Experimental Transmission of Eastern Equine Encephalitis Virus by Strains of *Aedes albopictus* and *A. taeniorhynchus* (Diptera: Culicidae)

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ABSTRACT The vector competence of *Aedes taeniorhynchus* (Wiedemann) and four strains of *Aedes albopictus* (Skuse) was assessed for eastern equine encephalitis (EEE) virus isolated from *Ae. albopictus* collected in Polk County, Florida. Both species became infected with and transmitted EEE virus by bite after feeding on 1-d-old chicks that had been inoculated with EEE virus (viremia = $10^{10.1}$ plaque-forming units [PFU] per ml of blood). However, when fed on an older chick with a lower viremia (viremia = $10^{6.1}$ PFU per ml of blood), *Ae. albopictus* was significantly more susceptible to infection (90%, n = 61) than was *Ae. taeniorhynchus* (15%, n = 40). Transmission was also significantly more efficient by *Ae. albopictus* (36%, n = 44), than by *Ae. taeniorhynchus* (0%, n = 14). These data, combined with the recent isolation of EEE virus from *Ae. albopictus* and its opportunistic feeding behavior, indicate that *Ae. albopictus* could function as a bridge vector between the enzootic *Culiseta melanura* (Coq.)-avian cycle and susceptible mammalian hosts.

KEY WORDS *Aedes albopictus*, vector competence, eastern equine encephalitis virus

THE RECENT ISOLATION of eastern equine encephalitis (EEE) virus from *Aedes albopictus* (Skuse) collected in Polk County, Florida (Mitchell et al. 1992) has raised concern that this species may serve as a vector for this virus. Along the east and gulf coasts of the United States, EEE virus is maintained in an enzootic transmission cycle between *Culiseta melanura* (Coq.) and wild birds (principally passerines). Various mosquito species act as bridge vectors and are capable of transmitting EEE virus from infected birds to secondary hosts such as horses and humans (Scott & Weaver 1989). Infection in these secondary hosts may result in serious disease or death.

Laboratory studies have demonstrated that a strain of *Ae. albopictus*, derived from specimens collected in Houston, TX, was susceptible to infection with and able to transmit EEE virus by bite (Scott et al. 1990) and that a strain derived specimens collected in Lake Charles, LA, was highly susceptible to infection with EEE virus (Mitchell et al. 1993). However, numerous studies have demonstrated that geographic populations of *Ae. albopictus* can vary widely in their susceptibility to a variety of alphaviruses (Tesh et al. 1976, Beaman & Turell 1991, Turell & Beaman 1992, Turell et al. 1992). We therefore evaluated populations of *Ae. albopictus* derived from specimens collected at the same site as the specimens from which EEE virus was isolated for their ability to transmit EEE virus. We also evaluated the susceptibility of *Ae. taeniorhynchus* (Wiedemann), a species implicated as a potential bridge vector (Karabatsos 1985) as well as strains of *Ae. albopictus* previously shown to vary in their susceptiblitly to infections with other alphaviruses.

Materials and Methods

Mosquitoes. Four strains of *Ae. albopictus* were evaluated for their ability to transmit EEE virus. These included the POLK II and POLK X strains, derived from specimens collected in 1989 and 1992, respectively, from the same tire pile from which 14 EEE virus-infected *Ae. albopictus* were collected in 1991 (Mitchell et al. 1992). We also used the HOUSTON strain, derived from specimens collected in 1985 in the vicinity of Houston, TX; and the GENTILLY strain, derived from specimens collected in 1988 in the Gentilly suburb of New Orleans, LA. The former three strains were provided by G. B. Craig Jr., University of Notre Dame; whereas the GENTILLY strain was provided by J. Freier, Centers for Disease Control. The long-colonized

In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the U.S. Department of Health and Human Services, National Institute of Health.

VERO BEACH strain of *Ae. taeniorhynchus* was used for comparison.

Mosquitoes were maintained at 26°C as described by Gargan et al. (1983); female mosquitoes were 4–10 d old when used for infection trials.

Virus and Virus Assay. The FL91-4679 strain of EEE virus, isolated from *Ae. albopictus* collected in Polk County, Florida (Mitchell et al. 1992), was passaged three times in Vero cells before its use in these studies.

Serial 10-fold dilutions of specimens (chicken blood or triturated mosquito suspensions) were tested for infectious virus by plaque assay on Vero cell monolayers as described by Gargan et al. (1983), except that the second overlay, containing neutral red, was added 2 d later (rather than 4 d).

Determination of Vector Competence. Mosquitoes were allowed to feed on a chick (*Gallus gallus*) that had been inoculated intraperitoneally 24 or 48 h earlier with 0.1 ml of a suspension containing $10^{4.5}$ plaque-forming units (PFU) of EEE virus. As soon as most of the mosquitoes in a cage had completed feeding (≈20 min), the chick was transferred to a second cage containing a different strain of mosquitoes. This was repeated until up to four strains of mosquitoes had fed on the same chick. Immediately after mosquito feeding, a 0.2-ml blood sample was obtained from the jugular vein of each bird and diluted in 1.8 ml of diluent (10% fetal bovine serum in Medium 199 with Hanks' salts, $NaHCO_3$, and antibiotics) plus 10 units of heparin per ml to determine the viremia at the time of mosquito feeding. In addition, three to six engorged mosquitoes from each chick were triturated individually in 1 ml of diluent, frozen at −70° C, then thawed and assayed on Vero cell monolayers to determine the amount of virus ingested. The remaining engorged mosquitoes were transferred to 3.8-liter cardboard containers with netting on one end. Apple slices or a 7% sucrose solution was provided as a carbohydrate source, and an oviposition substrate was added 4 d after the infectious blood meal.

To determine transmission rates, mosquitoes were allowed to feed individually on susceptible 1- to 2-d-old chicks 10 and 11 d after the infectious blood meal (for mosquitoes that ingested $10^{10.1}$ PFU/ml) or 14 and 15 d after the infectious blood meal (for those that ingested ≤$10^{8.4}$ PFU/ml). Immediately after each transmission trial, mosquitoes were triturated individually in 1 ml of diluent and frozen at −70°C until tested for virus. Each chick that had been fed on by a mosquito was bled from the jugular vein 1 d after the transmission attempt as described above, except that a 0.1 ml sample of blood was diluted in 0.9 ml of diluent plus heparin.

Table 1. Viremia levels by age of chickens when inoculated with $10^{4.5}$ PFU of EEE virus

Age at inoculation	Days after inoculation	No. tested	Mean $\log_{10}$ PFU/ml (range)
≤1 d[a]	1	4	9.9 (9.6–10.1)
3 d[a]	1	5	8.4 (7.3– 9.1)
5–6 d	1	10	8.1 (7.2– 8.7)
5–6 d	2	9	6.8 (6.1– 7.7)

[a] All chicks were dead by 2 d after inoculation.

Results

Viremia levels in the donor chicks depended on their age at the time of infection (Table 1). Among chicks used to expose mosquitoes to EEE virus, ≤1-d-old chicks produced a high viremia (mean = $10^{10.1}$ PFU/ml), whereas 6-d-old chicks produced viremias of $10^{6.1}$ and $10^{8.4}$ PFU/ml in the two trials, respectively. Mosquitoes ingested an average of $10^{2.5}$ PFU less than the viremia titer. All of the chicks that were ≤3 d old at the time of viral inoculation were dead <48 h after inoculation, including those that had not been bled.

All strains of mosquito tested were highly susceptible to infection at the high ($10^{10.1}$) viral dose (Table 2). However, at either of the lower viral doses, each of the *Ae. albopictus* strains was more susceptible ($\chi^2 \geq 7.4$, df = 1, $P \leq 0.007$) to infection with EEE virus than was *Ae. taeniorhynchus*. There was no significant difference in susceptibility among the various *Ae. albopictus* strains at any of the doses tested.

Similarly, all strains tested at the high viral dose transmitted virus by bite (Table 3). However, although transmission rates for *Ae. albopictus* strains did not differ ($\chi^2 = 3.8$, df = 2, $P = 0.15$) by infectious dose from the overall transmission rate (40%, $n = 213$), the ability of *Ae. taeniorhynchus* to transmit EEE virus was related directly to the dose ingested. At the two lower viral doses ($10^{6.1}$ and $10^{8.4}$ PFU/ml), *Ae. taeniorhynchus* was a less efficient vector (Fisher's exact test, $P = 0.005$; $\chi^2 = 3.6$, df = 1, $P =$

Table 2. Susceptibility of selected strains of mosquitoes to infection with EEE virus, by dose ingested

Species	LOG_{10} PFU/ml of blood[a]		
	6.1[b]	8.4[b]	10.1[b]
Ae. albopictus			
POLK II	95 (22)	100 (15)	100 (66)
POLK X	87 (39)	100 (45)	NT
GENTILLY	NT	100 (70)	100 (34)
HOUSTON	NT	100 (33)	100 (36)
Total	90 (61)	100 (163)	100 (136)
Ae. taeniorhynchus	15 (40)	58 (45)	99 (97)

[a] Viremia level in donor chick.
[b] Percent infected (no. tested), NT = not tested.

Table 3. Transmission of virus by mosquitoes orally exposed to EEE virus, by dose ingested

Species	LOG_{10} PFU/ml of blood[a]		
	6.1[b]	8.4[b]	10.1[b]
Ae. albopictus			
POLK II	18 (17)	42 (12)	37 (43)
POLK X	48 (27)	52 (25)	NT
GENTILLY	NT	48 (33)	23 (22)
HOUSTON	NT	45 (11)	39 (23)
Total	36 (44)	48 (81)	34 (88)
Ae. taeniorhynchus	0 (14)	19 (16)	53 (57)

[a] Viremia level in donor chick.
[b] Percent transmitting (no. fed), NT = not tested.

0.058, for the two doses, respectively) than was *Ae. albopictus*.

To test for transovarial transmission, female *Ae. albopictus* were inoculated intrathoracically (Rosen & Gubler 1974) with EEE virus and allowed to feed on a hamster 7 d later. Eggs (second ovarian cycle) obtained after a blood meal 15 d after inoculation were allowed to hatch and mature to the adult stage. The mosquitoes were separated according to sex and placed in pools of up to 25 mosquitoes each. Pools were triturated in 2 ml of diluent and assayed as described above. No virus was recovered from 1,295 F_1 progeny (630 males, 665 females).

Discussion

All strains of *Ae. albopictus* were highly susceptible to infection with EEE virus, and 40% of the orally exposed mosquitoes transmitted virus by bite when allowed to refeed 10–15 d later. These results are similar to those reported by Scott et al. (1990), who reported a 40% (n = 15) transmission rate for the HOUSTON strain tested 8–15 d after feeding on a chick with a viremia of $10^{8.7}$ PFU/ml, and indicate that *Ae. albopictus* should be considered a good to excellent vector of EEE virus (Chamberlain et al. 1954). Although there were no significant differences in vector competence among the four strains tested in our study, each of these strains was more susceptible to infection and a more efficient transmitter of EEE virus than was *Ae. taeniorhynchus* when exposed to viremias $\leq 10^{8.4}$ PFU/ml. The 90% infection rate among *Ae. albopictus* that fed on a chick with a viremia of $10^{6.1}$ PFU/ml in our study is consistent with an ID_{50} of $10^{5.1}$ PFU/ml reported by Mitchell et al. (1993) for this species feeding on viremic snowy egrets, *Egretta thula* (Thayer and Bangs).

Various studies have shown that *Ae. albopictus* is an opportunistic feeder (Tempelis et al. 1970, Sullivan et al. 1971), and in a recent study in North America, 21% of identified blood meals were obtained from avian hosts and 79% from mammalian hosts, including humans (Savage et al. 1993). Because *Ae. albopictus* is an opportunistic feeder, this species could serve as a bridge vector (i.e., mosquitoes could be infected while feeding on an infected avian host and then transmit virus to a mammalian host).

Since first reported in Houston, TX, in 1985, *Ae. albopictus* has increased its range to include most of the southeastern United States. Within this area, *Ae. albopictus* has displaced *Ae. aegypti* (L.) on several occasions (Hobbs et al. 1991, Rai 1991, O'Meara et al. 1992). The continued expansion of its range and prevalence within that range and its susceptibility to many viruses currently transmitted in North and South America increases the risk that *Ae. albopictus* may become involved in the transmission of viruses in the Americas. Various studies indicate that this species is a competent laboratory vector of numerous arboviruses, including both those native and exotic in North America (Shroyer 1986, Mitchell 1991).

Thus, the results of our study, combined with the recent isolation of EEE virus from *Ae. albopictus*, its opportunistic feeding behavior, and continued expansion within the southeastern United States suggest that *Ae. albopictus* could function as a bridge vector between the enzootic *Cs. melanura*-avian cycle and susceptible mammalian hosts.

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