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Author: BIGLER, WILLIAM J.

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VENEZUELAN ENCEPHALITIS ANTIBODY STUDIES IN CERTAIN FLORIDA WILDLIFE

WILLIAM J. BIGLER

Division of Veterinary Public Health
Florida State Board of Health
Jacksonville, Florida

Abstract

Between November, 1966 and November, 1968 serum samples from 725 wild vertebrates from south Florida were examined for antibody to Venezuelan encephalitis virus by the hemagglutination-inhibition test. Antibodies to Venezuelan encephalitis were found in 29 sera from the cotton mouse, *Peromyscus gossypinus,* the cotton rat, *Sigmodon hispidus,* the rice rat, *Oryzomys palustris,* the black rat, *Rattus rattus,* and the white-tailed deer, *Odocoileus virginianus.* Recent circulation of Venezuelan encephalitis virus in habitats other than documented enzootic foci warrants extensive ecological studies in areas adjacent to the Everglades National Park.

Introduction

Venezuelan encephalitis (VE) virus has been studied in south Florida by the National Communicable Disease Center (NCDC) for nearly 10 years beginning in 1960 with a serological survey of the Seminole Indians of Brighton and Big Cypress Reservations.[1] In 1961 the NCDC team sampled the Big Cypress Reservation in an attempt to isolate VE virus. Since 1963 NCDC has intensively collected mosquitoes and vertebrates in the Everglades National Park and certain other hammocks outside the park.[2] In 1966 the Division of Veterinary Public Health, Florida State Board of Health, as part of the statewide arbovirus surveillance program considered it advisable to define the current parameters of VE virus in native wildlife populations outside the Everglades National Park. This report briefly summarizes the wildlife serological studies conducted between November, 1966 and November, 1968.

Materials and Methods

Wild vertebrates were collected from a variety of diverse ecological habitats throughout the southern portion of Florida. Two cross-state transects from Ft. Pierce to Bradenton and from West Palm Beach to Ft. Myers sampled small mammal populations at approximately 10 mile intervals. However, most collections sampled animals in areas of suspected or known VE virus activity. During October and November, 1968 rodents were collected in areas east of the Everglades National Park as part of an epidemiological investigation of a confirmed human case of Venezuelan encephalitis in Homestead, Florida. Deer blood specimens were obtained from the Southeastern Cooperative Wildlife Disease Survey, University of Georgia, Athens.

Small mammals were captured in live traps, identified as to species, sex and age, then anesthetized and bled by cardiac puncture. Wild birds were collected in mist nets and by shooting. In each instance, .5 to 5.0 ml of whole blood was obtained. Samples less than 2.0 ml in volume were diluted 1:2, 1:3 or 1:5 with phosphate buffered saline, PH 7.7, containing 1000 units of penicillin sodium and 400 micrograms of streptomycin sulfate per ml.

Hemagglutination-inhibition (HI) tests were performed by the Virology Section of the Florida State Board of Health Central Laboratory at Jacksonville or the Epidemiology Research Center at Tampa, according to a modification of the technique described by Clarke and Casals.[3] VE HI reactive sera were confirmed in neutralization tests by the Department of the Army at Ft. Detrick or the NCDC Arbovirus Reference Laboratory at Atlanta.

Results

The results of mammal sera tested for HI antibodies against VE virus are summarized in Table 1. Of these 453 sera only five reacted with the VE antigen at a dilution of 1:20 or greater. Two of the positive sera were from a 1½ and a 2½ year old white-tailed deer collected in January, 1968, approximately five miles northwest of Ochopee (Fig. 1). The older deer may have become infected as early as the summer of 1965. VE virus was isolated from a pool of Culex (melanoconian) sp. collected at Turner River Jungle Gardens, Ochopee in September, 1965.[2] The other three positives were from rodents collected in 1967: two cotton rats trapped near Pinecrest, 40 miles west of Miami, and one rice rat two miles southeast of Florida City. Mouse neutralization tests at Ft. Detrick showed log neutralization indices (LNI) of 3.0 and 2.5 for the cotton rats and 3.0 and 1.6 for the 2½ and 1½ year old deer respectively. The rice rat sera was not neturalization tested. None of 39 wild birds, mostly egrets, herons and assorted passerines, were reactive in the HI test.

The results of the rodent sera collected east of the Everglades National Park during October and November, 1968 are summarized in Table 2. Twenty-four of the 233 sera tested reacted against VE antigen in the HI test with titers ranging from 1:20 to 1:320. Tissue culture neutralization tests at NCDC on 21 of these sera showed LNI that ranged from 2.6 $>$ 3.3. Two black rat sera from FC II, and one cotton rat sera from FC IV, were not neutralization tested. Fifteen positive sera came from Grossman's Hammock, an island surrounded by sawgrass approximately 12 miles northwest of Homestead. None of the 23 rodents collected in this hammock in 1964 had

TABLE 1. *Wildlife sera from south Florida* tested for VE HI antibodies*

Species	Total Tested	Nov. 1966 Fort Lauderdale	Feb. 1967 Sunniland	March 1967 Florida City	March 1967 Key Largo	Aug. 1967 Pinecrest (Loop Road)	Aug. 1967 Alligator Alley	Nov. 1967 Jan. 1968 Copeland	April 1968 Transect I	Jul/Aug. 1968 Transect II
Cotton Mouse	35	0/1	0/6					0/21	0/6	0/1
Cotton Rat	306	0/39	0/17	0/4	0/7	2/17	0/9	0/16	0/114	0/83
Rice Rat	45			1/9					0/27	0/9
House Mouse	5									0/5
Black Rat	18	0/6		0/1	0/11					
Norway Rat	5	0/2			0/1					0/2
Raccoon	5	0/1			0/3			0/1		
Opossum	23	0/6	0/4			0/1	0/2	0/9		0/1
Marsh Rabbit	6								0/1	0/5
White-tailed Deer	5							2/5		
Totals	453	0/55	0/27	1/14	0/22	2/18	0/11	2/52	0/148	0/106

*Excludes Collections made east of Everglades National Park, October and November, 1968

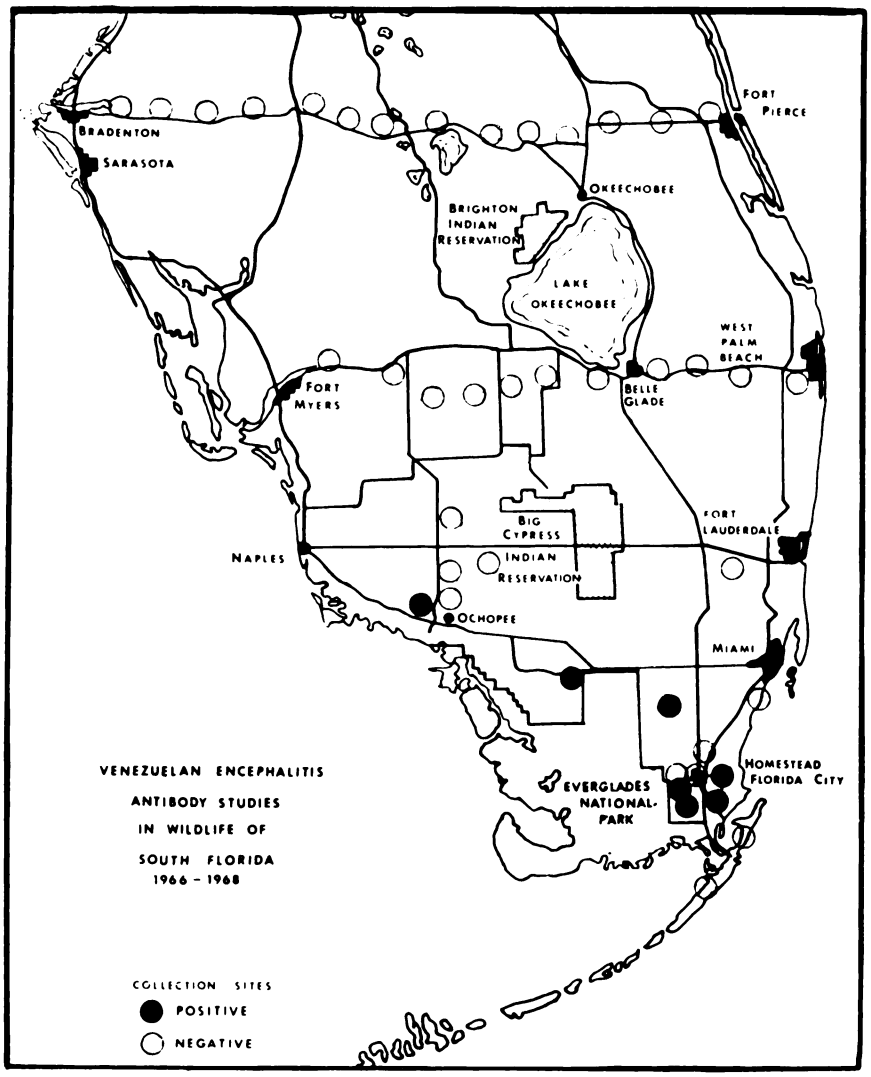

FIGURE 1. *Location of wildlife collection sites in south Florida.*

VE antibodies.[2] Five other positive sera were collected in a high-hammock within an agricultural area three miles southwest of Florida City (H III). Two positive black rats were collected along a drainage ditch transecting fallow fields four miles east of Florida City (FC II). The remaining two positive sera were collected along a series of diked roads and canals within the sawgrass marsh approximately five miles south of Florida City (FC IV).

TABLE 2. *VE HI antibodies in rodent sera collected east of Everglades National Park October and November, 1968*

SPECIES	HAMMOCK				FALLOW FIELDS				MARSH	URBAN	TOTAL
	Bauer Park	Grossman	Matheson	H III	FCIII	FC II	FC I	H II	FC IV	H I	
Cotton Mouse	0/8	13/18		0/3			0/1		1/3		14/33 (42.4%)
Cotton Rat	0/5		0/18	2/5	0/7	0/8	0/14		1/3	0/1	3/61 (4.9%)
Rice Rat	0/9			2/21		0/3	0/6	0/15			2/54 (3.7%)
House Mouse	0/5			0/15		0/1	0/1	0/18	0/1	0/4	0/45
Black Rat		2/2		1/1	0/7	2/17	0/2			0/11	5/40 (12.5%)
Totals	0/27	15/20 (75%)	0/18	5/45 (11.1%)	0/14	2/29 (6.9%)	0/24	0/33	2/7 (28.6%)	0/16	24/233 (10.3%)

Discussion

Significant HI titers and LNI in sera from wildlife sampled in the hammocks, agricultural areas, and in sawgrass marshes north and east of the Everglades National Park indicate VE virus is no longer confined to hammock foci within the park. The maintenance mechanisms of VE virus, i.e., the cotton mouse and cotton rat-Culex (melanoconian) sp. in enzootic hammocks, have been well documented,[2] however, very little is known about the various factors which contribute to the spread of VE virus outside these microcosms. Native wildlife populations in habitats adjacent to these everglades foci undoubtedly become involved in the circulation of VE virus during periodic epizootic sweeps. Extensive studies of the ecological distribution and abundance of certain vertebrates, particularly rodents, and mosquitoes in these areas are definitely indicated, the ultimate objective being to monitor VE virus activity with some degree of accuracy.

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