Journal of the American Mosquito Control Association, 19(1):33–38, 2003

ISOLATION OF EEE VIRUS FROM *OCHLEROTATUS TAENIORHYNCHUS* AND *CULISETA MELANURA* IN COASTAL SOUTH CAROLINA

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ABSTRACT. A 1-year arbovirus study was conducted at The Wedge Plantation located in coastal South Carolina to determine the occurrence and level of arbovirus activity in mosquito species inhabiting the site. Mosquito species composition and temporal abundance were also determined. A total of 45,051 mosquitoes representing 27 species in 9 genera was collected and identified during 130 trap-nights between August, 1997, and July, 1998. The most abundant species was *Culex salinarius* (n = 20,954) followed by *Ochlerotatus taeniorhynchus* (n = 12,185). Eastern equine encephalomyelitis virus (EEE) was isolated from 2 pools collected in August, 1997; one pool of *Oc. taeniorhynchus* (minimum infection rate [MIR] = 0.6/1,000) and a second of *Culiseta melanura* (MIR = 3.8/1,000). This report represents the first record of an EEE isolation from *Oc. taeniorhynchus* and *Cs. melanura* in South Carolina.

KEY WORDS Eastern equine encephalitis, surveillance, epidemiology, mosquitoes, horses

Knowledge of the natural history and epidemiology of eastern equine encephalitis in South Carolina is limited. However, cases in commercially important animals such as horses, donkeys, emus, pheasants, and quail are reported in the state every year (Alexander and Murray 1958, Brody and Murray 1959, Wozniak et al. 2001). Surveillance for equine encephalitis in the USA in 1971 showed that 52.2% (35/67) of all encephalitis cases caused by eastern equine encephalomyelitis virus (EEE) reported in horses were from North and South Carolina (Maness and Calisher 1981). A review of reported domestic animal and human encephalitis cases caused by EEE in South Carolina showed 458 horse cases reported in 1955–98, with most occurring in the Sandhills and Coastal Plain regions of the state (Table 1). The largest South Carolina epizootic resulting from EEE infection was reported in 1991 and involved at least 71 equine cases, with about 55% occurring in the lower coastal plain region (Clemson University Veterinary Diagnostic Center, Columbia, SC, unpublished data).

Human eastern equine encephalomyelitis cases in South Carolina are infrequent. McGowan et al. (1973) listed 3 human cases from South Carolina reported to the Centers for Disease Control and Prevention (CDC) during 1955–71, although no state records exist substantiating these cases (Tidwell et al. 1984). Unpublished information from the CDC recorded 2 serologically confirmed human cases caused by EEE from South Carolina, one in 1955 and another in 1969. From 1970 to 1990, 5 human cases were reported (Tidwell et al. 1984, Wright 1993). No human cases were reported between 1991 and 1995. However, there was one nonfatal human case in 1996 and 2 cases (one fatal) in 1997. All 3 individuals were residents of counties located in the Atlantic Coastal Plain (Horry County in 1996 and Charleston County in 1997) (Wozniak et al. 2001).

The first statewide mosquito-borne arbovirus surveillance program in South Carolina was conducted between 1996 and 1998 by the South Carolina Department of Health and Environmental Control (SCDHEC). During this study, EEE was detected in pools of *Anopheles crucians* Wiedemann, *Culex erraticus* (Dyar and Knab), *Anopheles punctipennis* (Say), *Anopheles quadrimaculatus* Say, *Culiseta melanura* (Coquillet), and *Coquilletidia perturbans* (Walker) collected in Allendale, Bamberg, Beaufort, Charleston, Clarendon, Colleton, Dillon, Hampton, Horry, Lee, and Sumter counties (Wozniak et al. 2001). The role of these mosquito species in the epidemiology of EEE in South Carolina remains unclear.

The purpose of our study was to determine the level of arbovirus activity in mosquitoes collected at The Wedge Plantation, Charleston County, SC, between 1997 and 1998. In addition, mosquito species composition and temporal abundance were also determined.

The Wedge Plantation is located 72 km northeast of Charleston, SC, at the northern end of Charleston County on the South Santee River, approximately 8 km inland from the Atlantic coast. This site consists of 202 ha of upland freshwater swamps and pine and hardwood forests and 404 ha of marsh habitat dominated by marsh elder (*Iva frutescens*),

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Table 1. Number of reported domestic animal and human cases of eastern equine encephalitis (EEE) virus in South Carolina (per county) from 1955 to 1998 (estimate).[1]

County	Horses	Birds	Emus	Donkeys	Dogs	Human	Total
Aiken	12	2	0	0	0	1	15
Anderson	1	0	0	0	0	0	1
Bamberg	2	0	0	0	0	1	3
Barnwell	16	0	0	0	0	0	16
Beauford	18	0	0	0	0	2	20
Berkeley	18	3	0	0	0	0	21
Calhoun	2	0	0	0	0	0	2
Charleston[2]	6	2	0	0	0	2	10
Chesterfield	1	0	0	0	0	0	1
Clarendon	15	1	0	0	0	0	16
Colleton	18	0	1	0	0	0	19
Darlington	5	2	2	0	0	1	10
Dillon	10	0	1	0	0	0	11
Dorchester	8	2	1	0	0	0	11
Florence	17	0	0	0	0	0	17
Georgetown	3	0	0	0	0	0	3
Greenville	2	0	0	0	0	0	2
Hampton	9	0	0	0	0	0	9
Horry	40	0	1	1	0	2	44
Jasper	4	0	0	0	0	0	4
Kershaw	28	0	1	0	0	0	29
Laurens	3	0	0	0	0	0	3
Lee	6	0	1	0	0	0	7
Lexington	8	1	0	0	0	0	9
Marion	11	0	0	0	0	0	11
Marlboro	0	0	1	0	0	0	1
McCormick	1	0	0	0	0	0	1
Orangeburg	10	0	1	0	0	0	11
Pickens	1	0	0	0	0	0	1
Richland	13	1	0	0	1	0	15
Spartanburg	1	0	0	0	0	0	1
Sumter	30	1	0	0	0	0	31
Williamsburg	7	0	0	0	0	0	7
York	3	0	0	0	0	0	3
Unknown Co.	129	2	0	0	0	2	133
Total	458	17	10	1	1	11	498

[1] Numbers based on Tidwell et al. (1984), Wright (1993), South Carolina Department of Health and Environmental Control, and Clemson University records.

[2] County where study area was located.

black rush (*Juncus roemeriatus*), broomsedge (*Andropogon virginicus*), foxtail grass (*Setaria* spp.), panic grass (*Panicum* spp.), glasswort (*Lilacopsis carolinensis*), saltgrass (*Distichlis spicata*), saltmarsh bulrush (*Scirpus robustus*), sea myrtle (*Baccaris halimifolia*), sea oxeye (*Borrichia frutescens*), and smooth cordgrass (*Spartina alterniflora*). The abundance of diverse mosquito habitats, site accessibility, and previous reports of arbovirus activity in the area were the main criteria for selection of The Wedge Plantation as our study site.

Adult female mosquitoes were collected for 2 consecutive days once a month from August 12, 1997, to July 21, 1998, using 6–8 CO_2-baited CDC miniature light traps (Sudia and Chamberlain 1962) (Hausherr's Machine Works, Toms River, NJ). Additionally, 1 Fay-Prince trap (Fay and Prince 1970) (John W. Hock Co., Gainesville, FL) and 2 gravid traps (Reiter 1983) (Hausherr's Machine Works and BioQuip, Gardenia, CA) were also used during our study to increase sample size and to attract other mosquito species less frequently collected in the CDC light traps. All CDC light traps were activated at dusk and mosquitoes retrieved the next morning. Fay-Prince and gravid traps were operated both day and night and captured mosquitoes retrieved every 8–10 h. Trap location was based on presence or absence of mosquito breeding sites, amount of vegetation, and accessibility to the area. Mesh bags containing collected mosquitoes were placed in a styrofoam cooler containing dry ice to kill the mosquitoes and preserve them during transport to the laboratory for virus testing.

In addition, mosquito larvae and pupae were surveyed and collected using a standard 1-pint mosquito dipper for 2 consecutive days once a month during August, October, and November, 1997, and March and May, 1998. Collection sites included woodland pools, ditches, and artificial containers located throughout the property. Immatures were

placed in mosquito breeding chambers (BioQuip), transported to the laboratory where they were transferred to pans inside 46-cm^3 mosquito cages (BioQuip), and reared to adulthood. Adults were collected 24–48 h postemergence using an insect aspirator (Hausherr's Machine Works), frozen, placed in petri dishes, and stored at −70°C until identified and tested for arboviruses.

Collected mosquitoes were stored in 15- × 100-mm Petri dishes at −70°C until processed. Female mosquitoes were identified to species using identification keys of Darsie and Ward (1981). During identification, mosquitoes were placed in petri dishes held on dry ice in a minicooler and viewed with a dissecting microscope. Specimens were pooled by species, trap location, and date of collection and stored in sterile cryovials at −70°C until tested. Approximately 5–10% of each mosquito pool was examined for parity status using the tracheation method (Meadows 1968).

Virus isolations and EEE and Saint Louis equine encephalitis (SLE) antigen-capture immunoassays (AC-EIA) were performed with the species-specific mosquito pools following previously published methods (Tsai et al. 1987, Day and Stark 1996, Wozniak et al. 2001). Mosquito pools for virus isolation consisted of 1–100 specimens. If 10–15 pools for virus isolation were obtained, additional pools of 40–50 mosquitoes were used for AC-EIA; one half of the homogenized volume of each pool was used for the EEE AC-EIA and the other half for the SLE AC-EIA.

Serological assays, in addition to Vero cell tissue cultures, were performed primarily to save resources and time in processing and testing the large amount of mosquito specimens collected during our study. Vero cell cultures demonstrating cythopathic effect were confirmed by virus neutralization testing using a panel of mouse hyperimmune ascitic fluids (MHIAFs) containing antibodies against EEE, SLE virus, California encephalitis virus (CE), western equine encephalomyelitis (WEE) virus, and Highlands J virus (HJ). Mosquito pools testing positive by viral AC-EIA were confirmed by antigen-capture inhibition testing using MHIAFs containing antibodies against EEE and SLE (Tsai et al. 1987, Day and Stark 1996, Wozniak et al. 2001). Adults reared from immature stages collected in the field were also tested using Vero cell cultures and AC-EIA using the same criteria as with the main collection. Finally, minimum infection rates (MIR) of arbovirus-positive mosquito species were calculated by mosquito species and month of collection.

A total of 45,051 adult mosquitoes representing 27 species in 9 genera were collected and identified between August 12, 1997, and July 21, 1998, during 130 trap-nights at The Wedge Plantation. Mosquito collections by species are shown in Table 2. *Culex salinarius* Coquillett was the most common species collected and tested, followed by *Ochlerotatus taeniorhynchus* (Wiedemann), *Oc. sollicitans* (Walker), and *An. crucians* complex, respectively. Mosquitoes were most abundant in the months of August 1997, and April, May, and July 1998 (Fig. 1).

Forty-six percent (20,847/45,051) of the mosquitoes were placed into 399 species-specific pools and tested for arboviruses. Additionally, a total of 964 larvae were reared to the adult stage in the laboratory and 90% of both male and female mosquitoes tested for arboviruses. Four species were collected as immatures during our study: *Aedes albopictus* (Skuse) (63%, n = 611), *Cx. restuans* Theobald (29%, n = 259), *Cx. territans* Walker (8%, n = 76), and *Cx. salinarius* (2%, n = 18). None of these larval pools were positive for arboviruses. A total of 2 adult mosquito pools, collected in August 1997 using CDC miniature light traps, were positive for the EEE virus, 1 pool from *Oc. taeniorhynchus* (Wiedemann) (MIR = 0.6/1,000) and another from *Culiseta melanura* (Coquillet) (MIR = 3.8/1,000). Additional pools corresponding to these 2 species tested by AC-EIA were all negative for EEE virus. Parity determination performed in August 1997 for *Oc. taeniorhynchus* specimens showed 66% (n = 37/56) parous and gravid females, whereas insufficient numbers of *Cs. melanura* specimens were obtained to determine parity in August samples.

Mosquito temporal abundance and species composition found at The Wedge Plantation are typical for the southeastern U.S. coastal saltmarsh/swamp habitats. Coastal areas of South Carolina generally support large population densities of *Oc. sollicitans* and *Oc. taeniorhynchus, Cx. salinarius,* and *An. crucians* complex (Mekuria et al. 1994). In fact, these 4 species represented 89% of the total collection in our study. Our data show a peak of abundance between April and May, mostly due to massive *Cx. salinarius* and *An. crucians* complex emergence, and another peak between July and August, mostly from emergent populations of *Oc. taeniorhynchus* and *Oc. sollicitans.* A 22-month survey to determine mosquito species composition and abundance at The Wedge Plantation and adjacent South Santee area reported 90% of their total collection contained all 4 species (Tidwell et al. 1984).

Tissue culture isolates of EEE virus from *Oc. taeniorhynchus* and *Cs. melanura* in August 1997 at The Wedge Plantation represent the first isolation of EEE from these 2 mosquito species in South Carolina. These isolations coincided with a number of human, horse, and bird cases reported throughout the state during the same year. Few studies have reported EEE in birds and mosquitoes in coastal South Carolina. Tidwell et al. (1984) conducted EEE surveillance with domestic chickens and mosquito collections between June and November 1981 at The Wedge Plantation and at a second nearby plantation. Eastern equine encephalomyelitis hemagglutination inhibition (HI) antibody titers of 1:20–1:160 were detected in 4 of 39 (10%) sentinel

Table 2. Adult mosquito species collected at The Wedge Plantation and tested for arboviruses, 1997–98.

Species	Number collected	Percent of total	Number tested	Number of pools	Virus isolation results (MIR)
Aedes					
albopictus	240	0.5	214	4	—
vexans	235	0.5	121	5	—
Anopheles					
crucians	2,812	6.2	1,913	36	—
quadrimaculatus	225	0.5	162	11	—
Coquilletidia					
perturbans	1,958	4.3	1,227	28	—
Culex					
erraticus	442	1.0	346	8	—
nigripalpus	9	>0.1	5	1	—
restuans	18	>0.1	15	2	—
salinarius	20,954	46.5	7,369	113	—
Culiseta					
inornata	3	>0.1	0	0	—
melanura	870	1.9	719	19	1 EEE (3.8/1,000)
Ochlerotatus					
atlanticus	112	0.2	103	6	—
canadensis c.	36	>0.1	17	2	—
fulvus pallens	1	>0.1	0	0	—
hendersoni	8	>0.1	0	0	—
infirmatus	116	0.3	98	4	—
mitchellae	1	>0.1	1	1	—
sollicitans	4,145	9.2	1,920	36	—
taeniorhynchus	12,185	27	6,120	102	1 EEE (0.6/1,000)
triseriatus	6	>0.1	0	0	—
Orthopodomyia					
signifera	1	>0.1	0	0	—
Psorophora					
ciliata	7	>0.1	2	2	—
columbiae	597	1.3	467	12	—
ferox	7	>0.1	5	3	—
howardii	8	>0.1	1	1	—
Uranotaenia					
sapphirina	55	>0.1	22	3	—
Total	45,051	100±	20,847	399	2 EEE

chickens, indicating local EEE transmission. None of the mosquito pools collected during their study, including those collected inside chicken cages, tested positive for EEE in suckling mice or serological assays. The small number of mosquito samples (n = 2,003) and time of collection (mid-September to late November) may have reduced the chance of isolating the virus. Durden et al. (1997) studied the prevalence of wild bird blood parasites and EEE and SLE in 2 southeastern coastal areas, including our study site. Although no EEE was isolated from 218 bird sera, serum neutralizing antibodies to EEE were found in 3% (4/121) of the samples collected. The positive sera were from Carolina wrens (*Thryothorus ludoviciantus*) and northern cardinals (*Cardinalis cardinalis*), which are considered nonmigrating permanent residents in the area.

The occurrence of EEE infection in domestic animals and humans along with the isolation of the virus from *Cs. melanura* and *Oc. taeniorhynchus* suggest that an active transmission cycle was occurring in this region of coastal South Carolina between July and August 1997. During 1997, 15 cases of EEE infection in domestic animals (12 horses, 2 emus, and 1 donkey) were reported in the state. Seven cases occurred between July and August in Colleton, Dorchester, and Charleston counties, all located in the coastal region of South Carolina (Clemson University Veterinary Diagnostic Center, Columbia, SC, surveillance data). Two human cases (one fatal) were reported from Charleston County during the same period. The fatal case, a 76-year-old woman, occurred 5 km south of The Wedge Plantation with an August 7 date of onset. The non-

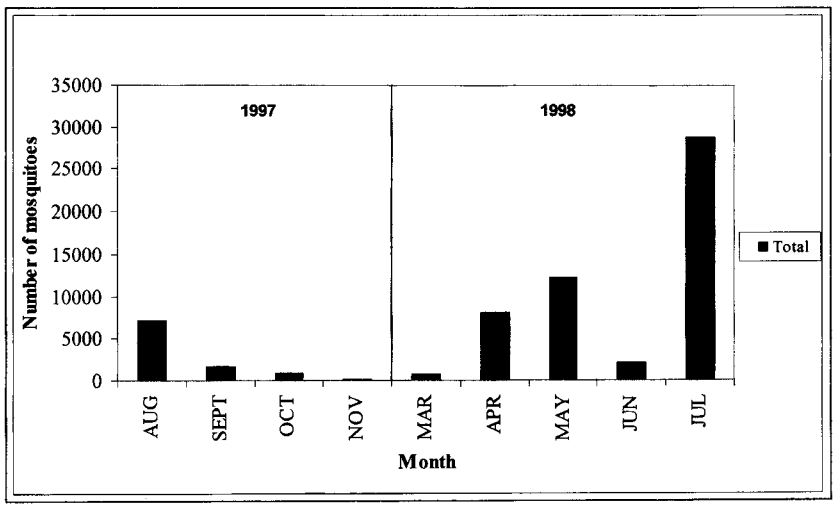

Fig. 1. Seasonal distribution of adult mosquitoes collected at The Wedge Plantation, 1997–98.

fatal case, an 81-year-old man, occurred about 65 km southwest of our study site with a September 17 date of onset (SCDHEC, Reportable Diseases Case Reports, 1997). During our study, EEE was detected in 2 mosquito pools, 1 in *Cs. melanura* and another in *Oc. taeniorhynchus,* both collected on August 12–13, 1997. Arbovirus surveillance in mosquito populations at The Wedge Plantation has been seldom reported. Wozniak et al. (2001) reported EEE in *An. quadrimaculatus* in June 1996 and in *Cs. melanura* in April 1998, both tested by virus AC-EIA. No virus isolation was attempted on these samples.

Culiseta melanura has been widely studied and is considered the primary enzootic vector of EEE in the USA (Moore et al. 1993). Peak monthly collections of *Cs. melanura* at The Wedge Plantation in 1997 and 1998 were in August 1997 (37%) and in April 1998 (53%). In temperate regions, *Cs. melanura* populations have 2 or 3 peaks of adult abundance, typically one each during May–June, July–August, and August–September (Scott and Weaver 1989).

Ochlerotatus taeniorhynchus is probably the most important pest mosquito in coastal South Carolina. In Charleston County, 53.8–79.3% of all mosquitoes collected from 1989 to 1991 were *Oc. sollicitans* and *Oc. taeniorhynchus,* with the latter being the most numerous (Mekuria et al. 1994). *Oclerotatus taeniorhynchus* females are persistent, opportunistic feeders that often migrate in large numbers to areas where they become serious pests (Carpenter and LaCasse 1974, Edman 1985). This species is a good experimental vector of EEE and it has been implicated as a bridging vector in several localities (Karabatzos 1985, Turell et al. 1994). In addition, *Oc. taeniorhynchus* populations in coastal areas of Charleston and Georgetown counties have shown resistance to malathion, a commonly used pesticide in the region (Mekuria et al. 1994).

Although our study included only 12 months of mosquito activity at The Wedge Plantation, it gives additional information about temporal distribution of mosquito vector populations and arbovirus activity in coastal South Carolina. The fact that EEE was never detected before in populations of *Cs. melanura* and *Oc. taeniorhynchus* from EEE endemic areas demonstrates the need for surveillance in South Carolina because little data exists about vector involvement in the local transmission of EEE.

This project was financially supported by the South Carolina Bureau of Laboratories with the collaboration of the University of South Carolina School of Public Health. We thank Dr. Harold Dowda, Dr. Edward O. Oswald, and Dr. Yilma Mekuria for their support and advice during the course of this project and Dr. Nick Karabatsos for his technical assistance. We are grateful to Mr. William Wills, Dr. Jerome Goddard (Mississippi State Department of Health), and Dr. Abelardo C. Moncayo (WHO Center for Tropical Diseases) for kindly reviewing this article.

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