TRANSMISSION OF EASTERN (EEE) AND WESTERN (WEE) ENCEPHALITIS TO BOBWHITE SENTINELS IN RELATION TO DENSITY OF *CULISETA MELANURA* MOSQUITOES

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INTRODUCTION. Eastern (EEE) and western (WEE) equine encephalitis viruses are transmitted within swamps of the Pocomoke River in Maryland by *Culiseta melanura* (Coquillett), a mosquito of fresh water swamps and bogs (Joseph and Bickley, 1969). The density of female *C. melanura* is consistently greater at some swamp sites than at others (Williams, Watts and Reed, 1971). Transmission of these viruses might be expedited in regions where vector density is high, since Hayes, LaMotte and Hess (1960) showed that the incidence of antibody to EEE virus was higher in sentinel chickens kept inside a swamp (65 percent) where *C. melanura* abounded than in chickens kept nearby but outside the swamp (35 percent). In 1968, we investigated the relationship between vector density and transmission of viruses to wild and to sentinel birds. Results using bobwhite quail sentinels are reported here.

MATERIALS AND METHODS. The study area was located at the Pocomoke Cypress Swamp, Worcester County, Maryland. Sites within the swamp were at 50–100 ft from the Pocomoke River (40Q), at 1,320 ft from the river (20Q) and at 2,640 ft from the river (1Q). A fourth site was situated on an upland area less than 20 ft from the swamp but at least 460 ft from any other site (Fig. 1).

Mosquitoes were collected at sites with light traps (Williams, Watts and Reed, 1971) and subsequently examined for virus. Pools of 1–25 mosquitoes were ground in TenBroeck grinders with 1.5 cc of diluent (Eagle's basal medium containing 20 percent heat inactivated fetal bovine serum, bicarbonate and antibiotics). Ground suspensions were centrifuged for 15 minutes at 5,000 x g, and 0.1 ml aliquots of supernatant were inoculated into tubes of primary hamster kidney cell culture (Grand Island Biological Company, N. Y.), which were examined at

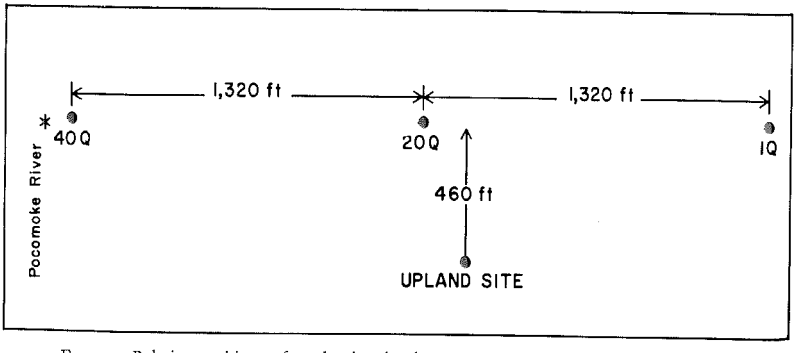

FIG. 1.—Relative positions of study sites in the Pocomoke Cypress Swamp, Maryland.

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least 7 days for cytopathic effect. Isolates were identified by neutralization with specific WEE and EEE rabbit antisera.

Bobwhite quail (*Colinus virginianus*) were purchased when 4–6 weeks old, were maintained in the laboratory until 2–2½ months of age, and were sampled for pre-existing neutralizing antibody prior to use. Two cages of 10 quail were placed at each study site in early June 1968. Alternate cages of birds were bled each week thereafter, and blood plasmas were used in tube-neutralization tests for EEE and WEE antibodies by the procedure described by Williams *et al.* (1971).

RESULTS. Virus isolation was attempted from all species of mosquitoes collected (Table 1), but virus was recovered only from female *C. melanura*. First isolations were from *C. melanura* collected on 29 July 1968 (WEE virus) and on 6 August 1968 (EEE virus). The incidence of infection in *C. melanura* was similar for both viruses during early August (Table 2), after which EEE infection was greater than WEE infection. Periods of high mosquito infection were 29 July–13 August for WEE and 12 August–3 September for EEE, but infected mosquitoes were found up to 23 October in 1968. Assuming one infected mosquito per pool (1–25 mosquitoes), virus infection in *C. melanura* between 29 July–23 October averaged one in 844 mosquitoes for WEE, one in 516 mosquitoes for EEE, and one in 320 mosquitoes for both viruses taken together. EEE and WEE were never isolated from the same mosquito pool, but we do not know if the isolation method used was capable of detecting both viruses simultaneously. Reisolation from original pools was accomplished for 17 of 18 EEE virus isolates and for 9 of 11 WEE virus isolates.

Most isolations of EEE and of WEE were made at 1Q and 20Q, sites of high *C. melanura* density. In average terms of mosquitoes collected per isolation, infection with EEE virus was more prevalent at sites of high vector density, whereas infection with WEE virus was not related to site densities of *C. melanura* (Table 3).

In the sentinel bobwhite quail, WEE antibody appeared simultaneously at all study sites on 29 July, whereas EEE antibody first appeared deep in the

TABLE 1.—Female mosquitoes used for virus isolation.

	Number	
	23 April–22 July	29 July–23 Oct.
Culiseta melanura	5,848	9,495
Other species:		
Aedes atlanticus	12	9
canadensis	591	72
cantator	781	31
sollicitans	7	10
taeniorhynchus	1	7
triseriatus	3	2
vexans	49	15
Anopheles bradleyi-crucians	47	61
punctipennis	9	12
quadrimaculatus	1	3
Culex salinarius	185	50
territans	3	2
Mansonia perturbans	1	8
Uranotaenia sapphirina	0	13
	1,690	295

TABLE 2.—Virus infection in *Culiseta melanura*.

Period	Pools Tested	Number of Isolates WEE	Number of Isolates EEE	Number of Mosquitoes Tested	Infection Rate WEE	Infection Rate EEE
1 May–9 July	140	0	0	2,723	0	0
14–15 July	111	0	0	2,718	0	0
21–22 July	20	0	0	407	0	0
29 July	15	2	0	302	1:151	0
5–6 Aug.	30	1	1	730	1:730	1:730
12–13 Aug.	26	2	2	611	1:306	1:306
19 Aug.	38	1	2	923	1:923	1:462
26–28 Aug.	42	1	3	881	1:881	1:294
2–3 Sept.	85	1	5	1,929	1:1,929	1:386
9–10 Sept.	59	1	2	1,372	1:1,372	1:686
16 Sept.–23 Oct.	137	2	3	2,538	1:1,269	1:846
29 Oct.–18 Nov.	13	0	0	209	0	0

swamp (1Q) on 6 August, but not until 14 August at 20Q and upland or 20 August at the river (40Q) (Fig. 2). Most sentinels produced antibodies by 4 September. EEE antibody appeared rapidly at 1Q, whereas the appearance of EEE antibody lagged at 40Q.

DISCUSSION. Antibody was detected in sentinel quail at the time infected *C. melanura* mosquitoes first appeared (29 July for WEE; 6 August for EEE). However, an isolation of WEE virus was made from the blood of a sentinel English sparrow on 22 July 1968 in a concurrent study (Williams, *et al.*, 1971). Since neutralizing antibody may appear in bobwhite 6 days after infection (Watts and Williams, 1972), transmission probably started on or before 16 July for WEE and 31 July for EEE. Peak summer densities of *C. melanura* mosquitoes occurred approximately 16 July 1968 (Williams, Watts and Reed, 1971). Therefore, transmission of WEE virus probably started when vector density in the study area was very high. The simultaneous appearance of WEE antibody at all sites may have resulted from dispersal of WEE virus via the feeding or flight activities of so many mosquitoes. In contrast, EEE virus transmission probably began when there were fewer *C. melanura* in the swamp, and a longer period of time elapsed before EEE virus could be introduced from the deep swamp (near 1Q) into border areas via dispersing mosquitoes or birds.

This study demonstrates that transmission of viruses is indeed rapid in swamp habitats where vector density is high. However, transmission rates in some circumstances (i.e., EEE on the upland; WEE on the upland and at 40Q) could

TABLE 3.—Virus isolations from *Culiseta melanura* by site.

	Site			
	1Q	20Q	40Q	Upland
Number of mosquitoes collected 29 July–23 Oct.	4,852	2,911	1,129	429
Number of isolations				
WEE virus	3	5	1	2
EEE virus	9	8	1	0
Infection rate (mosquitoes per isolation)				
WEE virus	1:1,617	1:582	1:1,129	1:215
EEE virus	1:539	1:364	1:1,129	....

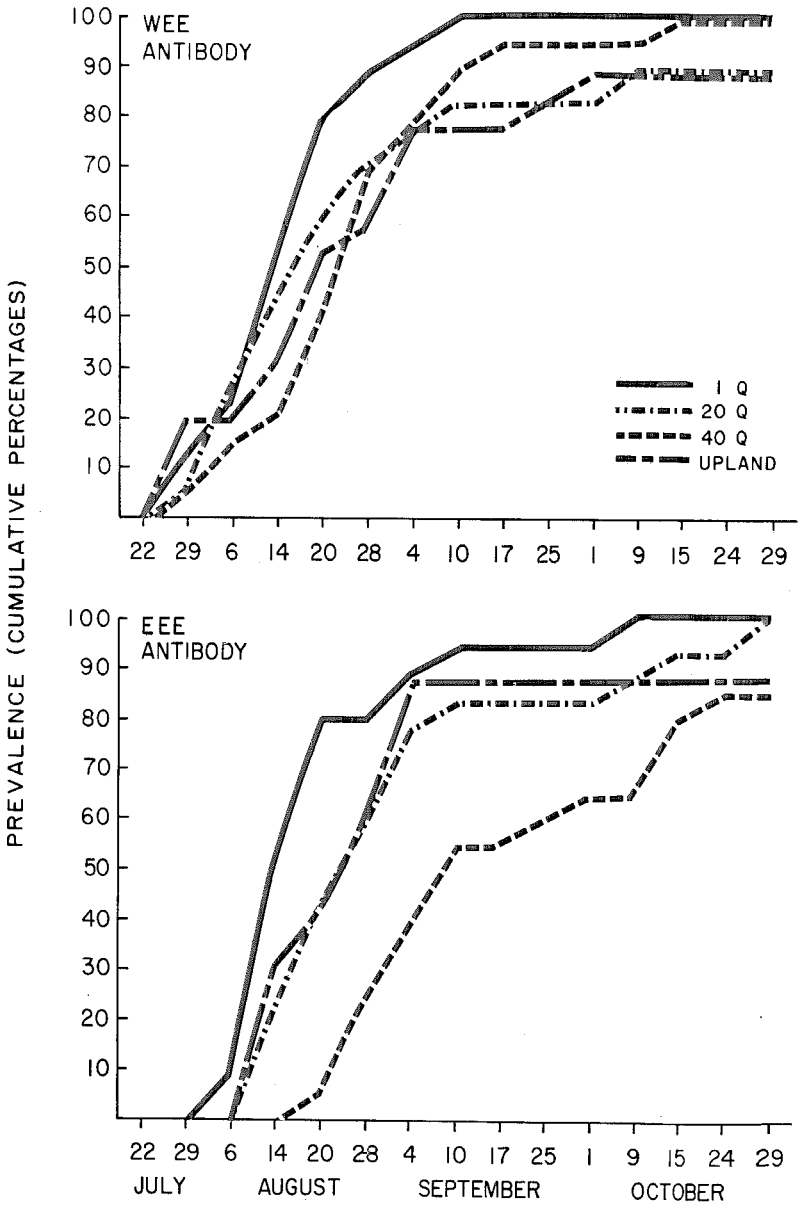

FIG. 2.—Appearance of neutralizing antibodies in sentinel bobwhite.

not be explained in terms of overall vector density or by the prevalence of virus-infected mosquitoes. A considerable number of wild birds were kept on the upland as sentinels, including English sparrows that can have long term and high level viremias (Williams, *et al.*, 1971). Possibly the wild-bird sentinels increased the potential for virus transmission to quail caged on the upland by providing the few *C. melanura* present there with a copious supply of virus.

SUMMARY. The relationship between overall density of vector mosquitoes and transmission of EEE and WEE viruses to sentinel bobwhite quail was studied in the Pocomoke Cypress Swamp, Maryland, and on an upland border of the swamp, in 1968. *Culiseta melanura* was the only mosquito found infected with the viruses. First isolations came from mosquitoes collected on 29 July (WEE) and on 6 August (EEE), and the corresponding neutralizing antibodies appeared in sentinel quail on those dates. WEE antibody was detected simultaneously at all sites, regardless of vector density, whereas EEE antibody occurred first where *C. melanura* typically occurred at high density. Probably transmission of WEE virus began when vector density was high throughout the region, while transmission of EEE virus began later when vector density was low at most sites.

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