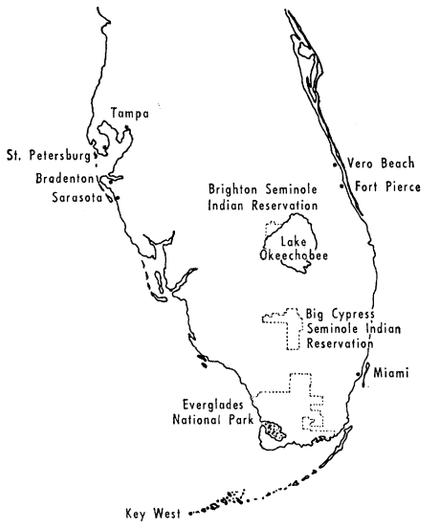

Fig. 1. Location of Seminole Indian Reservations and Everglades National Park in southern Florida.

reservation. A summary of these results is given in Table 2.

The low incidence of antibodies to several viruses, such as the eastern and western equine encephalitis viruses of Casals' group A, which are known to have widely scattered nonhuman activity in Florida, was unexpected. The higher percentage of antibodies to St. Louis encephalitis virus (SLE), VEE, and Bunyamwera group viruses indicates intensive and extensive exposure of the Seminole Indians to infection by mosquito-transmitted arboviruses, ecologically associated with the local habitat (biotope) in which they live. While exposure to SLE virus appears to have been substantial in both reservations—in the palmetto, grass, and cattle land of Brighton, as well as in the Big Cypress swamps and Everglades which extend southward continuously to the mangrove shores of the Caribbean Sea—the high incidence of immunity to VEE virus was concentrated in the Big Cypress habitat.

Where significant titers (1/20 to 1/160) for SLE virus appeared, certain other group B hemagglutinins were inhibited, but only to an extent indicative of group reactivity. Also listed in Table 2 are the minimum ages of donors of sera positive for different viruses. This information indicates that exposure to most of the viruses for which there is evidence of infection has been relatively recent and, for certain viruses, extensive.

These results indicate that SLE virus was frequently active and that it might be maintained in a natural cycle in the subtropical sylvan area of south-central Florida. On the other hand, the localization of VEE antibodies in proximity to the Everglades is evidence of a more selective association of the Big Cypress Indians with the Everglades habitat. Here, because of the striking difference between adults and children in the incidence of VEE virus, this virus appears to have been sporadic in appearance, possibly expanding under favorable conditions from a maintenance focus of infection deeper in the Everglades or introduced by some unknown mechanism of dissemination from its known endemic-epidemic foci in the southern Caribbean littoral and northern South America. Prior to this observation of VEE antibodies in the Big Cypress Seminole Indians, there had been no indication of human infection with this type of arbovirus in the United States, or, in fact, north of Panama.

The incidence of inhibitory sera for Cache Valley virus is substantial. Simultaneously with the demonstration of Bunyamwera group antibodies in the Seminole Indians, indicating that, as in Africa and South America, Bunyamwera group viruses do infect man, Casals and Whitman (*4*) demonstrated that the Cache Valley virus belongs antigenically in the Bunyamwera group.

The significance of finding Bunyamwera group antibodies in the Indian population was obvious (*5*) and it appeared imperative to follow up the serological surveys with actual searches for virus in accordance with principles defined by the WHO Study Group (*6*). A field station was therefore established at the Big Cypress Reservation headquarters in March 1961 (*7*) and a search for viruses in mosquitoes, birds, and other animals was begun on a systematic basis. Numerous strains of Bunyamwera group viruses were isolated from mosquitoes, providing a clearer definition of the significance of the Bunyamwera group antibodies in the Indian population. No further evidence of VEE virus activity was obtained.

The recent occurrence of central nervous system disease of SLE virus etiology in the Tampa Bay area (*8*) has reinforced our apprehensions concerning the high incidence of SLE antibodies in the indigenous population of the contiguous area of rural south-central Florida; evidently, mosquito-borne arbovirus activity in rural habitats of south Florida may be associated with subsequent epidemic occurrence of arbovirus disease in man.

The results of this work stimulated the extension of our investigations southward, deeper into a variety of habitats in the Everglades. What was initiated as an arbovirus exploration in 1960 has now emerged as a continuing study of substantial public health significance.

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20 May 1964

Venezuelan Equine Encephalitis Virus from South Florida

Abstract. *Venezuelan equine encephalitis virus was isolated three times from the* Culex (Melanoconion) *species of mosquitoes collected in south Florida in June, July, and October, 1963. Specific antibody was present in 16 of 28* Peromyscus gossypinus *(cotton mice) and 3 of 16* Sigmodon hispidus *(cotton rats) sampled from one of the infected sites in January 1964.*

Studies by Work in 1960 (*1*) revealed a significant incidence of Venezuelan equine encephalitis (VEE) antibodies in Seminole Indians of the Big Cypress reservation, located about 50 km south of Clewiston, Florida, on the northern edge of the Everglades. This finding stimulated attempts to

isolate the virus of VEE from mosquitoes in that area. However, 210,000 mosquitoes thus far tested from the reservation, collected mainly in the early summer and fall of 1961, failed to yield this particular virus. Since the antibodies may have been a reflection of an infrequent northern extension of virus activity (*1*), collection of mosquitoes was shifted to habitats in Everglades National Park, approximately 45 to 100 km south of the reservation, where ecological niches possibly more favorable for the persistence or seasonal recurrence of VEE might be found.

The collections of mosquitoes were made three times during the active season of 1963, from 30 May to 7 June, 23 to 30 July, and 23 to 27 October. Each collection included mosquitoes from several different types of habitats over a considerable geographic area: mangrove swamps and channels, grassy everglades, hardwood and palm hammocks, pinelands, and cypress swamp. Guided by results of the earlier collections, additional mosquito sampling, in conjunction with the trapping of wild rodents, was done in selected sites from 22 through 27 January 1964.

Miniature battery-operated light traps developed at the Communicable Disease Center (*2*) were used for making the mosquito collections. Methods already described were used in mosquito handling and testing for virus (*3*). Antigen production and hemagglutination methods were essentially as described by Clarke and Casals (*4*). Complement-fixation tests were performed according to the standard methods of the Communicable Disease Center (*5*). Neutralization tests by intracerebral inoculation were conducted in weanling mice.

Approximately 135,000 mosquitoes were captured during the survey, of which about one-half have been tested in suckling mice, as shown in Table 1. Three isolations of a virus apparently identical to that of VEE have been made, all from *Culex* mosquitoes of the subgenus *Melanoconion*. Serological data supporting the identity of the prototype virus strain (FE3–7c) are given in Table 2. The other two strains reacted in a similar manner. No serological relationships were demonstrable in hemagglutination-inhibition (HI) or complement-fixation tests with strain FE3–7c and antisera for group B, group C, California, Tensaw, Cache Valley, Guaroa, Turlock, Anopheles A, Flanders, Colorado tick fever, vesicular stomatitis, lymphocytic choriomeningitis, and encephalomyocarditis viruses.

Table 1. Mosquitoes collected from the Everglades National Park and vicinity, 1963–64, and isolation of VEE virus.

Time of collection	No. collected	No. tested		No. of virus isolations
		C. (*Melanoconion*) sp.	Other species*	
30 May to 7 June	38,873	3,103	35,770	1†
23 to 30 July	62,000	680	9,883	1‡
23 to 27 October	27,000	706	11,979	1†
22 to 27 January	6,642	334	6,308	0

* These 22 species included *Aedes atlanticus, infirmatus, sollicitans, taeniorhynchus, triseriatus; Anopheles atropos, crucians, quadrimaculatus, walkeri; Culex bahamensis, nigripalpus, quinquefasciatus, salinarius; Culiseta melanura; Deinocerites cancer; Mansonia indubitans, titillans; Psorophora confinnis, howardii; Uranotaenia lowii, sapphirina; Wyeomyia mitchellii.* † From pools of 117 and 25 *C.* (*Melanoconion*) sp., respectively, Mahogany Hammock, 31 May to 1 June and 23–24 October 1963. ‡ From a pool of 29 *C.* (*Melanoconion*) sp., Royal Palm area, 25 July 1963.

Since the virus was isolated twice from mosquitoes in Mahogany Hammock, indicating it to be a particularly favorable site, live rodents were trapped there during the January 1964 period. Of 28 *Peromyscus gossypinus* (cotton mice) captured, 16 (57 percent) had a significant titer of HI antibody, ranging from 1:20 to 1:640, against a Colombian strain (38873) of VEE virus. Three of 16 *Sigmodon hispidus* (cotton rats) were also positive.

This association of VEE virus with *Culex* mosquitoes and rodents is in keeping with findings in Trinidad, Brazil, and Panama (*6*). It is not known which of several possible species of the subgenus *Melanoconion* occurring in the Everglades area were harboring the virus. Since the females of most of this group are indistinguishable morphologically, they were pooled for testing without attempting identification by species. However, of 225 males captured at Mahogany Hammock in October 1963, and identified

Table 2. Serological studies relating strain FE3-7c to Venezuelan equine encephalitis virus. (Names and numbers in parentheses relate to the collections of the Rockefeller Foundation Virus Laboratories, New York.)

Antigen of FE3-7c				Antiserum to FE3-7c			
Serum	Neut* HT† / HO	HI HT / HO	CF HT / HO	Antigen	Neut* HT / HO	HI HT / HO	CF HT / HO
FE3-7c	3.1	640	64	FE3-7c	3.1	640	64
VEE (Col. 38873)	2.5 / 3.5	640 / >2560	256 / 256	VEE (Col. 38873)	3.6 / 3.1	160 / 640	64 / 64
VEE (BeAn 8007)	>5.7 / 7.3	1280 / 1280	256 / 1024	VEE (BeAn 8007)	3.7 / 3.1	160 / 640	64 / 64
Pixuna (BeAr 35645)	2.7‡ / 5.4	20 / 160	N.D.	Pixuna (BeAr 35645)	1.6‡ / >5.5	0 / 640	8 / 64
EEE§ (N. Jersey)	0.0 / 4.8	20 / >2560	0 / 256	EEE (N. Jersey)	0.8 / 3.1	0 / 640	0 / 64
WEE‖ (Fleming)	0.1 / 3.1	0 / 2560	0 / 256	WEE (Fleming)	0.0 / >5.5	0 / 640	0 / 64
WEE (Highlands J)	0.0 / >3.0	0 / 320	0 / 128	WEE (Highlands J)	0.0 / 3.1	0 / 640	0 / 64
Mayaro (Uruma)		40 / 160	0 / 16	Mayaro (Uruma)		0 / 640	0 / 64
Chikungunya (Ross)		0 / 80	0 / 512	Chikungunya (Ross)		0 / 640	0 / 64
O'nyong-nyong (Gulu)		N.D.	0 / 256	O'nyong-nyong (Gulu)			0 / 64
Semliki (Uganda)		0 / 40	0 / 256	Semliki (Uganda)		0 / 640	0 / 64
Sindbis (AR 1055)		0 / 80	0 / 128	Sindbis (AR 1055)		0 / 640	0 / 64

* Neutralization test. † Heterologous titer/homologous titer. ‡ Neutralization test performed in suckling mice. § Eastern equine encephalitis virus. ‖ Western equine encephalitis virus.

by examination of the terminalia, 116 were *pilosus* (Dyar and Knab); 49 were *opisthopus* Komp; 2 were *atratus* Theob.; 2, were *iolambdis* Dyar; 2 were *erraticus* Dyar and Knab; and 1 was probably *mulrennani* Basham (damaged specimen).

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Venezuelan Equine Encephalitis Virus in Veracruz, Mexico, and the Use of Hamsters as Sentinels

Abstract. *Venezuelan equine encephalitis virus was recovered in the state of Veracruz, Mexico, during July and August 1963 from young, weaned hamsters, and from baby mice used as sentinel animals, and from* Culex *mosquitoes. Hamsters of 5 to 10 weeks of age became infected in nature and were nearly as susceptible as suckling mice to subcutaneous inoculation of VEE virus.*

Venezuelan equine encephalitis (VEE) virus is an arthropod-borne virus (arbovirus) pathogenic for man and horses which has hitherto been known to occur only in northern South America and Panama. As recently as 1961 and 1962, several thousand persons in the Guajira peninsula of Venezuela and Colombia and a few hundred people in Panama have been infected (*1*). The disease is characterized mainly by fever, myalgia, and respiratory and gastrointestinal symptoms, the central nervous system being affected only rarely in man. In equine epizootics, encephalitis and death have been more common manifestations than in human epidemics (*2*).

During July and August 1963, VEE virus was discovered on the tropical eastern coast of Mexico in the state of Veracruz, less than 1600 km from Texas, Louisiana, and Florida, thus establishing its presence in North America and exposing evidence of a potential danger to the health of man and certain domestic animals in Mexico and possibly in the United States.

The initial isolations of VEE virus in Mexico were from hamsters used as bait in mosquito traps. On 23 July 1963, two hamsters, 6 weeks of age, were placed in a small trap made of metal and wire screen. On 29 July, one hamster was dead and the other ill. Suspensions of pooled heart, lung, and kidney tissues from both hamsters yielded agents which upon intracranial inoculation to suckling mice killed them in 30 to 48 hours. The agent from the sick hamster (designated 63U2 virus) could be passed through a 450-mμ Millipore filter. After three passages of this virus in mice a complement-fixing antigen was obtained from the brains and this antigen reacted with VEE mouse antiserum. Antigen-antibody titers with 63U2 antigen and VEE antiserum were 1:64/1:512; the titer of this VEE antiserum against VEE virus was also 1:512. To a lesser extent, 63U2 complement-fixing antigen also reacted with mouse antiserums to the Mucambo and Pixuna viruses which are both related to VEE virus. However, the complement-fixing antigen did not react with antiserums that were specific for eastern encephalitis virus, western encephalitis virus, Oriboca virus, or a Bunyamwera group virus from Mexico.

After three passages in mice the infectivity of 63U2 virus for suckling mice inoculated intracranially was neutralized by antiserums made against VEE viruses from Trinidad, Colombia, and Panama and TC-83 vaccine; the neutralization indices ($\log_{10}$) against 63U2 (and against the homologous VEE virus) were 3.2 (>5.7), 2.0 (3.2), 2.4 (4.6), and >3.9 (not tested). That the 63U2 virus represents a new strain of VEE virus is supported by the fact that it was isolated and cultured in laboratories where VEE virus had never previously been used. The procedures used for the complement-fixation and neutralization tests are described elsewhere (*3*).

Other strains of VEE virus were also detected in 17 out of 19 additional hamsters, 5 to 10 weeks of age, which were used as sentinels during August 1963. The hamsters died or were found ill 3 to 14 days (usually 3 to 7 days) after being placed in mosquito traps or in wire cages accessible to mosquitoes. Heart, lung, and kidney tissues were removed, pooled, and frozen on dry ice in the field for subsequent examination. Each strain was successfully isolated from tissue suspensions which were injected intracranially and subcutaneously into suckling mice; the strains were identified by complement-fixation tests with mouse antiserums made from the 63U2 strain and a serum from a convalescent patient who contracted a laboratory infection with 63U2 virus. One to 2 months later each strain was again isolated by inoculation of frozen tissue suspensions into primary cultures of chicken embryo cells in fluid medium and identified by neutralization in cell cultures with rabbit antiserum to 63U2 virus. The sensitivity of hamsters to the 63U2 strain of VEE virus inoculated subcutaneously was shown in simultaneous titrations in mice and hamsters; subcutaneous LD_{50} (50 percent lethal dose) titers per 0.01 ml for the virus after the third passage in suckling mice were $10^{-9.7}$ in 1- to 3-day-old mice, $10^{-8.5}$ in hamsters 45 days of age, and $10^{-8.3}$ in hamsters 100 days of age, and after the fifth passage in suckling mice, $10^{-9.8}$, $10^{-9.0}$, and $10^{-8.5}$, respectively. This susceptibility of hamsters to 63U2 virus plus the ease with which they could be housed and cared for in cages exposed in nature over periods of days to weeks made them effective and convenient sentinels for the Mexican strains of VEE virus.

Although some of the materials collected in 1963 have not yet been tested for virus, 17 additional strains of VEE virus have been recovered in chicken embryonic cell cultures from brains of baby mice used as sentinels in the endemic area (the mice were exposed 20 to 24 hours in coarse wire-mesh cages 1 to 2 m above ground). One strain has been isolated from a suspension of 53 *Culex iolambdis* and *coronator* inoculated into suckling mice. This recovery