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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*) *taeniorhynchus.* 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 encephalitic disease 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*) *taeniorhynchus,* 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. taeniorhynchus*, but not by *Cx. taeniopus*.[3,8–10] 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.[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.[2] Engorged, adult females were placed into a 45.7-cm^3 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 on 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

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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.[12] 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 F_{15}–F_{20} generations.

Viruses and infection. Five strains of VEEV were used for experimental infection of *Cx. 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.[13] 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.[14] 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.[15] Adult Swiss-Webster mice were anesthetized by isoflurane inhalation, then inoculated with approximately 10^3 plaque-forming units (PFU) of virus administered subcutaneously in the right thigh. This dose has been shown to approximate the maximum amount of virus delivered in nature by a single bite from an infected mosquito.[16] At 6–24 hours post-infection, animals were anesthetized for up to 90 minutes with sodium pentobarbital (50 mg/kg) administered intraperitoneally. Blood was collected from the retro-orbital sinus in heparinized glass capillary tubes and transferred to five volumes of phosphate-buffered saline. Erythrocytes were removed by centrifugation to yield an approximately 1:10 dilution of plasma and virus titration was performed immediately by plaque assay on Vero cells.

Anesthetized mice were offered to cohorts of 50 adult, female, 10–15-day-old *Cx. taeniopus* that had been sugar-starved for at least 12 hours. After one hour of feeding, the mice were removed and euthanized, and engorged mosquitoes were incubated for 24–26 days at 28°C with a 14:10 hour light:dark cycle. The extrinsic incubation period was chosen to maximize comparisons with previously published studies that used similar lengths of time and also to enable the possibility of slow virus dissemination, which has been seen after low-titer blood meal infection of *Cx. taeniopus*.[3,4,17] A sucrose solution (5–10%) was provided *ad libitum*. All work with vertebrate animals was approved by the University of Texas Medical Branch Institutional Animal Care and Use Committee.

Virus assays. Virus titers from mouse plasma were determined by standard plaque assay on Vero cells. Plasma were serially diluted in phosphate-buffered saline and inoculated onto confluent Vero cell monolayers. Virus was adsorbed onto cells for 60 minutes at 37°C with occasional rocking, then monolayers were overlayed with 0.4% agarose in minimum essential medium (MEM) supplemented with 2.5% fetal bovine serum. After 48 hours, cells were fixed with 10% formalin and stained with crystal violet for visualization of virus plaques.

Infection of mosquito bodies, legs/wings, and saliva was determined separately for each mosquito by a cytopathic effect assay. Virus detection in mosquito bodies, leg/wings, and saliva is 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
Strains of Venezuelan equine encephalitis virus used in experimental infections of *Culex taeniopus*

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

* 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 2.8 $\log_{10}$ PFU/mL, which resulted in 30% of the saliva samples containing virus after incubation. The highest blood meal titer for this strain was 7.6 $\log_{10}$ PFU/mL, which when combined with the next highest of 7.1 $\log_{10}$ PFU/mL, yielded an overall saliva infection proportion of 82%. A slightly lower blood meal titer (6.0 $\log_{10}$ 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 $\log_{10}$ PFU/mL. For this group, 36% of mosquito salivas contained infectious virus. At the highest titer tested for this virus strain, 6.5 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ PFU/mL and resulted in 55% of saliva samples containing virus. The highest blood meal titer tested was 6.2 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ PFU/mL, nearly half (43%) of the saliva samples contained virus. At 6.1 $\log_{10}$ 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 $\log_{10}$ 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.[18,19] 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.[3,17,20] In contrast, epizootic vectors do not appear to exhibit subtype-specific infection barriers to the same extent as enzootic vector mosquitoes.[8,10,20]

TABLE 2

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

Virus strain	Bloodmeal titer ($\log_{10}$ plaque-forming units/mL)	No.	Body infection, no. (%)	Leg/wing infection, no. (%)	Saliva infection, no. (%)
Trinidad Donkey (Epizootic I-AB)	7.4	9	1 (11)	0	0
68U201 (Enzootic I-E)	2.8	10	3 (30)	2 (20)	3 (30)
	5.7	10	7 (70)	3 (30)	3 (30)
	6	16	15 (94)	15 (94)	14 (89)
	7.1–7.6	11	11 (100)	11 (100)	9 (82)
Mx01-22 (Epizootic I-E)	1.9	11	9 (82)	5 (45)	4 (36)
	2.9	20	5 (25)	5 (25)	5 (25)
	3.1	18	12 (66)	7 (39)	3 (17)
	5.3–5.9	12	12 (100)	12 (100)	8 (66)
	6.5	20	19 (95)	18 (90)	16 (80)
CPA152 (Epizootic I-E)	2.0	9	7 (77)	4 (44)	5 (55)
	3.0	11	10 (91)	3 (27)	1 (9)
	4.2	16	16 (100)	9 (56)	6 (38)
	5.7	14	14 (100)	12 (86)	10 (71)
	6.2	8	8 (100)	8 (100)	7 (88)
CPA201 (Epizootic I-E)	< 1.7	20	10 (50)	6 (30)	1 (5)
	2.5	14	10 (71)	6 (43)	6 (43)
	4.2	20	19 (95)	14 (70)	10 (50)
	5.3	11	11 (100)	10 (91)	7 (64)
	6.1	8	8 (100)	8 (100)	5 (63)

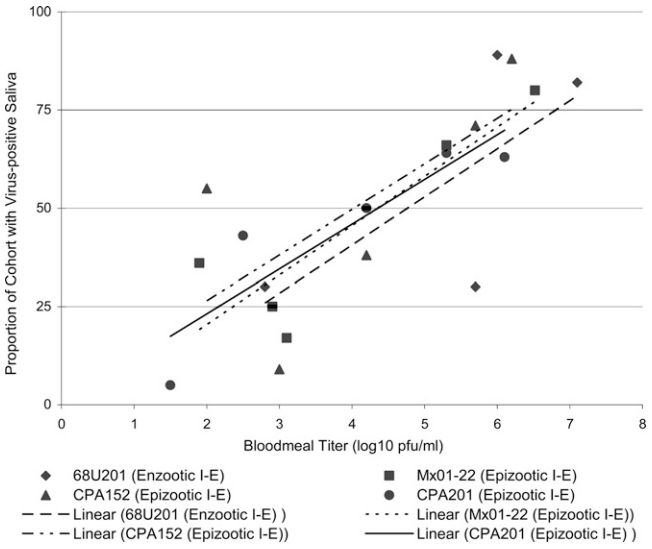

FIGURE 1. Transmission potential of equine-virulent subtype IE Venezuelan equine encephalitis virus by *Culex taeniopus* mosquitoes. Mosquitoes were orally infected on viremic mice. Presence of virus in saliva secretions is considered an indication of viral infection of the salivary glands and is interpreted as transmission potential. Best fit linear trendlines were calculated for each data set to aid in comparison between virus strains.

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 infectious 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.[16] 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*.[17] 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.[24] 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.[3,5]

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.[21] Mosquito mortality resulting from alphavirus infection has been previously reported for *Culiseta melanura* mosquitoes intrathroracically inoculated[25] 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*: *Phlebovirus*) 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 epizootic IAB, enzootic IE, and equine-virulent IE have been evalutated 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.[32] 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. taeniorhynchus* 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,[5] 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. taeniorhynchus* mosquitoes and if there are nonvaccinated horses in the region, the aggressive biting behavior and long flight range of *Ae. taeniorhynchus* 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.[1,33,34] 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.[23] 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).[35] 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.[7] 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*.

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REFERENCES

1. Weaver SC, Ferro C, Barrera R, Boshell J, Navarro JC, 2004. Venezuelan equine encephalitis. *Annu Rev Entomol 49:* 141–174.
2. Ferro C, Boshell J, Moncayo AC, Gonzalez M, Ahumada ML, Kang W, Weaver SC, 2003. Natural enzootic vectors of Venezuelan equine encephalitis virus, Magdalena Valley, Colombia. *Emerg Infect Dis 9:* 49–54.
3. Weaver SC, Scherer WF, Cupp EW, Castello DA, 1984. Barriers to dissemination of Venezuelan encephalitis viruses in the Middle American enzootic vector mosquito, *Culex* (*Melanoconion*) *taeniopus*. *Am J Trop Med Hyg 33:* 953–960.
4. Scherer WF, Weaver SC, Taylor CA, Cupp EW, Dickerman RW, Rubino HH, 1987. Vector competence of *Culex* (*Melanoconion*) *taeniopus* for allopatric and epizootic Venezuelan equine encephalomyelitis viruses. *Am J Trop Med Hyg 36:* 194–197.
5. Cupp EW, Scherer WF, Lok JB, Brenner RJ, Dziem GM, Ordonez JV, 1986. Entomological studies at an enzootic Venezuelan equine encephalitis virus focus in Guatemala, 1977–1980. *Am J Trop Med Hyg 35:* 851–859.
6. Garman JL, Scherer WF, Dickerman RW, 1968. A study of equine virulence of naturally occurring Venezuelan encephalitis virus in Veracruz with description of antibody responses. *Bol Oficina Sanit Panam 65:* 238–252.
7. Brault AC, Powers AM, Ortiz D, Estrada-Franco JG, Navarro-Lopez R, Weaver SC, 2004. Venezuelan equine encephalitis emergence: enhanced vector infection from a single amino acid substitution in the envelope glycoprotein. *Proc Natl Acad Sci USA 101:* 11344–11349.
8. Kramer LD, Scherer WF, 1976. Vector competence of mosquitoes as a marker to distinguish Central American and Mexican epizootic from enzootic strains of Venezuelan encephalitis virus. *Am J Trop Med Hyg 25:* 336–346.
9. Coffey LL, Weaver SC, 2005. Susceptibility of *Ochlerotatus taeniorhynchus* and *Culex nigripalpus* for Everglades virus. *Am J Trop Med Hyg 73:* 11–16.
10. Turell MJ, O'Guinn ML, Navarro R, Romero G, Estrada-Franco JG, 2003. Vector competence of Mexican and Honduran mosquitoes (Diptera: Culicidae) for enzootic (IE) and epizootic (IC) strains of Venezuelan equine encephalomyelitis virus. *J Med Entomol 40:* 306–310.
11. Brault AC, Powers AM, Holmes EC, Woelk CH, Weaver SC, 2002. Positively charged amino acid substitutions in the E2 envelope glycoprotein are associated with the emergence of Venezuelan equine encephalitis virus. *J Virol 76:* 1718–1730.
12. Dziem GM, Cupp EW, 1983. Laboratory bionomics of *Culex* (*Melanoconion*) *taeniopus*. *Mosq News 43:* 170–175.

13. Estrada-Franco JG, Navarro-Lopez R, Freier JE, Cordova D, Clements T, Moncayo A, Kang W, Gomez-Hernandez C, Rodriguez-Dominguez G, Ludwig GV, Weaver SC, 2004. Venezuelan equine encephalitis virus, southern Mexico. *Emerg Infect Dis 10:* 2113–2121.
14. Powers AM, Brault AC, Kinney RM, Weaver SC, 2000. The use of chimeric Venezuelan equine encephalitis viruses as an approach for the molecular identification of natural virulence determinants. *J Virol 74:* 4258–4263.
15. Fernandez Z, Moncayo AC, Carrara AS, Forattini OP, Weaver SC, 2003. Vector competence of rural and urban strains of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) from Sao Paulo State, Brazil for IC, ID, and IF subtypes of Venezuelan equine encephalitis virus. *J Med Entomol 40:* 522–527.
16. Smith DR, Carrara AS, Aguilar PV, Weaver SC, 2005. Evaluation of methods to assess transmission potential of Venezuelan equine encephalitis virus by mosquitoes and estimation of mosquito saliva titers. *Am J Trop Med Hyg 73:* 33–39.
17. Scherer WF, Cupp EW, Dziem GM, Breener RJ, Ordonez JV, 1982. Mesenteronal infection threshold of an epizootic strain of Venezuelan encephalitis virus in *Culex* (*Melanoconion*) *taeniopus* mosquitoes and its implication to the apparent disappearance of this virus strain from an enzootic habitat in Guatemala. *Am J Trop Med Hyg 31:* 1030–1037.
18. Weaver SC, Scherer WF, Taylor CA, Castello DA, Cupp EW, 1986. Laboratory vector competence of *Culex* (*Melanoconion*) *cedecei* for sympatric and allopatric Venezuelan equine encephalomyelitis viruses. *Am J Trop Med Hyg 35:* 619–623.
19. Scherer WF, Weaver SC, Taylor CA, Cupp EW, 1986. Vector incompetency: its implication in the disappearance of epizootic Venezuelan equine encephalomyelitis virus from middle America. *J Med Entomol 23:* 23–29.
20. Turell MJ, Barth J, Coleman RE, 1999. Potential for Central American mosquitoes to transmit epizootic and enzootic strains of Venezuelan equine encephalitis virus. *J Am Mosq Control Assoc 15:* 295–298.
21. Deardorff ER, Forrester NL, Travassos da Rosa AP, Estrada-Franco JG, Navarro-Lopez R, Tesh RB, Weaver SC, 2009. Experimental infections of potential Mexican reservoir hosts with Venezuelan equine encephalitis virus. *Emerg Infect Dis 15:* 519–525.
22. Dietz WH Jr, Alvarez O Jr, Martin DH, Walton TE, Ackerman LJ, Johnson KM, 1978. Enzootic and epizootic Venezuelan equine encephalomyelitis virus in horses infected by peripheral and intrathecal routes. *J Infect Dis 137:* 227–237.
23. Walton TE, Alvarez O Jr, Buckwalter RM, Johnson KM, 1973. Experimental infection of horses with enzootic and epizootic strains of Venezuelan equine encephalomyelitis virus. *J Infect Dis 128:* 271–282.
24. Weaver SC, 1986. Electron microscopic analysis of infection patterns for Venezuelan equine encephalomyelitis virus in the vector mosquito, *Culex* (*Melanoconion*) *taeniopus*. *Am J Trop Med Hyg 35:* 624–631.
25. Cooper LA, Sina BJ, Turell MJ, Scott TW, 2000. Effects of initial dose on eastern equine encephalomyelitis virus dependent mortality in intrathoracically inoculated *Culiseta melanura* (Diptera: Culicidae). *J Med Entomol 37:* 815–819.
26. Scott TW, Lorenz LH, 1998. Reduction of *Culiseta melanura* fitness by eastern equine encephalomyelitis virus. *Am J Trop Med Hyg 59:* 341–346.
27. Moncayo AC, Edman JD, Turell MJ, 2000. Effect of eastern equine encephalomyelitis virus on the survival of *Aedes albopictus*, *Anopheles quadrimaculatus*, and *Coquillettidia perturbans* (Diptera: Culicidae). *J Med Entomol 37:* 701–706.
28. Faran ME, Turell MJ, Romoser WS, Routier RG, Gibbs PH, Cannon TL, Bailey CL, 1987. Reduced survival of adult *Culex pipiens* infected with Rift Valley fever virus. *Am J Trop Med Hyg 37:* 403–409.
29. Weaver SC, Lorenz LH, Scott TW, 1992. Pathologic changes in the midgut of *Culex tarsalis* following infection with western equine encephalomyelitis virus. *Am J Trop Med Hyg 47:* 691–701.
30. Weaver SC, Scott TW, Lorenz LH, Lerdthusnee K, Romoser WS, 1988. Togavirus-associated pathologic changes in the midgut of a natural mosquito vector. *J Virol 62:* 2083–2090.
31. Guzman H, Ding X, Xiao SY, Tesh RB, 2005. Duration of infectivity and RNA of Venezuelan equine encephalitis, West Nile, and yellow fever viruses dried on filter paper and maintained at room temperature. *Am J Trop Med Hyg 72:* 474–477.
32. Brault AC, Powers AM, Weaver SC, 2002. Vector infection determinants of Venezuelan equine encephalitis virus reside within the E2 envelope glycoprotein. *J Virol 76:* 6387–6392.
33. Sudia WD, Fernandez L, Newhouse VF, Sanz R, Calisher CH, 1975. Arbovirus vector ecology studies in Mexico during the 1972 Venezuelan equine encephalitis outbreak. *Am J Epidemiol 101:* 51–58.
34. Scherer WF, Ordonez JV, Dickerman RW, Navarro JE, 1976. Search for persistent epizootic Venezuelan encephalitis virus in Guatemala, El Salvador and Nicaragua during 1970–1975. *Am J Epidemiol 104:* 60–73.
35. Gonzalez-Salazar D, Estrada-Franco JG, Carrara AS, Aronson JF, Weaver SC, 2003. Equine amplification and virulence of subtype IE Venezuelan equine encephalitis viruses isolated during the 1993 and 1996 Mexican epizootics. *Emerg Infect Dis 9:* 161–168.