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SCIENCE

Culex (Melanoconion) aikenii: Natural Vector in Panama of Endemic Venezuelan Encephalitis

Abstract. *Experiments performed in an endemic area of Venezuelan equine encephalitis in the Panama Canal Zone demonstrated transmission of Venezuelan equine encephalitis virus from naturally infected* Culex aikenii *mosquitoes to laboratory hamsters. Results of experiments indicate that* Culex aikenii *is an efficient natural vector and the principal species of mosquito transmitting Venezuelan equine encephalitis in this endemic zone.*

Venezuelan equine encephalitis (VEE) is a disease of man and equines, widespread throughout Middle America, from Florida south to Ecuador and the Guianas. Two forms of the disease are known, an endemic one present in wet, lowland, coastal areas and the epidemic form which reportedly occurs in explosive outbreaks in drier areas of Central and northern South America and involves thousands of humans and equines. *Culex* mosquitoes of the subgenus *Melanoconion* have come under suspicion as vectors of VEE virus in several endemic areas of the Caribbean region (*1*). Evidence has been based mainly on the frequency of isolation of virus from laboratory mice inoculated directly with macerated wild-caught mosquitoes. However, proof of either natural or experimental transmission through the bites of these mosquitoes has been lacking. Therefore, no conclusive evidence has been produced on the vectorship of these or any other species of mosquitoes in the transmission of endemic VEE.

The discovery, through exposure of sentinel hamsters, of a focus of intense VEE activity in the Panama Canal Zone in August 1970 led to development of a project aimed at determining whether *Culex* (*Melanoconion*) mosquitoes were active in the transmission of VEE virus in that area. Work was carried out along the Chilibre River, which empties into the Chagres River about 500 m upstream from the area of Juan Mina, between Madden Dam and the Canal Zone town of Gamboa. All-night collections with human bait and sporadic hand-collections from sentinel hamsters showed that the most common mosquito biting man and hamsters during the study period was *Culex* (*Melanoconion*) *aikenii* (Aiken and Rowland), 1906 (*2*). This preliminary report presents results of experiments on VEE transmission with wild-caught *C. aikenii* and laboratory hamsters.

Female mosquitoes used in these experiments were captured singly while attempting to bite human subjects exposed at stations along the Chilibre River, where sentinel hamsters had recently died of VEE virus infection. Collections were made daily, between the hours of 6:30 p.m. and 8:45 p.m., from 19 August through 13 September. Immediately after each collecting period all live mosquitoes were identified by species at the Juan Mina field station of the Gorgas Memorial Laboratory. All specimens of *C. aikenii* were subsequently liberated in groups of 80 or less inside Barraud-type cloth cages. Laboratory-bred hamsters were exposed individually to the bites of the mosquitoes held in the cloth cages. Special precautions were taken to prevent possible contamination of normal hamsters held at the field station with VEE virus from infected hamsters and mosquitoes. Each morning after exposure of the hamsters, engorged mosquitoes were separated and transferred from the cages to glass jars lined with plaster of Paris, in which they were held for 24 hours before being stored in liquid nitrogen. Mosquitoes that were not engorged were maintained in similar glass jars during the day and given access to the same hamster in the evening; additional females captured that evening were added to make a total of 80 specimens. With the exception of a few missing specimens from every group, presumably eaten or destroyed by the hamsters, all mosquitoes used in these experiments were frozen in liquid nitrogen along with tissue samples from hamsters which became ill or died after exposure to the bites of wild-caught *C. aikenii*.

Table 1. Results of exposures of hamsters to the bites of wild-caught *C. aikenii*. Six hamsters were infected (+) with VEE; five were not (−).

Hamster No.	Mosquitoes (No.)		Infection
	Allowed to feed	Engorged	
LH-6555	1161	273	−
LH-6556	180	56	+
LH-6564	210	62	+
LH-6622	411	236	+
LH-6623	284	209	+
LH-6625	889	160	−
LH-6626	66	24	+
LH-6631	106	15	+
LH-6767	70	43	−
LH-6768	579	85	−
LH-6769	271	40	−

Attempts to isolate virus from hamsters and mosquitoes were made at the central laboratory by inoculation of suckling mice. Each sample of serum or plasma was diluted in four volumes of phosphate-buffered saline solution containing antibiotics. Bovine albumin was

included in the diluent used for the trituration of hamster tissues and mosquitoes. Specimens from sick or dead hamsters were processed individually, whereas mosquitoes were pooled in groups of ten or less. We made a preliminary identification of viral isolates by testing crude mouse brain antigens with reference antiserum prepared against the No. 3880 strain of VEE virus (*3*), by means of the complement-fixation technique.

Table 1 summarizes results of exposures of hamsters to the bites of wild-caught *C. aikenii* females. A total of 4227 *C. aikenii* were given access to 11 hamsters, and 1203 (28 percent) became engorged. Six of the 11 hamsters exposed came down with VEE infections. In the case of two of the positive hamsters, VEE virus was isolated from one of several pools of mosquitoes which fed on these hamsters 2 to 4 days before death. Processing of mosquitoes allowed to feed on the other four hamsters which developed infections has not yet been completed.

In order to determine the vector potential of the species, 30 wild-caught *C. aikenii*, which had engorged on the blood of three infected hamsters, were held at ambient temperatures (24° to 28°C) for 8 to 16 days, after which they were each given access to a single clean hamster. Twelve of the 30 mosquitoes transmitted VEE virus for an overall transmission rate of 40 percent. The infection rate in 25 of these mosquitoes tested individually for presence of virus was 92 percent.

Results of these experiments show that *C. aikenii* was responsible for at least some of the VEE being transmitted in the study area. This conclusion is reached from the fact that VEE virus was isolated from clean laboratory-bred hamsters after exposure to wild-caught mosquitoes captured along the Chilibre River and, in some cases, from mosquitoes which engorged on their host 2 to 4 days before its death, the period of time which corresponds to the usual incubation of the virus in hamsters. The high rate of VEE transmission obtained in these experiments suggests that this species is a very efficient vector of the virus in nature. The high population density of *C. aikenii* as compared with other mosquito species, coupled with epidemiological information gathered in the study area but not pertinent to this report, indicates that this culicine species was the most important, if not the only, mosquito vector of VEE virus in this endemic area during the study period.

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References and Notes

1. P. Galindo, S. Srihongse, E. de Rodaniche, M. A. Grayson, *Amer. J. Trop. Med. Hyg.* **15**, 385 (1966); T. H. G. Aitken, L. Spence, A. H. Jonkers, W. G. Downs, *J. Med. Entomol.* **6**, 207 (1969); R. W. Chamberlain, W. D. Sudia, T. H. Work, P. H. Coleman, V. F. Newhouse, J. G. Johnston, *Amer. J. Epidemiol.* **89**, 197 (1969).
2. At present this group of mosquitoes poses a taxonomic problem. J. N. Belkin [*Mosquito Syst. Newsl.* 2, 59 (1970)] relegated the name *Culex* (*Melanoconion*) *aikenii* (Aiken and Rowland), 1906, to the status of a *nomen dubium* and resurrected the names *ocossa* Dyar and Knab and *panocossa* Dyar from the synonymy of *aikenii* to designate two closely related forms which he considered distinct species. Work being carried out at Gorgas Memorial Laboratory has shown that systematic relationships between the two forms and their taxonomic status need further clarification. For this reason we decided to continue the use of the old, well-established name of *Culex aikenii*, until the above-mentioned studies are completed.
3. K. M. Johnson, A. Shelokov, P. H. Peralta, G. J. Dammin, N. A. Young, *Amer. J. Trop. Med. Hyg.* **17**, 432 (1968).
4. Supported by PHS grant AI-02984.

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