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# SEROLOGIC SURVEY OF NEOTROPICAL BATS IN GUATEMALA FOR VIRUS ANTIBODIES

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ABSTRACT: Neotropical bats were collected from different life zones in Guatemala in 1983 and 1984 to determine the presence and distribution of antibody against 10 viruses. Bats were collected with mist nets at 13 sites in eight departments and 332 serum specimens were obtained for testing for neutralizing (N) antibody by the plaque-reduction neutralization test. Eighty-seven (26%) of the 332 bats from 16 (38%) of 42 bat species sampled were serologically positive for five of six arboviruses and for two other viruses tested. Antibodies against Venezuelan equine encephalitis (VEE) variant I-A/B, eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, vesicular stomatitis, Tacaribe, and Rio Bravo viruses were detected in resident species of bats. However, N antibodies against the enzootic strain of VEE (Mena II, variant I-E) or Nepuyo viruses were not detected.

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Key words: Bats, arboviruses, viruses, Guatemala, neotropical bats, serologic survey.

## INTRODUCTION

Members of the order Chiroptera have a wide distribution, perhaps the most extensive range among mammals except for Rodentia. The tropical climate of Central America supports a variety of species. As many as 100 species of bats eventually may be found in Guatemala (Jones, 1966).

Microbial pathogens that are found in bats or bat guano from roosts include bacteria, fungi, and viruses. Bats also harbor an array of ectoparasites and endoparasites that may cause disease or be vectors of one or more disease agents (Hill and Smith, 1984).

Bats are infected by at least 28 different viruses, and antibodies against another 32 viruses have been discovered (Hill and Smith, 1984; Karabatsos, 1985). Many of the viruses often are not pathogenic to bats; many also are not pathogenic or are only mildly pathogenic to humans (Hill and Smith, 1984).

Sulkin (1962) and Donalson (1970) have implicated bats as possible carriers of vesicular stomatitis (VS) viruses over long distances; Tesh et al. (1969) reported that among wild animals, bats had the highest prevalence of antibody against VS-New Jersey (VSNJ) virus. Venezuelan equine encephalitis (VEE) viruses have been isolated from neotropical bats in Venezuela (Kubes and Rios, 1939), Ecuador (Gutierrez, 1972), Colombia (Jonkers, 1972), and Mexico (Correa-Giron et al., 1971; Scherer et al., 1971). Antibodies against VEE have been found in bats from Texas (USA) (Sudia et al., 1975), Mexico, and Guatemala (Sudia et al., 1971; Seymour et al., 1973). Seymour et al. (1973) proposed that bats may serve as alternate hosts to maintain virus transmission in the enzootic cycle.

Other viruses such as St. Louis encephalitis (SLE), western equine encephalitis (WEE), and yellow fever (YF) could be important to the public and veterinary health in Central America, but their frequency and distribution are not well known (Acha and Szyfres, 1987). The role of bats in the transmission cycles and ecology of these viruses is not known. The apparent insignificance of the contribution of bats to the maintenance of SLE virus may be a result of lack of scrutiny rather than lack of importance (McLean and Bowen, 1980).

Other viruses isolated from mammals in Guatemala include Nepuyo (NEP) (Scherer et al., 1976), Patois (Scherer et al., 1985), and Jutiapa (Karabatsos, 1985). Tacaribe (TCR) virus also has been isolated and antibody against this virus found in bats



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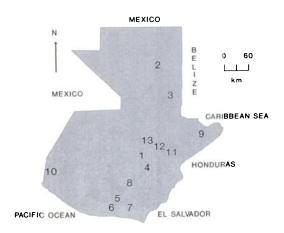


FIGURE 1. Trapping locations for bats collected in the various departments of Guatemala, 1983–84. Department of Baja Verapaz, Finca Raxija (Site 1); Department of El Peten, Tikal (Site 2) and Fincas Caldera and La Union (Site 3); Department of El Progreso, San Agustin Acasaguastlan (Site 4); Department of Escuintla, Finca Las Mercedes (Site 5), Finca Santillana del Mar (Site 6), and Auto Safari Chapin (Site 7); Department of Guatemala, Parque Naciones Unidas (Site 8); Department of Izabal, Finca Rio Frio (Site 9); Department of San Marcos, Finca El Eden (Site 10); and Department of Zacapa, Jesus Maria (Site 11), Finca Chahuites (Site 12), and Finca Montes de Moran (Site 13).

(Price, 1978). However, the public health importance of these viruses has not been established (Scherer et al., 1985; Calisher et al., 1971). Except for rabies, most viruses found in bats occur only in specific and frequently very localized regions of the world.

The importance of bats in the ecology of Guatemala and the lack of information on their possible role as reservoir hosts of viral diseases prompted this investigation. Our objective was to determine the serologic prevalence and distribution of 10 viruses in bats from Guatemala.

#### MATERIALS AND METHODS

Guatemala is the most western and northern of the Central American countries, situated between a latitude of 13°46' and 17°58'N and a longitude of 88°13' and 92°12'W, with an area of 108,900 km<sup>2</sup>. It is on the transitional limits of both the nearctic and neotropical regions and has a great variety of ecosystems (Land, 1970; Holdridge et al., 1971).

Bats were collected from 13 sites in eight departments in Guatemala (Fig. 1). Equipment and procedures used to capture and handle bats were similar to those described by Sudia et al. (1970). Mist nets were placed at ground level in open and sheltered sites within habitats selected to sample a variety of bat species. Net placement was the same during each sampling period, and nets were operated for one to three nights at a site from dusk to dawn. Nets were checked between 0500 and 0600 hr. Ectoparasites were removed from all captured bats and preserved in 70% ethanol. Bats were bled from the heart with a 1-ml syringe and 25 to 27 gauge needle after immobilization with ethyl ether. Blood (0.1 to 0.2 ml) from each bat was mixed with 0.9 ml of field diluent, consisting of M199 nutrient medium (Gibco, Grand Island, New York, USA) with antibiotics and 20% heat-inactivated (56 C for 30 min) fetal bovine serum (McLean et al., 1985a). Blood specimens were kept on wet ice and allowed to clot. Specimens were centrifuged at 900  $\times$  G for 15 min; the serum was stored in screw-capped, labeled vials and held at -70 C for shipment to the Centers for Disease Control laboratory in Fort Collins, Colorado (USA), for testing.

Taxonomic study skins and skulls of these bats were prepared in the field and later deposited in the mammal collection of the U.S. National Museum, Smithsonian Institution in Washington, D.C. for final identification (Hall, 1981).

Heat-inactivated (56 C for 30 min) serum specimens were tested for antibody against VEE (TC-83 strain, variant I-A/B and Mena II strain, variant I-E), SLE (MSI-7 strain), eastern equine encephalitis (EEE, NJ-160 strain), WEE (Fleming MI-2959B strain), VS-Indiana (VSI, laboratory strain), VSNI (Greentree strain), TCR (11573 strain), NEP (P55MI strain) and Rio Bravo (RB, M64 strain) viruses by the plaque-reduction neutralization test (McLean et al., 1985b). Serum samples were mixed with equal volumes of each of the challenge viruses diluted to contain approximately 100 plaque-forming units (PFU) per 0.1 ml. After overnight incubation at 4 C, 0.1 ml of the mixture was added to monolayers of Vero cell cultures (American Type Culture Collection, Rockville, Maryland, USA) grown in six-well plastic plates. After 1 hr of absorption at 37 C, inoculated cultures were overlaid with nutrient medium containing 1% Noble agar (Difco Laboratories Inc., Detroit, Michigan, USA) and 1:25,000 neutral red (Gibco). Cultures were incubated at 37 C with 5% CO, for 3 to 7 days or until plaques could be counted. Reduction of plaque counts by 80% or more as compared with controls was considered positive.

	VEE-TC	83°	VS N	J	VS I	N	SLE		EEE		
Bat species	Positive/ tested <sup>e</sup>	% <sup>d</sup>	Positive/ tested	%	Positive/ tested	%	Positive/ tested	%	Positive/ tested	%	
Artibeus intermedius	0/23	0	0/23	0	0/23	0	1/21	5	1/23	4	
Artibeus jamaicensis	16/105	15	6/105	6	4/103	4	4/97	4	9/105	9	
Artibeus lituratus <sup>e</sup>	0/12	0	0/12	0	2/12	17	2/12	17	3/12	3	
Artibeus phaeotis	0/25	0	1/25	4	3/25	12	2/19	11	0/25	0	
Carollia brevicauda	1/16	6	0/16	0	0/16	0	0/16	0	0/16	0	
Carollia subrufa	1/6	17	0/6	0	0/6	0	0/6	0	0/6	0	
Desmodus rotundus	0/18	0	4/18	22	0/18	0	0/16	0	0/18	0	
Glossophaga soricina	0/16	0	0/16	0	0/16	0	1/14	7	5/16	3	
Phyllostomus discolor	1/9	11	0/9	0	0/9	0	0/9	0	0/9	0	
Rhynchonycteris naso	0/4	0	0/4	0	0/4	0	0/3	0	2/4	50	
Sturnira lilium	2/54	4	4/54	7	0/54	0	4/53	8	4/54	8	
Sturnira ludovici	1/9	11	0/9	0	0/9	0	0/9	0	0/9	0	
Vampyrodes caraccioli	0/1	0	1/1	100	NT	NT	NT	NT	0/1	0	
Other species	0/30	0	0/30	0	0/29	0	0/29	0	0/30	0	
Unknown species	0/4	0	0/4	0	0/4	0	0/4	0	0/4	0	
Total	22/332	7	16/332	5	9/328	3	14/308	5	24/332	7	

 TABLE 1.
 Neutralizing antibody<sup>\*</sup> against selected viruses found in different species of bats, Guatemala, 1983-84.

• Neutralizing antibody in plaque-reduction neutralization test in Vero cell culture.

<sup>b</sup> VEE-TC83 = Venezuelan equine encephalitis virus (TC-83 strain), VSNJ = vesicular stomatitis virus (New Jersey strain), VSIN = vesicular stomatitis virus (Indiana strain), SLE = St. Louis encephalitis virus, EEE = eastern equine encephalitis virus. NT = not tested.

Number positive/number tested.

<sup>d</sup> Percent positive

\* One each of this species also was positive for neutralizing antibody against western equine encephalitis and Tacaribe viruses.

#### RESULTS

Five hundred bats were captured using mist nests at 13 study sites in Guatemala, but only 332 blood samples were obtained. Eighty-seven (26%) of the 332 individual bats from 16 (38%) of 42 bat species collected at 11 of the 13 sites had antibodies against one or more of the different viruses tested (Tables 1, 2, 3).

Twenty-two (6.6%) of the 332 bats examined for VEE virus, TC-83 strain, were neutralizing (N)-antibody positive (Table 1); however, no antibody-positive bats were detected for the enzootic strain of VEE virus, Mena II. Six bat species were seropositive for VEE virus TC-83 strain, and the species with the highest prevalence was *Artibeus jamaicensis*, with 16 bats positive (Table 1); 14 of these bats were collected in Finca Santillana del Mar, Masagua, Escuintla and the other two came from Jesus Maria, Zacapa sites (Table 2). Nine (2.7%) of 328 bats examined for the VSI virus strain were positive. All were species of the genus *Artibeus*. For the VSNJ virus strain, 16 (4.8%) of 332 bats examined were positive; *A. jamaicensis* had the highest antibody prevalence for this virus (Table 1). The area with the highest prevalence was El Peten with 6.7% of the bats positive for VSI virus (Table 2).

Fourteen (4.5%) of 308 bats tested for antibody to SLE virus were positive (Table 1). These bats were collected in Tikal and Finca Raxija (Table 2). The genus *Artibeus* had the highest prevalence (Table 1), and all SLE N-antibody positive bats were fruit feeders.

Six species of bats were seropositive for EEE virus (Table 1). The Department of Escuintla had the highest prevalence and the study site with the next highest prevalence was Tikal, with six positive bats (Table 2).

The only N-antibody positive bat for

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		VEE-TC83 <sup>b</sup>		VS-N	J	VS-IN	J	SLE		EEE	
Department	Study site	Posi- tive/ tested <sup>c</sup>	%ª	Posi- tive/ tested	%	Posi- tive/ tested	%	Posi- tive/ tested	%	Posi- tive/ tested	%
Baja Verapaz	Finca Raxija	0/8	0	0/8	0	0/8	0	2/8	25	0/8	0
El Peten	Tikal	3/105	3	3/105	3	7/104	7	12/94	13	6/105	6
	Fincas Caldera and Union	0/1	0	1/1	100	0	0	0/0	0	0/1	0
El Progreso	San Agustin	1/53	2	3/53	6	0/53	0	0/49	0	1/53	2
Escuintla	Finca Las Mercedes	1/19	5	1/19	5	0/19	0	0/19	0	0/19	0
	Finca Santillana del Mar	14/55	25	3/55	5	1/54	2	0/52	0	10/55	18
	Auto Safari Chapin	0/11	0	0/11	0	0/11	0	0/10	0	2/11	18
Izabal	Finca Rio Frio	0/20	0	3/20	15	1/20	5	0/19	0	2/20	10
San Marcos	Finca El Eden	0/10	0	0/10	0	0/10	0	0/10	0	1/10	10
Zacapa	Jesus Maria	2/13	15	0/13	0	0/13	0	0/13	0	0/13	0
	Finca Chahuites	1/34	3	2/34	6	0/33	0	0/31	0	2/34	6
	Montes de Moran	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0
Total		22/332	7	16/332	5	9/328	3	14/308	5	24/332	7

TABLE 2. Neutralizing antibody<sup>4</sup> against selected viruses found in bats, Guatemala, 1983-84.

\* Neutralizing antibody in plaque-reduction neutralization test in Vero cell culture.

<sup>b</sup> VEE-TC83 = Venezuelan equine encephalitis virus (TC-83 strain), VSNJ = vesicular stomatitis virus (New Jersey strain), VSIN = vesicular stomatitis virus (Indiana strain), SLE = St. Louis encephalitis virus, EEE = eastern equine encephalitis virus.

<sup>c</sup> Number positive/number tested.

<sup>d</sup> Percent positive.

WEE virus was an A. lituratus collected in Tikal. Antibody against TCR virus was only detected from one A. lituratus from Tikal, El Peten. No N-antibody positive results were obtained for NEP virus.

Fifty (19%) of 271 bats examined for RB virus were N-antibody positive (Table 3); the highest prevalence (50%) was in the genus *Artibeus*. Most of the RB-antibody positive bats were captured in Tikal (Table 3).

### DISCUSSION

To avoid unnecessary stress, some bats such as pregnant and lactating females, weak bats, very young bats, as well as individuals from some species for which adequate samples had already been collected, were released without being bled. Thus blood samples were taken only from 332 of the 500 captured bats.

Most bats that were N-antibody positive for VEE virus were collected in the Department of Escuintla (Fig. 1), near La Avellana, a VEE virus-enzootic area (Seymour et al., 1973). Only three seropositive bats were detected in El Progreso and Zacapa (Fig. 1), a disease-epidemic area in the 1969 VEE outbreak (Sudia et al., 1971). Tikal, where repeated human infections have occurred since 1966 (Scherer et al., 1970), only had three sero-positive bats. These findings, together with the work of Correa-Giron et al. (1971) and Seymour et al. (1973), are evidence that an enzootic VEE cycle exists. It was surprising that only serologic reactions to TC-83 strain, and not to the enzootic strain Mena II, were detected, even though the enzootic strain exists in Guatemala (Sudia et al., 1971). The enzootic strain may be more prevalent in the coastal habitats which were not sampled during this investigation. One

	Study site	Species <sup>6</sup>													
Department		Ai	Aj	Al	Ap	At	Ср	Cb	Dr	Ef	Gs	Pd	Sl	Uk	Total
Baja Verapaz	Finca Raxija	1	1	0	0	1	0	0	0	1	0	0	2	0	6
El Peten	Tikal	3	3	4	8	0	2	5	0	0	1	0	10	1	36
El Progreso	San Augstin	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Escuintla	Las Mercedes Santillana del Mar	1 0	0 2	0 0	3 0	0 0	0 0	4 2							
Zacapa Total number	Finca Chahuites of positive bats	0 5	1 7	0 4	0 8	0 1	0 2	0 5	0 1	0 1	0 1	0 3	0 12	0 1	1 50

TABLE 3. Neutralizing antibody<sup>4</sup> against Rio Bravo virus in bat species, Guatemala, 1983-84.

Neutralizing antibody in plaque-reduction neutralization test in Vero cell cultures.

<sup>b</sup> Ai = Artibeus intermedius, Aj = Artibeus jamaicensis, Al = Artibeus lituratus, Ap = Artibeus phaeotis, At = Artibeus toltecus, Cp = Carollia perspicillata, Cb = Carollia brevicauda, Dr = Desmodus rotundus, Ef = Eptesicus fuscus, Gs = Glossophaga soricina handleyi, Pd = Phyllostomus discolor, Sl = Sturnira lilium parvidens, Uk = Unknown species.

No neutralizing antibodies against Rio Bravo virus was found among any bats tested at the Fincas Caldera and Union study site in the Department of El Peten, at the Auto Safari Chapin study site of the Department of Escuintla, at the Finca Rio Frio study site of the Department of Izabal, at the Finca El Eden study site of the Department of San Marcos, or at the Jesus Maria and Montes de Moran study sites of the Department of Zacapa.

explanation for the presence of antibody against the epizootic variant I-A/B virus may be that the vaccine virus that was derived from an epizootic variant I-A/B virus has been circulating in natural cycles within Guatemala as was discovered previously in Louisiana (USA) (Pedersen et al., 1972).

Epizootics due to EEE virus are rare in Central and South America, although epizootics in North America are frequent. These differences may be attributed to the habits of the respective vectors. It has been suggested that EEE virus has a sylvatic cycle in the subtropical and tropical regions of the American continent (Galindo et al., 1966). McLean et al. (1985a) believe that mammals may be important hosts for EEE virus. Based on serologic results, bats roosting in places commonly used by mosquitoes can serve as natural hosts for EEE and other viruses (Main, 1979). We found 24 (7.2%) of the 332 bats with EEE N-antibody; 12 of these bats were collected in Escuintla (Table 2, Fig. 1) at the same site where another cluster of bats also was found to be VEE virus antibody positive. Bats with EEE antibody also were found in El Peten (Fig. 1), an area where the virus had

been isolated previously from humans (Scherer et al., 1976). According to Ordonez et al. (1971), the only mammals found positive for EEE virus were sentinel hamsters (*Mesocricetus auratus*). Our report is the first of an enzootic activity of EEE virus during non-epizootic periods in Guatemala. However, little is known about the natural history of EEE virus in this country.

Western equine encephalitis occurs in the same geographic areas as SLE and may even share the same vector species (Monath, 1980). Mixed SLE-WEE epizootics are the rule in the western United States, but generally one or the other virus predominates (Monath, 1980). Western equine encephalitis virus has been isolated from bats (Constantine, 1970). Western equine encephalitis N-antibody also was detected in A. jamaicensis from Haiti (McLean et al., 1979). One A. lituratus bat from Tikal was found positive for WEE N-antibody. Thus, it appears that bats occasionally may become infected with WEE, especially during epizootics of the disease in other hosts. No previous WEE activity has been reported in Guatemala. Bats in Tikal also had antibodies to SLE virus, which would

confirm the presence of the two viruses sharing the same ecosystem, and be evidence that enzootic activity of both viruses is possible in the dry tropical forest of El Peten.

Serologic surveys of humans in the neighboring countries of Belize, El Salvador, and Mexico have provided evidence for SLE virus activity in those countries (Bertram, 1971; Spence, 1980). We found evidence of antibody to SLE virus in bats from El Peten and Baja Verapaz (Table 2, Fig. 1) which is a range extension of this virus.

The mode of VS virus transmission in this region is not understood, although in some situations the virus could be transmitted by direct contact among animals (Webb and Holbrook, 1989). High antibody rates to VSI virus in bats have been observed in leishmaniasis-endemic areas (Srihongse, 1969). Shelokov and Peralta (1967) suggested that sand flies could be a common vector. Also, we found a high prevalence of antibody to VS viruses, specifically to VSI virus in El Peten (Table 2, Fig. 1); this site also is endemic for cutaneous leishmaniasis (Navin et al., 1988). The other regions where leishmaniasis has been reported in Guatemala are El Progreso and Zacapa (Fig. 1); both of these areas also had bats positive for antibody against VSNJ virus (Table 2). However, VS-antibody positive bats were captured in Escuintla, which is not within the leishmaniasis-endemic regions.

Bats could be maintenance hosts and even possible carriers of VS viruses over long distances (Donalson, 1970; Tesh et al., 1970). We found antibodies to VSI virus in three species of frugivorous *Artibeus*, while antibodies to VSNJ virus were found in both hematophagous and nonhematophagous bats (Table 1). Based on our results, we further propose that bats may be maintenance hosts of VS viruses, and that vampire bats may be transmitters of the viruses.

Other viruses in the TCR group are

known to produce infections in rodents and sometimes severe and often fatal hemorrhagic fevers in humans (Shelokov, 1964; Calisher et al., 1970). But, Price (1978) believes that there is no current evidence of TCR virus infection in humans. Tacaribe virus has been isolated from A. lituratus palmarum and A. jamaicensis trinitatis (Downs et al., 1963). Antibodies to TCR virus also have been found in 25 species of bats in Trinidad (Price, 1978). The antibody-positive bat found in this study was an A. lituratus collected in the dry tropical forest of Tikal (Table 1, Fig. 1). Even though TCR virus does not have any public health importance, it should be differentiated from rabies in bats that have bitten humans (Constantine, 1970).

We were unable to detect N antibody against NEP virus in any of the bats collected in Guatemala, probably because none of the study sites were in coastal marshes or near marshy areas. Nepuyo virus may be widely distributed on marshy coastal areas (Scherer et al., 1985); however, the incidence is completely unknown.

Our findings confirm the presence of RB virus in the bats of Guatemala (Table 3). Most past virus isolates had been obtained from salivary glands and a few were from brown fat (Constantine and Woodall, 1964; Baer and Woodall, 1966). Tadarida brasi*liensis* is believed to be the natural host for RB virus (Constantine, 1970). This bat is distributed from the southern United States into Argentina (Nowak and Paradiso, 1983); therefore, the virus probably is widely distributed too. Based on our findings, antibody to the virus is present in different species of neotropical bats (Table 3), including Eptesicus fuscus, a species in which the virus was previously found (Constantine, 1970; Karabatsos, 1985).

All the bats reported here as positive for antibody to RB virus are known to roost in caves just as *T. brasiliensis* and *E. fuscus* do. Since RB virus is believed to be transmitted via aerosol, cave roosting increases the probability for transmission of this virus among neotropical bats.

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