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ARBOVIRUSES ASSOCIATED WITH MOSQUITOES FROM NINE FLORIDA COUNTIES DURING 1993

C. J. MITCHELL,[1,3] C. D. MORRIS,[2] G. C. SMITH,[1] N. KARABATSOS,[1]
D. VANLANDINGHAM[1] AND E. CODY[2]

ABSTRACT. Mosquitoes were collected for virus isolation tests from 36 sites in Bradford, Lake, Leon, Manatee, Orange, Osceola, Pasco, Putnam, and Sarasota counties, FL, from April 6 through October 11, 1993. A total of 158,129 adult specimens were collected in 726 trap nights using CDC light traps, usually baited with dry ice. At least 35 species were represented, although 60% of the collections was made up of 3 species (*Aedes infirmatus,* 6.5%; *Anopheles crucians,* 14.4%; and *Culex nigripalpus,* 39.4%). Four of the 36 collecting sites were located at waste-tire sites, where 254 trap nights yielded 27,455 specimens (17.4% of 9-county total). Forty-three virus strains were isolated from 2,812 mosquito pools consisting of 158,129 specimens. The viruses isolated include eastern equine encephalitis (EEE), 5 strains; Everglades (EVE), 2 strains; Keystone (KEY), 6 strains; Tensaw (TEN), 21 strains; trivittatus (TVT), one strain; Shark River (SR), one strain; and Flanders (FLA), one strain. In addition, 2 strains that are either KEY or Jamestown Canyon (JC) virus, and 4 ungrouped viruses remain to be identified. Twenty-one (48.8%) of the 43 virus strains were isolated from mosquitoes collected at waste-tire sites as follows: EEE (1), KEY (5), KEY/JC (1), TEN (13), and one ungrouped virus. The vector relations of the viruses are discussed and the potential importance of waste-tire sites as breeding habitats and harborages for vector and nuisance species is emphasized.

INTRODUCTION

The isolation of eastern equine encephalitis (EEE) virus from *Aedes albopictus* (Skuse) collected at a waste-tire site in Polk County, FL, during June 1991 focused attention on the potential adverse impact such sites could have on public and veterinary health in Florida through the production of vector mosquitoes (Mitchell et al. 1992). Strong lobbying by state and local vector control interests prompted the Florida legislature to pass HB 459-H in 1992. This bill stipulated that at least 10% of funds from the waste-tire fee, allocated to the Solid Waste Management Trust Fund, be reallocated for the specific purpose of abating and providing mosquito control relating to waste-tire sites, other tire piles, and other waste debris sites identified by local mosquito control agencies as mosquito-breeding areas. In addition, the 1992 Florida legislature added a provision to HB 459-H to allocate $400,000 to the Department of Agriculture and Consumer Science to fund research on mosquitoes and their control at waste-tire sites during calendar year 1993. The latter funds were used to support 9 mosquito research projects during the year, including a major portion of our own.

The main purposes of our project were to evaluate the role that mosquitoes that breed in waste-tire piles play in the ecology of arboviruses, especially EEE and St. Louis encephalitis (SLE) viruses, in Florida, and to provide a service to mosquito control agencies interested in having mosquitoes from arbovirus foci tested for the presence of viruses.

MATERIALS AND METHODS

Mosquitoes were collected using Centers for Disease Control (CDC) miniature light traps, usually baited with dry ice. A collection consisted of specimens from 1 to 10 traps operated in the same general area on the same date. Collections were made by cooperators who worked for local mosquito control agencies. Mosquito populations were sampled approximately every 2 wk and samples were shipped on dry ice overnight to the Florida Medical Entomology Laboratory in Vero Beach. The sex of mosquitoes was determined and males were discarded. Females were identified to species or species group, classified as non-blooded, blooded, or gravid, pooled in lots of 100 or less, and placed in shell vials that were stoppered, labeled, and stored on dry ice. Each week, accumulated pools were shipped overnight on dry ice to the CDC laboratory in Fort Collins, CO, for further processing and testing.

Mosquito pools were triturated in 2 ml of BA-1 diluent (1× M199 containing Hanks balanced salt solution, 0.05 *M* Tris, pH 7.6, 1% bovine serum albumin, 0.35 g/liter sodium bicarbonate, 100 units/ml penicillin, 100 μg/ml streptomycin, 1 μg/ml amphotericin B, and 10 mg/liter phenol red) using cold mortars and pestles. Suspensions

[1] Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, P. O. Box 2087, Fort Collins, CO 80522.

[2] Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida, 200 9th Street SE, Vero Beach, FL 32962-4657.

[3] Author to whom correspondence and reprint requests should be addressed.

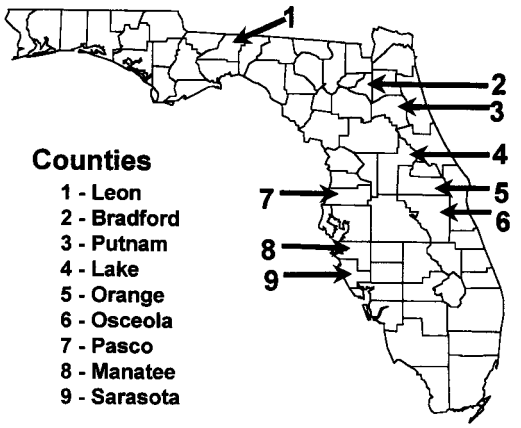

Fig. 1. Outline map of Florida counties identifying the 9 counties where mosquito collections were made during 1993.

were centrifuged in Eppendorf tubes at 14,000 rpm for 2 min. Supernatants were poured into 1-dram screw-cap vials and stored at −70°C until tested.

Specimens were tested for virus in Vero cell culture grown in 6-well plates. Specimens were inoculated in 0.1-ml quantities into 2 wells each, adsorbed for 1 h at 37°C, then the cells were overlayed with 1% Noble agar in M199 + 2% fetal bovine serum (FBS), 2.0 g $NaHCO_3$, 150 μg/ml of diethylaminoethanol–dextran and 1: 40,000 neutral red plus 100 units/ml penicillin, 100 μg/ml streptomycin, 250 μg gentamicin/ml, and 4.5 μg amphotericin B/ml. Cell cultures were incubated at 37°C and examined for 10 days for plaques.

Virus-positive cell cultures were harvested in 2 ml of BA-1 and frozen at −70°C until passed into fluid cultures of Vero cells in 25-cm^2 flasks. When early cytopathic effects were noted, infected cells were scraped from the surface of the flask or removed by trypsinization and resuspended in phosphate-buffered saline, pH 7.4, containing 5% FBS. Twelve-well spot slides were prepared, air-dried, and fixed in cold acetone. These were tested in an indirect fluorescent antibody, assay (Wulff and Lange 1975) against a battery of National Institutes of Health- and CDC-hyperimmune ascitic grouping fluids. Usually, viral type-specific monoclonal antibodies against common or suspected viruses also were included in the test to definitively identify isolates at this stage. Otherwise, antigenically grouped viral isolates were tested by neutralization (N) assay in Vero cell culture against reference polyclonal immune reagents prepared against specific members of the antigenic group. Homologous N titers were predetermined for reference reagents used in the identifying N tests.

In addition, antibody was prepared to the Shark River (SR) isolate. The SR virus was ultimately identified by cross-neutralization tests including all members of the Patois complex of bunyaviruses.

RESULTS

A total of 125 collections consisting of 726 trap nights were made from 36 sites in Bradford, Lake, Leon, Manatee, Orange, Osceola, Pasco, Putnam, and Sarasota counties from April 6 to October 11, 1993 (Fig. 1 and Table 1). Eighty-eight percent of the 158,129 mosquitoes collected came from 4 counties: Pasco, 13.0%; Manatee, 15.5%; Orange, 19.0%; and Sarasota, 40.9% (Table 2). At least 35 species were represented, although 60% of the collections was made up of 3 species (*Aedes infirmatus* Dyar and Knab, 6.5%; *Anopheles crucians* Wiedemann, 14.4%; and *Culex nigripalpus* Theobald, 39.4%). An ad-

Table 1. CDC light trap mosquito collections by county, Florida, 1993.

County	Inclusive dates	No. of sites	No. of collections	No. of trap nights	Total mosquitoes
Bradford	June 24–July 10	1	4	35	1,159
Lake	April 6–7	3	5	5	6,856
Leon	May 27–Oct. 10	7[1]	36	180	7,216
Manatee	June 5–Sept. 19	2	6	26	24,517
Orange	May 19–Oct. 5	5[1]	17	159	29,979
Osceola	Sept. 20–21	1	3	11	1,946
Pasco	May 26–Sept. 30	3[1]	17	145	20,546
Putnam	June 29–July 8	5	6	25	1,229
Sarasota	June 4–Oct. 11	9	31	140	64,681
Totals	April 6–Oct. 11	36	125	726	158,129

[1] Includes 2 waste-tire sites in Leon County and one each in Orange and Pasco counties.

ditional 8% of the specimens was identified only to the genus *Culex*.

Four of the 36 collecting sites in the 9 counties were located at waste-tire sites in Leon, Orange, and Pasco counties (Table 1). Thirty collections made at the 4 sites between May 26 and October 2, 1993, consisted of 254 trap nights and yielded 27,455 mosquitoes (17.4% of 9-county total). All *Aedes aegypti* (Linn.) collected during this study (414 specimens) came from the waste-tire sites. Collections at the waste-tire sites also accounted for 78.6% of *Ae. albopictus,* 52.2% of *Aedes triseriatus* (Say), 9.7% of *Culiseta melanura* (Coquillett), and 58.0% of *Coquillettidia perturbans* (Walker) collected during the study. Otherwise, with the exceptions of *Aedes fulvus pallens* Ross, *Anopheles punctipennis* (Say), *Culex restuans* Theobald, *Orthopodomyia signifera* (Coquillett), *Psorophora howardii* Coquillett, *Uranotaenia lowii* Theobald, and species of *Wyeomyia,* all species collected elsewhere (Table 2) also were represented in the collections from the waste-tire sites.

Forty-three virus strains were isolated from 2,812 mosquito pools consisting of 158,129 specimens. Twenty-one (48.8%) of the 43 virus strains were isolated from mosquitoes collected at waste-tire sites. These results, along with minimum infection rates (MIRs) calculated by site and month of collection, are summarized in Table 3. The majority (86.0%) of the 43 isolates came from 4 counties: Manatee, 9.3%; Sarasota, 14.0%; Orange, 23.3%; and Pasco, 39.5% (Table 4). Virus was not isolated from the relatively small numbers of specimens tested from Osceola (1,946) and Putnam (1,229) counties.

The 43 virus strains isolated consist of 2 alphaviruses (EEE and Everglades [EVE]), 4 bunyaviruses (Keystone [KEY], Tensaw [TEN], trivitattus [TVT], and SR virus), and one virus in the family Rhabdoviridae (Flanders [FLA]). In addition, 2 bunyavirus isolates reacted with both KEY and Jamestown Canyon (JC) antibodies and have not been definitively identified. Four other viral strains currently are ungrouped. Almost one-half of the isolates (21/43) are TEN virus, and most of these (90.5%) came from *An. crucians* (Table 5). A significant percentage (18.6%) of the virus strains was isolated from mixed pools of *Aedes* species. Five strains of EEE virus were isolated, 3 from *Cs. melanura,* and one each from *Culex erraticus* (Dyar and Knab) and *Culex* species (Table 5). No flavivirus was isolated from the collections. Viruses isolated from mosquitoes collected at waste-tire sites included strains of EEE (1), KEY (5), KEY/JC (1), TEN (13), and one ungrouped virus (Table 3).

DISCUSSION

All viruses isolated by us have been reported from Florida previously (Karabatsos 1985). Among these, only EEE and EVE viruses are of public health or veterinary importance. Five strains of EEE virus were isolated from mosquitoes collected in Lake, Orange, and Pasco counties. Three of the strains came from the enzootic vector, *Cs. melanura,* and MIRs calculated by month and by site ranged from 4.0 to 38.5 per 1,000 in this species. These compare with monthly MIRs of 0.2 to 2.4 in the Tampa Bay area during 1963–70 (Wellings et al. 1972). Reasons for these differences are not apparent. In general, EEE virus activity in Florida during 1993 was not unusual as indicated by human and equine cases of EEE and seroconversions in sentinel avians. Among 5 human cases of EEE confirmed in the United States during 1993, one came from Holmes County in the Florida panhandle, well outside of our collection area (Centers for Disease Control 1994). Within the 9 counties where mosquito collections were made, 20 equine cases of EEE were reported as follows: Lake (4), Leon (4), Orange (3), Osceola (2), Pasco (5), and Putnam (2).

Two strains of EVE virus were isolated from *Aedes* species collected in Sarasota and Manatee counties. Everglades virus is a subtype of Venezuelan equine encephalitis (VEE) virus and has been reported only from Florida (Calisher and Karabatsos 1988). The EVE virus is maintained in a mosquito/rodent cycle and usually is associated with *Culex* (*Melanoconion*) mosquitoes in hardwood hammocks surrounded by sawgrass and shallow fresh water (Chamberlain et al. 1964, 1969). Minimum infection rates of 6.3 and 30.3 observed in *Aedes* species in our study compare favorably with MIRs of 5–20 reported for *Culex* (*Melanoconion*) species (Chamberlain et al. 1969). Previous isolations of EVE virus in southern Florida from *Aedes atlanticus/tormentor, Aedes taeniorhynchus* (Wiedemann), *An. crucians,* and *Cx. nigripalpus,* as well as from *Culex* (*Melanoconion*) species (Sudia et al. 1969), and experimental transmission of EVE virus by *Ae. triseriatus* and *Ae. taeniorhynchus* (Karabatsos 1985) suggest that several mosquito species may become involved in the transmission cycle.

Shark River virus, isolated from *Cx.* (*Mel.*) *opisthopus* (Komp), collected in Manatee County, often is found in the same habitat as EVE virus. In fact, the prototype strains of both viruses came from Mahogany Hammock, Everglades National Park, FL (Chamberlain et al. 1964, Fields et al. 1969). Shark River virus is cycled mainly between *Culex* (*Melaniconion*)

Table 2. Mosquitoes collected in 9 Florida counties during April–October, 1993, and tested for the presence of virus.

Species	Number collected	Percentage of total by county				
		Bradford	Lake	Leon	Manatee	Orange
Ae. aegypti	414					93.0
Ae. albopictus	3,341			26.8	<0.1	53.6
Ae. atlanticus/tormentor	1,073	11.8	5.8	1.8	2.6	6.5
Ae. dupreei	54			100.0		
Ae. fulvus pallens	123			100.0		
Ae. infirmatus	10,342	1.3	10.3	4.8	8.4	5.8
Ae. mitchellae	34	5.9				14.7
Ae. sollicitans	3					
Ae. taeniorhynchus	3,360				99.5	0.1
Ae. triseriatus	182	0.5		33.0	0.5	14.8
Ae. vexans	710	0.1	5.4	49.9		42.7
Ae. sp.	786	0.8		15.5		83.7
An. crucians	22,692	2.8	5.8	7.1	2.1	42.8
An. punctipennis	2			100.0		
An. quadrimaculatus	979			21.8	4.4	56.4
An. sp.	19			100.0		
Cq. perturbans	6,236	1.0	0.7	8.4	2.2	18.8
Cx. erraticus	5,127	0.1	0.3	21.3	8.2	16.0
Cx. nigripalpus	62,332	0.1		1.5	22.7	8.0
Cx. opisthopus	9,167				3.4	
Cx. pilosus	21					28.6
Cx. quinquefasciatus	20			5.0	5.0	40.0
Cx. restuans	500	100.0				
Cx. salinarius	1,298	0.4		2.8	19.6	38.9
Cx. sp.	12,714		19.4	0.4	25.3	41.7
Cx. melanura	2,355	4.5	56.9	8.7	0.3	12.8
Ma. dyari	1,156				35.7	9.4
Ma. titillans	5,696		<0.1		6.6	33.6
Or. signifera	1					
Ps. ciliata	147	3.4			2.7	17.7
Ps. columbiae	5,263		0.2	0.1	8.0	11.8
Ps. ferox	1,315	0.5	0.5	19.0	1.3	
Ps. howardii	6			16.7	16.7	33.3
Ps. sp.	3					
Ur. lowii	254			0.8		7.5
Ur. sapphirina	384	1.8		32.6		8.9
Wy. mitchellae	9					
Wy. vanduzeei	1					
Wy. sp.	10				10.0	90.0
Totals	158,129	1,159	6,856	7,216	24,517	29,979
% of total		0.7	4.3	4.6	15.5	19.0

mosquitoes and rodents, and, in addition to the southeastern USA, has been found in Mexico and Guatemala (Scherer et al. 1972).

Two other bunyaviruses isolated in this study, TVT and KEY, are cycled between mammalian hosts and *Aedes* mosquitoes. In Florida, TVT virus has been most frequently associated with *Ae. infirmatus* and KEY virus with *Aedes atlanticus* (Dyar and Knab) (Taylor et al. 1971). Both viruses have been recovered elsewhere from naturally infected mosquito larvae; TVT from *Ae. trivittatus* (Andrews et al. 1977), and KEY from *Ae. atlanticus* (Le Duc et al. 1975). Experimental studies support these findings and emphasize the potential importance of vertical transmission in maintaining endemic foci (Tesh 1984). Our results, which confirm the involvement of *Aedes* mosquitoes in these transmission cycles, include a single isolate of KEY virus from *Ae. albopictus* collected in Orange County (Table 3). Pre-

Table 2. Extended.

Percentage of total by county				Percentage by species
Osceola	Pasco	Putnam	Sarasota	
	6.8		0.2	0.3
	18.1	0.7	0.7	2.1
0.6	27.4	1.5	42.0	0.7
				<0.1
				<0.1
1.0	19.8	1.8	46.8	6.5
35.3	29.4	14.7		<0.1
	100.0			<0.1
	<0.1		0.3	2.1
	36.3	2.2	12.6	0.1
	1.5	0.4		0.4
				0.5
1.2	31.4	1.2	5.5	14.4
				<0.1
1.3	7.6	1.9	6.6	0.6
				<0.1
7.0	54.0	3.8	4.1	3.9
1.0	16.9	1.1	35.1	3.2
0.7	1.5	0.3	65.1	39.4
			96.6	5.8
33.3	38.1			<0.1
	35.0	5.0	10.0	<0.1
				0.3
3.5	8.4	8.9	17.6	0.8
	3.6		9.6	8.0
	3.1	2.0	11.8	1.5
8.0	0.1		46.8	0.7
2.3	38.9	0.1	18.5	3.6
100.0				<0.1
	68.7	2.0	5.4	0.1
6.1	39.3	1.3	33.3	3.3
0.2	1.4	0.4	76.6	0.8
			33.3	<0.1
100.0				<0.1
0.4			91.3	0.2
	11.7		45.1	0.2
			100.0	<0.1
			100.0	<0.1
				<0.1
1,946	20,546	1,229	64,681	
1.2	13.0	0.8	40.9	

viously, KEY virus also was isolated from this species collected in adjacent Polk County (Mitchell et al. 1992). Although Tesh (1980) demonstrated vertical transmission of KEY virus by *Ae. albopictus* in the laboratory, other experimental studies (Grimstad et al. 1989) suggest that some strains of *Ae. albopictus* are unlikely to transmit KEY virus at significant levels, if at all. The Florida results may represent strain differences in mosquito or virus, or may simply reflect the mammalophilic feeding habits of *Ae. albopictus* (Savage et al. 1993, Niebylski et al. 1994).

The TEN virus was isolated principally from *An. crucians* (19/21 isolates) (Table 3). These results agree with those of previous studies conducted in Florida (Wellings et al. 1972) and elsewhere in the southeastern United States (Calisher et al. 1986). The TEN virus is cycled mainly between *Anopheles* mosquitoes and mammalian hosts. It has not been shown to be a human pathogen despite one dubious report of a single human illness in Indiana attributed to a TEN virus infection (McGowan et al. 1973) based on serologic testing of a single serum sample (C. H. Calisher, unpublished data). Indiana is well outside the range of established TEN virus activity (Calisher et al. 1986), and a history of travel by the suspect case to virus-endemic areas was lacking.

A single strain of FLA virus was isolated from *Cs. melanura* collected in Sarasota County. Flanders is a rhabdovirus, originally isolated from *Cs. melanura* collected in Flanders, NY (Whitney 1964). It has been isolated from *Cx. nigripalpus* collected in southern Florida (Boyd 1972), elsewhere in the United States, and Canada (Karabatsos 1985).

The absence of SLE-virus-infected mosquitoes in our collections is noteworthy because we tested 62,332 specimens of *Cx. nigripalpus,* the main epidemic vector of this virus in Florida. This mosquito made up 39.4% of our total collection. Saint Louis encephalitis virus activity as measured by seroconversions in sentinel chickens was higher than normal in Florida in 1993. Data from the Tampa Branch Laboratory, Florida Department of Health and Rehabilitative Services (L. M. Stark, unpublished data) indicate that 252 of 1,821 (13.8%) sentinel chickens seroconverted during 1993. Because 88% of the *Cx. nigripalpus* tested in our study came from Manatee and Sarasota counties, it is worth noting that 6 of 9 SLE sentinel seroconversions in Manatee County and 11 of 16 in Sarasota occurred after our last mosquito collections were made in these counties on September 19 and October 11, respectively. Therefore, the absence of SLE-infected *Cx. nigripalpus* in our study is probably due to the cessation of mosquito collecting before the peak season of SLE virus activity in southern and central Florida. Statewide, only 5 SLE cases were reported from Florida during 1993: 2 cases from Lee County and 3 from Collier County (Centers for Disease Control and Prevention 1994).

Our results emphasize the importance of waste-tire sites as mosquito-producing areas and as harborages for adult mosquitoes. Although

Table 3. Virus isolations from Florida mosquitoes during 1993 and minimum infection rates (MIR) by site and month of collection.

Species	Virus	Collection date	County	County site no.	Strain (FL93-)	MIR[1]
Ae. albopictus	KEY[2]	Aug. 4	Orange	1	2972	1.3
Ae. atlanticus/tormentor	KEY/JC[2,3]	Sept. 27	Pasco	1	4551	22.7
	TEN[2]	Sept. 28	Pasco	1	4664	22.7
Ae. atlanticus/infirmatus	KEY[2]	May 20	Orange	1	1198	22.2
Ae. sp.	KEY[2]	June 23	Pasco	1	2260	1.2
	KEY	June 27	Sarasota	8	1885	3.6
	KEY[2]	Aug. 2	Pasco	1	3519	1.8
	KEY[2]	Aug. 4	Orange	1	2990	27.8
	EVE	July 9	Sarasota	6	2473	30.3
	EVE	July 24	Manatee	2	2214	6.3
	TVT	July 24	Manatee	2	2226	6.3
	TEN[2]	Sept. 27	Pasco	1	4545	11.8
An. crucians	TEN	May 20	Orange	2	1228	0.8
	TEN	May 20	Orange	2	1233	
	TEN	May 20	Orange	2	1259	
	TEN[2]	May 28	Pasco	1	1295	1.1
	TEN[2]	May 28	Pasco	1	1300	
	TEN[2]	June 4	Orange	1	1474	21.7
	TEN	June 8	Leon	3	1564	3.1
	TEN[2]	June 9	Pasco	1	1596	2.3
	TEN[2]	June 9	Pasco	1	1597	
	TEN[2]	June 9	Pasco	1	1602	
	TEN[2]	June 9	Pasco	1	1605	
	TEN	June 22	Orange	3	1772	0.4
	TEN	June 26	Bradford	1	2015	5.3
	TEN	June 26	Bradford	1	2016	
	TEN	July 10	Bradford	1	2030	3.9
	TEN[2]	Sept. 27	Pasco	1	4533	3.2
	TEN[2]	Sept. 27	Pasco	1	4535	
	TEN[2]	Sept. 28	Pasco	1	4645	
	TEN[2]	Sept. 28	Pasco	1	4648	
Cx. erraticus	EEE	June 9	Pasco	2	1637	12.8
	Ungrouped	June 20	Sarasota	4	1730	23.8
	Ungrouped[2]	Aug. 2	Pasco	1	3599	2.8
Cx. nigripalpus	KEY/JC[3]	Aug. 7	Sarasota	7	3984	0.7
	Ungrouped	Aug. 20	Sarasota	9	4238	0.5
Cx. opisthopus	Ungrouped	Aug. 7	Manatee	2	4080	7.0
	SR	Sept. 18	Manatee	2	5083	13.3
Cx. (*Cux*) spp.	EEE	April 7	Lake	1	939	0.5
Cx. melanura	EEE	April 7	Lake	2	969	4.0
	EEE[2]	June 4	Orange	1	1476	38.5
	EEE	June 22	Orange	3	1814	8.8
	FLA	June 6	Sarasota	1	1454	21.7

[1] MIR = Minimum infection rate per 1,000 tested from site during month indicated.
[2] Isolated from mosquitoes collected at waste-tire sites.
[3] Specific identification pending.

Table 4. Distribution by county of viruses isolated from Florida mosquitoes during 1993.

Virus	Bradford	Lake	Leon	Manatee	Orange	Pasco	Sarasota	Total
EEE		2			2	1		5
EVE				1			1	2
KEY					3	2	1	6
KEY/JC						1	1	2
TEN	3		1		5	12		21
TVT				1				1
SR				1				1
FLA							1	1
Ungrouped				1		1	2	4
Totals	3	2	1	4	10	17	6	43

only 4 of 36 collecting sites were waste-tire sites, and only 17% of the total mosquitoes collected came from these sites, almost one-half (48.8%) of the 43 virus strains were isolated from mosquitoes collected at waste-tire sites. These sites accounted for all of the *Ae. aegypti* collected during the study, and for high percentages of *Ae. albopictus* (78.6%) and *Ae. triseriatus* (52.2%). Waste-tire sites also accounted for significant proportions of enzootic (*Cs. melanura,* 9.7%) and epidemic/epizootic (*Cq. perturbans* 58.0%) vectors of EEE virus, indicating their proximity to suitable breeding habitats for these species. One isolate of EEE virus came from *Cs. melanura* collected at a waste-tire site in Orange County. Collectively, these results justify concerns about the public and veterinary health threats posed by mosquitoes associated with waste-tire sites, especially when the sites are located in or near enzootic EEE foci. These threats, coupled with the production of nuisance mosquitoes at waste-tire sites, confirm the wisdom of the Florida legislature's decision to allocate funds for mosquito control in waste-tire sites.

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Table 5. Distribution of virus isolations in Florida during 1993 by mosquito species.

	Mosquito species[1]										Total
Virus	ALB	ATI	ATT	ASP	CRU	ERR	NIG	OPI	CXS	MEL	Isolates
EEE						1			1	3	5
EVE				2							2
KEY	1	1		4							6
KEY/JC			1				1				2
TEN			1	1	19						21
TVT				1							1
SR								1			1
FLA										1	1
Ungrouped						2	1	1			4
Totals	1	1	2	8	19	3	2	2	1	4	43

[1] ALB = *Ae. albopictus,* ATI = *Ae. atlanticus/infirmatus,* ATT = *Ae. atlanticus/tormentor,* ASP = *Ae.* sp., CRU = *An. crucians,* ERR = *Cx. erraticus,* NIG = *Cx. nigripalpus,* OPI = *Cx. opisthopus,* CXS = *Cx.* sp., MEL = *Cs. melanura.*

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