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Potential Sympatric Vectors and Mammalian Hosts of Venezuelan Equine Encephalitis Virus in Southern Mexico

Jesús Sotomayor-Bonilla,[1,2] Carlos Antonio Abella-Medrano,[3] Andrea Chaves,[4] Paulina Álvarez-Mendizábal,[1,2] Óscar Rico-Chávez,[1,2] Sergio Ibáñez-Bernal,[3] Melinda K. Rostal,[5] Rafael Ojeda-Flores,[2,3] Arturo Barbachano-Guerrero,[6,7] Gustavo Gutiérrez-Espeleta,[4] A. Alonso Aguirre,[8] Peter Daszak,[5] and Gerardo Suzán[1,2,9] [1]Departamento de Etología, Fauna Silvestre y Animales de Laboratorio, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Av. Universidad 3000, Delegación Coyoacán, México DF, CP 04510; [2]Asociación Mexicana de Medicina de la Conservación, Kalaan-Kab AC, Ciclistas 68-3, Delegación Coyoacán, México DF, CP 04220; [3]Red Ambiente y Sustentabilidad, Instituto de Ecología A. C. Carretera Antigua a Coatepec 351, El Haya, Xalapa, Veracruz, México, CP 91070; [4]Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica, CP 11501-2060; [5]EcoHealth Alliance, 460 W 34th St., New York, New York 10001, USA; [6]Laboratorio de Medicina de Conservación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Calle Plan de San Luis y Díaz Mirón, s/n, Delegación Miguel Hidalgo, México DF, México, CP 11340; [7]Department of Microbiology and Immunology, State University of New York, Upstate Medical University, 766 Irving Ave., Syracuse, New York 13210, USA; [8]Department of Environmental Science and Policy, George Mason University, 4400 University Dr., MS 5F2, Fairfax, Virginia 22030, USA; [9]Corresponding author (email: gerardosuz@gmail.com)

ABSTRACT: Arboviruses are important zoonotic agents with complex transmission cycles and are not well understood because they may involve many vectors and hosts. We studied sympatric wild mammals and hematophagous mosquitoes having the potential to act as hosts and vectors in two areas of southern Mexico. Mosquitoes, bats, and rodents were captured in Calakmul (Campeche) and Montes Azules (Chiapas), between November 2010 and August 2011. Spleen samples from 146 bats and 14 rodents were tested for molecular evidence of Venezuelan equine encephalitis virus (VEEV), eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and West Nile virus (WNV) using PCR protocols. Bat (*Artibeus lituratus*, *Carollia sowelli*, *Glossophaga soricina*, and *Sturnira parvidens*) and rodent (*Sigmodon hispidus* and *Oryzomys alfaroi*) species were positive for VEEV. No individuals were positive for WNV, EEEV, or WEEV. A total of 1,298 mosquitoes were collected at the same sites, and five of the mosquito species collected were known VEEV vectors (*Aedes fulvus*, *Mansonia indubitans*, *Psorophora ferox*, *Psorophora cilipes*, and *Psorophora confinnis*). This survey simultaneously presents the first molecular evidence, to our knowledge, of VEEV in bats and rodents from southern Mexico and the identification of potential sympatric vectors. Studies investigating sympatric nonhuman hosts, vectors, and arboviruses must be expanded to determine arboviral dynamics in complex systems in which outbreaks of emerging and reemerging zoonoses are continuously occurring.

Key words: Bats, Calakmul, Mexico, Montes Azules, mosquitoes, rodents, sylvatic cycle, Venezuelan equine encephalitis virus.

Arboviruses are a highly diverse group with complex and poorly understood transmission cycles, involving many species of mosquito vectors and vertebrate hosts, including humans (Weaver and Reisen 2010). Many zoonotic arboviruses have been reported in areas with lower human population density close to important protected areas in southern Mexico (Adams et al. 2012). Montes Azules Biosphere Reserve, Chiapas, Mexico (16°9′46″N, 90°41′18″W) and Calakmul Biosphere Reserve, Campeche, Mexico (18°26′1″N, 89°36′61″W) represent two high-priority regions for conservation because of their biological importance, and both areas have been severely disturbed by human encroachment, promoting new interactions among arboviruses, vectors, and small mammals. Rodents and bats are the most diverse and abundant mammal orders, and their contribution to arbovirus dynamics can vary widely. Mosquito assemblages in Calakmul and Montes Azules are unknown, despite more than 200 mosquito species having been described in southern Mexico and Central America (Chaverri 2009). We investigated the presence of arboviruses in small mammal (bats and rodents) and identified the mosquito species richness at concurrent times in both protected areas.

Small mammal and mosquito specimens were collected in Montes Azules and Calakmul. Small mammals were captured as

previously described (Sotomayor-Bonilla et al. 2014). For reverse transcriptase-PCR (RT-PCR) screening, 150 bats and 15 rodents were anesthetized with isoflurane and euthanized with an overdose of sodium pentobarbital, following specific guidelines (Sikes and Gannon 2011), and with the approval of the Institutional Animal Care and Use Subcommittee of the Veterinary Faculty of the Universidad Nacional Autonoma de Mexico (UNAM). We extracted RNA with Trizol® (Fisher Scientific, Gaithersburg, Maryland, USA), according to the manufacturer's protocol. Then, samples were analyzed with RT-PCR for the presence Venezuelan equine encephalitis virus (VEEV), eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and West Nile virus (WNV; Lanciotti et al. 2000; Linssen et al. 2000). Positive controls were purchased from National Veterinary Services Laboratories (US Department of Agriculture, Ames, Iowa, USA), and distilled water was used as the negative control.

The RT-PCR products were stained with Gel Red™ (Biotium, Inc., Fremont, California, USA) and visualized by 2% agarose gel electrophoresis. Samples that showed bands with the expected size (VEEV: 194 base pairs [bp], EEEV: 262 bp, WEEV: 195 bp, WNV: 400 bp) were considered positive.

Samples with positive bands were subsequently reamplified, and the amplicons with adequate concentrations were sequenced with the same diagnostic primers in a commercial laboratory at the Instituto de Fisiologia Celular (UNAM, Mexico City, Mexico), to confirm PCR results.

Adult mosquitoes were collected from Montes Azules and Calakmul during July and August 2011, using two Centers for Disease Control (CDC) miniature blacklight traps (John W. Hock Company, Gainesville, Florida, USA) and one modified Disney trap (Dorval et al. 2007) with 10 fruit-eating bats (*Artibeus lituratus*), which was used in two sites in Calakmul and three sites in Montes Azules. The CDC miniature traps were operated from 1800 to 0600 hours, and the modified Disney trap was active for 24 h. Both trap types were placed 1.5 m aboveground. Mosquitoes were euthanized with triethylamine and immediately frozen. We identified species with several open-access keys (Harbach and Howard 2007; WRBU 2012). Then, we calculated the mosquito species richness.

We analyzed spleen samples from 150 (of 603) bats and 15 rodents representing 16 bat and seven rodent species, respectively (Table 1). Five bats and two rodents were positive for VEEV, yielding a prevalence of 3% (5/150; 95% confidence interval: 92.1–97.9) in bats and a prevalence of 14% (95% confidence interval: 0.017–0.405; Table 1) in rodents.

In Calakmul, 2/85 bats were VEEV-positive: one *Artibeus lituratus* bat and one *Glossophaga soricina* bat. In Montes Azules, 3/65 bats were VEEV-positive: one bat of each *A. lituratus*, *Carollia sowelli*, and *Sturnira parvidens* species. Sequencing confirmed the results of 2/5 positive PCR tests from *G. soricina* in Calakmul and *S. parvidens* in Montes Azules. The nucleotide identity percentage of the partial E2 region of viruses collected was 96–98%. When performing a comparison among detected sequences and Genbank sequences (National Center for Biotechnology Information, Bethesda, Maryland, USA) by BLAST analysis (NCBI 2016), the viruses demonstrated the highest identity with VEEV subtype IAB. One *Sigmodon hispidus* rat and one *Oryzomys alfaroi* rat from Calakmul and Montes Azules, respectively, were positive for VEEV (Table 1). No bats or rodents were found positive for EEEV, WEEV, or WNV.

We collected 1,298 adult mosquitoes, belonging to nine genera and 24 species using the CDC traps and manual aspiration from Disney traps. A total of 199 specimens were identified to genera and 1,099 specimens to species, including 555 and 743 specimens from Calakmul and Montes Azules, respectively. These sites shared 46% (11/24) of the mosquito species (*Aedes fulvus*, *Aedes infirmatus*, *Aedes tormentor*, *Anopheles apicimacula*, *Culex bastagarius*, *Culex nigripalpus*, *Culex thriambus*, *Mansonia indubitans*, *Psorophora cyanescens*, *Psorophora ferox*, *Uranotaenia geometrica*), suggesting the two sites

TABLE 1. Number of bats and rodents sampled in Calakmul, Campeche, and Montes Azules, Chiapas, Mexico, between November 2010 and August 2011 and tested for Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, and West Nile virus using PCR.

Species	Location		Totals
	Calakmul	Montes Azules	
Bats			
Artibeus jamaicensis	21	7	28
Artibeus lituratus	18[a]	23[a]	41
Artibeus phaeotis	1	0	1
Carollia perspicillata	1	0	1
Carollia sowelli	0	14[a]	14
Desmodus rotundus	0	1	1
Glossophaga commissarisi	1	1	2
Glossophaga soricina	29[a,b]	6	35
Hylonycteris underwoodi	0	1	1
Platyrrhinus helleri	0	2	2
Pteronotus davyi	1	0	1
Pteronotus parnellii	6	0	6
Saccopteryx bilineata	0	1	1
Sturnira parvidens	6	6[a,b]	12
Sturnira ludovici	0	2	2
Uroderma bilobatum	0	1	1
Total bats	85	65	150
Rodents			
Heteromys gaumeri	4	0	4
Liomys pictus	1	0	1
Oryzomys alfaroi	0	3[a]	3
Ototylomys phyllotis	1	0	1
Peromyscus yucatanicus	1	0	1
Rattus rattus	1	1	2
Sigmodon hispidus	3[a]	0	3
Total rodents	11	4	15

[a] Venezuelan equine encephalitis virus PCR–positive small mammal species.

[b] Positive result confirmed by sequencing.

have similar mosquito species assemblages. Supplementary Material Table S1 describes the mosquitoes identified per region. Nine mosquito species, including *Ps. ferox* and *Ma. indubitans*, were collected from the traps using bats as bait. Eight mosquito species collected are known arboviral vectors, including mosquitoes of the genera *Aedes*, *Culex*, *Anopheles*, *Mansonia*, and *Psorophora* (WRBU 2012). Arboviruses were not tested in mosquito specimens.

Serologic evidence of VEEV subtype IE has been previously described in rodents and domestic animals from southeastern Mexico (Ulloa et al. 2003; Deardorff et al. 2011). In Chiapas, VEEV activity has been reported in domestic animals near our study site (Ulloa et al. 2003). However, we were unable to find published VEEV evidence circulating in wildlife near the study sites, despite the fact that, in other countries, evidence of infection has been found in wild rodents, bats, marsupials, and carnivores (Weaver et al. 2004). Little work has been done in Mexico at different sites, but several wild and domestic mammals have been identified with specific strains of VEEV (Aguirre et al. 1992).

We found molecular evidence of VEEV in four bat species and two rodent species; moreover, samples from *G. soricina* and *S. parvidens* were VEEV-IAB confirmed by sequencing. We were not able to confirm three of the bat PCR amplicons or the two rodent PCR amplicons possibly because of sample degradation. Although we detected VEEV RNA, we cannot determine the role of small mammals in transmission dynamics of the enzootic and epizootic cycles. Further research should investigate whether these bat and rodent species are accidental hosts, dead-end hosts, reservoirs, or amplifiers.

The VEEV has been reported in bats in southeastern Mexico, the Caribbean Islands, and in Central and South America (Scherer et al. 1971; Price 1978; Ubico and McLean 1995; Estrada-Franco et al. 2004). Furthermore, Ubico and McLean (1995) found antibodies against VEEV-IAB in the genera *Artibeus*, *Carollia*, and *Sturnira* in Guatemala. In 2000, one *Oryzomys couesi* and six *S. hispidus* (of 34 wild rodents) sampled in Chiapas had neutralizing antibodies against VEEV (Deardorff et al. 2011). Other rodent genera (*Zygodontomys*, *Heteromys*, *Proechymis*, and *Peromyscus*) have been suggested to be primary sylvatic hosts of enzootic VEEV (Weaver et al. 2004). Experimentally, five rodent species from southeastern Mexico (*Baiomys musculus*, *Liomys salvini*, *Oligoryzomys fulvescens*, *O. couesi*, and *S. hispidus*) developed sufficient viremia to infect mosquitoes, and all of them, except for *B.*

musculus, survived infection with the ability to reproduce (Deardorff et al. 2009).

We collected 24 mosquito species; of which, the four most abundant species (*Ps. ferox*, *Ae. tormentor*, *An. apicimacula*, and *Cx. nigripalpus*) were found in both regions. Previous work related to mosquito ecology and epidemiology in Mexico has been focused on *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*, the principal vectors of zoonotic arboviruses. Interestingly, *Ae. aegypti* and *Aedes taeniorhynchus* were not found in the sites we studied, despite a notable increase in the distribution of this mosquito species within rural Guatemala and in Chiapas (Diéguez-Fernández et al. 2006; Deardorff et al. 2011). Alternative collection methods will improve capture-rate success for other mosquito species, and the study of different life stages will help to develop novel research strategies (Wijayanti et al. 2016).

We present original molecular evidence of VEEV-IAB in bats and rodents from southeastern Mexico. This was a pilot study designed to provide a basis for further research to determine reservoir competence and the role of these mammal groups in VEEV dynamics. Integrated studies involving arboviruses of public health concern, synanthropic wild reservoirs, and mosquito vectors need to be performed to understand how this tripartite process occurs in different temporal and spatial scales (Reisen 2010).

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SUPPLEMENTARY MATERIAL

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