

## Short Report: Dengue Virus in Bats from Southeastern Mexico

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**Abstract.** To identify the relationship between landscape use and dengue virus (DENV) occurrence in bats, we investigated the presence of DENV from anthropogenically changed and unaltered landscapes in two Biosphere Reserves: Calakmul (Campeche) and Montes Azules (Chiapas) in southern Mexico. Spleen samples of 146 bats, belonging to 16 species, were tested for four DENV serotypes with standard reverse transcriptase polymerase chain reaction (RT-PCR) protocols. Six bats (4.1%) tested positive for DENV-2: four bats in Calakmul (two *Glossophaga soricina*, one *Artibeus jamaicensis*, and one *A. lituratus*) and two bats in Montes Azules (both *A. lituratus*). No effect of anthropogenic disturbance on the occurrence of DENV was detected; however, all three RT-PCR-positive bat species are considered abundant species in the Neotropics and well-adapted to disturbed habitats. To our knowledge, this study is the first study conducted in southeastern Mexico to identify DENV-2 in bats by a widely accepted RT-PCR protocol. The role that bats play on DENV's ecology remains undetermined.

Dengue fever is an important public health concern in the tropics,<sup>1–4</sup> and ecological and epidemiological studies are needed to assess the role of bats and other mammals in a possible sylvatic maintenance cycle.<sup>5</sup> Dengue viruses (DENVs) comprise four antigenically distinct but genetically related serotypes of the *Flavivirus* genus (*Flaviviridae* family).<sup>1</sup> DENVs are positive-sense single-stranded RNA viruses that cause one of the most common infectious diseases in humans in tropical regions.<sup>2</sup> Their transmission includes an urban endemic/epidemic cycle between *Aedes aegypti* mosquitoes and humans as the reservoir host and a sylvatic enzootic cycle between non-human primates and arboreal mosquitoes of the genus *Aedes*.<sup>3</sup> The urban cycle is well-documented in the Neotropics, with four serotypes reported in urban areas,<sup>1–4</sup> whereas the sylvatic cycle has been shown in West Africa and peninsular Malaysia.<sup>5</sup> Thus far, the sylvatic cycle has not been described in the Neotropics. However, in Bolivia, DENV seroconversions among the indigenous Ayoreo people were found in a remote area where *Ae. aegypti*, the primary vector, was absent.<sup>6</sup> This finding suggests a possible sylvatic cycle involving a different mosquito species or cross-reaction with antibodies to another flavivirus. In French Guiana, all four DENV serotypes have been identified by molecular methods in 92 wild mammals (bats, rodents, and marsupials) in all settings investigated: periurban, rural, and sparsely populated areas.<sup>7</sup> This finding suggests that primarily urban DENV strains could infect wildlife in non-urban forested areas.<sup>7</sup> The role of wildlife in DENV transmission remains unknown.

Bats are important reservoirs of many viruses, such as rabies viruses, Nipah viruses, and coronaviruses.<sup>8–12</sup> *Flaviviridae* are the second most frequently reported viral family in the order *Chiroptera* (13% frequency; second only to rhabdoviruses)<sup>9</sup>; however, their role in the dynamics of DENVs remains poorly

understood. DENV have been reported in large frugivorous bats (*Pteropus* genus) in Australia.<sup>13</sup> More recently, polymerase chain reaction (PCR) and antigen detection (direct immunofluorescent assay) of DENV were described in bats from DENV-endemic islands in China.<sup>14</sup> Seroconversion in bats (DENV-1, -2, and -3) has been reported in urban areas in Costa Rica and Ecuador.<sup>15</sup> Bats captured during a dengue fever outbreak along the Gulf and Pacific Coasts of Mexico were found to be DENV-seropositive (enzyme-linked immunosorbent assay [ELISA]), have DENV NS1 protein, and be positive on reverse transcriptase (RT) -PCR for DENV-2.<sup>16</sup> Molecular evidence of DENV-1, -2, and -3 in bats from French Guiana was reported, including a phylogenetic sequence from a *Carollia perspicillata* bat consistent with DENV-1.<sup>7</sup> Qualitative detection of the NS1 antigen of DENVs in bat serum samples and plaque reduction neutralization tests has been reported in bats from southeastern Mexico from disturbed sites near human settlements in Campeche (S. Cabrera-Romo and others, unpublished data) and Mérida,<sup>17</sup> which is the largest city in the Yucatan Peninsula.

In this study, samples were collected from bats trapped in two biosphere reserves located in southeastern Mexico. High bat diversity and large tracts of continuous forest characterize these areas. Additionally, DENV-1, -2, and -4 have been reported in human populations. The aim of this study was to show the presence of DENV serotypes in bats within the biosphere reserves and adjacent areas with anthropogenic changes using RT-PCR. Bats were collected in Montes Azules Biosphere Reserve (Chiapas; 16°9'46" N, 90°41'18" W) and Calakmul Biosphere Reserve (Campeche; 18°26'1" N, 89°36'61" W) from two landscape types categorized as undisturbed forest (UD), comprised of primary forest with no human disturbance, and disturbed forest (D), defined as a transitional zone between primary forest and agricultural/livestock areas or human settlements. In both regions, the sites were located at least 20 km from significant human populations where dengue has been reported (Xpujil in Campeche State and Benemérito de la América in Chiapas State). Bats were

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TABLE 1

Number of bats sampled by species by site type collected and analyzed for DENV between 2010 and 2011 from Calakmul and Montes Azules

Bat species	Calakmul		Montes Azules	
	UD	D	UD	D
<i>Artibeus jamaicensis</i>	14*	8	7	
<i>A. lituratus</i>	2	16*	13*	7*
<i>A. phaeotis</i>	1			
<i>Carollia perspicillata</i>	1			
<i>C. sowelli</i>			3	11
<i>Desmodus rotundus</i>				1
<i>Glossophaga commissarisi</i>	1			1
<i>G. soricina</i>	7*	22*	2	4
<i>Hylonycteris underwoodi</i>			1	
<i>Platyrrhinus helleri</i>				1
<i>Pteronotus davayi</i>	1			
<i>P. parnellii</i>	5	1		
<i>S. bilineata</i>				1
<i>Sturnira lilium</i>		6	2	4
<i>S. ludovici</i>			1	1
<i>Uroderma bilobatum</i>				1
Total	32	53	29	32

\* A positive PCR result for DENV-2.

captured using four mist nets (5 × 9 m) in foraging sites. Nets were opened at dusk and remained open for 4 consecutive hours. Each site was sampled three times between November of 2010 and August of 2011. Field guides were used for the taxonomic species identification of all bats captured.<sup>18</sup>

In total, 146 bats were euthanized following the Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research<sup>19</sup> with the approval of the Institutional Animal Care and Use Subcommittee of the Veterinary Faculty of Universidad Nacional Autónoma de México. One hundred forty-six spleen specimens were collected and preserved in liquid nitrogen, and RNA from all samples was extracted using TRIzol LS Reagent (Thermo Fisher Scientific Inc., Carlsbad, CA) according to the manufacturer's instructions. RT-PCR was carried out for DENV (DENV-1, -2, -3, and -4) as described previously using highly specific and sensitive primers.<sup>20</sup> The DENV-positive control strains used in the RT-PCR for DENVs (D1/AO/XX/1988 Angola, D2/CR/13538/2007 Limón, D3/CR/14532/2007 Corredores, and D4/DM/814669/1981 Dominica) were donated by the Dengue Reference Center, Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA; Costa Rica). Nuclease-free water was used as a negative control. RT-PCR products were visualized by 2% agarose gel electrophoresis and stained with Gel Red (Biotium Inc., Hayward, CA). Positive and negative controls resulted as expected.

Samples from 16 bat species were analyzed. Six DENV-2-positive bats were detected (prevalence of 4.1%; 95% confidence interval ± 3.22) (Table 1). In Calakmul, four bats were PCR-positive: two *Glossophaga soricina*, one *Artibeus jamaicensis*, and one *A. lituratus*. Two bats, *G. soricina* and *A. jamaicensis*, were captured in the UD sites, and two bats, *G. soricina* and *A. lituratus*, were captured in a D site. In Montes Azules, two *A. lituratus* were positive to DENV-2: one bat from a UD site and one bat from a D site. These three bat species (*A. jamaicensis*, *A. lituratus*, and *G. soricina*) are considered highly abundant bat species in the Mexican Neotropics,<sup>21–23</sup> coinciding with the relative abundance found in this study (*A. lituratus*: 179 [22%]; *G. soricina*: 118 [14.5%];

and *A. jamaicensis*: 111 [13.6%]). No significant difference was detected comparing the presence of DENV-2 and the type of site (UD and D) using a test of equal or given proportions in  $R^{24}$  ( $\chi^2 = 0.1738$ , degrees of freedom = 1,  $P = 0.6768$ ).

This preliminary study provides additional evidence of DENV exposure in Neotropical bats.<sup>7,15–17</sup> Unfortunately, because of sample degradation, we could not amplify a different region of the viral genome for phylogenetic analysis from the positive samples. Although DENV infection was serologically recognized in 26 bats of the same three species (*A. jamaicensis*, *A. lituratus*, and *G. soricina*) from Merida City,<sup>17</sup> this detection is the first detection of the DENV-2 genome by molecular diagnostic methods in bats from south-eastern Mexico. DENV-1, -2, and -3 have been reported in bats from French Guiana by RT-PCR, where DENV was found in *A. planirostris* and *C. perspicillata*.<sup>7</sup> Along the Gulf Coast of Mexico, DENV-2 was identified in *Myotis nigricans*, *Carollia sowelli*, and *A. jamaicensis*.<sup>16</sup> However, this detection is the first molecular detection of the DENV-2 genome in *A. lituratus* and *G. soricina*.

Old World DENV-2 strains diverged relatively recently and spread to the Neotropics by human carriers and the transportation of infected monkeys and mosquitoes.<sup>5</sup> The encroachment of human settlements and agricultural areas on UD areas could promote new interactions among DENV vectors and potential hosts, increasing the possible development of sylvatic DENV.<sup>5</sup> Large-scale research of anthropogenic change and pathogen evolution using an epidemiologic framework is vital to develop comprehensive policies across public health, economic development, and conservation biology.<sup>5</sup>

This study adds additional evidence for the presence of DENV-2 in three bat species (*A. jamaicensis*, *A. lituratus*, and *G. soricina*). However, the extent to which bats are involved in DENV transmission and their ability to act as competent hosts remain undetermined. Because these species are the three most abundant species in the region, we may be seeing random spillover into dead-end hosts. Understanding the ecology of DENV in bats is important before implicating bats as a threat to public health. Sylvatic DENV in any species could evolve from biological interactions between wild reservoirs and sylvatic vectors.<sup>5,7</sup> Lastly, it may arise from socio-economic factors, such as human encroachment into natural areas in locations where medical and health services are absent.<sup>25</sup> Future investigations of DENV in Neotropical bats and other potential wild reservoirs will improve the understanding of the ecological dynamics of these viruses in nature.

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