



APPENDIX F

Eastern Equine Encephalitis and Salt Marsh Mosquitoes

*Suffolk County Vector Control & Wetlands
Management Long Term Plan & Environmental
Impact Statement*



**Task 4: Current operations
Eastern Equine Encephalitis and Salt
Marsh Mosquitoes**

Prepared for:

**Suffolk County Department of Public Works
Suffolk County Department of Health Services
Suffolk County, New York**

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**SUFFOLK COUNTY VECTOR CONTROL AND WETLANDS MANAGEMENT
LONG - TERM PLAN AND ENVIRONMENTAL IMPACT STATEMENT**

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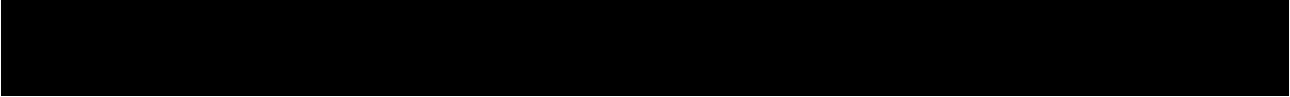
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List Abbreviations and Acronyms

CDC	Centers for Disease Control and Prevention
EEE	Eastern Equine Encephalomyelitis
EEEV	Eastern Equine Encephalitis Virus

1. Introduction

Eastern equine encephalitis virus (EEEV) is a mosquito transmitted pathogen that occurs naturally in a wide variety of birds along the eastern flyway of the United States (Morris, 1988, Scott and Weaver, 1989). The virus produces the clinical disease eastern equine encephalomyelitis (EEE) in humans with a 50 percent mortality outcome and is virtually 100 percent fatal in horses (Morris, 1988). Chamberlain (1958) felt that EEEV reached highest levels in coastal areas where freshwater swamps joined salt marsh habitat. *Culiseta melanura*, a bird feeding mosquito that uses acid water swamps as habitat, has since been identified as the primary enzootic vector in Georgia (Chamberlain et al., 1958), Maryland (Dalrymple et al., 1972), New Jersey (Burbutis and Jobbin, 1957), New York (Morris, et al. 1980), Connecticut (Wallis, 1959), and Massachusetts (Grady et al., 1978). *Culiseta melanura* appears to be a fixed avian feeder (Edman et al., 1972) and is probably not responsible for the transmission of EEEV to either humans or equine hosts. Although the epidemic vectors probably vary over the geographic range of this virus, salt marsh mosquitoes play an important role in areas where tidal marshland impinges on the breeding habitat utilized by *Cs. melanura*. Epidemiological data collected during the 1959 outbreak of EEE in New Jersey led Hayes et al. (1962) to hypothesize that the salt marsh mosquito, *Ochlerotatus sollicitans*, served as the primary epidemic vector in the coastal zone where all of the human cases occurred. Crans (1977) used Koch's postulates to show that *Oc. sollicitans* met basic criteria to indirectly prove vector status and suggested that the species should be controlled for the prevention of human disease whenever EEEV is found to be active.

Considerable epidemiological evidence has been gathered since that time to underscore the potential for salt marsh mosquitoes to acquire EEEV in areas where freshwater swamp habitat drains into the salt marsh ecotone. Much of the new information takes on added importance when the results of early EEE research are re-examined.

2. The History of EEEV in the Northeastern United States

Descriptions in the veterinary literature suggest that EEEV was probably cycling between mosquitoes and birds to the north and south of Suffolk Co. prior to the turn of the 20th century (Cohen and Sussman, 1957). Goldfield and Sussman (1968) reported that several hundred horse deaths with symptoms consistent with EEE were reported by New Jersey veterinarians as early as 1905. They list additional equine episodes, probably, caused by EEE, in 1912, 1920, 1928, and 1933. Beadle (1952) provides similar historical evidence for EEEV on Long Island. The first definitive evidence associating equine deaths with EEEV was obtained when Ten Broeck and Merrill (1933) isolated the type specimen for EEEV from a horse that died from the disease in Burlington County, NJ. The location where the isolation was obtained is less than 150 miles from Suffolk County, NY. EEEV was recognized as a fatal pathogen of wild ring-necked pheasants in Connecticut five years after the initial virus isolation (Tyzzer et al., 1938) and was linked to numerous large-scale commercial pheasant die-offs in New Jersey in subsequent years (Beaudette and Hudson, 1945; Beaudette and Black, 1948). Unlike native species, exotic birds such as the ring-necked pheasant lack natural immunity to EEEV and succumb to the virus fairly early in the amplification cycle. The first evidence for human involvement with this mosquito-borne pathogen came when 34 human cases accompanied the 1938 epizootic in Massachusetts (Fothergill et al., 1938). The severity of the pathogen was underscored when 67 percent of those infected died from the disease (Callisher, 1994). Notable outbreaks of EEE involving 10 or more cases in the northeast have taken place in Massachusetts (1938, 1947, 1955, and 1956) and New Jersey (1959 and 1965). Lesser numbers have occurred sporadically in the intervening years. Calisher (1994) reports an incidence of zero to 36 human cases, with an average of seven, per year in the eastern United States since 1955. During the period 1964 to 1992 the average dropped to 4.9 cases per year, which is thought to be due to vector control intervention.

3. The Ecology of EEEV

EEEV is perpetuated in eastern North America in a benign maintenance cycle that involves the bird feeding mosquito, *Cs. melanura*, as the primary enzootic vector and passerine birds as the amplification hosts. The term epiorntic is often used to describe the amplification phase of this virus because birds are the only vertebrate hosts involved in this portion of the cycle (Howard et al., 1994). When EEEV reaches epiorntic proportions in the northeastern region, clinical disease is frequently reported from horses. Epiorntics leading to human infections are sporadic and considerably less frequent than equine involvement. Since *Cs. melanura* does not accept mammalian hosts, mosquitoes that accept both mammals and birds play function as bridge vectors to pass the virus from avians to mammals during recognized outbreak periods.

Two rather distinct ecological cycles have been described for EEEV in the northeastern United States. An inland cycle that emanates from red maple swamps (*Acer rubrum* L.) and surrounding wet woodlands has been reported from Massachusetts (Grady et al., 1978), Connecticut (Wallis, 1974), upper New York state (Morris et al., 1980) and New Jersey (Crans et al., 1986). The inland cycle involves *Cs. melanura* as the amplification vector and *Aedes vexans*, *Oc. canadensis*, or *Coquillettidia perturbans* as vectors to mammalian hosts (Callisher, 1994). A similar inland cycle has been described from the central flyway where *Cq. perturbans* functioned as a bridge vector in Michigan (McLean et al., 1985). For as yet unknown reasons, the inland cycle claims large numbers of equine cases, but only rarely involves humans.

A coastal cycle of EEEV amplification occurs almost annually from Maryland to New Jersey in areas where Atlantic white cedar swamps (*Chamaecyparis thyoides* [L.]) drain into the salt marsh ecotone originally described by Chamberlain (1958). The coastal cycle involves *Cs. melanura* as the amplification vector and *Oc. sollicitans* as the primary vector to mammalian hosts. Kandle (1960), Hayes et al. (1962), Goldfield (1968), and Crans (1977, 1986) all present convincing evidence that the salt marsh mosquito, *Oc. sollicitans*, functions as the primary vector to humans. Unlike the inland cycle where horses are the primary mammalian victims, EEE episodes in coastal areas often involve human cases.

Culiseta melanura exhibits a bivoltine life cycle in the zone where human outbreaks have occurred that creates a seasonal cycle of virus amplification that contributes to salt marsh mosquito involvement (Mahmood and Crans, 1998). Unlike most mosquito species, *Cs. melanura* overwinters as a larva and does so in subterranean habitats where cold water temperatures slow down larval growth considerably. The first generation of adults emerges during the month of May from larvae that have spent the winter in their 4th instar. These mosquitoes host seek in the canopy and blood feed on adult birds that are nesting at that time of year. No juveniles are present in the bird population when the first generation of *Cs. melanura* is building. A high proportion of the adult birds that are being fed upon on the ecotonal edge of salt marsh-cedar swamp habitat has antibody to EEEV from a prior year's infection, preventing widespread amplification during late May and early June. Crans et al. (1994) found evidence for EEEV recrudescence in a small proportion of adult birds at this time of year. If that is the case, EEEV is present, but is maintained at very low levels due to the low number of susceptible hosts. The eggs from the first generation of adult mosquitoes are laid in cedar swamp habitat during the months of May and June. Cold habitat water retards development of the second generation of *Cs. melanura*, slowing down emergence of new adults until sometime in July and August. When this generation of *Cs. melanura* emerges, young of the year birds lacking immunity to EEEV make up a high proportion of the avian population. This sets the stage for virus amplification directly in the saltmarsh-cedar swamp ecotone, historically described as the primary EEEV focus. Salt marsh mosquitoes appear in broods during the summer months that are generated by lunar tides and typically move inland to search for blood (Headlee, 1945). Whenever amplification of EEEV coincides with an inland migration of *Oc. sollicitans*, newly emerged mosquitoes pass directly through the area of greatest virus activity as they penetrate the upland in quest of blood setting the stage for contact with viremic birds and potential vector involvement.

4. Criteria for Establishing Vector Involvement

Reeves et al. (1962) used a set of guidelines to establish *Culex tarsalis* as a vector of western equine encephalitis in California, and Sudia and Chamberlain (1964) employed a similar approach to establish *Culex nigripalpus* as the vector of St. Louis encephalitis in Florida. Sudia et al. (1969, 1975) repeated the process when they investigated an outbreak of mosquito-borne Venezuelan equine encephalitis in southern Florida and underscored the importance of separating speculation from fact with guidelines to establish vector involvement. Although specific methodologies varied, each of these researchers used the following criteria to incriminate a mosquito species as a vector of mosquito-borne disease:

1. Evidence that the mosquito can function as a bridge vector by accepting both the reservoir and the human as blood meal hosts.
2. Evidence that the mosquito can become experimentally infected by feeding on a viremic host.
3. Evidence that the mosquito can successfully transmit the virus to a susceptible host by bite.
4. Isolation of the virus from the mosquito during an epidemic or epizootic.

Data to support any one of the criteria is evidence for possible vector involvement. Satisfying all of the criteria establishes the mosquito as a vector on epidemiological grounds. Considerable evidence has been published to indicate that *Ochlerotatus sollicitans* has fulfilled each of the criteria and should be viewed as a potential vector of EEEV whenever and wherever the virus is active.

4.1 Evidence that *Oc. sollicitans* Feeds on Birds as well as Mammals

Numerous studies have shown that birds function as the primary reservoirs and sole amplification hosts for EEEV in nature (Stamm, 1963; Emord and Morris, 1984; Scott, 1988). As a result, competent bridge vectors for mammalian pathogens that originate in avians must accept both classes of animals as blood meal hosts. A precipitin test is a serologic test in which an antibody reacts with a specific soluble antigen to form a precipitate. Early studies using precipitin tests to determine blood meal hosts with *Oc.*

sollicitans suggested that the mosquito had a strong preference for mammals which would preclude it as an efficient bridge vector for EEEV (Thompson et al., 1963, Schaefer and Steelman, 1969, Edman 1971). Sprenger et al. (1990) later showed that blood-feeding patterns determined solely by precipitin testing of wild-caught specimens may not be indicative of the true host preference for a given species and that the methods used to collect blooded mosquitoes often further bias the results. Chamberlain et al. (1954) were the first to show that *Oc. sollicitans* was probably not a fixed mammalian feeder when they experimentally infected specimens by having them feed on a viremic chick. Nayer and Sauerman (1977) supplied further evidence when they successfully fed wild caught specimens on a chicken, dove and owl during experiments designed to test the effects of blood source on oocyste development. Because of the low incidence of avian feedings from field collected *Oc. sollicitans*, Crans et al. (1996) collected host-seeking mosquitoes from the field and offered them a restrained bird and restrained mammal in outdoor cages to determine if the species would accept or reject an avian host. In their studies, a majority of mosquitoes accepted the bird when it was the only host offered, suggesting that *Oc. sollicitans* was an opportunistic feeder rather than a fixed mammalophilic species as field collections suggested. When *Oc. sollicitans* was offered a choice between mammal and bird in the same cage, one third of the test sample fed on the bird even though a mammal was present as an alternative. Crans and Sprenger (1996) later showed that *Oc. sollicitans* usually searches for a blood meal host at ground level in open fields which probably limits contact with many of the bird species that exhibit EEEV viremias during epornitic periods. Their studies point toward birds that nest or forage on the ground as key sources for infection of *Oc. sollicitans* during epornitic periods as well as birds that are unable to roost due to illness. Callisher (1994) presents a strong argument for salt marsh wading birds as a source of virus for salt marsh mosquitoes that forage at ground level in salt marsh habitats.

4.2 Evidence that *Oc. sollicitans* can become infected by feeding on a viremic host.

The susceptibility of *Oc. sollicitans* to EEEV was established by Centers for Disease Control and Prevention (CDC) workers in the early 1950s when multiple suspect vectors were compared for their ability to become infected with arboviruses. Chamberlain et al.

(1954) obtained a 100 percent infection rate when *Oc. sollicitans* were fed on a viremic chick under laboratory conditions. Less than 4.6 logs of EEEV resulted in the infection of 80 percent of the test sample when *Oc. sollicitans* were fed on a viremic pigeon. The results of those studies led Schaeffer and Arnold (1954) to rank *Oc. sollicitans* as the most susceptible mosquito vector known with an estimated infection threshold of 2.0-3.0 logs of virus. More recently, Turell (1998) compared EEEV infection rates in *Oc. sollicitans* with those of *Oc. taeniorhynchus*, a salt marsh mosquito implicated as a vector of EEEV in the southern United States. In his studies, 79 percent of the *Oc. sollicitans* became infected when fed upon a viremic chick compared to only 42 percent of the *Oc. taeniorhynchus* in his test sample.

Because of this species' high vector competence, Sudia et al. (1956) selected *Oc. sollicitans* for experimental investigations with animals that circulated very low levels of virus. During their studies, they successfully infected 41 percent of the *Oc. sollicitans* that fed on an experimentally infected horse. Horses are considered to be dead-end hosts for this virus because they circulate levels that are considered too low to re-infect mosquitoes. The fact that *Oc. sollicitans* can pick up enough virus to initiate replication from a dead-end host underscores its ability as an exceptionally competent vector for this virus.

4.3 Evidence that *Oc. sollicitans* can successfully transmit the virus by bite.

Early studies conducted by CDC workers showed that *Oc. sollicitans* is fully capable of transmitting EEEV by bite. Chamberlain (1956) was the first to point out that a mosquito's ability to transmit by bite is considerably more important than its ability to become infected because many species possess innate barriers that prevent them from passing the virus on. His rationale was drawn from studies with co-workers (Chamberlain et al., 1954) where they rated *Oc. sollicitans* as "excellent" after 75 percent of the specimens in their test sample successfully transmitted EEEV to susceptible chicks. He then compared *Oc. sollicitans* with 18 other potential vectors of EEEV and ranked it second on his list of potential vectors with a 100 percent infection rate and 75 percent transmission rate. Most of the mosquitoes tested in these studies yielded a less than 50 percent transmission rate. *Aedes vexans*, an recognized vector of EEEV in the

northeast, showed only a 63 percent infection rate and 13 percent transmission rate in his ranking. Table 1 compares the Long Island mosquitoes that Chamberlain included in his comparative rankings and underscores the high vector competence exhibited by this extremely abundant salt marsh pest.

The most significant transmission studies, however, were conducted by Sudia et al. (1956) in their investigations with dead end hosts. This group successfully transmitted EEEV from horse to horse and horse to chick and was able to show evidence for virus replication and dissemination in the mosquitoes after each transmission attempt.

Table 1. Comparative EEEV Infection and Transmission Rates for Long Island Mosquitoes (Taken from Chamberlain 1956).

Mosquito Species	Percent Infected	Percent Transmitting
Oc. triseriatus	100	86
Oc. sollicitans	100	75
Cx. restuans	45	33
Ps. confinnis	100	22
Cq. perturbans	94	18
Ps. ciliata	83	18
Ps. ferox	100	15
Ae. vexans	63	13
An. quadrimaculatus	79	0
Cx. salinarius	3	0

4.4 Isolation of EEEV from Wild-caught *Oc. sollicitans* during Epidemics and Epizootics

New Jersey experienced an outbreak of EEE in 1959 that produced 32 human cases with 22 deaths (Goldfield and Sussman, 1968). *Ochlerotatus sollicitans* was suspected as the probable vector because all 32 of the affected humans had either recently visited the New Jersey shore or resided in a zone that was sharply limited to the coastline. Hayes et al. (1962) incriminated *Oc. sollicitans* as the probable vector of EEEV during the 1959 outbreak without the benefit of virus isolations from field caught specimens. They based their conclusion on the exceptionally high numbers of salt marsh mosquitoes that were present during the outbreak together with the striking coastal distribution of human cases. The disease next appeared in New Jersey in 1965, and two isolations of EEEV were obtained from *Oc. sollicitans* collected less than five miles from the residence of the

single human case that occurred that year (Goldfield et al., 1966). Since that time, EEEV was repeatedly isolated from *Oc. sollicitans* during epidemic episodes in New Jersey. In a summary of 10 years of investigation, Goldfield and Sussman (1970) reported that EEEV was isolated from *Oc. sollicitans* in the four years that human cases took place and in none of the other years during the decade.

EEEV has also been isolated from *Oc. sollicitans* during epizootics that did not lead to human involvement. In 1982, a concerted effort was made to collect and test *Oc. sollicitans* during a New Jersey epizootic among horses to determine if infected salt marsh mosquitoes could be collected and if infection rates could be quantified (Crans et al., 1986). EEEV was isolated from 70 pools of *Cs. melanura* during that epizootic with activity extending from mid-July to early October. During that same time frame, nearly 34,000 *Oc. sollicitans* were tested yielding three EEEV isolations with an overall virus isolation rate of 1:11,309. Andreadis et al. (1998) obtained 36 EEEV isolations from eight species of mosquitoes during a 1996 epizootic in Connecticut. Most of their isolations were made from red maple swamp habitat associated with the traditional inland cycle Connecticut normally experiences, but two isolations were obtained from pools of *Oc. sollicitans* collected from the relatively small amount of salt marsh habitat found in that state.

5. Distribution of Atlantic White Cedar Swamps in Suffolk County

Edinger et al. (2002) report that Atlantic white cedar swamps are restricted to the coastal lowland zone in New York State, which includes Long Island. The reference site for Atlantic white cedar swamps is given as the Cranberry Bog County Park. This abandoned cranberry bog is adjacent to Sweezy and Cheney Ponds, in the 460-acre park, and is the largest of Long Island's surviving Atlantic white cedar swamps. This a somewhat anomalous white cedar swamp, in that it is not associated with salt marshes. Atlantic white cedars can be found in coastal plain poor fens, but they are not the dominant vegetation.

Cashin Associates (2004) reported that this community type can be found within Cranberry Bog County Park and within Cedar Swamp (along the perimeter of Cheney Pond and Cedar Pond, on the north side of County Road 51 southwest of downtown Riverhead behind the County Complex). The coastal plain Atlantic white cedar swamp is a predominantly evergreen or mixed evergreen/deciduous swamp that occurs on organic soils along streams and in poorly drained depressions and kettle holes of the Long Island coastal plain. The peat deposits that are prevalent typically overlie a substrate of sand. Atlantic white cedar (*Charmaecyparis thyoides*) comprises over 50 percent of the canopy cover in these communities. Red maple (*Acer rubrum*) trees are often co-dominant in impure Atlantic white cedar stands, although lesser black gum and pitch pine may be present as well. Atlantic white cedars require full sunlight and moist soils to thrive. However, long-term soil saturation associated with damming, or, conversely, dessication associated with the draining of surface water bodies can result in tree loss. Changes in long-term precipitation patterns will have a similar impact. Moreover, degraded water quality (from stormwater discharges and/or polluted groundwater) can adversely affect the health of Atlantic white cedar trees. Ironically, a major threat to Atlantic white cedar regeneration and sustainability is the inhibition of light penetration caused by the screening effect of the trees' own foliage on potential seedlings. As individual trees are lost or become stressed, opportunities arise for competing species to gain dominance. Forest fires, chronic flooding, and windthrow, however, occasionally open these stands to larger expanses of sunlight, thereby allowing the stand to regenerate.

Atlantic white cedar forests were once prevalent on Long Island (Spring-Rushia and Stewart, 1996), but are now considered to be rare with only a few significant occurrences on Long Island. This community type has a global ranking of G3G4 and a State ranking of S1 making it “especially vulnerable” in the State of New York (Edinger et al., 2002).

Contacts were made with local natural resources experts to determine if other Atlantic white cedar swamps are identifiable in Suffolk County (Table 2).

Table 2. Atlantic White Cedar Swamps in Suffolk County

Town	Location
Babylon	Belmont State Park ¹
Brookhaven	None ²
East Hampton	None ³
Huntington	None ⁴ ; perhaps on the east side of Crab Meadow ⁵
Islip	
Riverhead	None ⁶
Shelter Island	None known ⁷
Smithtown	Blydenberg Park ⁸
Southampton	Cranberry Bog County Park ⁹ Cedar Swamp ¹⁰ Along Route 24, at and around Hubbard Creek ¹¹ Owl Pond in Flanders Bay drainage area ¹²
Southold	

¹ J. Guarino, Senior Environmental Analyst, Town of Babylon, Department of Environmental Control, personal communication, 2005

^{2, 8} J. Turner, Director of Environmental Protection, Town of Brookhaven, personal communication, 2005

³ B. Frank, Chief Environmental Analyst, Town of East Hampton, personal communication, 2005

⁴ J. Dieterich, Department of Maritime Services, Town of Huntington, personal communication, 2005

⁵ M. Myles, Department of Planning, Town of Huntington, personal communication, 2005

⁶ J. Hall, Town of Riverhead Planning Department, personal communication, 2005

^{7, 12} L. Bavaro, Peconic Estuary Program, SCDHS Office of Ecology, personal communication, 2005

⁹ Edinger et al., 2002

¹⁰ Cashin Associates, 2004

¹¹ M. Brusseau, Cashin Associates, personal observations

Although there are not many Atlantic white cedar swamps in the County, there are also some red maple swamps in close proximity to salt marshes. A prime example of this is

the Mastic-Shirley area, where Cashin Associates found stands of red maple swamps in the fresh water wetlands immediately north and east of the salt marshes found along the shoreline (CA-CE, 2005). There are also red maple swamps in Amityville near the shoreline, as well, according to Town officials. It is clear that similar guilds of birds inhabit each swamp, and that *Cs. melanura* can thrive in both. Therefore, coastal red maple swamps may very well serve the same function in areas of Suffolk County that coastal stands of Atlantic white cedar swamps appear to serve in New Jersey – that is,, to create opportunities for salt marsh mosquitoes to come into contact with EEE-infected birds and so become vectors. Suffolk County therefore appears to have been fortunate not to have experienced human cases of EEE spread by this mechanism, given the competence of *Oc. sollicitans* as a vector of EEE.

6. Conclusions Regarding the Role of Salt Marsh Mosquitoes as Vectors of EEE

Examination of scientific literature compiled over a 50 year period clearly shows that the salt marsh mosquito, *Oc. sollicitans*, is a competent vector of EEEV. The mosquito fulfills each of the criteria necessary to prove vector status and appears to be the most efficient vector ever tested for this important mosquito-borne arbovirus. Most states along the eastern seaboard specifically target this mosquito whenever equine cases, pheasant die-offs or virus isolations signal EEEV activity. This approach appears to have reduced the amount of active transmission to horses and minimized human cases in recent years (Crans and McCuiston, 1993, Calisher, 1994). Suffolk County has suitable habitat to support amplification of EEEV in *Cs. melanura*, although the prime areas for this are red maple swamps, not Atlantic white cedar swamps; Suffolk County Department of Health Services frequently obtains evidence for virus amplification during late summer and fall (D. Ninivaggi, Suffolk County Vector Control, personal communication, 2005). Epornitics in ducks (Dougherty and Price, 1960), die-offs in pheasants (Whitney 1960), and equine cases (Bast et al. 1973) show that conditions are favorable for this virus to cycle in mosquitoes and birds in Suffolk County. Although environmental issues must be addressed, targeting salt marsh mosquitoes in the interest of public health would appear to be logical. Available evidence clearly shows that this mosquito is a primary vector of EEEV.

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