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ARBOVIRUS SURVEILLANCE IN FLORIDA: WILD VERTEBRATE STUDIES 1965-1974

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Abstract: Wildlife species from 38 of Florida's 67 counties were surveyed over a 10 year period for the presence of antibody to the five major arboviruses circulating in the state. The routine screening of 7891 sera from wild birds and mammals via the hemagglutination-inhibition (HI) test with selected reactors subjected to serum neutralization testing has 1) provided information regarding geographic distribution and seasonality of circulation of these viruses 2) identified enzootic foci of infection and those species of wildlife most commonly infected and 3) documented the potential value of certain wild mammals as indicators of St. Louis Encephalitis and Venezuelan equine encephalomyelitis virus activity prior to the detection of human cases. Limited studies of Tamiami and Tensaw virus on sera from mammals collected for other purposes provided additional baseline information on the activity of these viruses in Florida mammals. Isolations of eastern equine encephalomyelitis virus were made from the heart of a loggerhead shrike (*Lanius excubitor*), Tensaw virus from the brain of a gray fox (*Urocyon cinereoargenteus*), and Keystone virus from the heart of a bluejay (*Cyanocitta cristata*).

INTRODUCTION

The possibility of an epidemic of arthropod-borne encephalitis in man has concerned the Florida Division of Health (FDH) for many years. Since St. Louis encephalitis (SLE) virus epidemics in the Tampa Bay Area during 1959, 1961 and 1962 the FDH has maintained an extensive statewide arbovirus surveillance program.

The program consisted of the collection and serologic testing of serum specimens from humans, horses, dogs, domestic birds and wild vertebrates.[17] In addition mosquitoes and tissues from wild vertebrates collected from specific areas were submitted for virus isolation attempts. Data from this surveillance system has 1) helped to accurately map the geographic distribution of the five major arboviruses in the state, 2) identified enzootic foci of infection, 3) defined the seasonality of arbovirus circulation, 4) identified the species of wild and domestic vertebrates and mosquitoes commonly infected and 5) evaluated the potential of certain vertebrates as indicators of current virus activity.

This report summarizes the results of wild vertebrate studies conducted by the Veterinary Public Health Section of the FDH during the 10-year period 1965-1974. Other facets of the arbovirus surveillance program will be reported elsewhere.

MATERIALS AND METHODS

FDH maintains close liaison with physicians, veterinarians, diagnostic laboratories, county health departments and other government agencies in order to identify arbovirus infections in man and animals within the state. Laboratory confirmed cases of encephalomyelitis in humans are usually followed by an epidemi-

ologic investigation which includes collection of serum samples from wild vertebrates. These investigations are supplemented by the routine testing of mammal and bird sera obtained in connection with other zoonoses studies conducted by the FDH. In addition Dr. Frank Hayes of the Southeastern Cooperative Wildlife Disease Study. University of Georgia, Athens, graciously provided 75 white-tailed deer, *Odocoileus virginianus,* sera collected throughout Florida.

The collection, identification and processing of all wild animals followed essentially routine procedures. Birds were collected in Stoddard quail traps, mist nets, drugged with alpha-chlorolose treated bait or shot. Medium sized mammals were trapped either with number 3 steel leg-hold traps or in wire box traps. Rodents were captured in metal box traps.

Species, sex and age were recorded for every animal. A blood sample, 0.5 to 5.0 ml, was then obtained from the heart, femoral artery, jugular vein or cephalic vein. Birds collected by shooting (>300) were bled from the jugular or wing vein or the auricle of the heart at necropsy. Deer and rabbits collected by shooting were also bled by cardiac puncture at necropsy. Samples less than 2.0 ml were diluted 1:2 to 1:10 in pH 7.7 phosphate buffered saline containing penicillin sodium (1000 units/ml) and streptomycin (500 μg/ml) or in 1% bovin albumin diluent, pH 8.0. The serum samples were stored at 0 C until tested.

A micro-titer hemagglutination-inhibition (HI) test[12] was used to screen each serum sample for reactivity against all or some of the following viruses: SLE, eastern equine encephalomyelitis (EEE), western equine encephalomyelitis (WEE), Venezuelan equine encephalomyelitis (VEE) strains Fe3-7C and TC-83, California encephalitis complex (CE) strains BFS-283, LaCrosse and Keystone and Tensaw (TEN). In addition certain collections of mammal sera obtained as part VEE surveys or rabies studies were tested for antibodies to Tamiami (TAM) virus in complement fixation (CF) tests.[12] Selected serum samples with HI titers of 1:10 or greater were subjected to appropriate weanling mouse or tissue culture serum neutralization (SN) tests at the FDH central laboratory, Jacksonville, the FDH Epidemiology Research Center, Tampa, the Center for Disease Control Arbovirus Reference Laboratory, Atlanta, Georgia, the U.S. Department of Agriculture diagnostic laboratory, Ames, Iowa, or the U.S. Army Biological Warfare Center, Fort Detrick, Maryland. In mouse SN tests a log neutralization index $\geqq$ 1.7 was considered positive, while in tissue culture tests a 90% plaque reduction was positive.

Virus isolation attempts were conducted at the FDH central laboratory by intracerebral and intraperitoneal inoculation of 2 to 4 day old suckling white mice with 20% suspensions of blood clots and tissues from selected mammals and birds, and, during 1965, brains from animals submitted for rabies examination. Pooled samples of brain, heart, lung, liver and spleen were assayed from the mammals while only the heart was tested from birds. The blood clots and tissues were stored at —70 C until assayed. The physiological buffered saline described above with 25% normal rabbit serum was used to prepare the inocula. Virus isolants were identified by CF tests of crude antigen against known antisera. Crude antigen was approximately 20% suspension of brain in 0.4% bovine albumin borate saline pH 9.0.

RESULTS

Mammalian Serology

The serologic results on serum samples collected from 16 genera of mammals are presented in Table 1. Of 2596 sera tested for SLE HI antibody, 17 (0.7%) were reactive with nine sera (53%) confirming in SN tests.

Among the group A viruses, VEE reactors dominated with 108 of 2569 sera (4%) reactive in the HI test and 65 of 69 (94%) confirming in SN tests. Reactivity in the HI test to EEE virus was detected in 11 of 2596 sera (0.4%) with three of four confirming in SN tests. One

of 1564 sera (0.06%) tested by HI was reactive to WEE, however it did not confirm by SN.

All mammalian sera were not routinely tested for HI antibody against CE and TEN or CF antibody against TAM. Of 161 sera from raccoons, *Procyon lotor*, opossums, *Didelphis marsupialis*, cotton rats, *Sigmodon hispidus*, black rats, *Rattus rattus*, and marsh rabbits, *Sylvilagus palustris*, three were reactive to CE and 37 to TEN. None of these sera were tested against TAM. The reactors included 3 of 144 (2%) raccoons to CE, 35 of 144 (24%) raccoons to TEN, and 2 of 3 marsh rabbits to TEN. In addition, 139 rodent sera yielded 3 of 114 (3%) cotton rat reactors to CE plus 40 of 114 (35%) cotton rats and 4 of 27 (15%) rice rats to TAM. None of the HI or CF reactive sera were submitted for SN testing.

TABLE 1. Summary of wild mammal sera tested for arbovirus antibody during statewide survey of Florida 1965-1974.

Species	SLE	VEE	EEE	WEE
Didelphis marsupialis	2/199[1] (2/2)[2]	1/184 (1/1)	1/199 (1/1)	0/159
Dasypus novemcinctus	1/11 (1/1)	2/11 (2/2)	0/11	0/6
Sylvilagus spp.	0/46	0/43	0/46	0/46
Peromyscus gossypinus	1/248 (1/1)	34/248 (23/24)	0/248	0/179
Sigmodon hispidus	7/578 (0/7)	14/578 (13/13)	1/578 (0/1)	1/497 (0/1)
Oryzomys palustris	1/172 (0/1)	4/172 (3/3)	0/172	0/124
Rattus spp.	0/106	9/98 (3/3)	0/106	0/75
Mus musculus	0/108	0/107	0/108	0/103
Procyon lotor	5/917 (5/5)	39/917 (15/18)	1/917	0/196
Urocyon cinereoargenteus	0/13	0/13	5/13	0/1
Mephitis mephitis	0/15	0/15	1/15	0/12
Odocoileus virginianus	0/143	5/143 (5/5)	1/143 (1/1)	0/126
Other[3]	0/40	0/40	1/40 (1/1)	0/40
TOTAL	17/2596 (9/17)	108/2569 (65/69)	11/2596 (3/4)	1/1564 (0/1)

[1] Number of HI reactors/total tested
[2] Number of SN confirmations/total tested
[3] Includes *Spilogale ambervalis, Neotoma floridana, Lynx rufus* and *Sus scrofa.*

Avian Serology

The HI and SN test results on serum samples obtained from 25 families of wild birds are given in Table 2. Of 5295 sera tested for SLE antibody 25 (0.5%) were reactive with six of those confirming in SN tests.

Among the group A arboviruses neither of the 2 of 4774 sera reactive for VEE in the HI test confirmed in SN tests. Of 606 sera tested for WEE HI antibody, 11 (1.8%) were reactive. However, all seven of the HI reactive sera selected for SN tests failed to confirm. HI reactivity to EEE was detected in 70 of 5295 sera (1.3%) with 18 of 36 (50%) confirming in SN tests.

Virus Isolation Attempts

Between 1965 and 1967, a total of 333 isolation attempts (156 from mammals, 177 from birds) yielded three viruses. In January 1965 TEN was isolated from the brain of a gray fox, *Urocyon cinereoargenteus,* from Marion County submitted for rabies examination. Two other isolants were from the hearts of birds collected in 1966. The first was EEE from a loggerhead shrike, *Lanius excubitor,* in Orange County in June and the second was CE (Keystone serotype) from a blue jay, *Cyanocitta cristata,* in Alachua County in November. The latter was shot during an investigation of EEE in pheasants. Precipitin tests confirmed that the CE isolant was from a passerine bird and not a laboratory contaminant.[1]

[1] Edman, J. O. Florida Medical Entomology Laboratory, Vero Beach, Florida. Personal communication.

TABLE 2. Summary of wild bird sera tested for arbovirus antibody during statewide survey of Florida 1965-1974.

Family	SLE	VEE	EEE	WEE
Pelicanidae (Pelicans)	1/3[1] (0/1)[2]	0/3	0/3	—
Cathartidae (Vultures)	0/3	0/3	0/3	—
Phasianidae (Quail)	0/6	0/4	0/6	0/3
Ardeidae (Herons)	1/187 (0/1)	0/92	4/187 (4/4)	1/109
Scolopacidae (Woodcock)	0/1	0/1	1/1 (1/1)	0/1
Rynchopidae (Skimmers)	0/9	0/9	0/9	—
Columbidae (Doves)	10/3637 (5/10)	0/3616	4/3637	0/21
Cuculidae (Anis)	0/10	0/10	0/10	—
Strigidae (Owls)	0/5	0/3	1/5 (1/1)	0/2
Caprimulgidae (Goat suckers)	1/6 (1/1)	0/5	0/6	0/1
Picidae (Woodpeckers)	1/51 (0/1)	0/24	2/51	1/27
Tyrannidae (Flycatchers)	0/20	0/8	0/20	0/12

[1] Number of HI reactors/total tested
[2] Number of SN confirmations/total tested

TABLE 2 — Continued

Family	SLE	VEE	EEE	WEE
Hirundinidae (Swallows)	0/8	0/8	0/8	—
Corvidae (Jays)	2/102 (0/2)	0/60	15/102 (6/10)	2/42 (0/2)
Paridae (Titmice)	0/9	0/5	0/9	0/4
Troglodytidae (Wrens)	0/29	0/19	0/29	0/10
Mimidae (Mockingbirds)	4/236 (0/4)	1/152 (0/1)	24/236 (1/11)	4/94 (0/2)
Turdidae (Robins)	0/37	0/8	0/37	0/29
Sylviidae (Kinglets)	0/2	0/1	0/2	0/2
Laniidae (Shrikes)	0/11	0/3	2/11 (1/1)	0/8
Vireonidae (Vireos)	0/29	0/21	0/29	0/6
Parulidae (Warblers)	0/19	0/16	0/19	0/3
Plocidae (Weaver finches)	2/223 (0/2)	1/223 (0/1)	3/223 (0/1)	3/22 (0/3)
Icteridae (Blackbirds)	2/324 (0/2)	0/237	0/324	0/95
Fringillidae (Sparrows)	1/328 (0/1)	0/243	14/328 (4/7)	0/115
Total	25/5295 (6/25)	2/4774 (0/2)	70/5295 (18/36)	11/606 (0/7)

DISCUSSION

During the three epidemics of SLE in the Tampa Bay area in 1959, 1961 and 1962 there were 315 clinical and confirmed cases with 55 deaths. Human cases were not observed again until the fall of 1969 when three cases were reported in Polk county.[1] No human cases were reported from 1970 through 1974.

Prior to the detection of three human cases of SLE in the fall of 1969, evidence of SLE activity was noted in several collections of wildlife sera. HI and SN antibodies to SLE were detected in 1) a cotton mouse, *Peromyscus gossypinus,* and two opossums collected from Dade county during a VEE investigation in October and November 1968, 2) five raccoons collected from Manatee county during the investigation of a rabies epizootic in March and April 1969,[5] 3) a chuck-will's-widow, *Caprimulgus carolinensis,* from Dade county collected in July 1969, and 4) a mourning dove, *Zenaidura macroura,* collected from a flock maintained in Orange county for SLE surveillance in September 1969. (Fig. 1). HI and SN antibodies to SLE were also detected in horses and domestic fowl[2] and military guard dogs[18] from Dade county in late 1968 and early 1969. These data were confirmed by other studies in Highlands county (Central

[2] Nichols, J. B. 540 67th St., Holmes Beach, Florida. Unpublished observations.

Ridge) and Sarasota and Hillsborough counties (Tampa Bay area) where HI and SN antibodies to SLE were found in raccoons and opossums collected in 1968 and 1969.[3] SLE virus was also isolated from the blood of a raccoon trapped in Hillsborough county in October, 1969.[22] These findings plus those from Texas[20] support the contention that certain species of wildlife can serve as indicators of SLE activity. SLE HI-positive sera from rodents collected from transects across south Florida were not confirmed in SN tests. These reactions may represent serologic crossing with Cowbone Ridge virus[7] or another group B agent.

The endemic Everglades strain of VEE virus has rarely been associated with clinical illness in humans.[4,9] However, serological surveys of selected human populations in the Everglades and Big Cypress regions indicate that subclinical infections may be common.[21,23] Until March of 1967 when HI antibodies were detected in a rice rat from Florida City[8] it was commonly believed that VEE virus activity was restricted to the Everglades National Park. The first naturally occurring human

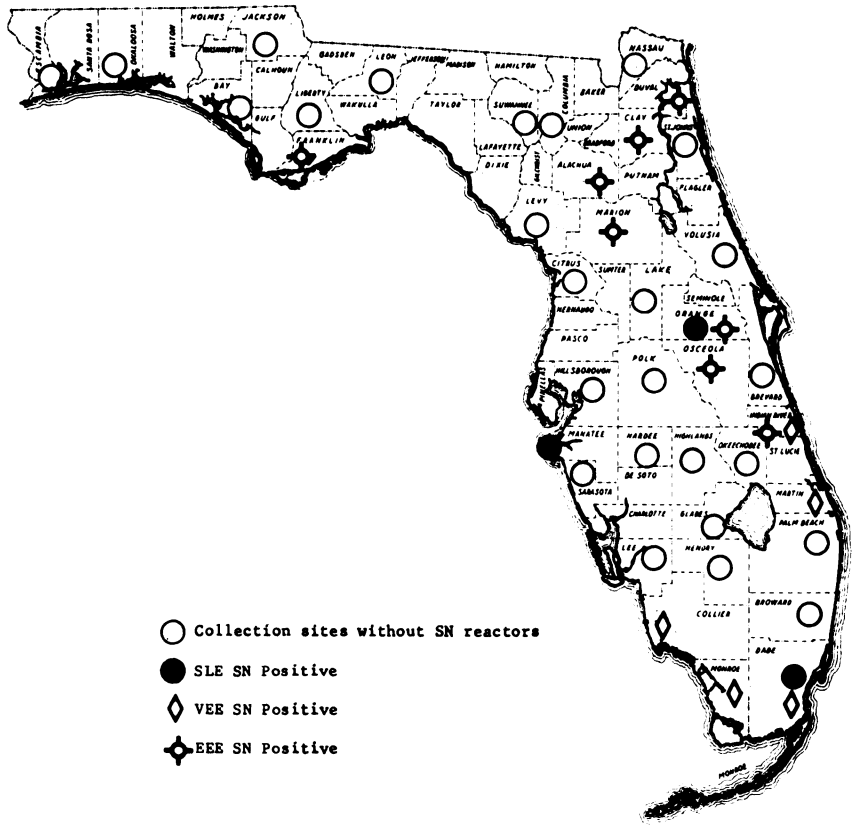

FIGURE 1. Counties sampled and results of serum neutralization (SN) tests.

[3] Wellings, F. M. Florida Division of Health, Tampa, Florida. Unpublished observations.

case of VEE originated in Florida City in October 1968. Since then VEE HI and SN antibodies have been found in eight species of mammals. The virus ranges from Collier County on the gulf coast to Indian River County on the central east coast (Fig. 1). Cotton mice and rats have already been successfully utilized to detect fluctuations in VEE activity in endemic areas.[6]

In Florida EEE has been isolated from mosquitoes, horses, humans[4] or wild birds every month of the year. Human cases generally occurred between June and September in areas where field studies of mosquitoes, domestic fowl, egrets and horses have documented EEE activity.[2,10,17] During the present study EEE HI and SN antibodies have been detected in up to 34% of the wild bird populations sampled following human infections. EEE antibodies were not commonly found in samples from Florida wild mammal populations. EEE virus and antibodies have only been found in animals trapped along the gulf coast. It would be interesting if the annual introduction of strains of EEE from other areas via migrant birds could be related to the infections of coastal mammals.[8,16]

No naturally occurring human cases of WEE have been recognized in Florida. The lack of WEE HI antibodies in wild birds and mammals also suggests that the virus is not widely distributed in the state. However, several investigators have recovered WEE virus from wild birds, commercial game birds, a horse, sentinel chickens and mice and mosquitoes collected from the north-central portion of Peninsular Florida.[13,14,22]

Sporadic cases of CE in children have been documented in Florida.[11] The present study and serologic surveys elsewhere have shown mammals and not birds to be involved in the maintenance cycle of CE, although certain serotypes of the virus can produce viremia in chickens.[19] Consequently, the isolation of CE from the blue jay was of great interest. In a follow-up study CE SN antibodies were not detected in 18 other birds, however were found in one of four gray squirrels, *Sciurus carolinensis* collected on the same farm. Twenty blue jays that were subsequently inoculated subcutaneously with 43 or 430 mouse lethal $dose_{50}$ of the isolant failed to develop detectable viremia or neutralization antibodies. At present, it is not known if wild birds are important in the dissemination of certain serotypes of CE or if the isolation from the blue jay was a fortuitous circumstance.

Since neither TAM or TEN appear to have public health significance in Florida at the present time, studies of these viruses have been limited.[15] Data from the present study indicate that TAM virus may be active in rodents from many areas of Florida. These and other studies[22] document TEN virus activity in several species of Florida mosquitoes and wild mammals including carnivores, rodents and lagomorphs.

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