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POTENTIAL FOR CENTRAL AMERICAN MOSQUITOES TO TRANSMIT EPIZOOTIC AND ENZOOTIC STRAINS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS

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ABSTRACT. Experimental studies were undertaken to compare the vector competence of *Culex* (*Melanoconion*) *taeniopus* Dyar and Knab, *Culex* (*Melanoconion*) *ocossa* Dyar and Knab, and *Psorophora confinnis* (Lynch Arribalzalga) from Central America for epizootic (IAB) and enzootic (IE) strains of Venezuelan equine encephalitis (VEE) virus. Virus infection and dissemination rates were significantly higher in *Cx. taeniopus* orally exposed to IE as compared to those orally exposed to IAB virus. In contrast, both infection and dissemination rates were similar in *Cx. ocossa* exposed to either IAB or IE strains of VEE virus. Thus, susceptibility to epizootic and enzootic strains of VEE virus seems to be species specific within the subgenus *Culex* (*Melanoconion*). Both species transmitted each strain of VEE virus after intrathoracic inoculation, indicating that a midgut barrier affected vector competence in these species. *Psorophora confinnis* was equally susceptible to both IAB and IE viruses, but apparently had a salivary gland barrier, as only 1 of 16 mosquitoes with a disseminated infection transmitted VEE virus by bite.

KEY WORDS *Culex* (*Melanoconion*) *ocossa*, *Culex* (*Melanoconion*) *taeniopus*, Venezuelan equine encephalitis virus, transmission

INTRODUCTION

Venezuelan equine encephalitis (VEE) virus is responsible for sporadic epizootics of severe disease in Central America (Walton and Grayson 1989). Epizootics have extended from central South America (e.g., Peru) to as far north as Texas. Recent epidemics in Colombia and Venezuela (Weaver et al. 1996, Rivas et al. 1997) have led to an increased interest in understanding the epidemiology of VEE and identification of potential vectors of both enzootic and epizootic strains of this virus. Studies by Scherer et al. (1986, 1987) indicate that although members of the subgenus *Culex* (*Melanoconion*) are highly susceptible to enzootic (IE) strains of VEE virus, they are virtually refractory to the epizootic IAB strain. In contrast, a study by Kramer and Scherer (1976) indicated that *Aedes taeniorhynchus* (Wiedemann) was a more efficient vector of epizootic IAB strains than enzootic IE strains of VEE virus.

In this study, we compared the susceptibility of several Central American mosquito species to epizootic and enzootic strains of VEE virus. We allowed field-collected mosquitoes from Panama and Belize to feed on VEE virus-infected hamsters and determined rates of infection, dissemination, and transmission for individual females.

MATERIALS AND METHODS

Mosquitoes

Adult female mosquitoes were collected in dry ice-baited Centers for Disease Control and Prevention (CDC) miniature light traps (John W. Hock Co., Gainesville, FL). Mosquitoes were collected near Gamboa, Panama, in July 1994 (*Culex* (*Melanoconion*) *ocossa* Dyar and Knab), and near Punta Gorda and Freetown, Belize, August 23 and 24, 1994 (*Culex* (*Melanoconion*) *taeniopus* Dyar and Knab and *Psorophora confinnis* (Lynch Arribalzalga)), respectively. Mosquitoes were transported to a BL3+ laboratory at the Unites States Army Medical Research Institute of Infectious Diseases at Fort Detrick, MD, where they were provided apple slices and held at 26°C for 1–3 days until exposed to VEE virus.

Virus and virus assay: We used a 2nd baby hamster kidney cell culture passage of an infectious clone (V3000) of the epizootic VEE subtype IAB Trinidad donkey strain (Davis et al. 1989). 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). Earlier studies (Kramer and Scherer 1976, Scherer et al. 1986, 1987) found differences in the susceptibility of mosquitoes for epizootic and enzootic strains of VEE virus. Therefore, we also used the 68U201 strain of the enzootic subtype IE. This strain was isolated from a sentinel hamster in Guatemala in 1968 (Scherer et al. 1970).

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 2nd overlay, containing neutral red, was applied 2 days after the initial assay.

Determination of vector competence

Anesthetized adult female Syrian hamsters that had been inoculated intraperitoneally 28–48 h earlier with 0.2 ml of a suspension containing $\approx 10^4$ plaque-forming units (PFU) of either the IAB or IE

Table 1. Infection and dissemination rates of epizootic and enzootic strains of Venezuelan equine encephalitis virus in mosquitoes collected in Panama and Belize.

	Virus[1]					
	IAB strain (epizootic)			IE strain (enzootic)		
Species	No. tested	Infection rate[2] (%)	Dissemination rate[3] (%)	No. tested	Infection rate[2] (%)	Dissemination rate[3] (%)
Culex taeniopus	41	7	0	17	100	53
Culex ocossa	53	91	21	71	82	30
Psorophora confinnis	17	65	47	18	50	28

[1] Viremias for the IAB strain ranged from $10^{7.5}$ to $10^{8.0}$ PFU/ml of blood, whereas those for the IE strain ranged from $10^{7.2}$ to $10^{8.2}$ PFU/ml of blood.
[2] Percentage of mosquitoes containing virus.
[3] Percentage of mosquitoes containing virus in their legs.

strain of VEE virus were placed individually on top of cages containing 100–300 field-collected mosquitoes each. Immediately after feeding, a 0.2-ml sample of blood was obtained from each hamster by cardiac puncture and added to 1.8 ml of diluent (10% fetal bovine serum in Medium 199 with Earle's salts and antibiotics). The blood suspension was frozen at $-70°C$ until assayed on Vero cell monolayers to determine the viremia at the time of mosquito feeding. After feeding on an infectious hamster, engorged mosquitoes were transferred to 3.8-liter cardboard screen-topped cartons. Mosquitoes were maintained on apple slices or a 7% sucrose solution (carbohydrate source) and held at 26°C and a 16:8 h (light: dark) photoperiod.

Mosquitoes were tested for their ability to transmit virus 7–22 days after the infectious blood meal. These mosquitoes were allowed to feed on susceptible hamsters either individually or in groups of 2–5 mosquitoes. Immediately after each transmission trial, mosquitoes were cold-anesthetized, identified to species, and their legs and bodies triturated separately in 1 ml of diluent. These suspensions then were frozen at $-70°C$ until tested for virus.

Infection was determined by recovery of virus from the mosquito body tissue samples. We considered a mosquito that had virus recovered from its body, but not its legs, to have a nondisseminated infection limited to its midgut. In contrast, if virus was recovered from both body and leg suspensions, we considered the mosquito to have a disseminated infection (Turell et al. 1984a). Because VEE virus infection is consistently fatal to hamsters, death of these animals was used to indicate virus transmission. Transmission was verified by isolation of virus from brain tissue. Any hamster that survived 21 days after being fed on by a mosquito with a disseminated infection was challenged with 10^4 PFU of the appropriate strain of VEE virus to determine its immune status.

Because field-collected mosquitoes do not readily take 2 blood meals, we also inoculated a small number of individuals of each species intrathoracically (Rosen and Gubler 1974) with 0.3 μl containing $\approx 10^{1.5}$ PFU of 1 of the 2 strains of VEE virus. These mosquitoes were held 7–18 days and then allowed to feed individually on hamsters as described above.

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 Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

RESULTS

Viremias ranged from $10^{7.2}$ to $10^{8.2}$ and $10^{7.5}$ to $10^{8.0}$ PFU/ml of blood for each of the 4 hamsters infected with the IE and the IAB strain, respectively, during the mosquito feedings. For each of the mosquito species tested, infection and dissemination rates were similar when compared by day after feeding (7–14 days) or by the titer of the infectious blood meal. Thus, data for each species were pooled for analysis. *Culex* (*Mel.*) *taeniopus* was highly susceptible to the IE strain, but nearly refractory to the IAB strain (Table 1). In contrast, *Cx.* (*Mel.*) *ocossa* was equally susceptible ($\chi^2 \leq 1.28$, df = 1, $P \geq 0.26$) to both strains of VEE virus (Table 1). Although none of the 41 *Cx.* (*Mel.*) *taeniopus* developed a disseminated infection after ingesting the IAB strain of VEE virus, the 1 intrathoracically inoculated mosquito that took a blood meal transmitted virus. Only 1 female *Cx.* (*Mel.*) *taeniopus* with a disseminated IE infection took a 2nd blood meal. This mosquito transmitted VEE virus by bite. Transmission rates for *Cx.* (*Mel.*) *ocossa* with disseminated IE or IAB VEE virus infections (after either oral exposure or intrathoracic inoculation) were 4 of 6 (67%) and 3 of 6 (50%), respectively.

Infection and dissemination rates in *Ps. confinnis* were similar with both strains of VEE virus ($\chi^2 \leq 0.69$, df = 1, $P \geq 0.41$) (Table 1). However, transmission was inefficient, because only 1 of 16 refeeding females with a disseminated infection (after either oral exposure or intrathoracic inoculation) transmitted virus by bite.

DISCUSSION

Although *Cx.* (*Mel.*) *taeniopus* was highly susceptible to an IE strain of VEE virus, this species was nearly refractory to infection or virus dissemination with an epizootic IAB strain. This is in agreement with studies by Scherer et al. (1986, 1987). This refractoriness to epizootic VEE virus seemed to be due to a combination of midgut infection and midgut escape barriers (Kramer et al. 1981). However, the ability of an inoculated female to transmit the epizootic IAB strain of VEE virus indicated that this species does not have a salivary gland barrier and could transmit virus if it developed a disseminated infection. Studies by Turell et al. (1984b) and Vaughan and Turell (1996) indicate that the concurrent ingestion of virus and microfilariae from a dually infected rodent could allow the virus to bypass both midgut infection and escape barriers. Because filarial infections tend to be lifelong in a rodent, the prevalence of microfilaremias in a region determines the prevalence of dual virus and microfilariae infections. Because filarial infections are hyperendemic in many places in Central and South America (Orihel 1964, Sousa et al. 1974, Godoy et al. 1980), most mosquitoes ingesting virus from a viremic host would also be coingesting microfilariae. Thus, despite the low laboratory infection and dissemination rates reported in this and previous studies for *Cx.* (*Mel.*) *taeniopus,* it is still possible that this species could be involved in the transmission of epizootic as well as enzootic strains of VEE virus.

In contrast to the results with *Cx.* (*Mel.*) *taeniopus,* female *Cx.* (*Mel.*) *ocossa* were equally susceptible to both strains of VEE virus. Likewise, both infection and dissemination rates in *Culex* (*Melanoconion*) *vomerifer* Komp were similar for females orally exposed to enzootic and epizootic strains of VEE virus (Turell, unpublished data). Thus, there does not seem to be a consistent pattern within the subgenus *Culex* (*Melanoconion*) for susceptibility to enzootic, but refractoriness to epizootic, strains of VEE virus.

Although moderately susceptible, *Ps. confinnis* had a major salivary gland barrier as only 1 (6%) of the 16 feeding individuals with a disseminated infection transmitted VEE virus by bite. This was not expected because *Ps. confinnis* was implicated as a vector of VEE virus during several outbreaks (Sellers et al. 1965; Sudia et al. 1971a, 1971b, 1975). The viremias to which mosquitoes were exposed in our study, $10^{7.2}$–$10^{8.2}$ PFU/ml of blood, are consistent with those observed in burros (Gochenour et al. 1962) or horses (Kissling et al. 1956, Sudia et al. 1971a) inoculated with an epizootic strain of VEE virus or in bats inoculated with the enzootic IE strain (Seymour et al. 1978). Despite our increasing knowledge about the natural history of epizootic and enzootic strains of VEE virus, the vertebrate reservoirs and maintenance vectors remain unknown for most of its distribution. Additional studies are needed to clarify the host–vector relationships and to define the enzootic maintenance cycle.

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