Published in final edited form as:
J Wildl Dis. 2013 July ; 49(3): . doi:10.7589/2012-12-318.

Serologic Evidence of Flavivirus Infection in Bats in the Yucatan Peninsula of Mexico

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Abstract

We captured 140 bats of seven species in Merida City in the Yucatan Peninsula of Mexico in 2010. Serum was collected from each bat and assayed by plaque reduction neutralization test (PRNT) using six flaviviruses: West Nile virus, St. Louis encephalitis virus, and dengue viruses 1–4. Flavivirus-specific antibodies were detected in 26 bats (19%). The antibody-positive bats belonged to three species: the Pallas's long-tongued bat (*Glossophaga soricina*), Jamaican fruit bat (*Artibeus jamaicensis*), and great fruit-eating bat (*Artibeus lituratus*), and their flavivirus antibody prevalences were 33%, 24%, and 9%, respectively. The PRNT titers were usually highest for dengue virus 2 or dengue virus 4, but none of the titers exceeded 80. These data could indicate that most of the antibody-positive bats had been infected with dengue virus. However, because all titers were low, it is possible that the bats had been infected with another (perhaps unrecognized) flavivirus not included in the PRNT analysis, possibly a virus more closely related to dengue virus than to other flaviviruses. Each serum sample was assayed for flavivirus RNA by reverse transcription PCR, but all were negative.

Keywords

Bats; Chiroptera; dengue virus; flavivirus; Mexico; surveillance

Bats are one of the most abundant and geographically widespread groups of vertebrates. There are 1,116 recognized species of bats and they inhabit every continent (except Antarctica) and almost every ecosystem (Wilson and Reeder, 2005; Calisher et al., 2006). Bats are often found in areas inhabited by humans and harbor a diverse range of viruses of medical importance, including filoviruses, SARS-like and other coronaviruses, henipaviruses, and lyssaviruses.

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Dengue virus (DENV), West Nile virus (WNV), and St. Louis encephalitis virus (SLEV) are recognized human pathogens in the genus *Flavivirus*, family *Flaviviridae*. The role of bats in the transmission and ecology of DENV, WNV, and SLEV is not well understood, but several studies have demonstrated that bats of some species are susceptible to infection by these viruses (Sulkin et al., 1966; Paul et al., 1970; de Thoisy et al., 2009). Dengue virus exists as four serotypes (DENV-1 to DENV-4), and all are present in the Yucatan Peninsula of Mexico (Lorono-Pino et al., 2004). Serologic data indicate that WNV and SLEV also occur in this region (Farfan-Ale et al., 2006). Nevertheless, there is no information on the infection or prevalence of antibody to these viruses or any other flaviviruses in bats in the Yucatan Peninsula. Dengue virus–specific nucleic acid and antibodies were detected in bats in the nearby states of Veracruz, and DENV-specific antibodies were detected in bats in the states of Colima and Jalisco in southwest Mexico (Aguilar-Setien et al., 2008). No other investigators have examined whether flaviviruses infect bats in Mexico. Our goal was to determine the occurrence of selected flaviviruses in bats in Merida, the largest city in the Yucatan Peninsula.

We collected whole blood from 140 free-ranging bats captured using mist nets at five study sites, July–October 2010. One site was within the central business district (CBD); the other four were directly to the north, south, east, or west of the CBD. The site within the CBD was in the Parque Zoologico El Centenario (Merida zoo; 20°58′7.6″N, 89°38′26.0″W). The northern site (Santa Gertrudis Copo; 21°02′34.9″N, 89°35′930.1″W) was on a private property containing a sheep pen, an enclosure of chickens, low scrub, and sparse trees. The southern site (Parque Metropolitano; 20°53′50.8′N, 89°39′31.1″0W) was established in a public park with abundant trees and other vegetation and three small caves. The eastern site (Acuaparque; 20°56′950.8″N, 89°34′40.4″W) was in a public park containing a manmade lake, recreational facilities, low scrub, and native grasses. The western site (Parque Hundido; 20°58′936.8″N, 89°39′28.5″W) was a public park with numerous trees, native grasses, and several ponds.

We trapped four nights a week (one site per night) using three mist nets (6×2 m with 16-mm mesh openings) at each site. Nets were placed over water, along fruiting trees, and at the entrances of caves whenever possible. Nets were set from dusk (~7:00 PM) until 11:00 PM. Captured bats were placed in cloth bags until processed. Bats were identified to species, age class (juvenile or adult), and sex (Medellín et al., 1997). Taxonomy followed Wilson and Reeder (2005). Approximately 100 μL of blood was collected from each bat by nonlethal cardiac puncture or from the ulnar vein. Blood samples were diluted 10-fold using phosphate-buffered saline containing 0.75% bovine albumin fraction V, 500 units/mL penicillin, 500 μg/mL streptomycin, and 12.5 μg/mL amphotericin, and placed on ice. Samples were transported to the laboratory, centrifuged, and stored at –70 C.

Sera were assayed by plaque reduction neutralization test (PRNT) following standard methods (Beaty et al., 1995). The PRNTs were done using WNV (strain NY99-35261-11), SLEV (strain TBH-28), DENV-1 (strain Hawaii), DENV-2 (strain New Guinea C), DENV-3 (strain H-87), and DENV-4 (strain H-241). The PRNTs were performed in six-well plates containing confluent monolayers of African green monkey kidney (Vero) cells. Sera were initially tested at a dilution of 1:20, and those that reduced the number of plaques by ≥70% were titrated. Titers were expressed as the reciprocal of serum dilutions yielding ≥90% reduction in the number of plaques ($PRNT_{90}$). Mouse hyperimmune ascitic fluids containing antibodies to WNV, SLEV, and DENV1–4 were used as positive controls and sera from uninfected mice served as negative controls.

Sera were also assayed by reverse transcription PCR (RT-PCR) for flavivirus RNA. Briefly, 100 μL of each serum sample was added to 0.9 mL of Trizol (Invitrogen, Carlsbad,

California, USA) and total RNA was extracted in accordance with the manufacturer's instructions. Complementary DNAs were generated using Superscript III reverse transcriptase (Invitrogen) and random hexamer primers (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The PCRs were performed using *Taq* polymerase (Invitrogen) and flavivirus-specific primers that target an ~800-nucleotide region of NS5 gene (Maher-Sturgess et al., 2008). The RT-PCR products were examined by 1% agarose gel electrophoresis and visualized with ethidium bromide.

We captured 140 bats belonging to two families, five subfamilies, and seven species (Table 1). The most highly represented species were the Jamaican fruit bat (*Artibeus jamaicensis*) and great fruit-eating bat (*Artibeus lituratus*), which comprised 56% and 23% of captures, respectively. Eighty-nine (64%) bats were adults and 51 (36%) were juveniles. Fifty-nine bats were trapped in Parque Hundido, 42 in Parque Metropolitano, 21 in Acuaparque, 14 in the Merida zoo, and four in Santa Gertrudis Copo. All bats were nonmigratory and appeared healthy.

Twenty-six bats (19%) had evidence of flavivirus infection (Tables 1 and 2). The bats with antibodies belonged to three species: the Pallas's long-tongued bat (*Glossophaga soricina*), Jamaican fruit bat, and great fruit-eating bat. The species with the highest flavivirus antibody prevalence was the Pallas's long-tongued bat (33%). Antibody prevalences for the Jamaican fruit bat and great fruit-eating bat were 24% and 9%, respectively. Fifteen (58%) of the bats with flavivirus-specific antibodies were adults; 11 (42%) were juveniles.

The $PRNT_{90}$ titers were usually highest for DENV-2 or DENV-4, although all of the titers could be considered low because none exceeded 80 (Table 2). Low antibody titers were also observed in bats from Costa Rica and Ecuador; these bats were assayed by PRNT using all serotypes of DENV and none had titers (when expressed as $PRNT_{80}$) above 80 (Platt et al., 2000). Likewise, low antibody titers were detected in bats from China that were assayed by microseroneutralization test using Japanese encephalitis virus (Cui et al., 2008).

Our PRNT data could indicate that most of the flavivirus-antibody–positive bats had been exposed to DENV but high levels of neutralizing antibodies were not generated because DENV replicates inefficiently in chiropterans. Another potential explanation is that efficient DENV replication does occur in bats but the titers were low because bats do not produce high levels of neutralizing antibodies in response to DENV infection. In this regard, neutralizing antibodies to WNV were not detected in any bats following WNV inoculation, even though some were viremic (Davis et al., 2005). Experimental infection studies should be performed to assess the viremia profiles and antibody responses in bats after DENV inoculation. Flavivirus-specific neutralizing antibodies have been shown to persist at low titers in some vertebrate species long after the initial exposure (Gibbs et al., 2005), and therefore another explanation is that some of the infections were not recent. However, this is probably not a major reason because 42% of the flavivirus-antibody–positive bats were juveniles. A more likely reason is that the majority (if not all) of the antibody-positive bats were infected with another, perhaps unrecognized, flavivirus not included in the PRNTs and that the low titers are due to serologic cross-reactivity. Flaviviruses have a close antigenic relationship and therefore neutralizing antibodies to one flavivirus often cross-react with heterologous flaviviruses (Calisher et al., 1989). Other flaviviruses known to infect bats include Rio Bravo virus (RBV) and Tamana bat virus (Allen et al., 1970; Price, 1978). Additional flaviviruses were not included in the PRNTs because many of the bats were small and the volume of sera available was limited.

As stated earlier, 19% of bats had flavivirus-neutralizing antibody. Similar flavivirus antibody prevalences have been reported for bats elsewhere in Latin America. In a

serosurvey performed in Guatemala, 19% of bats had antibodies that neutralized RBV (Ubico and McLean, 1995). Evidence of flavivirus infection was detected in 23% of bats in Costa Rica that were assayed by PRNT using all DENV serotypes (Platt et al., 2000). Higher antibody prevalence (30%) was observed in bats in Ecuador. However, in the studies described above, titers were expressed as $PRNT_{80}$, and therefore the reported antibody prevalences would have presumably been slightly lower if the more stringent $PRNT_{90}$ had been used. In another study, 12% of bats in Colima, Jalisco, and Veracruz, Mexico, had antibodies that reacted with DENV antigen by enzyme-linked immunosorbent assay (Aguilar-Setien et al., 2008).

We did not detect flavivirus RNA by RT-PCR in any of the sera. Evidence of flavivirus infection has been demonstrated in bats assayed by RT-PCR in other studies (Zhang et al., 1998; Aguilar-Setien et al., 2008; de Thoisy et al., 2009). For example, DENV-2 RNA was detected in heart tissues from three species of bats in Veracruz (Aguilar-Setien et al., 2008). A notable difference between our study and the others cited here is that our RT-PCRs were performed using sera rather than tissues. Approval to perform terminal bleeds and harvest was not granted for our study.

We have provided serologic evidence that flaviviruses infect bats of some species in the Yucatan Peninsula. Our PRNT titers were often highest for DENV, but, because all of the titers were low, many (if not all) of these bats could have been infected with a flavivirus not included in the PRNTs. Experimental infection studies are needed to assess the potential of bats to serve as amplifying hosts of DENV. These experiments would also provide valuable information on the viremia profiles and antibody responses in bats after DENV inoculation. Such data would facilitate the interpretation of PRNT data obtained in flavivirus serosurveys among free-ranging bats.

Acknowledgments

We thank Virginia Ramirez-Sosa for assisting with trapping. We thank the Ayuntamiento de Merida for providing trapping permits. We acknowledge the Institutional Animal Care and Use Committees and the Institutional Biosafety Committees at the Universidad Autónoma de Yucatán and Iowa State University for approving the procedures described in this report. This study was supported by grant 5R21AI067281-02 from the US National Institutes of Health.

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Table 1

Flavivirus antibody prevalence estimates[a] for species of bats sampled in Merida, Yucatan Peninsula, of Mexico, 2010.

Species	Common name	No. tested	No. positive (%)
Artibeus lituratus	Great fruit-eating bat	32	3 (9)
Artibeus jamaicensis	Jamaican fruit bat	79	19 (24)
Diphylla ecaudata	Hairy-legged vampire bat	6	0 (0)
Glossophaga soricina	Pallas's long-tongued bat	12	4 (33)
Mimon cozumelae	Cozumelan golden bat	1	0 (0)
Sturnira lilium	Little yellow-shouldered bat	9	0 (0)
Vespertilionidae spp.	Vesper bat	1	0 (0)
Total		140	26 (18.6)

[a]Prevalences determined by plaque reduction neutralization test using West Nile virus, St. Louis encephalitis virus, and dengue viruses 1–4.

Table 2

Serology results for bats with neutralizing antibodies to flaviviruses in Merida, Yucatan Peninsula, Mexico, 2010.[a]

Serum ID	Species	Study site	Month of capture	PRNT$_{90}$ titer					
				DENV1	DENV2	DENV3	DENV4	WNV	SLEV
B1-002	*Artibeus jamaicensis*	Parque Hundido	September	20	20	__ [b]	20	—	—
B1-034	*Artibeus jamaicensis*	Parque Metropolitano	September	20	40	—	20	—	—
B1-041	*Glossophaga soricina*	Parque Hundido	September	20	20	—	40	—	—
B1-046	*Artibeus jamaicensis*	Parque Hundido	September	20	20	—	20	40	—
B1-048	*Artibeus jamaicensis*	Parque Hundido	September	20	20	—	40	—	—
B1-049	*Glossophaga soricina*	Parque Hundido	September	20	20	—	20	—	—
B1-052	*Artibeus jamaicensis*	Merida Zoo	September	20	20	—	20	—	—
B1-053	*Artibeus jamaicensis*	Merida Zoo	September	20	40	—	20	—	—
B1-056	*Artibeus jamaicensis*	Merida Zoo	September	20	20	—	20	40	—
B1-058	*Artibeus jamaicensis*	Merida Zoo	September	20	20	—	20	20	—
B1-060	*Artibeus lituratus*	Merida Zoo	September	20	20	—	20	—	—
B1-061	*Artibeus jamaicensis*	Merida Zoo	September	20	40	—	40	20	—
B1-062	*Artibeus jamaicensis*	Merida Zoo	September	20	40	—	20	—	—
B1-063	*Artibeus jamaicensis*	Merida Zoo	September	20	20	20	80	—	—
B1-074	*Glossophaga soricina*	Acuaparque	October	20	20	—	20	—	—
B1-076	*Artibeus lituratus*	Acuaparque	October	20	20	—	20	—	—
B1-081	*Artibeus lituratus*	Parque Hundido	October	40	40	—	80	20	—
B1-082	*Artibeus jamaicensis*	Parque Hundido	October	20	40	—	20	—	—
B1-086	*Artibeus jamaicensis*	Parque Hundido	October	20	20	—	20	—	—
B1-088	*Artibeus jamaicensis*	Parque Hundido	October	40	80	20	80	20	—
B1-089	*Artibeus jamaicensis*	Parque Hundido	October	20	80	—	20	—	—
B1-091	*Artibeus jamaicensis*	Parque Hundido	October	20	40	20	80	20	20
B1-092	*Artibeus jamaicensis*	Parque Hundido	October	20	40	—	20	—	—
B1-093	*Artibeus jamaicensis*	Parque Hundido	October	20	20	—	20	20	—
B1-114	*Glossophaga soricina*	Parque Metropolitano	October	20	20	—	20	—	—
B1-131	*Artibeus jamaicensis*	Parque Hundido	October	20	40	—	20	—	—

[a] PRNT$_{90}$ = plaque reduction neutralization test titer yielding ≥90% reduction in the number of plaques; DENV = dengue virus; WNV = West Nile virus; SLEV = St. Louis encephalitis virus.

[b] Dash = titer <20.