Vector Competence of Culex (Melanoconion) taeniopus for Equine-Virulent Subtype IE Strains of Venezuelan Equine Encephalitis Virus

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Abstract. The mosquito Culex (Melanoconion) taeniopus is a proven vector of enzootic Venezuelan equine encephalitis virus (VEEV) subtype IE in Central America. It has been shown to be highly susceptible to infection by this subtype, and conversely to be highly refractory to infection by other VEEV subtypes. During the 1990s in southern coastal Mexico, two VEE epizootics in horses were attributed to subtype IE VEEV. These outbreaks were associated with VEEV strains with an altered infection phenotype for the epizootic mosquito vector, Aedes (Ochlerotatus) taeniopyrhynus. To determine the infectivity for the enzootic vector, Culex taeniopus, mosquitoes from a recently established colony were orally exposed to VEEV strains from the outbreak. The equine-virulent strains exhibited high infectivity and transmission potential comparable to a traditional enzootic subtype IE VEEV strain. Thus, subtype IE VEEV strains in Chiapas are able to efficiently infect enzootic and epizootic vectors and cause morbidity and mortality in horses.

INTRODUCTION

Venezuelan equine encephalitis virus (VEEV) is an emerging pathogen in the American tropics and has been associated with multiple outbreaks of equine and human disease.1 Venezuelan equine encephalitis virus is a member of the genus Alphavirus (family Togaviridae) and is transmitted by mosquitoes. Strains of VEEV are classified as either enzootic, meaning they have not been associated with equine disease, or epizootic, meaning they are known to cause severe enzootic hemorrhagic fever in equids. Enzootic strains typically circulate among rodents in forest or swamp habitats. Epizootic strains, typically belonging to subtypes IAB and IC, circulate among equine amplification hosts in agricultural habitats, but are not known to persist in permanent foci. Enzootic and epizootic VEEV strains also categorically use mosquitoes from different species as vectors. Enzootic strains are believed to be transmitted almost exclusively by mosquitoes of the Spissipes section of the subgenus Melanoconion within the genus Culex.2 Subtype IE VEEV strains have historically been considered enzootic and the mosquito Culex (Melanoconion) taeniopus is the proven enzootic vector for enzootic strains of this subtype.3,5

In 1993, an outbreak of equine disease occurred in Chiapas, the southernmost state in Mexico that borders Guatemala. It was subsequently found that this outbreak was caused by subtype IE VEEV, which was not previously known to be virulent for horses.6 Experimental vector studies showed that virus strains isolated during the outbreak were better able to infect the proven epizootic vector, Aedes (Ochlerotatus) taeniopyrhynus, than were older enzootic subtype IE VEEV strains, prompting the hypothesis that the outbreak was the result of adaptation to a new vector.7

Typical epizootic VEEV strains of subtypes IAB and IC are efficiently transmitted by the epizootic vector Ae. taeniopyrhynus, but not by Cx. taeniopus.8–10 If subtype IE VEEV had adapted to use the former epizootic mosquito vector, would there be a corresponding loss of fitness observed in the enzootic mosquito vector? Because the equine-virulent IE strains were found to be more closely related, genetically, to enzootic IE strains than to epizootic subtypes IAB or IC, we hypothesized that the new, equine-virulent subtype IE VEEV strains would still be able to efficiently infect and be transmitted by Cx. taeniopus mosquitoes.1,11

To test this hypothesis, we established a colony of Cx. taeniopus from adult females collected in the epizootic region of Chiapas State, Mexico, and performed experimental infections using enzootic and equine-virulent subtype IE VEEV strains.

METHODS

Colonization of Culex (Melanoconion) taeniopus. Previously published laboratory colonization of Culex (Melanoconion) taeniopus guided our colonization process.12 Adult, female Cx. taeniopus were collected in hamster-baited, modified Trinidad-10 traps from a mangrove forest (15.4521N, 093.1461W) over the course of two days in June 2007.7 Engorged, adult females were placed into a 45.7-cm³ cage covered with wet towels and containing a terracotta clay flowerpot with holes drilled in the sides and placed upside down in a dish of water. Cotton balls saturated with 10% sucrose were placed daily on the mesh top of the cage as a carbohydrate source. The temperature ranged from 31°C during the day to 26°C during the night, humidity was maintained between 65% and 75% and natural light entered through windows. Because Cx. taeniopus females typically require two blood meals before laying egg rafts, a hamster was offered for one hour each evening.12 Neither this hamster nor the four hamsters used as bait became ill, which led to the conclusion that no VEEV-infected mosquitoes were present in the colony. All hamster use followed protocols reviewed and approved by the University of Texas Medical Branch Institutional Animal Care and Use Committee.

Egg rafts were placed individually into plastic cups with purified drinking water and several drops of food slurry (10% suspension in water of 1:1 active dry bakers yeast and pulverized rabbit chow). Upon hatching, larvae were given five drops food slurry every day until day three days post-hatch when they were transferred to rectangular (14 × 26 × 4 cm) plastic food storage containers and fed 1.0 mL of food slurry every 2 days.

For transport from Mexico, adults were packaged into 0.47-liter cardboard containers with mesh lids and larvae were placed into Whirl-Pack® bags (Nasco, Fort Atkinson, WI) with...
clean water and approximately 1 mL of food slurry. Cardboard containers and Whirl-Pack® bags were then placed into individual polystyrene boxes for insulation and packaged carefully into an insulated suitcase to be transported back to the insectary at the University of Texas Medical Branch for colonization as per Dziem and Cupp. Conditions in the insectary were designed to simulate the natural habitat: ambient temperature was maintained at 27°C, relative humidity was maintained at approximately 80%, and hour-long crepuscular periods were simulated at dusk and dawn with incandescent light bulbs. Mosquitoes used in these experiments are from estimated F₁₅–F₂₀ generations.

**Viruses and infection.** Five strains of VEEV were used for experimental infection of *C. taeniopus* (Table 1). Three strains of subtype IE VEEV isolated during or after the 1993 outbreak, with known or suspected equine virulence, were used: Mx01-22, CPA152, and CPA201. Strain Mx01-22 was isolated in 2001 from the heart of a sentinel hamster in coastal Chiapas and was passaged twice (once in C6/36 cells and once in Vero cells) before being used in this study. This strain has not yet been evaluated in horses, but it has been shown to be virtually identical, genetically, to other equine-virulent strains. Strain CPA152 was isolated in 1996 from the blood of a diseased horse in Oaxaca, Mexico, and was passaged three times (once in suckling mouse brain, once in rabbit kidney cells, and once in Vero cells) before being used in this study. Strain CPA201 was isolated in 1993 from the brain of a dead horse in Chiapas, Mexico and was passed three times (once in suckling mouse brain, once in rabbit kidney cells and once in baby hamster kidney cells) before being used in this study.

The enzootic subtype IE VEEV strain 68U201 and the epizootic subtype IAB VEEV strain Trinidad Donkey were included in the study as historical controls. Strain 68U201 was isolated from a sentinel hamster in 1968 in Guatemala and was passed once in suckling mouse brain and twice in baby hamster kidney cells before an infectious cDNA clone was created. The virus used in this study was derived from the infectious clone. The Trinidad Donkey strain was isolated from a donkey in Trinidad in 1930. It was passed at least seven times before being used in this study.

Viremia levels were estimated from previously published viremia curves so that desired blood meal titers could be obtained by infecting mice at selected times before the mosquito feeding attempt. Adult Swiss-Webster mice were anesthetized by isoflurane inhalation, then inoculated with an estimated F₁₅–F₂₀ generations.

**Virus assays.** Virus titers from mouse plasma were determined separately for each mosquito by a cytopathic effect assay. Virus detection in mosquito bodies, legs/wings, and saliva was considered a measure of infection, dissemination, and potential transmission, respectively. Mosquitoes were immobilized by chilling, then legs and wings were removed and pooled for each individual. Each mosquito’s proboscis was then inserted into a capillary tube containing mineral oil and the mosquito was allowed to salivate for up to one hour. Visualization of saliva bubbles in the mineral oil was attempted with a dissection microscope but saliva bubbles were rarely seen. Mosquito bodies were then placed in a separate tube and the saliva/oil was extracted by centrifugation into 120 μL of MEM. Bodies and legs/wings were suspended in 120 μL of MEM and homogenized with a TissueLyser® (Qiagen, Valencia, CA). Homogenates were then clarified by centrifugation and samples were applied to Vero cell monolayers and allowed to adsorb for 60 minutes at 37°C with occasional rocking. Cell culture medium was then added and samples were incubated at 37°C for three days and observed for cytopathic effect.

### Table 1

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Subtype</th>
<th>Source</th>
<th>Year</th>
<th>Location</th>
<th>Passage history</th>
<th>GenBank accession no.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trinidad Donkey</td>
<td>IAB</td>
<td>Donkey</td>
<td>1930</td>
<td>Trinidad</td>
<td>GP-1, V-6</td>
<td>L01442</td>
<td>Kinney and others, 1986</td>
</tr>
<tr>
<td>68U201</td>
<td>IE</td>
<td>Hamster</td>
<td>1968</td>
<td>Guatemala</td>
<td>SMB-3, V-2, BHK-1</td>
<td>U34999</td>
<td>Oberste and others, 1996</td>
</tr>
<tr>
<td>Mx01-22</td>
<td>IE</td>
<td>Hamster</td>
<td>2001</td>
<td>Chiapas, Mexico</td>
<td>C636-1, V-1</td>
<td>AY823299 (13)</td>
<td></td>
</tr>
<tr>
<td>CPA152</td>
<td>IE</td>
<td>Horse</td>
<td>1996</td>
<td>Oaxaca, Mexico</td>
<td>SMB-1, RK-1, C636-1</td>
<td>AF448535 (11)</td>
<td></td>
</tr>
<tr>
<td>CPA201</td>
<td>IE</td>
<td>Horse</td>
<td>1993</td>
<td>Chiapas, Mexico</td>
<td>SMB-1, RK-1, BHK-1</td>
<td>AF448537 (11)</td>
<td></td>
</tr>
</tbody>
</table>

*GP = guinea pig, V = Vero cells; SMB = suckling mouse brain; BHK = baby hamster kidney cells; C636 = C6/36 mosquito cells; RK = rabbit kidney cells.*


RESULTS

Epizootic and enzootic VEEV control strains. Infection, dissemination, and potential transmission proportions for Cx. taeniopus are shown in Table 2. A single cohort that fed on a high-titer blood meal of the epizootic subtype IAB VEEV strain was included as an historical control and yielded the expected results of low infection and no dissemination or transmission potential (virus in the saliva). As enzootic subtype IE VEEV controls, four cohorts were fed on a range of blood meal titers from mice infected with the 68U201 strain. The lowest blood meal titer for this strain was 7.6 log10 PFU/mL, which when combined with the next highest of 7.1 log10 PFU/mL, yielded an overall saliva infection proportion of 82%. A slightly lower blood meal titer (6.0 log10 PFU/mL) yielded a slightly higher saliva infection proportion of 89%. However, Fisher’s exact test showed that the difference in infection proportions between these two groups was not statistically significant (P = 0.96).

Equine-virulent subtype IE VEEV strains. For strain Mx01-22, the lowest titer blood meal used to infect mosquitoes was 1.9 log10 PFU/mL. For this group, 36% of mosquito salivas contained infectious virus. At the highest titer tested for this virus strain, 6.5 log10 PFU/mL, 95% of mosquitoes were found to be infected and 80% were estimated to be capable of transmitting based on infectious saliva. The average saliva infection proportion for the three intermediate groups (blood meal titers = 2.9–5.9 log10 PFU/mL) was 36%.

Infection of Cx. taeniopus with strain CPA152 yielded results similar to those for strain Mx01-22. The lowest blood meal titer tested for CPA152 was 2.0 log10 PFU/mL and resulted in 55% of saliva samples containing virus. The highest blood meal titer tested was 6.2 log10 PFU/mL and had a saliva infection proportion of 88%. The average saliva infection proportion for the three intermediate groups (blood meal titers = 3.0–5.7 log10 PFU/mL) was 41%.

The subtype IE VEEV strain CPA201 infected Cx. taeniopus to an extent similar to the previous two epizootic virus strains. At the lowest blood meal titer tested, which contained an undetectable amount of virus (< 1.7 log10 PFU/mL), half of the cohort became infected and 5% were found able to transmit the virus. At the next lowest blood meal titer of 2.5 log10 PFU/mL, nearly half (43%) of the saliva samples contained virus. At 6.1 log10 PFU/mL, the highest blood meal titer tested for this virus strain, 100% of mosquitoes developed disseminated infections and 63% of those contained virus in the saliva. The average saliva infection proportion for the three intermediate dose groups (blood meal titers = 2.5–5.3 log10 PFU/mL) was 51%. This proportion was not significantly different from the 36% and 41% average saliva infection proportions from the intermediate dose groups of the previous two VEEV strains. Saliva infection proportions of all three equine-virulent viruses and the enzootic control virus 68U201 are shown in Figure 1.

DISCUSSION

This study was designed to compare recently isolated, equine-virulent strains of subtype IE VEEV from Mexico to older, equine-avirulent strains of subtype IE VEEV with respect to their ability to infect the enzootic mosquito Culex taeniopus. The infectivity or transmissibility of VEEV by Cx. taeniopus was previously demonstrated to be unaffected by colonization.8,19 The low infection and lack of dissemination for epizootic IAB we measured are consistent with findings of previously published studies.4,17,19 Intrathoracic inoculation of Cx. taeniopus with intermediate titer epizootic subtype IAB VEEV results in complete cohort infection. Thus, the refractory nature of Cx. taeniopus to oral infection by epizootic subtype IAB VEEV is the result of a midgut infection/escape barrier and not a salivary gland infection barrier.4,17,20 In contrast, epizootic vectors do not appear to exhibit subtype-specific infection barriers to the same extent as enzootic vector mosquitoes.8,10,20

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Table 2

Infection, dissemination, and transmission proportions for Culex taeniopus mosquitoes infected with enzootic and equine-virulent Venezuelan equine encephalitis virus subtype IE.*

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Bloodmeal titer (log10 plaque-forming units/mL)</th>
<th>No.</th>
<th>Body infection, no. (%)</th>
<th>Leg/wing infection, no. (%)</th>
<th>Saliva infection, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trinidad Donkey (Epizootic I-AB)</td>
<td>7.4</td>
<td>9</td>
<td>1 (11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>68U201 (Enzootic I-E)</td>
<td>2.8</td>
<td>10</td>
<td>3 (30)</td>
<td>2 (20)</td>
<td>3 (30)</td>
</tr>
<tr>
<td></td>
<td>5.7</td>
<td>10</td>
<td>7 (70)</td>
<td>3 (30)</td>
<td>3 (30)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>16</td>
<td>15 (94)</td>
<td>15 (94)</td>
<td>14 (89)</td>
</tr>
<tr>
<td></td>
<td>7.1–7.6</td>
<td>11</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Mx01-22 (Epizootic I-E)</td>
<td>1.9</td>
<td>11</td>
<td>9 (82)</td>
<td>5 (45)</td>
<td>4 (36)</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>20</td>
<td>5 (25)</td>
<td>5 (25)</td>
<td>5 (25)</td>
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<tr>
<td></td>
<td>3.1</td>
<td>18</td>
<td>12 (66)</td>
<td>7 (39)</td>
<td>3 (17)</td>
</tr>
<tr>
<td></td>
<td>5.3–5.9</td>
<td>12</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>8 (66)</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>20</td>
<td>19 (95)</td>
<td>18 (90)</td>
<td>16 (80)</td>
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<tr>
<td>CPA152 (Epizootic I-E)</td>
<td>2.0</td>
<td>9</td>
<td>7 (77)</td>
<td>4 (44)</td>
<td>5 (55)</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>11</td>
<td>10 (91)</td>
<td>3 (27)</td>
<td>1 (9)</td>
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<tr>
<td></td>
<td>4.2</td>
<td>16</td>
<td>16 (100)</td>
<td>9 (56)</td>
<td>6 (38)</td>
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<tr>
<td></td>
<td>5.7</td>
<td>14</td>
<td>14 (100)</td>
<td>12 (86)</td>
<td>10 (71)</td>
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<tr>
<td></td>
<td>6.2</td>
<td>8</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>CPA201 (Epizootic I-E)</td>
<td>&lt;1.7</td>
<td>20</td>
<td>10 (50)</td>
<td>6 (30)</td>
<td>1 (5)</td>
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<tr>
<td></td>
<td>2.5</td>
<td>14</td>
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<td>8</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>5 (63)</td>
</tr>
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</table>
The proportions of infection for the 68U201 VEEV strain in Cx. taeniopus salivary glands were consistent with those of previously published studies.¹⁷,¹⁹ High proportions of virus detection in the saliva were found after oral exposure to the modest viremia titers most likely to be encountered in wild rodents (2.0–4.0 log_{10} PFU/mL),²¹ although none of the cohorts were found to have 100% infection of saliva samples as reported in a previously published study.¹⁹ However, that study measured actual transmission to uninfected hamsters, which may be more sensitive than the ex vivo approach used here if the 50% lethal dose for the hamster is lower than one Vero cell infective unit. It has been shown that the in vitro salivation technique, although commonly used to minimize vertebrate animal experimentation, is less sensitive than in vivo transmission and can miss 30–50% of saliva samples subsequently found to be positive by newborn mouse brain injection.¹⁰ The reduced sensitivity of the salivation technique is likely caused by the reduced sensitivity of Vero cells compared with newborn mice.

There was no difference observed in infection rates between any of the three equine-virulent subtype IE VEEV strains tested and the enzootic subtype IE VEEV control strain 68U201. The latter strain is often used as an enzootic control in experimentation and is considered avirulent in horses on the basis of its close genetic relatedness to strains that have been experimentally evaluated in horses.²²,²³ All four viruses were detected in the saliva of mosquitoes that imbibed low-titer blood meals. Some variability existed between and within groups, although the level and degree did not differ from that seen previously with experimental infection of Cx. taeniopus.²⁴ A Spearman rank correlation test confirmed that variation in the proportion of infection detected in saliva was correlated with variation in blood meal titer. In this application of the Spearman test, it functions as a test of homogeneity.

Electron microscopic studies found evidence of VEEV dissemination in Cx. taeniopus as soon as two days after ingestion of high-titer infectious blood meals, and virus was found in the salivary glands as early as four days after infection.²⁴ Additionally, transmission has been reported as early as eight days post-infection, although high proportions of transmission do not occur until day 17.³ The rate of dissemination to the salivary glands appears to be a function of blood meal titer, with lower titer infections taking longer to reach the salivary glands.²⁵ The long Cx. taeniopus life span observed in the field and the laboratory, combined with the ingestion of up to eight blood meals throughout their lifespan would ensure that the slow dissemination associated with low-titer blood meals did not preclude transmission by this mosquito.²⁵

It was casually observed that mosquitoes infected with high-titer infectious blood meals had higher mortality rates during the 25-day extrinsic incubation period than those infected with lower-titer blood meals; up to 50% mortality was seen for cohorts infected with ≥6.0 log_{10} PFU/mL, and 0–20% mortality was seen for cohorts infected with lower titers. This difference was observed repeatedly during these experiments, although the biological significance is unknown because natural hosts of VEEV rarely circulate 6.0 log_{10} PFU/mL of virus.²³ Mosquito mortality resulting from alphavirus infection has been previously reported for Caliseta melanura mosquitoes intrathoracically inoculated or orally infected,²⁶ and for Coquillettidia perturbans orally infected with eastern equine encephalitis virus.²⁷ In another study, Culex pipiens orally infected with Rift Valley fever virus (Bunyaviridae: Phleboviridae) were shown to have significantly increased mortality.²⁸ It has been shown that western and eastern equine encephalitis viruses can cause pathologic changes to the midgut of their respective mosquito vectors Cx. tarsalis and Cs. melanura.²⁹,³⁰ Ultrastructural studies of Cx. taeniopus may show whether the mortality observed in this study was caused by similar midgut disruption or a different mechanism of pathology.

We occasionally observed that the saliva sample and body of a particular mosquito were positive for virus, but the corresponding leg/wing pool were not. This finding is most likely explained by desiccation of the legs and wings during the time spent completing dissection and salivation of the remaining cohort or incomplete trituration of these body parts that have a high chitin content. Isolation of infectious VEEV from dried filter paper after up to 40 days has been shown in the laboratory, arguing against the former explanation.³¹ However, that study tested virus that was suspended in cell culture medium and it is possible that biochemical changes within the hemolymph of the mosquito legs and wings after separation from the body may reduce elution or infectivity of virions therein. In future studies we will place legs and wings immediately into diluent to prevent desiccation.

Genetically engineered VEEV reciprocal chimeras of enzootic IAB, enzootic IE, and equine-virulent IE have been evaluated for infection efficiency in the epizootic vector Ae. taeniorhynchus. Chimeras containing the structural genes of a donor strain and the nonstructural genes of a parent strain and chimeras comprised of a donor E2 gene inserted into a parent backbone all infected Ae. taeniorhynchus with efficiency comparable to the unaltered donor strain.²⁴ It is not known whether a reciprocal relationship exists in the enzootic vector species Cx. taeniopus. Now that a breeding colony is available, these studies and others are possible to assess the genetic
determinants of their extremely high oral susceptibility to subtype IE and the stark contrast with subtype IAB.

Although equine-virulent subtype IE VEEV strains have been shown to be more infectious to the epizootic vector Ae. taeniorynchus than enzootic subtype IE VEEV strains, our study provides no evidence for a corresponding loss of fitness in the enzootic vector Cx. taeniopus. Although these equine-virulent subtype IE VEEV strains have similar mutations in the E2 gene as epizootic subtype IAB VEEV strains, they do not exhibit the same inability to orally infect Cx. taeniopus. This characteristic sets them apart from other VEEV strains and suggests that infection efficiency of different mosquitoes may involve genomic regions outside the E2 gene. Equine-virulent virus that is efficiently transmitted by epizootic vectors and maintained in natural foci by enzootic vectors could represent a new class of VEEV worthy of continued study. Additionally, it appears that equine virulence may not be a reliable marker for low infection efficiency of subtype IE VEEV in this mosquito.

Based on this work, we suggest that Cx. taeniopus mosquitoes maintain VEEV in circulation foci in coastal Chiapas, where there remain intact mangrove swamps large enough to support their populations. The catholic feeding pattern of these mosquitoes, in conjunction with the proximity of human settlements and horse stables to these swamps, would enable occasional transmission of subtype IE VEEV to a horse or person near the coast. If subsequent viremia is sufficient to orally infect Ae. taeniorynchus mosquitoes and if there are non-vaccinated horses in the region, the aggressive biting behavior and long flight range of Ae. taeniorynchus may quickly perpetuate an epizootic outbreak.

This model differs from the paradigm in several important aspects. First, epizootic IAB and IC VEEV strains are thought to periodically emerge and then undergo extinction when they kill or immunize most equids. There is no evidence that they are maintained by enzootic mosquitoes during inter-epizootic periods. In contrast, equine-virulent subtype IE VEEV strains in Chiapas appear to be maintained by enzootic mosquito vectors, which due to their larval habitats of permanent or semipermanent bodies of water, are present in relatively stable populations. Second, epizootic IAB and IC strains are known to cause high viremia in horses but enzootic subtypes IE and ID do not. Subtype IE VEEV strains circulating in Chiapas have been shown to cause equine disease in the absence of high viremia, and experimental infection studies have been equivocal with respect to the ability of these strains to induce high viremia (Adams AP and Bowen R, unpublished data). Lastly, traditional epizootic VEEV strains are thought to be best transmitted by epizootic vector species and enzootic strains by enzootic vector species. In Chiapas, equine-virulent subtype IE VEEV strains can be efficiently transmitted by epizootic and enzootic vector species. Thus, the changing ecology of coastal Chiapas is associated with changing ecology of VEEV. The existence of equine-virulent strains of virus that can be maintained by enzootic vector mosquitoes during inter-epizootic periods emphasizes the importance of equine vaccination. If Mexican horses are not regularly vaccinated, virulent subtype IE VEEV could spread to the United States, as it did during the 1971 subtype IAB VEEV outbreak. Because VEEV is not endemic in the United States, horses are not regularly vaccinated and an outbreak of virulent IE VEEV similar to the 1971 IAB VEEV outbreak could be devastating.

This work also highlights the variability in natural transmission cycles between virus strains and geographic regions. Lastly, it demonstrates the need for further studies with the enzootic vector species to discern how these virus strains are maintained and the genetic factors that determine infection efficiently in Cx. taeniopus.

Received September 15, 2009. Accepted for publication February 9, 2010.

Acknowledgments: We thank Jose Estrada-Franco for assistance with capture and importation of wild mosquitoes; Billy Philips for assistance with statistical analysis; and Nicole Arrigo, Joanie Kenney, and Jing Huang for assistance with mosquito rearing.

Financial support: Eleanor R. Deardorff was supported by a James W. McLaughlin Predoctoral Fellowship and by National Institutes of Health (NIH) training grant T32-AI060549. This research was supported by NIH grant AI071192 and AI25489.

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