Transmission of a Venezuelan Equine Encephalitis Complex Alphavirus by *Culex* (*Melanoconion*) *gnomatos* (Diptera: Culicidae) in Northeastern Peru

STEPHEN P. YANOVIAK,[1, 2, 3], PATRICIA V. AGUILAR,[1, 4] L. PHILIP LOUNIBOS,[3] AND SCOTT C. WEAVER[1, 2, 4, 5]

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ABSTRACT Venezuelan equine encephalitis (VEE) complex alphaviruses are serious health threats in the Americas and regularly infect humans living in or near Amazonian rain forests. As part of a larger surveillance program, we placed six hamster-baited mosquito traps in a disturbed white sand forest of northeastern Peru for 3 d. Virus isolations from hamster serum and trapped mosquito pools demonstrated that a VEE subtype IIIC alphavirus was transmitted to a hamster by the mosquito *Culex* (*Melanoconion*) *gnomatos* Sallum, Hutchings & Ferreira. This species, like the other seven proven VEE complex alphavirus vectors, is a member of the Spissipes section of this subgenus. The composition of mosquitoes collected at the site over the sampling period was typical for the region.

RESUMEN Encefalitis equina venezolana (EEV) es una enfermedad emergente e importante en las Américas, y con frecuencia afecta a los seres humanos que viven en o cerca de la cuenca amazónica. Como parte de una extensa investigación sobre la distribución de EEV en la amazonía peruana, seis trampas usando hamster como cebo fueron colocadas por tres días en un bosque secundario sobre suelos de arena blanca, ubicado a 10 Km al suroeste de la ciudad de Iquitos. Los virus aislados del suero de un hamster y de los mosquitos indicaron que EEV subtipo IIIC fue transmitido a un hamster por el mosquito *Culex* (*Melanoconion*) *gnomatos* Sallum, Hutchings & Ferreira. Esta especie, como las otras siete especies conocidos vectores del complejo alphavirus, es un miembro de la sección Spissipes del subgénero. La composición de los mosquitos colectados en el área fue típica de la región.

KEY WORDS arbovirus, alphavirus, encephalitis, transmission, vector

THE VENEZUELAN EQUINE ENCEPHALITIS (VEE) complex of alphaviruses (Togaviridae: *Alphavirus*) includes emerging arboviral pathogens that pose a serious health threat to equines and humans in the Americas (Walton and Grayson 1988, Weaver et al. 2004). In the Amazon basin of Peru, three different subtypes in the VEE complex circulate in sylvatic, enzootic cycles and regularly infect people, causing febrile illness that is underdiagnosed due to the lack of pathopneumonic features (Watts et al. 1998, Aguilar et al. 2004). Although these enzootic strains do not amplify in equines to produce widespread epidemics, they can cause fatal human disease (Weaver et al. 2004). The viruses in VEE subtype III are considered a distinct species (Mucambo virus) from VEE virus (Weaver et al. 2000), but they probably cause similar human disease.

Relatively little is known about the reservoir hosts and vectors of VEE complex alphaviruses in the Amazon basin of Peru. However, serosurveys and virus isolates from spiny rats (*Proechimys* spp.) strongly suggest that they are the principal reservoir hosts in this region (S.C.W., unpublished) as they are in other parts of South America (Barrera et al. 2002). Experimental susceptibility studies and field isolations suggest that a variety of mosquito species that are common in the western Amazon, especially *Culex* (*Melanoconion*) spp., potentially transmit these enzootic VEE complex viruses (Turell et al. 2000). However, few field-based studies have thoroughly incriminated VEE complex vectors in South America (but see Ferro et al. 2003), and none in Peru.

We documented the sublethal transmission of a VEE subtype IIIC virus to a sentinel hamster by *Culex* (*Melanoconion*) *gnomatos* Sallum, Hutchings & Ferreira in the western Amazon Basin. We also examined

[1] Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555–0609.
[2] Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555–0609.
[3] University of Florida, Florida Medical Entomology Laboratory, 200 9th Street SE, Vero Beach, FL 32962.
[4] Department of Microbiology and Immunology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555–0609.
[5] Corresponding author, e-mail: sweaver@utmb.edu.

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the composition of mosquitoes collected at the site, and variation in mosquito abundance and species richness was documented over the brief sampling period.

Materials and Methods

This project was conducted in a lowland moist forest fragment in northeastern Peru, ≈10 km south southwest of Iquitos (3.8552° S, 73.3435° W, elevation ≈120 m). The site is disturbed secondary forest on poorly drained white sand soils (locally called "varillal humedo"; Anderson 1981, Villacorta et al. 2002) and is surrounded by agriculture and suburban development typical of the area along the highway connecting Iquitos and Nauta (Mäki et al. 2001). Vásquez (1997) and Madigosky and Vatnick (2000) provide details about the flora and climate of the region.

As part of a larger arbovirus surveillance program, six modified Trinidad traps (Davies 1971, Ferro et al. 2003), each baited with one Syrian golden hamster, were positioned at the site on 25 February 2003. Traps were spaced >15 m apart in the forest and hung from low branches ≈1.2 m above the ground. Mosquitoes were collected from the traps with a mechanical aspirator each morning up to and including 28 February, when the traps were removed from the field ($n = 3$ consecutive collection days per trap). Mosquitoes were killed by freezing, sorted on a cold table (−15°C), and stored at −70°C.

A postorbital blood sample (0.1 ml) was taken from each hamster 7 d before its placement in the field and 3 d after it was removed from the field. Upon collection, the blood was diluted in 0.9 ml of phosphate-buffered saline, and plasma was separated by low-speed centrifugation (2,000 rpm for 30 s) and stored at −70°C. Hamsters were weighed immediately after blood collection. Frozen mosquito and serum samples subsequently were assayed for virus isolation and arbovirus antibodies.

We used several resources to identify mosquitoes to species, including the keys listed in Pecor et al. (2000) and Jones et al. (2004), and reference collections at the Walter Reed Biosystematics Unit (WRBU), Suitland, MD. Voucher specimens were deposited at WRBU and the Museo de Historia Natural Javier Prado in Lima, Peru.

Mosquito pools were triturated in 1 ml of Eagle's minimal essential medium (MEM) supplemented with 20% fetal bovine serum, penicillin, and streptomycin. Virus isolation from hamster serum or mosquito pools was attempted by inoculation into cultures of Vero monkey kidney cells and incubation at 37°C for 1 wk. When cytopathic effects were observed, a sample of MEM from the culture was collected for RNA extraction and plaque assay. A reverse transcription-polymerase chain reaction (RT-PCR) assay followed by phylogenetic analyses was used to identify and characterize alphaviruses present in the area (Aguilar et al. 2004).

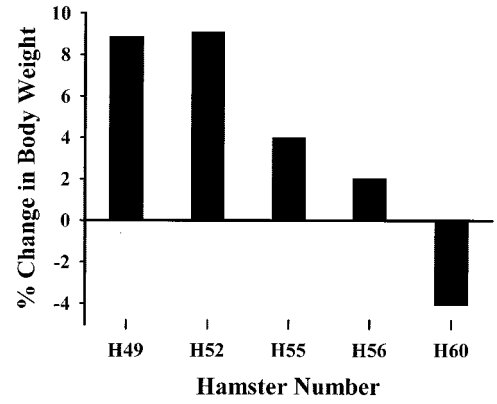

Fig. 1. Percentage of change in hamster mass between 17 February and 3 March 2003. Hamster 25 showed zero change and is not plotted. The mean ± 1 SD initial weight of hamsters (on 17 February) was 96 ± 5.3 g, range 88–100 g.

Results

Hamster 60 (hereafter H60) was found dead on 11 March 2003. The behavior of this hamster during the first week of March did not indicate a viral infection or a general decline in health. However, this was the only hamster of the six to lose weight after being placed at the field site (Fig. 1). The cause of death of this hamster is unknown, but it was probably not the direct result of arboviral infection in the field. The incubation period for all viruses known to circulate in the region is far less than the 11 d the animal spent in a protected laboratory before death (Scherer et al. 1963, 1975).

In total, 352 mosquitoes (20 species) were collected in the six traps over the 3 d at the study site (Table 1). Mean ± 1 SD mosquito abundance and number of species collected per trap per day were 19.6 ± 16.3 and 5.8 ± 2.7, respectively. A VEE subtype IIIC alphavirus was isolated from the H60 serum sample collected on 3 March. Therefore, all mosquitoes collected from the H60 trap were sorted by species and day, and pools were tested for virus by inoculation of Vero cells and observation for cytopathic effects. All mosquito pools collected on 26–27 February, the first 2 d of hamster exposure, were negative for virus. The collection from 28 February had two pools of infected mosquitoes composed of 19 *Cx.* (*Mel.*) *gnomatos*, and five *Culex amazonensis* (Lutz) (Table 1). The titer of the *Cx. amazonensis* pool (1.2×10^2 plaque-forming units [PFU]/ml) was inconsistent with VEE complex alphavirus transmission-competent mosquitoes, suggesting that one or more mosquitoes in this pool fed on the hamster late during the 24-h collection period, when viremia had begun. In contrast, the titer of the *Cx. gnomatus* pool (3.5×10^5 PFU/ml) was consistent with that of a competent, infected VEE complex alphavirus vector (Cupp et al. 1979, Weaver et al. 1984).

The virus isolates from H60 and the *Cx. gnomatus* pool were analyzed further using RT-PCR to sequence the N-terminal region of the PE2 envelope glycoprotein precursor gene as described previously (Aguilar

Table 1. Mosquitoes collected in hamster-baited traps

Taxon	Frequency[a]	Total abundance[b]	Abundance in H60 on 28 Feb. (titer)[c]
Coquillettidia arribalzagae (Theobald)	1	1	0
Cx. (*Aed.*) *amazonensis* (Lutz)	7	23	5 (2.1)
Cx. (*Cx.*) *coronator* Dyar & Knab	1	1	0
Cx. (*Cx.*) *declarator* Dyar & Knab	1	1	0
Cx. (*Cx.*) *quinquefasciatus* Say	1	1	1 (<0.7)
Cx. (*Mel.*) *gnomatos* Sallum, Hutchings & Ferreira	16	119	19 (5.6)
Cx. (*Mel.*) *ocossa* Dyar & Knab	1	1	0
Cx. (*Mel.*) *pedroi* Sirivanakarn & Belkin	8	15	4 (<0.7)
Cx. (*Mel.*) *portesi* Senevet & Abonnenc	14	29	1 (<0.7)
Cx. (*Mel.*) *spissipes* (Theobald)	3	4	0
Cx. (*Mel.*) *vomerifer* Komp	12	63	0
Cx. (*Mel.*) "morphospecies 1"	16	52	7 (<0.7)
Cx. (*Mel.*) sp. indet.	1	1	0
Limatus flavisetosus de Oliveira-Castro	3	5	0
Ochlerotatus serratus (Theobald)	2	7	0
Psorophora albigenu (Peryassu)	5	6	0
Ps. ferox (von Humboldt)	1	1	0
Wyeomyia aphobema Dyar	1	1	0
Wy. flui Bonne-Wepster & Bonne	2	2	0
Wy. pseudopecten Dyar & Knab	8	19	0

[a] Number of trap nights a species was collected during the 3-d study (maximum 18).
[b] Number of individuals of each species collected during the study.
[c] Number of mosquitoes of each species present in trap H60 on 28 Feb. 2003. Parentheses represent virus titers of mosquitoes pooled by species (values are Log_{10} PFU/pool; mosquito pools from H60 on all other trap dates tested negative for virus).

et al. 2004). Comparison of the derived sequences indicated that they were identical, and phylogenetic analyses placed this sequence as a closely related sister group to strain 71D1252 isolated in the Iquitos region in 1971 from a mosquito pool (Scherer and Anderson 1975) and strain 54-001 isolated from a sentinel hamster there in 2002 (Aguilar et al. 2004) (Fig. 2). These results indicate that the new isolates are members of VEE complex subtype IIIC, an enzootic strain with unknown human pathogenicity.

Discussion

VEE continues to be a serious health threat in the Americas, especially in northern South America (Watts et al. 1998, Aguilar et al. 2004), and many aspects of the basic ecology of the enzootic cycle of the virus remain unknown. Here, we provide the first confirmed transmission of VEE subtype IIIC by *Cx. gnomatos* in the western Amazon basin. This mosquito species previously was shown to be susceptible to VEEV subtypes IAB, IC, ID, and IE (Turell et al. 2000), but no transmission events involving *Cx. gnomatos* were recorded in the Amazon basin before this study. The medical importance of the VEE subtype IIIC alphavirus is unknown; it has not been isolated from febrile patients in the Iquitos area, but most enzootic VEE complex viruses cause a dengue-like illness that is underdiagnosed (Watts et al. 1998, Aguilar et al. 2004). Because human infection with several other VEE subtypes can be fatal (Weaver et al. 2004), further research on the public health significance of the subtype IIIC strains is needed.

Our identification of natural transmission of a VEE complex alphavirus by *Cx. gnomatos* is consistent with repeated isolations of subtype III VEE complex strains from this species in the Iquitos region of Peru (M. J. Turell, USAMRIID, personal communication). The incrimination of this species brings to eight the total number of enzootic mosquito vectors identified for this virus taxon (Weaver et al. 2004). All of these vectors are members of the *Culex* (*Melanoconion*) subgenus, a highly diverse taxon restricted to the New World. Furthermore, all are members of a single section within the subgenus, the Spissipes section, comprised of only 23 species. The physiological and/or ecological traits shared by this group that allow them to serve as efficient enzootic vectors deserve further study.

The species composition of mosquitoes collected in the six sentinel hamster traps was consistent with the known mosquito fauna of the region (Need et al. 1993, Pecor et al. 2000, Mendez et al. 2001, Jones et al. 2004). The mosquito fauna collected was also typical for sentinel hamster traps, which tend to attract fewer individuals and a greater proportion of *Culex* (*Melanoconion*) spp. than do CDC light traps and human landing collections (cf. Mendez et al. 2001, Ferro et al. 2003, Jones et al. 2004; S.Y., unpublished data). Long-term data from a variety of habitat types around Iquitos show greater variation in mosquito composition and species richness than we observed in this short study (Need et al. 1993, Jones et al. 2004; S.Y., unpublished data). The single *Culex quinquefasciatus* Say in our collection probably originated from nearby human dwellings.

As deforestation and other anthropogenic disturbances increasingly modify tropical landscapes and species distributions (Dale et al. 1994, Nepstad et al. 1999), the need to understand the ecology of virus circulation and document the taxa involved in transmission cycles is also increasing. Here, we contribute to understanding of the VEE sylvatic cycle in the western Amazon. Determining the specific ecological requirements of *Cx. gnomatos* and other known VEE vectors would be an appropriate extension of this project.

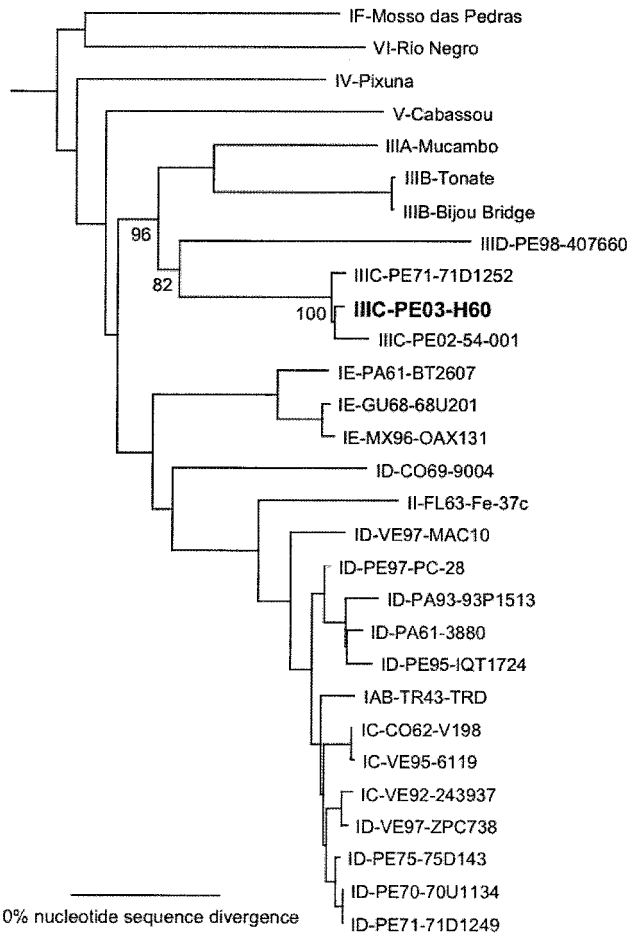

Fig. 2. Phylogenetic tree derived from partial PE2 envelope glycoprotein gene sequence of the H60 isolate and VEE complex alphavirus sequences representing all major subtypes and lineages, and strains circulating in the Peruvian Amazon basin. Eastern equine encephalitis virus sequences were used to root the tree. The neighbor joining program with the HKY85 distance formula, implemented in PAUP 4.0 (Swofford 1998) was used to generate the tree, and maximum parsimony and maximum likelihood analyses yielded the same overall topology. Numbers indicate bootstrap values for groups to the right. Most subtype I and III virus strains are denoted by abbreviated country and year of isolation, followed by strain designation.

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