), in the same region where an outbreak of a highly lethal hemorrhagic fever occurred 50 years ago, should be a warning sign. In 1967, the outbreak was preceded by a large-scale deforestation in the area and an increase in the abundance of rodents around and inside people's homes (Goldsmith and Shields 1971). In this report, the positive rodent was captured in the kitchen of a rural village, in close proximity with people. In addition, *P. mexicanus*, *M. musculus* and *R. rattus*, which were captured in our survey, have a long history as reservoirs and transmitters of diseases to humans (Kosoy *et al.* 2015; Williams *et al.* 2018), and have been reported as carriers of antibodies to *Arenavirus* in the area or other places of the world (Sudia *et*

[al. 1975](#); [Bowen et al. 1997](#); [Salazar-Bravo et al. 2004](#); [Milazzo et al. 2010](#); [Cajimat et al. 2012](#); [Li et al. 2015](#)).

It is important to continue carrying out molecular analyzes to detect *Arenavirus* in mammals (increasing the sample number) that have previously been found to serve as reservoirs of zoonotic virus hemorrhagic fever (VHF) in order to determine recidivism and prevalence of viruses that cause hemorrhagic fevers, and thereby be able to respond to outbreaks of VHF in Mexico.

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