Invest Clin 18(1): 63-68, 1977.

Arbovirus studies of bats from Zulia state: Serological survey for Venezuelan, eastern and western Encephalitis virus antibodies. February 1976.

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Abstract. One hundred and forty five bats of 13 species collected during February 1976 at three sites in the State of Zulia, Venezuela, were negative for hemagglutination-inhibition (HI) and neutralization antibodies to Venezuelan encephalitis virus. One hundred and thirty seven of these were also negative for HI antibodies to both Eastern and Western encephalitis viruses. Due to small series for most species in the sample, negative results are difficult to interpret.

Estudios sobre arbovirus en murciélagos del Estado Zulia: encuesta serológica para determinar anticuerpos contra encefalitis venezolana, encefalitis del este y encefalitis del oeste. Febrero de 1.976.
Invest Clin 18(1):63-68, 1977.

Resumen: Ciento cuarenta y cinco sueros de murciélagos de 13 especies, capturados durante febrero de 1976 en tres localidades del Estado Zulia, Venezuela, no presentaron anticuerpos contra encefalitis venezolana mediante las pruebas de inhibición de la hemaglutinación (IH) y neutralización. Ciento treinta y siete de ellos fueron también negativos para encefalitis del este y del oeste, utilizando la prueba IH. Debido a la pequeña cantidad de animales que representaban la mayoría de las especies, los resultados negativos son difíciles de interpretar.

INTRODUCCTION

Natural infections of bats with Venezuelan encephalitis (VE) virus have been demonstrated through the isolation of the virus from bats in Mexico, Guatemala, Colombia and Ecuador (4, 5, 6, Seymour and

Dickerman, unpublished results), and the detection of VE virus specific antibodies in bats from Mexico, Guatemala, Colombia and Panama (3, 4, 5, 6, Seymour and Dickerman, idem). Experimental inoculation studies with several species of Neotropical bats indicate that some develop relatively high levels of viremia and suggest they may have considerable biological potential to act as amplifying hosts of VE virus (Seymopur, Dickerman and Martin, unpublished results). Data are not yet available regarding the involvemenet of bats in the natural cycles of VE virus in Venezuela. Thus, preliminary serological surveys were made on bats collected in February 1976 from three sites in the State of Zulia.

Because of the ocurrence of Eastern encephalitis (EE) virus activity at San Carlos shorty after our visit, plasmas remaining after studies of VE virus were completed and were screened for the presence of HI antibodies to EE and Western encephaltis (WE) viruses.

MATERIAL AND METHODS

At San Carlos (Distrito Colon), bats were caught in Japanese "mist" nest place under fruit trees in the garden of a small house along Rio Escalante. In the Rio Catatumbo region (Distrito Colon), bats were netted at sites 18 and 8 kilometers north of Rio Catatumbo and were caught by hand in culverts under the highway 9 kilometers north and 7 kilometers south of the river crossing. Nets were placed along side channels of the Rio Guasare at the edges of cleared fields and in second growth of tropical wet forest at Las Delicias (Distrito Mara) Fig. 1.

Bloods were taken by cardiac puncture in plastic disponsable syringes wetted with heparin, 50 units per ml in sterile saline. Some bloods were diluted 1:1 or 1:2 in sterile normal saline. Specimens were refrigerated until centrifuged and plasmas were frozen at - 5 oC until transported to New York City.

Hemagglutination-inhibition (HI) techniques of Clarke and Casals (1) adapted for microplates were utilized, with plasmas extracted by acetone and used in dilution of 1:6 to 1:10. Suckling mouse brain antigen to enzootic Guatemala strain 68U201 was used in HI tests. Neutralization (N) tests were carried using primary chick embryo cell cultures as described previously (7). Plasmas were N tested with Venezuelan epidemic strain E541/73 isolated by Ryder from blood of an 8-year-old boy (Dividive, Distrito Páez), exhibiting symptoms of encephalitis who was bled 25 October 1973. Selected plasmas were also N tested with enzootic Guatemalan strain 68U201. Lowest plasma dilutions in N tests were 1:4 to 1:2.

Strain 68U230 of EEV virus isolated from Guatemala and WE strain 1985-60 isolated at the Rocky Mountain Laboratory, Montana, USA, were used in HI tests for these viruses.

After exsanguination bats were preserved in 10% formalin and were

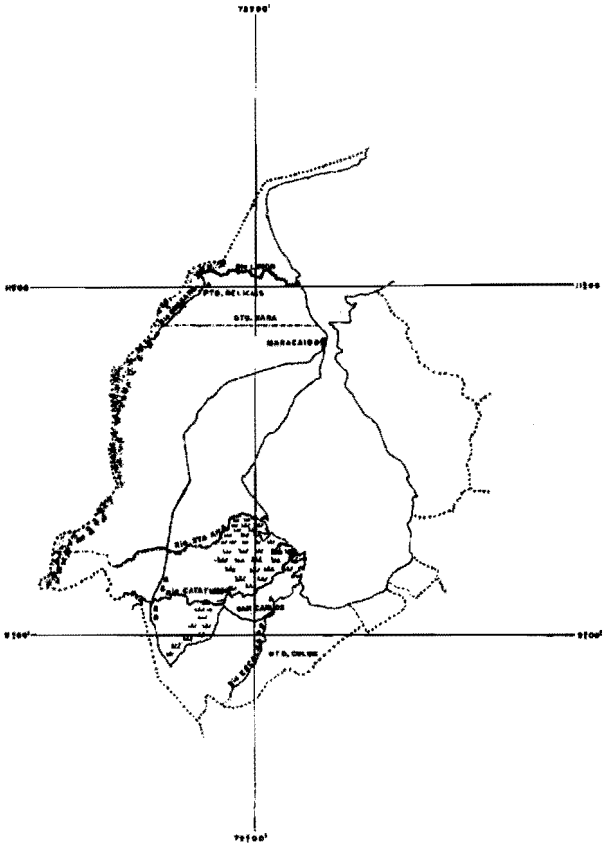

Fig. 1. Map of the State of Zulia indicating sites of collection.

deposited in the collection of the American Museum of Natural History, New York, where they were identified by Dr. Karl Koopman, Associate Curator of mammals.

RESULTS

All of 145 individuals of 13 species were negative by HI and N tests for antibodies to VE virus when strains 68U201 and E541/73 respectively were used as antigens (Table I). Six plasmas negative by HI but with $\log_{10}$ neutralization indexes (LNI) of 1.0 to 1.3, and six plasmas with LNI ≤ 0.8 were rerun in a plaque reduction neutralization test using strain 68U201 as the antigen. Again all plasmas had LNI values of ≤ 0.9 and all were considered negatives.

TABLE I

NUMBER OF BATS COLLECTED DURING FEBRUARY 1976 AT THREE SITES IN ESTADO ZULIA, VENEZUELA AND TESTED AND NEGATIVE* FOR HEMAGGLUTINATION INHIBITION (HI) AND NEUTRALIZATION (N) ANTIBODIES TO VENEZUELAN ENCEPHALITIS VIRUS

Species	San Carlos (Distrito Colón)	Río Catatumbo (Distrito Colón)	Puerto Delicias, Río Guasare (Distrito Mara)
Noctilio albiventris			1
Macrophyllum macrophyllum		17	
Phyllostomus discolor	2		
Phyllostomus hastatus	1		
Glossophaga soricina	4		
Carollia perspicillata		22	17
Sturnira lilium		3	1
Uroderma bilobatum	2	4	1
Vampyrops helleri		1	
Artibeus jamaicensis	26	1	24
Artibeus lituratus	3	5	7
Lonchophylla robusta			1
Desmodus rotundus			2
Total	38	53	54

*Negative HI test = 1:6-1:10 and chick embryo cell culture plaque reduction.
N test = log neutralization 1:3 (plasma diluted 1:4 to 1:12).

Likewise none of 35 bat plasmas from San Carlos, 52 from the Rio Catatumbo, region nor of 50 from Puerto Delicias had HI antibodies to EE or WE viruses.

DISCUSSION

These data illustrate a problem encountered in studying the enzootiology of arboviruses in faunistically rich regions; namely that of obtaining sufficient series of all species to allow interpretation of information obtained from field samples. This is particulary true when working with an agent that may be highly local in its ecological distribution such as VE virus. The problem when studying bats is only slightly less than when working with even more diverse avifaunas (2).

Negative results are difficult to interpret, specially when as in this study only three of thirteen species collected are represented by more than 10 individuals. However based on previous knowledge that in active enzootic foci of VE virus some groups of bats are regularly found with specific antibody (Seymour and Dickerman, unpublished results), and that in experimental studies HI and N were relatively long lasting in three species studied (Seymour, Dickerman and Martin, unpublished results), it is probable that VE virus has not been active over a wide area at San Carlos or Las Delicias sites within the last 1-3 years. At those localities adequate series were obtained of Artibeus jamaicensis a species highly susceptible to infection with VE virus that retains antibodies for long periods.

In contrast, in the Rio Catatumbo region where collections were from scattered sites and numbers for any one site were small, negative data should not be interpreted as indicating lack of VE virus activity. More studies are needed in that region to obtain larger samples of species known elsewhere to be involved in enzootic cycles of VE virus.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Pedro Rangel and Florencio Añez for their assistance in the field; Major Hugo Fernández and the personnel of the Destacamento Militar at Puerto Delicias for their hospitality; and División de Endemias Rurales, Dirección de Malariología y Saneamiento Ambiental for use of facilities at Sinamaica.

The work was supported in part by United States Public Health Research Grant AIII383 from the National Institute of Allergy and Infectious Diseases and in part by Consejo Nacional de Investigaciones Científicas y Tecnológicas de Venezuela (CONICIT), Research Grant SI-027.

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