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ARBOVIRUSES IN NEW YORK STATE: SURVEILLANCE IN ARTHROPODS AND NONHUMAN VERTEBRATES, 1972–1977[1]

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ABSTRACT. During an arbovirus surveillance in New York State conducted from 1972 to 1977 a total of 918,047 wild-caught mosquitoes, approximately 60% *Aedes* species, were examined for virus in 20,616 pools. Five genera yielded 228 isolates: Eastern equine encephalomyelitis (EEE), 39; Highland J strain of Western equine encephalomyelitis (WEE), 7; California encephalitis complex (CAL), 73; Cache Valley (CV), 8; and Flanders (FLA), 96; there were also 5 unidentified virus strains. Both EEE and WEE were isolated primarily from *Culiseta melanura*, CV from *Aedes* and *Anopheles*, FLA from *Culex pipiens*, and CAL viruses from aedine mosquitoes. The CAL and FLA viruses had the widest geographic distribution, whereas EEE, WEE, and CV isolates were limited to central and/or southeastern New York. This surveillance, supplemented by virus isolation attempts and serological studies of domestic and wild vertebrates, identified the endemic and epidemic occurrence of several human pathogenic arboviruses and indicated potential vector species for CAL and EEE viruses, which are of the greatest public health importance among arboviruses known to occur in New York State.

Following the detection of human infections with California encephalitis (CAL) and Powassan (POW) viruses in New York State (Vianna et al. 1971, Smith et al. 1974) and outbreaks of Eastern equine encephalomyelitis (EEE) among horses in Long Island and upstate New York, which also resulted in a fatal human case (Bast et al. 1973, Morris et al. 1973), an extensive statewide surveillance for arboviruses was initiated in 1972 under the auspices of the Bureau of Disease Control and the Division of Laboratories and Research of the New York State Department of Health.

In this communication we summarize the overall results of arbovirus surveillance activities, with emphasis on attempts to isolate viruses from wild-caught mosquitoes, from 1972 through 1977. Certain data which have been reported elsewhere (Morris et al. 1975, Srihongse et al. 1978, Morris and Srihongse, 1978, Srihongse et al. 1979) are also included in the interest of presenting as complete a picture as possible.

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MATERIALS AND METHODS

FIELD INVESTIGATION. Study areas in New York State were organized into 5 regions, which were named after their principal cities from west to east: Buffalo, Rochester, Syracuse, Albany, and White Plains. In each region an entomologist was in charge of the collection and identification of the field specimens. Collections in 1972 were limited to a few counties in each region. From 1973 on, additional collection sites were explored, and by 1977 a total of 41 counties had been surveyed at least once: 5 in Buffalo, 8 in Rochester, 10 in Syracuse, 11 in Albany, and 7 in White Plains.

Field collections were usually limited to the summer months (June-September). Mosquitoes were collected mainly in CDC miniature light traps with or without dry ice as attractant. Arthropods were identified in the field and pooled in groups up to 200 by species, location, and date. The specimens were frozen on dry ice immediately after pooling and were kept frozen during storage and shipment to the laboratory.

Samples of blood and/or brain from horses with clinical evidence of central nervous system infection were collected by local veterinarians. Materials from sentinels, trapped birds, and other vertebrates were collected by our field teams. Techniques for obtaining blood and tissue from birds, including sentinel pheasants, have been described (Morris et al. 1973). Sentinels were exposed only at sites in the Buffalo, Syracuse, and White Plains regions where occurrence of EEE and/or St. Louis encephalitis (SLE) virus was anticipated.

LABORATORY METHODS. Virus isolations from triturated pools of mosquitoes, horse brain, and other vertebrate tissues, as well as from diluted blood samples, were attempted by intracerebral and intraperitoneal injections of 2- to 4-day-old white mice. The inoculated mice were observed for 2 weeks for signs of illness. Brain suspensions from sick mice were passed in new groups of suckling mice by the intracerebral route.

Virus strains used to prepare antigens and immune fluids were all New York State strains unless otherwise stated. These included EEE (69–7836), SLE (Parton), POW (64–7062), CAL (65–8569), CV (prototype), and Flanders (FLA, 61–7484). Reference virus strains and immune ascitic fluids for Western equine encephalomyelitis (WEE), which was represented by western (Fleming) and eastern (Highland J) strains, were kindly supplied by Dr. N. Karabatsos. New isolates were identified by hemagglutination-inhibition (HI, Clarke and Casals 1958), hemagglutination-reduction (HR, Srihongse et al. 1978) or complement-fixation (CF, Casey 1956) tests. In some instances neutralization tests were also performed in suckling mice or Vero cell cultures, using the constant-serum, varying-virus-dilution technique, and titration end points were calculated accordingly (Reed and Muench 1938).

Serum samples from sentinel, domestic, and wild vertebrates were tested for HI antibodies against EEE, WEE, SLE, POW, and CAL viruses. Nonspecific inhibitors were removed from bird sera by extraction with acetone with or without protamine sulfate treatment and from other sera by kaolin treatment; the sera were also adsorbed with goose red cells. An HI titer of 20 or greater against 4 units of antigen was considered a positive reaction. An individual sample positive for more than 1 antigen in the same serologic group was counted only for the antigen producing the higher-titered reaction. A fourfold or greater rise of HI titer in paired sera was considered evidence of current infection. For horse sera an EEE HI titer of 320 or greater in a single specimen was presumed to be evidence of current or recent infection, unless there was a history of vaccination.

RESULTS

INVERTEBRATE STUDIES. The number of wild-caught mosquitoes examined each year rose from only 983 pools of 12,083

Table 1. Arboviruses isolated from wild-caught mosquitoes, 1972–1977, by year, region, and genus.

Category	No. tested		No. isolates						
	Pools	Specimens	Total	EEE	WEE	CAL	CV	FLA	Unidentified
Year									
1972	983	12,083	11	0	0	5	0	6	0
1973	2,358	87,674	25	1	7	3	0	13	1
1974	3,827	143,647	26	8	0	13	0	5	0
1975	4,148	157,630	64	0	0	8	8	48	0
1976	5,750	290,524	54	9	0	17	0	24	4
1977	3,550	226,489	48	21	0	27	0	0	0
Total	20,616	918,047	228	39	7	73	8	96	5
Region									
Buffalo	1,576	54,003	7	0	0	7	0	0	0
Rochester	4,453	292,281	70	0	0	12	7	49	2
Syracuse	3,191	125,839	50	38	0	4	0	7	1
Albany	7,381	231,368	47	0	0	41	0	6	0
White Plains	4,015	214,556	54	1	7	9	1	34	2
Total	20,616	918,047	228	39	7	73	8	96	5
Genus									
Aedes	11,723	548,692	75	2	0	63	5	2	3
Anopheles	1,466	37,772	7	0	0	4	3	0	0
Coquillettidia	3,255	185,715	9	1	0	4	0	4	0
Culex	2,461	92,035	72	2	0	0	0	69	1
Culiseta	1,252	38,028	58	34	7	0	0	17	0
Mixed	459	15,805	7	0	0	2	0	4	1
Total	20,616	918,047	228	39	7	73	8	96	5

specimens in 1972 to 5,750 pools of nearly 300,000 specimens in 1976 (Table 1). During the 6-year surveillance period a total of 20,616 pools of 918,047 mosquitoes were collected and tested for virus in suckling mice. Aedine mosquitoes accounted for 60.1% of the collections. Nearly half (48.4%) were identified as *Aedes vexans* (Meigen) and 10.5% as *Ae. canadensis* (Theob.). The non-*Aedes* genera most commonly identified were *Anopheles, Culex, Culiseta,* and *Coquillettidia*. Two other genera tested were *Psorophora* and *Uranotaenia*.

From inoculation in suckling mice 228 virus isolates were obtained. Two pathogenic viruses, EEE and CAL, were detected almost every year (Table 1). Two other agents appeared only once each: WEE in 1973 and CV in 1975. All of our WEE isolates were found by HI to be more closely related to the Highland J strain than to the more pathogenic Fleming strain of WEE virus. Flanders virus, which has not yet been shown to be pathogenic for man, varied greatly in incidence from year to year. CAL and FLA viruses had the widest geographic distribution, but more than half of the CAL virus isolates were from the Albany region. This virus complex was isolated from mosquitoes collected in 20 of the 41 counties surveyed (Figure 1). The other viruses appeared to be geographically limited: WEE to Suffolk County in the White Plains region, CV to the Rochester region, and EEE to the Syracuse region.

From the 7 genera of mosquitoes tested, EEE and WEE isolates were most frequently obtained from *Culiseta*, CAL from species of *Aedes*, CV from *Aedes* and *Anopheles*, and FLA from *Culex* (Table 1). Minimal field infection rates of these viruses in the species of mosquitoes tested are listed in Table 2. *Cs. melanura* (Coq.) was the main species infected with EEE

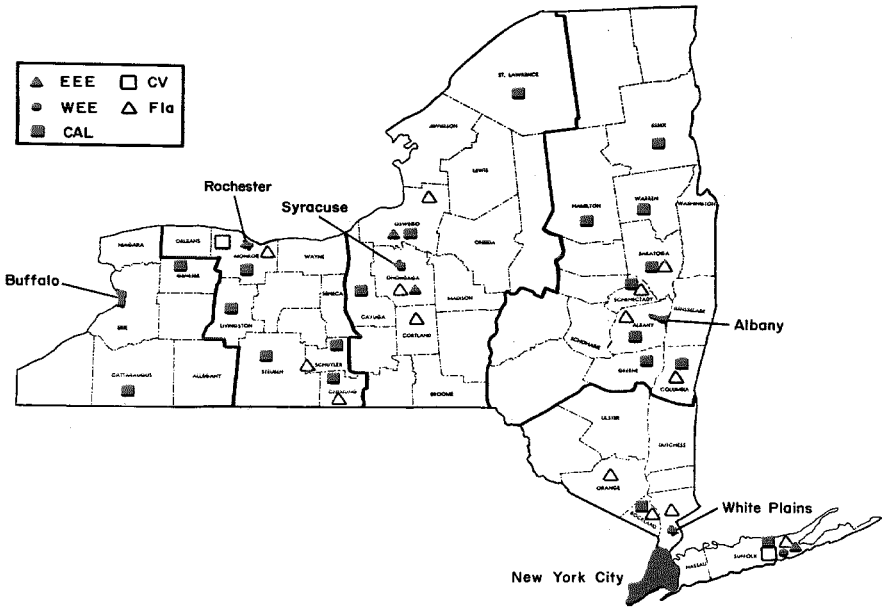

Figure 1. New York State counties where arboviruses were isolated from wild-caught mosquitoes, 1972–1977.

and WEE. Of nine *Aedes* species which yielded CAL virus isolates, the *Ae. communis* group had the highest minimal field infection rate, 1:1,095, during the 6-year-study period. However, in Warren County of the Albany region the rate was as high as 1:387 in *Ae. communis* mosquitoes collected during 1977.

EPIZOOTICS IN HORSES. After the first confirmed EEE outbreak in horses in upstate New York in 1971, virus infections in equines were detected annually 1972, 1 animal; 1973, 1; 1974, 10; 1975, 4; 1976, 37; 1977, 7; total, 60). The most extensive epizootic, involving 37 horses, occurred from August through October 1976 in the Syracuse region.

NONHUMAN VERTEBRATE STUDIES. From 1975 to 1977 a total of 124 sentinels were exposed: 43 chickens in Buffalo and 54 and 27 pheasants in the Syracuse and White Plains regions respectively. Positive reactions occurred only in the Syracuse region. In 1975, 4 sentinel pheasants showed SLE antibody conversions. In 1976 and 1977 EEE antibody conversions were detected in 7 of 14 and 3 of 20 pheasants respectively, and EEE virus was isolated from 2 of the preconversion bloods.

Birds and other vertebrates were collected occasionally for laboratory studies in each region during the summer months. From a total of 1,597 birds and 647 nonavian vertebrate tissues tested for viruses, 19 isolates of EEE were recovered in 1972 from outbreaks in 2 pheasant colonies, and 1 WEE isolate was obtained in 1973 from a yellow warbler in Suffolk county. In addition, 6 EEE isolates were obtained from the Syracuse region (1 in 1974, 5 in 1976). Four of these isolates were obtained from domestic pheasants and 1 each from a fox sparrow and a Savannah sparrow.

Plasma or serum from 5,803 birds and

Table 2. Minimal field infection rates of arboviruses in New York State, 1972–1977.

Virus	Mosquito species	No. mosquitoes tested	No. isolates	Minimal field infection rate (1:)
EEE	*Cs.melanura* (Coq.)	18,590	28	664
	Cx. restuans (Theob.)	3,682	1	3,682
	Cs.morsitans (Theob.)[3]	5,965	1	5,965
	Cx. pipiens (L.)	51,788	1	51,788
	Coq. perturbans (Walker)	176,064	1	176,064
WEE	*Cs. melanura*	18,590	7	2,656
CAL	*Aedes communis* group	20,807	19	1,095
	Ae. cinereus Meigen	7,413	5	1,482
	Ae. triseriatus (Say)	14,016	6	2,336
	Ae. stimulans group	27,524	9	3,058
	An. punctipennis (Say)	12,195	3	4,065
	Ae. cantator (Coq.)	17,276	3	5,759
	Ae. aurifer (Coq.)	13,551	2	6,775
	Ae. canadensis (Theob.)	58,855	7	8,408
	Ae. sollicitans (Walker)	19,768	1	19,768
	Coq. perturbans	176,064	4	44,016
	Ae. vexans (Meigen)	271,170	6	45,195
CV	*An. walkeri* Theob.	15,786	2	7,893
	Ae. stimulans group	27,524	2	13,762
	Ae. sollicitans	19,768	1	19,768
	Ae. vexans	271,170	2	135,585
FLA	*Cs. morsitans*[3]	5,965	7	852
	Cx. pipiens	51,788	56	925
	Cs. silvestris minnesotae Barr	1,275	1	1,275
	Cx. restuans	3,682	1	3,682
	Cs. melanura	18,590	5	3,718
	Cx. salinarius Coq.	9,526	2	4,763
	Ae. triseriatus	14,016	1	14,016
	Coq. perturbans	176,064	4	44,016
	Ae. vexans	271,170	1	271,170

[3] Listed in the Knight and Stone Catalog (1977) as *Cs. morsitans dyari* (Coquillett).

other vertebrates collected from 1973 to 1977 was tested by HI (Table 3). Of 4,428 avian samples tested, 359 reacted with alphavirus antigens, mostly with EEE virus, while 100 had antibodies to SLE, a flavivirus. Catbirds and robins had the highest EEE antibody rates among all birds tested. No antibodies to POW and CAL viruses were detected in wild birds.

Among 1,375 nonavian specimens tested, antibody to EEE virus was detected in some, especially deermice, but flavivirus and CAL antibodies were more prevalent. Chipmunks and deermice had higher rates for SLE than for POW; the reverse was true for woodchucks. Antibodies to CAL viruses were found, although at low rates, in several mammalian species.

DISCUSSION

This long-term collaborative surveillance for arboviruses provided information concerning the epidemic and endemic occurrence of several arboviruses important to public health in New York State. Three—EEE, POW, and CAL—proved to be pathogenic for man and or domestic animals.

Table 3. HI antibodies in birds and other wild vertebrates, 1973–1977.

Common name	No. tested	Percent positive	No. positive for				
			EEE	WEE	SLE	POW	CAL
Catbird	364	20.1	55	5	13	0	0
Sparrow	1,444	10.1	117	7	20	0	0
Robin	202	19.8	35	0	5	0	0
Other	2,418	8.4	127	13	62	0	0
All birds	4,428	10.3	334	25	100	0	0
Chipmunk	483	11.2	2	0	35	8	9
Deermouse	415	11.1	11	0	25	4	6
Woodchuck	140	41.4	0	0	12	43	3
Other	337	16.9	7	0	26	17	7
All nonavian	1,375	12.7	20	0	98	72	25
Total	5,803	10.9	354	25	198	72	25

Only 2 areas of the state have yielded evidence of EEE infections. On the eastern seaboard this virus occasionally caused outbreaks with high mortality in commercially reared pheasants and white Peking ducklings (Beaudette et al. 1954, Dougherty and Price, 1960). Upstate in the Oswego area, east and northeast of the Finger Lakes, EEE virus reappeared every year after the first confirmed outbreak in 1971, and it caused high mortality in horses during the summer of 1976 (Srihongse et al. 1978). Both these areas are part of major flight routes of migratory birds. *Cs. melanura* mosquitoes were implicated as the main vectors, with catbirds and robins as potential reservoir hosts. Our data do not permit conclusions as to whether the virus is reintroduced each year by migrating birds or persists in foci in local arthropod or vertebrate reservoirs. A recent attempt to analyze field data for evidence of transovarial transmission of EEE virus by *Cs. melanura* was inconclusive; however, the authors stated a preference against this hypothesis (Morris and Srihongse 1978).

The CAL viruses were widespread throughout New York State but were most prevalent in the Albany region, where 41 of the 73 isolates were obtained and where 73% of the laboratory-confirmed human cases of California encephalitis occurred (Deibel et al. 1979). Mosquito species with a high CAL minimum field infection rate, such as *Ae. cinereus* (Meigen) and *Ae. triseriatus* (Say), as well as those in the *Ae. communis* and *Ae. stimulans* group, are potential vectors of this virus complex in New York State. However, further studies are required to determine if these species can transmit the virus efficiently and if transovarial transmission is a mechanism for the overwintering of CAL viruses in this state.

One of the tickborne encephalitis viruses, POW, has caused serious CNS infections in man in New York State (Smith et al. 1974). Our surveillance activities did not include ticks, but in 1965 the virus was isolated from ticks (*Ixodes cookei*) and from woodchucks (*Marmota monax*) collected in 1964 in St. Lawrence County (Whitney and Jamnback 1965). Serologic evidence obtained during the 1972–1977 surveillance pointed to woodchucks as potential reservoirs of POW virus.

Arboviruses with relatively minor roles in New York State include WEE virus, which was isolated only in 1 year in Suffolk County on Long Island, and SLE virus, which has not been detected in insect vectors, although several human cases were diagnosed in 1975 (Deibel et al. 1979). Our HI results imply that certain species of avian and mammalian wildlife may be involved in SLE virus cycles in this state. However, flavivirus hemagglutinins are generally highly cross-reactive within the group, and cer-

tain strains of SLE have a tendency to produce nonspecific inhibition of hemagglutination, particularly when kaolin is used in the pretreatment procedure. Since wildlife bloods were routinely diluted for serologic study at the time of collection in the field, there was rarely sufficient material for confirmation of HI results by other more specific techniques. We recognize that some of these reactions may be nonspecific or may represent infection with another, closely related virus.

Two arboviruses without established pathogenicity for man or domestic animals were also encountered: CV virus, which was detected mainly in the Rochester region, and FLA virus, which was frequently isolated from *Culex* mosquitoes collected throughout the state.

This surveillance has identified regions where human pathogenic arboviruses are widespread and where increasing land use could create hazards to man and domestic animals.

Judged by the prevalence, annual reoccurrence, and pathogenic effects on human and/or equine populations, EEE and CAL viruses are the most important arthropod-borne viral pathogens known to occur in New York State. Studies of this ecology should help to elucidate their natural transmission cycles as a basis for more effective, less costly control measures.

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PREFERRED BREEDING MEDIA OF THE STABLE FLY, *STOMOXYS CALCITRANS*, IN NORTHWESTERN FLORIDA[1]

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ABSTRACT. Breeding media of the stable fly *Stomoxys calcitrans* (L.) were studied over a 41-month period (1972–1975) in 7 counties in northwestern Florida. Quantitative samples were collected routinely and the number of immatures was recorded for each type of breeding medium. The largest numbers of larvae and pupae were found in decomposing silage and green chop which had been used for feeding dairy animals. During these studies agricultural areas were found to be the primary source of stable flies in the northwestern Florida area.

The stable fly, *Stomoxys calcitrans* (L.), is a major pest of livestock and man throughout much of the world. It is a pest of considerable importance to livestock and dairy industries, and to tourist and recreational facilities along lakeshores and coastal areas.

Outbreaks of the stable fly (known as the "dog fly" in northwestern Florida') were reported in the literature as early as 1936 by King and Lenert. Early studies on the biology, breeding sites, and control operations around Panama City, Florida, were reported by Simmons and Dove (1941 and 1944) and Dove and Simmons (1942).

Although much research has been done on the stable fly, there is need for additional studies on the field biology of these flies. This knowledge would be especially helpful for planning and conducting control measures.

One of the major insect problems in the northwestern Florida area occurs when large numbers of stable fly adults accumulate along Gulf tourist beaches, particularly from Panama City to Pensacola, Florida. This area consists of 15 counties of which 8 border on the Gulf of Mexico. This study was carried out mainly in 7 counties: 3 coastal (Gulf, Bay, and Walton) and 4 inland (Washington, Jackson, Calhoun, and Holmes). This paper reports on breeding media and location of immature stable flies in this area over a 41-month period (1972–1975).

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