CALIFORNIA ENCEPHALITIS COMPLEX VIRUS ISOLATIONS FROM MOSQUITOES COLLECTED IN NORTHEASTERN NEW YORK, 1976–1977[1]

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ABSTRACT. Twenty-nine isolations of California encephalitis complex (CAL) viruses were obtained when suckling mice were inoculated with 3,609 pools of 123,464 mosquitoes collected in northeastern New York State during the summers of 1976 and 1977. Vero cell cultures subsequently inoculated with 1,092 of the negative pools yielded 2 additional isolates. These findings, together with results of parallel titrations of 8 virus-positive mosquito suspensions in both systems, indicated that the detection of CAL viruses in wild-caught mosquitoes was optimal when Vero cell cultures were used as a supplementary isolation system to suckling mice.

Serologic evidence obtained from patients with central nervous system infections in New York State between 1966 and 1977 showed that California encephalitis virus was the most prevalent arthropod-borne pathogen; it was predominant in the northeastern part of the state (Deibel et al. 1979). For this reason extensive surveys for potential vector species of California encephalitis group (CAL) viruses were conducted in the northeastern New York State during the summers of 1976 and 1977.

Certain arboviruses can be propagated more readily in African green monkey kidney (Vero) cell cultures than in suckling mice (Simizu et al. 1967), and this cell line has been used successfully to isolate CAL and other arboviruses (Hayes et al. 1976). A Vero cell was therefore chosen to supplement the customary suckling mouse system (American Committee on Arthropod-borne Viruses 1975) for our study.

MATERIALS AND METHODS

MOSQUITO COLLECTION. Adult mosquitoes were collected by miniature CDC light traps from early June through late August 1976 and 1977 in 9 counties of northeastern New York State. They were identified in the field, grouped by species and collection site, frozen, and stored at −70C for shipment on dry ice to the laboratory in Albany.

VIRUS ISOLATION AND IDENTIFICATION. Samples of up to 100 mosquitoes were triturated with 2–4 ml of phosphate-buffered saline supplemented with 7.5% bovine albumin plus 1,000 units of penicillin and 1,000 μg of streptomycin per mil. The mosquito suspensions were centrifuged at 1.000 x g for 30 min at 4°C, and 0.03-ml aliquots of each supernatant were inoculated intracerebrally and intraperitoneally into eight 2- or 3-day-old mice. A portion of each preparation was quickly frozen and stored at −70°C.

The mice were observed daily for 2 weeks for signs of illness. Suspensions of brain from mice that became sick were serially passed, and crude antigen was prepared in borate-buffered saline, pH 9.0, from the brains of subinoculated mice (Clarke and Casals 1958). Each mixture was stored overnight at 4°C and centrifuged at 1,000 x g for 20 min at 4°C. The supernatant was used in complement-fixation tests (Fulton and Dumbell 1949) and in neutralization tests utilizing a constant-serum, varying-virus dilution technique (Lennette and Schmidt 1969).

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The stored portion of each positive mosquito suspension was thawed, and part was used for reisolation attempts by the same procedure. The remainder was refrozen and stored at −70°C.

After 2 to 6 months, 0.1-ml aliquots of some of the virus-negative mosquito suspensions were each inoculated into 1 or 2 Vero cell culture tubes and incubated at 37°C for 1 hr. The cell monolayers were washed once with the maintenance medium, which consisted of Eagle's minimal essential medium supplemented with fetal bovine serum (2%), penicillin (100 units/ml), and streptomycin (100 μg/ml). The cultures were examined for cytopathogenic effects (CPE), and the maintenance medium was changed 2 or 3 times a week for 2 weeks. If CPE were noted, the cells and fluid were quickly frozen and thawed 3 times and centrifuged for 5 min at 1,000 x g. The supernatant was used for identification of virus by the neutralization technique.

Parallel titrations of 8 virus-positive mosquito suspensions were performed in suckling mice and Vero cells, using 0.03- and 0.1-ml inocula respectively. Mice were injected by the intracerebral route only. Titration end points were calculated (Reed and Muench 1938) and adjusted for a volume of 0.1 ml.

Hyperimmune ascitic fluid for use in complement-fixation tests was obtained from adult mice immunized with CAL strain 65–8569, isolated in New York State in 1965 (Whitney et al. 1969). Hyperimmune serum which neutralized 4 to 5 $\log_{10}$ mean lethal doses (LD_{50}) of homologous virus was obtained from rabbits immunized with the same strain. This serum was used in neutralization tests for identification of new isolates.

RESULTS AND DISCUSSION

A total of 3,609 pools of 123,464 mosquitoes processed and inoculated into suckling mice yielded 29 isolates of CAL virus from 6 of 9 counties sampled (Table 1). About 77% of the mosquitoes tested were aedine, and all but 3 CAL isolates were from this genus (Table 2). The exceptions were a single isolate from *Anopheles* spp. and 2 isolates from *Coquillettidia* spp. Minimal field infection rates varied from 1:596 in *Aedes cinereus* to 1:16,710 in *Ae. vexans*. The other *Aedes* species found infected with this virus were *Ae. canadensis*, members of the *Ae. communis* and *Ae. stimulans* groups and *Ae. triseriatus*, the latter a proven vector of La Crosse virus in the north-central United States (Thompson et al. 1972, Gauld et al. 1974).

Of the 2,596 mosquito suspensions tested in 1976 which did not yield virus in suckling mice, 1,092 were subsequently inoculated in Vero cell cultures. Two additional CAL virus isolates were obtained, one from the *Ae. stimulans* group, the other from a pool of *Aedes* mosquitoes

Table 1. Distribution by county of CAL virus isolates from mosquitoes collected in northeastern New York State, 1976–1977.

County	No. tested		No. isolates obtained in suckling mice
	Pools	Specimens	
Albany	495	14,688	5
Columbia	327	8,534	3
Essex	18	292	1
Greene	148	6,626	1
Rensselaer	8	26	0
Saratoga	1,923	65,304	6
Schenectady	233	9,540	0
Warren	198	9,602	13
Washington	259	8,852	0
Total	3,609	123,464	29

Table 2. Distribution by species of CAL virus isolates from mosquitoes collected in northeastern New York State, 1976–1977.

Species	No. tested		No. isolates	Minimal field infection rate
	Pools	Specimens		
Aedes canadensis (Theob.)	382	17,101	5	1:3,420
Aedes cinereus Meigen	44	2,384	4	1:596
Aedes communis group	236	7,420	10	1:742
Aedes stimulans group	279	9,546	1	1:9,546
Aedes triseriatus (Say)	263	2,441	2	1:1,220
Aedes vexans (Meigen)	437	16,710	1	1:16,710
Aedes spp.	592	39,910	3	
Anopheles spp.	392	3,385	1	1:3,385
Coquillettidia spp.	467	18,281	2	1:9,140
Culex spp.	271	4,051	0	
Culiseta spp.	209	2,048	0	
Other	37	187	0	
Total	3,609	123,464	29	

(species not determined) collected in Albany County. These agents produced focal areas of granulated cells, which rounded up and detached from the glass wall 6 to 9 days after primary inoculation and 2 to 3 days after first-passage inoculation. Reisolation attempts in Vero cell cultures were successful in both cases, whereas simultaneous reinoculation in suckling mice still produced no detectable evidence of infection.

The results of parallel titrations of virus-positive mosquito suspensions suggested that the Vero cell culture system detects CAL viruses more effectively than our standard suckling mouse inoculation system. Virus titers were higher in Vero cells than in suckling mice for 7 of the 8 suspensions, even when adjusted for the difference in volume of the inoculum (Table 3). Since the test material used for isolation attempts in Vero cells had been subjected to several months' storage and to additional freezing and thawing, the efficacy of this technique may be even greater with freshly processed specimens.

Our results show that CAL virus remains widespread in *Aedes* mosquitoes of northeastern New York, where infection is endemic in the human population (Deibel et al. 1979). The continuing occurrence of California encephalitis infection in humans of New York State emphasizes the need for rapid and effective surveillance for CAL viruses in mosquitoes. Virus isolation attempts in Vero cell cultures, to supplement those in suckling mice, could optimize the chances for detecting viruses of the CAL complex.

Table 3. Parallel titrations of CAL virus-positive mosquito suspensions

Suspension	Virus titers	
	Suckling mice[a]	Vero cells[b]
1	<1.5[c]	2.5
2	1.8	2.5
3	2.1	3.5
4	<1.5	1.5
5	3.2	4.0
6	<1.5	2.5
7	<1.5	1.0
8	<1.5	2.0

[a] Log_{10} LD_{50}/0.1 ml ic.
[b] Log_{10} $TCID_{50}$/0.1 ml.
[c] A few mice sick after inoculation with undiluted sample.

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AERIAL APPLICATIONS OF A SAND FORMULATION OF METHOPRENE FOR THE CONTROL OF SALT-MARSH MOSQUITO LARVAE

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ABSTRACT. Aerial tests of a sand formulation of methoprene indicate that a rate of 0.015 lb AI/acre is required for 95% control of field populations of *Aedes taeniorhynchus* (Wied.). Desired gross application rates were either 5 or 7.5 lb/acre, but in practice there was considerable variation due to the inability of the aircraft distribution equipment to apply uniformly the desired small amount of material.

Previous data by Rathburn and Boike (1975 and 1977) and Rogers et al. (1976) indicated that effective control of *Aedes taeniorhynchus* (Wied.) was obtained with granular formulations of methoprene up to one-half the recommended dosage rate for liquid formulations. The need for using granular formulations to penetrate the dense vegetation associated with mosquito larvae habitats in Florida is obvious since liquid formulations commonly deposit on the surface of marsh vegetation with little reaching the water surface and mosquito larvae. Although earlier tests were conducted with vermiculite-based granules, the sand formulation was selected for these tests because of availability, lower cost and the ability to treat a larger area with a single aircraft load.

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