

ISOLATION OF EASTERN EQUINE ENCEPHALITIS VIRUS FROM *Aedes sollicitans* DURING AN EPIZOOTIC IN SOUTHERN NEW JERSEY¹

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ABSTRACT. Eastern equine encephalitis virus (EEE) was isolated from the salt marsh mosquito, *Aedes sollicitans*, collected from coastal areas of New Jersey on 3 occasions during the late summer and fall of 1982. The isolations were made at a time when local *Culiseta melanura* were either undergoing a population increase or exhibiting high levels of EEE virus. Although no human cases were reported during the epizootic period, the data lend support to the hypothesis that *Ae. sollicitans* is capable of functioning as an epidemic vector in the coastal areas of New Jersey where human cases of EEE have been most common.

INTRODUCTION

Eastern equine encephalitis (EEE) is caused by a mosquito-borne virus that occurs naturally in a variety of wild birds along the eastern flyway of the United States. The mosquito, *Culiseta melanura* (Coquillett), is recognized as the primary enzootic vector (Burbulis and Jobbins 1957, Chamberlain et al. 1958, Wallis 1959, Nasci and Edman 1981). The epidemic vectors appear to vary from one geographic area to the next but have never been clearly defined during outbreak years (Feemster et al. 1958, Hayes et al. 1962, Chamberlain et al. 1969). The salt marsh mosquito, *Aedes sollicitans* (Walker), has been a primary suspect in coastal areas of the middle Atlantic states (Hayes et al. 1962, Altman et al. 1967, Goldfield et al. 1969). Crans (1977) reviewed the literature in this regard and concluded that sufficient evidence had been gathered to implicate this species as a potential vector in New Jersey.

In 1982, EEE virus reached epizootic proportions in New Jersey with 6 confirmed equine deaths due to the virus in late summer and early fall (Sofield et al. 1983). From July 13 to October 14, EEE virus was repeatedly isolated from *Cs. melanura* collected at coastal sites in the southern portion of the State. During the same period, a concerted effort was made to collect large numbers of *Ae. sollicitans* for virus isola-

tion attempts to see if the virus would transfer to the suspected epidemic vector. This paper presents the results of the virus isolation attempts that were made during the epizootic period.

MATERIALS AND METHODS

DESCRIPTION OF THE COLLECTION SITES. *Aedes sollicitans* were collected from 2 study areas where EEE virus was being monitored in *Cs. melanura* populations (Fig. 1). The first site encompassed the coastal zone from West Creek, Ocean County to Atlantic City, Atlantic County on the eastern coast of the State. *Culiseta melanura* collections (the main source for monitoring the level of EEE activity) were concentrated in a 3 ha stand of white pine within the Bass River State Forest, approximately 2.5 km northeast of the town of New Gretna. Additional specimens from the Green Bank State Forest, 5 km to the west, were tested for EEE virus but no population estimates were taken. *Aedes sollicitans* submitted for virus isolation attempts were trapped throughout the zone from West Creek to Atlantic City.

The second site was situated in Cape May County on the western coast of the State. *Culiseta melanura* collections were conducted in a 10 ha stand of white pine on the salt marsh-upland ecotone in the Belleplaine State Forest 3 km west of the town of Dennisville. In this area, all *Ae. sollicitans* submitted for virus isolation attempts were trapped within a 5 km radius of the Belleplaine State Forest study site where *Cs. melanura* populations were being monitored.

COLLECTIONS FOR VIRUS ISOLATION ATTEMPTS. *Culiseta melanura* were collected from a line of 50 resting boxes at each of the sites 3 days each week from June 3 to October 14. Specimens were frozen on dry ice at the collection site and sorted on a chill table in the laboratory before being pooled in groups containing not more than 100 specimens for virus isolation attempts. *Aedes sollicitans* were collected for virus isolation attempts as soon as EEE was detected in *Cs. melanura*. A back-pack aspirator with a gasoline

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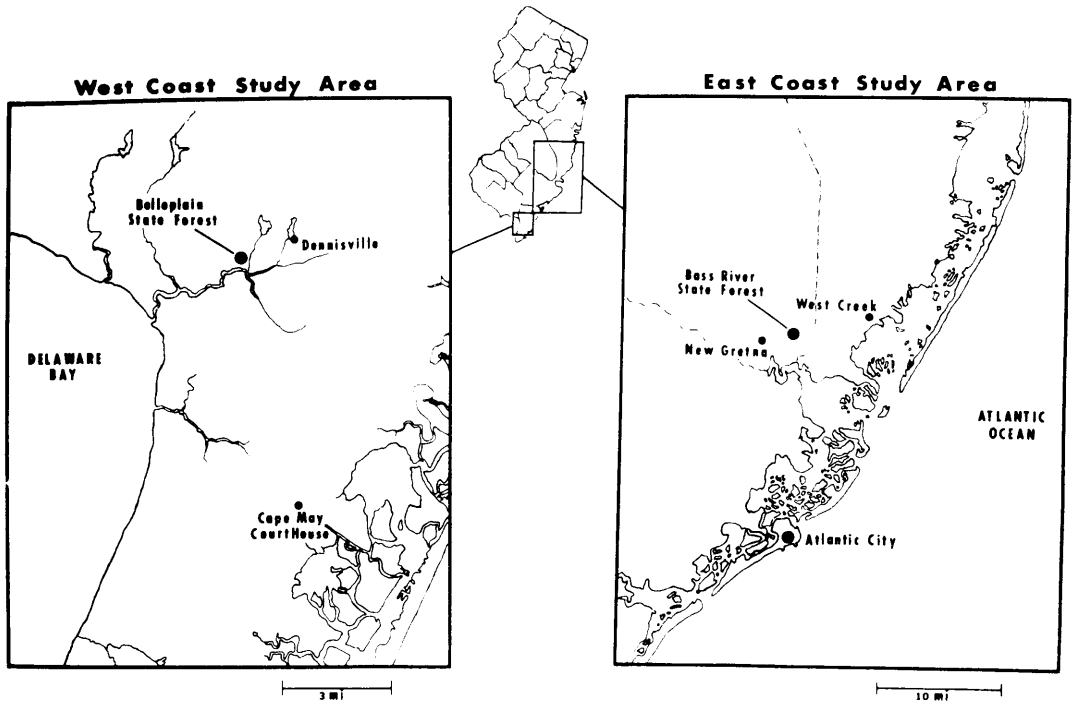


Fig. 1. Study areas in New Jersey where eastern equine encephalitis virus was monitored during 1982.

engine was used to collect resting mosquitoes from the vegetation during the day. A series of CDC light traps baited with dry ice were utilized to make night-time collections within the zones designated for study. Modified New Jersey light traps operated by 6 volt automobile batteries were fitted with live collection sleeves to collect additional specimens in some areas.

All *Ae. sollicitans* tested for EEE virus were frozen at -57°C prior to processing. The mosquitoes were sorted on wet ice at the Cape May County Mosquito Commission Laboratory and pooled in groups containing not more than 100 specimens. Unengorged, blooded and gravid specimens were pooled separately for testing. All material was kept frozen at -57°C until tests could be conducted.

TESTING PROCEDURES USED FOR VIRUS ISOLATION ATTEMPTS. All *Cs. melanura* collected in these studies were tested in White Leghorn chicks less than 12 hrs old by the methods described by Chamberlain et al. (1954). The brain suspensions from chicks showing symptoms were inoculated into fresh chicks, suckling mice and duck embryo tissue culture. The final virus identification was made by fluorescent antibody from the tissue culture preparations. *Aedes sollicitans* were tested by two methods. One portion from each collection date was tested in "wet" chicks paralleling the methodology used for the *Cs. melanura* samples. The remaining

specimens were tested in suckling mice by the methods described by Sudia and Chamberlain (1967).

RESULTS

EEE VIRUS IN *Cs. melanura*. Eastern equine encephalitis (EEE) virus was isolated from 70 pools of *Cs. melanura* in 1982 with activity extending from mid-July to early October. Figure 2 plots population curves for *Cs. melanura* and EEE isolations by week at the 2 sites that were monitored.

The virus of EEE was first detected in *Cs. melanura* from the Belleplain State Forest on the western coast of the State on July 13. Low recovery rates indicated that virus activity was minimal at that site until early August when resting box collections of *Cs. melanura* increased 10-fold. The high populations apparently favored amplification of EEE virus. From August 5 to August 12, 60% of the pools were positive with 1 isolation for every 130 *Cs. melanura* tested. Four confirmed equine cases of EEE occurred 45 km to the north of the study site during this period, indicating that virus amplification was taking place in other areas of the State. An equine case was also reported from a coastal area 30 km south of the study site 2 weeks after the dramatic increase in virus activity.

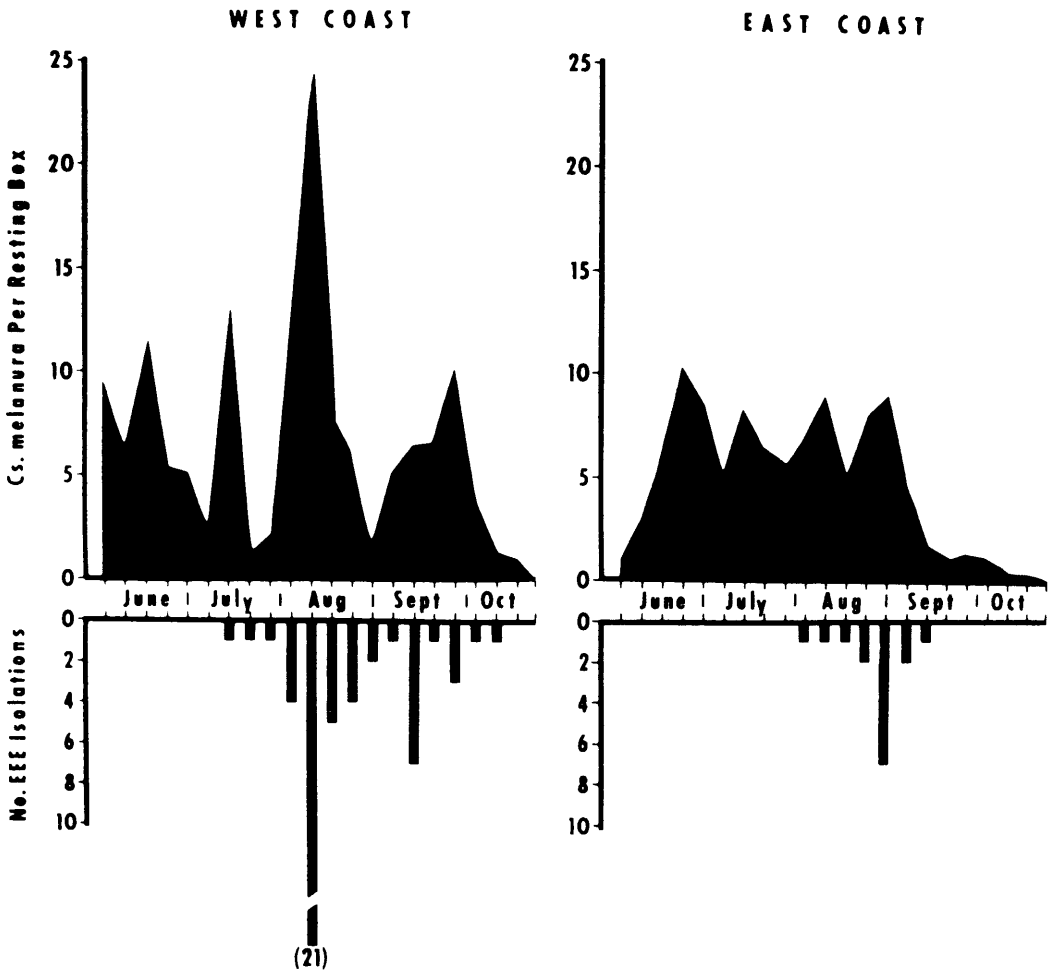


Fig. 2. Resting box populations of *Culiseta melanura* and isolations of eastern equine encephalitis by week on the eastern and western coasts of New Jersey during 1982.

Culiseta melanura populations declined at the west coast site during late August, but EEE virus remained evident in the samples. Virus activity then showed a second peak in the middle of September. From September 13 to 20, 55% of the pools from the Belleplaine State Forest were positive with 1 isolation for every 89 *Cs. melanura* tested. Populations of *Cs. melanura* declined rapidly in October but virus was still detectable in the samples that were tested. The last EEE isolation of the season at that site was recovered on October 8, the latest date that sufficient numbers of mosquitoes could be collected for testing.

Eastern equine encephalitis virus appeared later on the eastern coast of the State than on the western coast. *Culiseta melanura* did not exhibit a marked population increase in the Bass River State Forest and EEE was only recovered at a rate of 1 isolation for every 1000 mos-

quitoes tested during August. In the first week of September, when populations were declining, 40% of the pools from the east coast site were positive with 1 isolation for every 140 *Cs. melanura* tested. An equine case was reported from the eastern coast late in August, indicating that virus may have been amplified earlier than the study site data indicated. Populations of *Cs. melanura* in the Bass River State Forest declined rapidly during September and virus activity could not be followed beyond mid-month when low populations prohibited further testing.

VIRUS ISOLATIONS FROM *Ae. sollicitans* DURING THE EPIZOOTIC PERIOD. From July 29 to October 19, nearly 34,000 specimens from 470 pools of *Ae. sollicitans* were tested for EEE virus. Table 1 lists the specimens that were tested by site, physiological status and test method. Three of the pools tested positive for EEE virus. All positive pools were obtained from the wet chick

system with confirmation in suckling mice on the second passage.

Table 2 lists pertinent collection data for the *Ae. sollicitans* that yielded EEE in these investigations. The first isolation was obtained from a pool of 52 *Ae. sollicitans* collected from the Belleplain State Forest on August 4. The specimens yielding the isolation were collected at the same time that *Cs. melanura* samples were showing increased virus activity at that site. The second isolation was obtained from a pool of 18 *Ae. sollicitans* collected August 13 on the eastern coast of the State. The specimens were collected in a New Jersey light trap that was operated less than 50 m from the resting box line used to monitor *Cs. melanura* in the Bass River State Forest. Resting box data showed that the *Cs. melanura* populations on the east coast were peaking when the EEE isolation was made from *Ae. sollicitans*. Virus isolations from *Cs. melanura*, however, did not show a marked rise until late in the month. The final isolation from *Ae. sollicitans* was recovered from the Belleplain State Forest on September 21 when a pool of 100 specimens yielded EEE virus. The collection was made at a time when EEE was being recovered from *Cs. melanura* at a rate of 1 isolation for every 89 mosquitoes tested, the highest rate observed during the season.

DISCUSSION

Data collected during 1982 suggest that EEE virus can be acquired by *Ae. sollicitans* when amplification is occurring in the avian cycle perpetuated by *Cs. melanura*. The first isolation

from *Ae. sollicitans* was made at a time when local populations of *Cs. melanura* were undergoing a marked population increase. Wallis et al. (1974) reported that an epizootic of EEE in Connecticut coincided with a similar increase in *Cs. melanura* populations. Two of the isolations from *Ae. sollicitans* were made when *Cs. melanura* were yielding the highest infection rates of the season. Goldfield and co-workers (1966, 1968, 1969) isolated EEE during 3 outbreak years but did not indicate if the isolations accompanied or followed episodes of virus activity in *Cs. melanura*.

All of the isolations from *Ae. sollicitans* were recovered from pools of unengorged specimens suggesting that the infected mosquitoes were actively seeking a host at the time of capture. Ovarian dissections made on specimens that were taken from the study sites on the dates that the infected pools were collected showed that the biting populations of *Ae. sollicitans* had parous rates ranging from 75 to 100%.

The results of these investigations provide additional support to the hypothesis that *Ae. sollicitans* functions as an epidemic vector of EEE in coastal areas of New Jersey. Virus isolations were minimal with only 3 of 470 pools yielding virus, but *Ae. sollicitans* occurs in tremendous numbers and causes severe annoyance to humans within the zone that human cases have been reported. No human cases were reported during this epizootic period but the isolation rates obtained from *Ae. sollicitans* fall within the range of those obtained during human outbreaks of EEE in New Jersey. The overall isolation rate for the specimens tested in

Table 1. Numbers of *Aedes sollicitans* tested for EEE virus in wet chicks and suckling mice during 1982.

Area	Physiological state			
	Unengorged	Engorged	Gravid	Total
West coast site				
Wet chick	14,530 (174)**	67 (13)	433 (12)	15,030 (199)
Suckling mouse	10,491 (111)	24 (2)	52 (5)	10,567 (118)
East coast site				
Wet chick	6,247 (109)*	40 (15)	27 (9)	6,314 (133)
Suckling mouse	2,017 (20)	0	0	2,017 (20)

Numbers in parentheses indicate number of pools that were tested in each category.

* Single pool positive.

** Two pools positive.

Table 2. *Aedes sollicitans* collections that tested positive for EEE virus in 1982.

Collection date	Study site	No. specimens in pool	Physiological status
August 4	West coast (Belleplain Forest)	52	Unengorged
August 13	East coast (Bass River Forest)	18	Unengorged
September 21	West coast (Belleplain Forest)	100	Unengorged

1982 was 1:11,309. Veazey et al. (1980) reported isolation rates of 1:1597 in 1965 (1 human case), 1:6959 in 1957 (1 human case) and 1:11,088 in 1968 (12 human cases). *Aedes sollicitans* can apparently acquire virus when EEE is being perpetuated in the avian cycle by *Cx. melanura*. This epidemiological evidence suggests that *Ae. sollicitans* should be monitored and controlled whenever EEE virus is known to be active.

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References Cited

- Altman, R., M. Goldfield and O. Sussman. 1967. The impact of vector-borne viral diseases in the middle Atlantic states. *Med. Clinics N. Am.* 51:661-671.
- Burbutis, P. P. and D. M. Jobbins. 1958. *Culiseta melanura* and eastern equine encephalomyelitis in New Jersey. *Proc. N.J. Mosq. Exterm. Assoc.* 44:68-78.
- Chamberlain, R. W., R. K. Sikes and R. E. Kissling. 1954. Use of chicks in eastern and western equine encephalitis studies. *J. Immunol.* 73:106-114.
- Chamberlain, R. W., W. D. Sudia, P. P. Burbutis and M. D. Bogue. 1958. Recent isolations of arthropod-borne viruses from mosquitoes in the eastern United States. *Mosq. News* 18:305-308.
- Chamberlain, R. W., W. D. Sudia, P. H. Coleman, J. G. Johnston and T. H. Work. 1969. Arbovirus isolations from mosquitoes collected in Waycross, Georgia. 1963, during an outbreak of equine encephalitis. *Am. J. Epidemiol.* 89:82-88.
- Crans, W. J. 1977. The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey. *Mosq. News* 37:85-89.
- Feemster, R. E., R. E. Wheeler, J. B. Daniels, H. D. Rose, R. E. Kissling, R. O. Hayes, R. E. Alexander and W. A. Murray. 1958. Field and laboratory studies on equine encephalitis. *New England J. Med.* 259:107-113.
- Goldfield, M., O. Sussman, R. P. Kandle. 1966. A progress report on arbovirus studies in New Jersey. *Proc. N.J. Mosq. Exterm. Assoc.* 53:47-51.
- Goldfield, M., O. Sussman, W. Gusciora, R. Kerlin, W. Carter and R. P. Kandle. 1968. Arbovirus activity in New Jersey during 1967. *Proc. N.J. Mosq. Exterm. Assoc.* 55:14-19.
- Goldfield, M., O. Sussman, R. Altman and R. P. Kandle. 1969. Eastern encephalitis in New Jersey during 1968. *Proc. N.J. Mosq. Exterm. Assoc.* 56:56-63.
- Hayes, R. O., I. D. Beadle, A. D. Hess, O. Sussman and M. J. Bonese. 1962. Entomological aspects of the 1959 outbreak of eastern encephalitis in New Jersey. *Am. J. Trop. Med. Hyg.* 11:115-121.
- Nasci, R. S. and J. D. Edman. 1981. Vertical and temporal flight activity of the mosquito *Culiseta melanura* (Diptera: Culicidae) in southeastern Massachusetts. *J. Med. Entomol.* 18:501-504.
- Soffield, R. K., J. McNelly and W. J. Crans. 1983. Epidemiological investigations of mosquito populations following an outbreak of eastern equine encephalitis among horses in New Jersey. *J. Fla. Entomol. Assoc.* 51:21-23.
- Sudia, W. D. and R. W. Chamberlain. 1967. Collection and processing of medically important arthropods for arbovirus isolation. National CDC Publication. Atlanta, GA 30333.
- Wallis, R. C. 1959. *Culiseta melanura* (Coquillett) and eastern equine encephalitis in Connecticut. *Mosq. News* 19:157-158.
- Wallis, R. C., J. J. Howard, A. J. Main, Jr., C. Frazier and C. Hayes. 1974. An increase of *Culiseta melanura* coinciding with an epizootic of eastern equine encephalitis in Connecticut. *Mosq. News* 34:63-65.
- Veazey, J., D. Adam and W. Gusciora. 1980. Arbovirus surveillance for eastern encephalitis in New Jersey during the years 1960-1969. *Proc. N.J. Mosq. Control Assoc.* 67:160-173.