Experimental Transmission of Venezuelan Equine Encephalomyelitis Virus by a Strain of Aedes albopictus (Diptera: Culicidae) from New Orleans, Louisiana

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ABSTRACT

Experimental studies were undertaken to ascertain the vector competence of a strain of Aedes albopictus (Skuse) collected in New Orleans, LA, Gentilly strain, for an epizootic (Trinidad donkey) strain of Venezuelan equine encephalomyelitis (VEE) virus. This strain of Aedes albopictus was significantly more susceptible to infection with VEE virus than were any of the four strains tested previously, including two from North America and two from South America. Likewise, dissemination (44 of 180, 24.4%) and transmission (40 of 88, 45.5%) rates were significantly higher in the Gentilly strain than in any of the strains previously tested. Analysis of the results of the present study along with those of a previous study with a second alphavirus, chikungunya (CHIK) virus, indicated that, although all three strains of Aedes albopictus tested were more susceptible to VEE virus than to CHIK virus, susceptibility to infection and dissemination with one alphavirus appeared to be directly related to susceptibility to infection and dissemination with the other virus and may indicate shared receptor sites for these two alphaviruses in Aedes albopictus.

KEY WORDS

Insecta, Aedes albopictus, vector competence, Venezuelan equine encephalomyelitis virus

The recent introduction of Aedes albopictus (Skuse) into the Americas has raised a concern that these mosquitoes may serve as a vector for indigenous as well as exotic viruses (Knudson 1986). Laboratory studies have demonstrated the ability of this species to transmit numerous arboviruses, including some native to the Americas (Shrover 1986, Hawley 1988). In addition, Aedes albopictus has displaced populations of Aedes aegypti (L.) in the southern United States (Rai 1991) and has demonstrated an ability to flourish in tree holes as well as in artificial containers (Hawley 1988).

Venezuelan equine encephalomyelitis (VEE) virus, of the genus Alphavirus, family Togaviridae, has caused sporadic epizootics of severe disease. This disease occurs primarily in Central America, and infection is usually fatal in horses and occasionally so in man (Walton & Grayson 1989). Epizootics have occurred as far south as Ecuador and Peru in northern South America and as far north as southern Texas in 1969–1972.

A comparison of several North and South American strains of Aedes albopictus for their ability to transmit VEE virus under laboratory conditions indicated that, although all strains tested were competent vectors, none was a particularly efficient vector. North American strains, however, were significantly less efficient vectors of VEE virus than were South American strains, with transmission rates (after oral exposure) of 3 and 24% for the North and South American strains, respectively (Beaman & Turell 1991). However, a recent evaluation of 10 strains of Aedes albopictus for their susceptibility to infection with chikungunya (CHIK) virus, another member of the genus Alphavirus, indicated that a strain from New Orleans, LA (Gentilly strain), was the most susceptible (Turell et al. 1992a). Therefore, we evaluated the potential for this Aedes albopictus strain to transmit VEE virus. We also compared the relative susceptibility of selected strains of Aedes albopictus for VEE and CHIK viruses to determine if susceptibility to one alphavirus was related to susceptibility to a second alphavirus.

Materials and Methods

Mosquitoes. The Gentilly strain of Aedes albopictus, obtained from J. Freier, Centers for Disease Control, was derived from specimens collected...
Table 1. Infection, dissemination, and transmission rates by day of extrinsic incubation in the Gentilly strain of *Ae. albopictus* after ingestion of 10^4.5 PFU of Venezuelan equine encephalomyelitis virus (combined data from two infectious feeding trials)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Day of extrinsic incubation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Number tested</td>
<td>40</td>
</tr>
<tr>
<td>Infection rate</td>
<td>78%</td>
</tr>
<tr>
<td>Dissemination rate</td>
<td>73%</td>
</tr>
<tr>
<td>Dissemination (I)a</td>
<td>94%</td>
</tr>
<tr>
<td>Transmission rate, %c</td>
<td>50 (8)</td>
</tr>
</tbody>
</table>

a Percentage of all mosquitoes with virus in their legs.

b Percentage of infected mosquitoes with virus in their legs.

c Percentage transmitting (total number feeding, including uninfected mosquitoes).

in the Gentilly suburb of New Orleans, LA. They were used in the F_7_ generation of colonization. Mosquitoes were maintained at 26°C using procedures described by Gargan et al. (1983); female mosquitoes were 4–10 d old when used for infection trials.

**Virus and Virus Assay.** A second BHK cell culture passage of an infectious clone (V3000) of the epizootic VEE subtype IA Trinidad donkey strain (Davis et al. 1989) was used throughout these studies. This clone is biologically similar to the parent Trinidad donkey strain and has similar pathogenicity in mice, hamsters, and guinea pigs (Davis et al. 1991).

Serial 10-fold dilutions of specimens 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 (rather than 4) d later.

**Determination of Vector Competence.** Mosquitoes were allowed to feed on one of two anesthetized female Syrian hamsters that had been inoculated intraperitoneally 48 h earlier with 0.2 ml of a suspension containing 10^3.5 plaque-forming units (PFU) of VEE virus. Immediately after feeding, three engorged mosquitoes from each hamster were individually triturated in 1 ml of diluent (10% fetal bovine serum in Medium 199 with Hanks’ salts and antibiotics), frozen at −70°C, then thawed and assayed on Vero cell monolayers to determine the amount of virus ingested. The remaining engorged mosquitoes were placed in two 3.8-liter cardboard containers (one per hamster) 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. At 7-d intervals after the infectious blood meal, transmission attempts were made by allowing a sample of mosquitoes to feed on susceptible hamsters. On the day 7 trial, mosquitoes were tested either individually or in pools of three mosquitoes each, whereas in all other trials (days 14, 21, 28, and 35), all mosquitoes were tested individually. Immediately after each transmission trial, mosquitoes were cold-anesthetized, their legs and bodies triturated separately in 1 ml of diluent, and frozen at −70°C. Infection was determined by the recovery of virus from the mosquito body tissue samples at ≥7 d after the infectious blood meal. If virus was recovered from both body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). Because infection with VEE virus is virtually 100% fatal for hamsters, hamster death was used as the criterion for viral transmission. Isolation of virus from liver or brain tissue samples, or both, verified transmission. Any hamster that survived 21 d after being fed upon by a mosquito with a disseminated infection was challenged with 10^3.5 PFU of VEE virus to determine its immune status.

**Results**

Mosquitoes ingested an average of 10^4.5 PFU of VEE virus during each of the two infectious feedings. Because infection, dissemination, and transmission results were nearly identical for the two feedings, these data were combined for further analysis and are presented in Table 1. The Gentilly strain of *Ae. albopictus* was highly susceptible to infection with VEE virus, and infection rates at each of the time intervals tested did not differ significantly (χ^2 = 5.2, df = 4, P = 0.26) from the mean of 85% (153 of 180). Dissemination rates were also high, with 97% of the infected mosquitoes having a disseminated infection. Again, there was no significant (Fisher’s exact test, P = 0.57) association between virus dissemination and period of extrinsic incubation for specimens tested at 7–35 d after virus exposure. Likewise, no consistent difference in transmission rates was observed by time after the infectious blood meal (days 7–35) (χ^2 = 7.1, df = 4, P = 0.13). Overall, 40 (45%) of 88 refeeding mosquitoes transmitted VEE virus.

**Discussion**

The Gentilly strain of *Ae. albopictus* was highly susceptible to infection with VEE virus, and 45% (40 of 88) of the orally exposed mosqui-
Arboviruses. For example, the Houston strain was more susceptible to all four serotypes of dengue viruses than were Brazilian strains of *Ae. albopictus* (Miller & Ballinger 1988).

The Gentilly strain was significantly more susceptible to infection with both VEE and CHIK viruses than were any of five other American strains of *Ae. albopictus* tested (Beaman & Turell 1991, Turell et al. 1992a). In addition, strains of *Ae. albopictus* collected in the Gentilly area of New Orleans differed significantly in photosensitivity, susceptibility to freezing, and in their susceptibility to infection with *Dirosa* *sapidissima* from *Ae. albopictus* collected elsewhere (G. Craig & G. Scales, unpublished data). Although these differences may be the result of founder effects during the dispersal of *Ae. albopictus* across the United States, they are also indicative of multiple introductions. Multiple introductions of this species have almost certainly occurred, as evidenced by the interdiction of 11 tires infested with *Ae. albopictus* (Craven et al. 1988). Based on their study, they estimated that >2,000 tires containing *Ae. albopictus* larvae were imported into the United States from Asia alone during 1985–1986. Regardless of whether the Gentilly strain represents a separate importation of a potentially more efficient vector or merely the product of a founder effect, the great susceptibility of this strain to two alphaviruses increases the risk of transmission of these arboviruses by *Ae. albopictus* within the United States.

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