

VENEZUELAN EQUINE ENCEPHALITIS VIRUS-VECTOR STUDIES FOLLOWING A HUMAN CASE IN DADE COUNTY, FLORIDA, 1968

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Venezuelan equine encephalitis (VEE) virus was first isolated in the Everglades region of south Florida in 1963 by Chamberlain *et al.* (1969), three years after VEE antibodies were discovered by Work (1964) in the Seminole Indians living in that area. From 1960 to the present, no clinical disease due to VEE virus was observed in south Florida. However, in late October 1968, the first naturally acquired human clinical case of VEE in the United States was reported by Ehrenkranz *et al.* (in press) in a female resident of Homestead, Florida. The onset of illness occurred in early September.

The Arbovirus Ecology Laboratory (AEL) of the National Communicable Disease Center (NCDC) has been studying the ecology of VEE in south Florida since 1961. As a part of these studies, mosquitoes were collected in the Everglades National Park (ENP) in October 1968, prior to the report of the human case. Following the report, Florida State Board of Health officials requested the AEL to conduct virus-vector studies in Dade County, Florida, to determine the continued presence and geographical distribution of VEE virus in the county.

DESCRIPTION OF THE AREA. Dade County was divided into nine areas for convenience of survey and discussion (Figure 1). Areas 1, 3, and 4, with the exception of Homestead and the Air Force Base, contain mainly dry agricultural land which is irrigated by overhead sprinklers. The fields are bordered by flooded Everglades. Area 2 is a densely populated

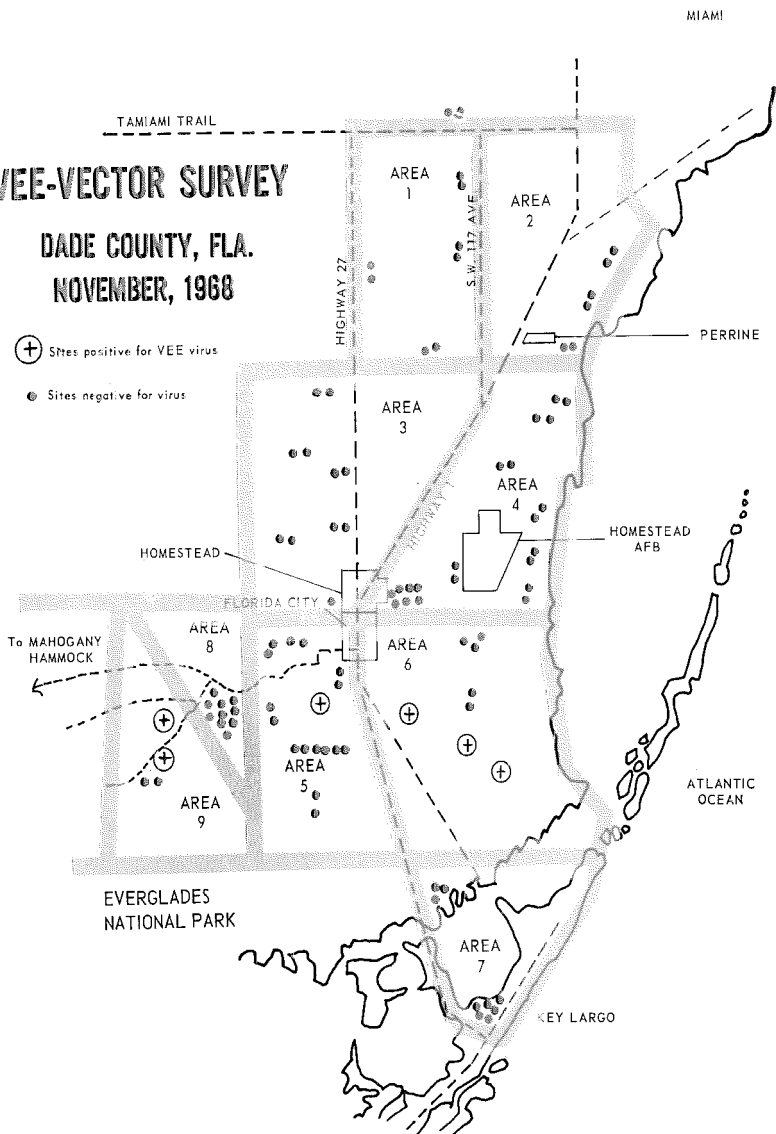
suburb of Miami and contains little unused land. Some scrub areas exist near the shore. Areas 5 and 6 contain large tracts of land which are usually flooded; these tracts are laced with drainage canals which are fished extensively by residents of Homestead and Florida City. Area 7 is mostly salt marsh which produces hordes of *Aedes taeniorhynchus* (Wied.). Area 8 is Royal Palm Hammock in the ENP; this hammock contains subtropical hardwoods and scattered palms. Area 9 consists of the eastern portion of private land within the Park and is primarily grassland, except for a hedgerow of trees and a canal bordering the old road to Flamingo. Areas 7, 8, and 9 are sparsely settled, except for personnel at small military installations and some Boy Scouts temporarily inhabiting a campground.

METHODS. Battery-operated CDC light traps (Sudia and Chamberlain, 1962) supplemented by dry ice (Newhouse, *et al.*, 1966) were used to collect the mosquitoes. Standard NCDC methods for the collection, field handling, and laboratory processing of specimens for virus isolation were used (Sudia and Chamberlain, 1967). Mosquito collections were made on a routine basis at Royal Palm and Mahogany Hammocks within the Park on October 15-16, 1968, as part of a continuing ecological study. Special virus-vector studies were conducted November 11-15, 1968, following the report of the human case. In these special studies, an effort was made to collect mosquitoes at suitable habitats throughout the inhabited portions of Dade County, with

VEE-VECTOR SURVEY

DADE COUNTY, FLA.

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FIG. 1.—Areas and locations of collection sites which were surveyed for VEE virus in Dade County, Florida, November 5–11, 1968.

emphasis on locations where the VEE patient might have been bitten by mosquitoes (Figure 1). These locations included her residence in Homestead and the vicinity of sites where she had been fishing before the onset of illness.

The mosquitoes routinely collected in October, together with 88 pools of *Culex* (*Melanoconion*) spp. collected in November, were tested for virus by intracerebral inoculation of litters of mice 2-3 days old. Brains of mice that died were passed into additional litters of newborn mice. Passage mouse brain material was used in complement fixation tests (Casey, 1965) by Dr. Charles Calisher, NCDC, to identify the isolates.

All mosquitoes collected in November were tested for virus in duck embryo cell (DEC) monolayer cultures (Coleman, unpublished data). The DEC cultures were inoculated with mosquito suspensions and observed for plaque formation daily for 10 days. Representative plaques from each isolate were subcultured in DEC monolayer cultures. Virus from these cultures was used in DEC neutralization tests for identification.

RESULTS. A total of 99,081 mosquitoes, representing 26 species, were collected in south Florida in October and November 1968, and tested for virus in 1,511 pools. Four of the five mosquito species from which VEE has been previously isolated

in south Florida were predominant (Table 1). *Culex nigripalpus* Theobald (63.4 percent) and *Anopheles crucians* Wied. (14.1 percent) are both permanent fresh water species. *Aedes taeniorhynchus*, a brackish water mosquito, was collected but the population was at a seasonal low (12.2 percent). The numbers of *Culex* (*Mel.*) spp. collected were also low (4.7 percent).

In October, eight VEE isolations were made in newborn mice, all from Mahogany Hammock, ENP (Table 2). Five of these isolations were made from *C. nigripalpus*. In November, ten VEE virus isolations were made in Dade County, all from *Culex* (*Mel.*) spp. One isolation was made in Area 5, five isolations at three sites in Area 6 and four isolations were made from two sites in Area 9. The six sites which produced VEE virus lie essentially on a line due east from Royal Palm in the Park (Figure 1). All of the November VEE virus isolations were made in both tissue culture and newborn mice.

Both the October and November collections yielded a number of other viruses, including Shark River, Mahogany Hammock, Gumbo Limbo, and Guama Group viruses. These isolations were made in newborn mice but not in DEC cultures. Two St. Louis encephalitis virus strains were isolated in newborn mice in October from the Park. The details on non-VEE

TABLE 1.—Mosquitoes from South Florida tested for virus October and November, 1968.

Mosquito Species	October	November		Total
	Ev. Natl. Park	Ev. Natl. Park	Dade County	
<i>Aedes atl.-tor.</i>	82 (7)*	28 (7)	164 (12)	274 (26)
<i>Aedes taeniorhynchus</i>	5,479 (58)	1,914 (23)	4,718 (74)	12,111 (155)
<i>Anopheles crucians</i>	4,385 (61)	5,782 (82)	3,842 (81)	14,009 (224)
<i>Culex nigripalpus</i>	30,178 (306)	21,402 (216)	11,319 (135)	62,899 (657)
<i>Culex</i> (<i>Mel.</i>) spp.	1,826 (38)	959 (23)	1,903 (65)	4,688 (126)
Other Species (21)**	980 (58)	2,207 (76)	1,913 (189)	5,100 (323)
Total	42,930 (528)	32,292 (427)	23,859 (556)	99,081 (1,511)

* Number of mosquitoes collected (Number of pools tested).

** Includes:

Aedes aegypti, *infirmatus*, *sollicitans*, *triseriatus*, *vexans*; *Anopheles atropos*, *quadrimaculatus*, *walkeri*; *Culex salinarius*; *Culiseta melanura*; *Deinocerites cancer*; *Mansonia indubitans*, *perturbans*, *titillans*; *Psorophora ciliata*, *confinis*, *ferox*; *Uranotaenia lowii*, *sapphirina*; *Wyeomyia mitchellii* and *vanduzeei*.

TABLE 2.—VEE Virus isolations from mosquitoes, South Florida October and November, 1968.

Mosquito Species	October	November		Total
	Ev. Natl. Park	Ev. Natl. Park	Dade County	
<i>Anopheles crucians</i>	1	0	0	1
<i>Culex nigripalpus</i>	5	0	0	5
<i>Culex (Mel.)</i> spp.	2	0	10	12
Other Species	0	0	0	0
Total	8	0	10	18

virus isolates will be the subject of a later report.

Thus far, 92 VEE virus strains have been isolated from mosquitoes in south Florida since 1963 (Table 3). Sixty-nine strains have been isolated from *Culex (Mel.)* spp.; 12 from *Culex nigripalpus*, 8 from *Aedes taeniorhynchus*, 2 from *An. crucians*, and 1 from *A. atlanticus tormentor*. VEE virus has been isolated from *Culex (Mel.)* spp. each year since 1963.

DISCUSSION. The Everglades region of south Florida is characterized by grasslands dotted with many wooded islands or hammocks. The grasslands are usually flooded during the rainy season from May to December, but during the dry season (January-April) the water level recedes and many areas become dry. The eastern portion of this region is made habitable or fit for agricultural use by a number of drainage canals, generally bordered by a road and a hedgerow.

The past VEE ecology studies were restricted to relatively few of the many hammocks present, preventing an accurate determination of the distribution of VEE virus in south Florida. The advent of the first naturally acquired human case

stimulated expansion of vector studies beyond the National Park to include the entire eastern portion of Dade County from the Tamiami Trail to the Florida Keys.

Finding VEE virus in several areas of Dade County for at least two months after the patient was infected demonstrates suitability of the area for virus persistence and suggests that the patient may have contracted the infection in that area while fishing. The fact that no virus was isolated near her residence lends substance to this presumption.

The question of the vector to man is still unresolved. *Culex (Mel.)* spp. are highly suspect because 69 of the 92 mosquito isolations have been made from them. Eight species of *Melanoconion* are found in south Florida, and since the females cannot be identified with certainty, the relative importance of the various species in the transmission of VEE virus is still unknown. The distribution and feeding habits of *Culex (Mel.) erraticus* D. and K., *Culex (Mel.) pilosus* D. and K., and *Culex (Mel.) peccator* D. and K. are known to some degree; however, much less is known about the other spe-

TABLE 3.—Isolation of VEE virus from potential vector species collected in South Florida, 1963-1968.

Mosquito Species	1963	1964	1965	1966	1967	1968	Total
<i>Aedes atl.-tor.</i>		1					1
<i>Aedes taeniorhynchus</i>	2		3	3			8
<i>Anopheles crucians</i>		1				1	2
<i>Culex nigripalpus</i>		1		3	3	5	12
<i>Culex (Mel.)</i> spp.	5	15	3	17	17	12	69
Total	7	18	6	23	20 ^a	18 ^a	92 ^a

^a Data incomplete.

cies in this subgenus, and their man-biting habits have been little studied. That some species are rodent feeders may be surmised from the high VEE antibody rates found in cotton rats and cotton mice (Chamberlain, *et al.*, 1969). The additional VEE isolations reported here tend to substantiate the enzootic vector role for the *Culex (Melanoconion)* mosquitoes.

Chamberlain *et al.*, 1969, suggested that *A. taeniorhynchus* could be the accessory vector most likely to carry the virus from a rodent cycle to man. *Culex nigripalpus*, although not as notorious a biter as *A. taeniorhynchus*, nevertheless is known to feed upon man. Five isolations of VEE virus were made from *C. nigripalpus* in the ENP in October, one month after the patient was presumed to have become infected. Seven other isolations have been made since 1963. In view of these findings, *Culex nigripalpus* should now be added as a potential accessory vector of VEE in south Florida, along with *A. taeniorhynchus*. It may be significant that the human case occurred in the fall, at a time when *C. nigripalpus* has been reported to alter its feeding preference from birds to mammals (Edman and Taylor, 1968).

Four basic criteria should be met to prove vector status of a particular species: (1) isolation of the disease-producing agent from wild-caught specimens, (2) demonstration of its ability to become infected by feeding upon a viremic host, (3) demonstration of its ability to transmit by bite, and lastly, (4) field evidence confirming association of the infected arthropod with the vertebrate population in which the infection is occurring. In respect to VEE in south Florida, information thus far has been accumulated only on the first and the fourth criteria. Studies required to fulfill the second and third criteria are underway in our laboratory.

SUMMARY. A virus-vector study was made in south Florida in October and November 1968, following the occurrence of a naturally acquired human case of Venezuelan equine encephalitis in the

area. In October, eight VEE isolations were made: five from *Culex nigripalpus*, two from *Culex (Melanoconion)* spp., and one from *Anopheles crucians*, all collected in the Everglades National Park. In November, ten VEE isolations were made, all from *C. (Melanoconion)* spp. collected in southeastern Dade County. Several isolations were made from the general vicinity where the patient was exposed to mosquitoes while fishing. However, the vector to man in south Florida is still undetermined.

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