713 Reprinted from Venezuelan Encephalitis
Scientific Publication No. 243
Pan American Health Organization

ENDEMIC VECTORS OF VENEZUELAN ENCEPHALITIS

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There are 41 species belonging to 11 genera of mosquitoes that have been reported naturally infected with endemic viruses of the VE complex (*2, 3, 4, 6, 7, 14*). Of these, 20 belong to the genus *Culex* and 13 to the subgenus *Melanoconion*. Of a total of 406 isolations reported from naturally infected mosquitoes, 286 or 70 per cent were obtained from *Culex* (*Melanoconion*) females. In addition, the only two species of mosquitoes that have been proved to be efficient natural vectors of endemic VE are *Culex* (*Melanoconion*) *aikenii* (*8*) and *C.* (*M.*) *portesi* (*3*).

Based on these facts, the conclusion may be reached that some *Culex* species of the subgenus *Melanoconion* are the principal natural vectors of endemic VE viruses throughout their range of distribution. However, the number of isolations obtained from wild-caught specimens belonging to other taxa, coupled with experimental work demonstrating the efficiency of mosquitoes other than *Culex* in the transmission of these viruses (*12*), appears to indicate that other groups besides species of *Culex* (*Melanoconion*) may be involved at least as secondary vectors in the natural transmission of endemic viruses of the VE complex.

It would seem that the critical factors which determine vectorship of a particular species of mosquito are its accessibility to the blood of vertebrates circulating moderate to high titers of viruses and the threshold of infection for each species of mosquito. Since wild rodents are known to develop fairly high viremias of VE for several days (*10, 16*) and have been frequently found infected in nature (*6, 7, 14*), mosquitoes that habitually feed on these vertebrates are to be considered prime suspects as natural vectors of the virus. It must be pointed out, however, that recent experimental work (*10*) has demonstrated that other vertebrates, such as sloths and herons, are capable of circulating fairly high titers of virus for more than one day. These titers were found to be sufficiently high to infect at least the more efficient mosquito vectors such as *Culex aikenii* (*11*). It is thus possible that secondary natural transmission chains, other than the usual rodent-mosquito-rodent cycles, may exist in some areas.

The discussion that follows will deal exclusively with *Culex* (*Melanoconion*) mosquitoes, which we consider to be the principal vectors of endemic VE viruses.

The subgenus *Melanoconion* of *Culex* is a taxon almost exclusively of neotropical distribution with a few incursions into the nearctic region. It includes approximately 130 described species, of which fewer then 10 per cent have been reported naturally infected with VE virus. Most of the species that have been found harboring the virus in nature belong to two closely related categories, the *spissipes* and *aikenii* groups. Some morphologic characteristics of the larvae

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[2] All work on which this report is based has been partly financed by Grant AI.02984 from the National Institute of Allergy and Infectious Diseases.

appear to bridge the gap separating these two groups and also seem to indicate that they are more highly evolved taxa within the subgenus. Described species of the *spissipes* group are: *spissipes, taeniopus, epanastasis* (= *crybda*), *vomerifer, paracrybda, portesi, pseudotaeniopus, pereyrai,* and *delpontei.* Of these, all but the last three have been found carrying VE virus in nature. The *aikenii* group is made up of the following species: *atratus, aikenii, dunni, zeteki, commevynensis,* and *caribeanus.* Only *aikenii* and *dunni* have thus far been reported naturally infected with VE virus (*14*).

Our information on *C. aikenii* has been drawn largely from forthcoming papers on the colonization and ecology of the species (*1, 9*). *Culex* (*Melanoconion*) *aikenii* (Aiken and Rowland, 1906) was described from British Guiana. As is now known, it occurs from Mexico south to Brazil and Ecuador. It appears to be composed of at least two morphologic forms that have recently been given the rank of distinct species (*5*). We consider that the taxonomic status of these forms is still uncertain and until work now in progress is completed, we are considering the whole complex as a single species under its present accepted name.

C. aikenii occurs in very humid localities with abundant open spaces, as in sunny, swampy pastures cut by slowly flowing, meandering streams. The species requires a mean temperature of 26.6°C and very high relative humidity in its microhabitat. The immature stages are usually found intimately associated with the aquatic, floating aroid known as *Pistia stratiotes* Linn., or water lettuce, although they have been collected on several occasions in the absence of *Pistia* from a mixed association dominated by the water pier (*Ludwigia natans*), accompanied by water weeds (*Hydrilla verticillata*) and water ferns (*Salvinia auriculata* and *Azolla* sp.). The habits of the immature stages of *C. aikenii* described below for *Pistia* communities appear to be the same in *Ludwigia* associations.

Larvae and pupae very seldom come up to the open surface of the water to breath, usually resting under the *Pistia* leaves where they draw oxygen from air pockets formed along the grooved surface of the leaf. Larvae remain motionless for days under the leaf, unless disturbed, maintaining an almost horizontal position. This behavior appears to have developed as a protective mechanism against the numerous predators that constitute an inherent part of the ecosystem sustained by the *Pistia* communities. A similar mechanism has been described for *Mansonia* spp., *Ficalbia* spp., and *Aedeomyia* spp., and for *Culex poicilipes* larvae which breed in similar situations (*13, 15*). In the laboratory, *C. aikenii* larvae are greatly dependent on *Pistia* for development and, unless ready to pupate, die within a few days if deprived of at least the *Pistia* leaves. The factors connected with this dependence of the larvae on the plants are unknown at present, but it is possible that nutritional requirements may be one of them since special microflora and microfauna develop around the *Pistia* leaves. The larval period extends for an average of 10 days, at a mean temperature of 26.6°C. Mortality of the pupae increases greatly in the absence of *Pistia,* but a fair percentage of apparently normal adults manage to emerge without water lettuce. The pupal period lasts from 48-60 hours at the above-mentioned mean temperature.

Sexual activity and blood sucking begin simultaneously about 60-72 hours after emergence. While insemination is not a prerequisite for blood feeding of females, it has been noted that most blooded specimens have already undergone copulation. Mating takes place during the short crepuscular period at dusk and at dawn. Copulation has not been closely observed, but before it unusual activity of the males has been noted over the *Pistia* plants.

Blood feeding of the females is restricted to the hours between dusk and dawn. All night captures with human bait, carried out once a month for one year, indicate that peaks of activity are quite variable during the night and that such peaks may be associated with microclimatic factors that were not recorded.

Direct observations made using a number of live baits showed that *C. aikenii* is readily attracted in numbers to such animals as cows, horses, pigs, chickens, rodents, and marsupials. Precipitin-test surveys, conducted in collaboration with Dr. C. H. Tempelis of the University of California at Berkeley, indicate that the species has a wide range of hosts, including poikilothermic animals, with a decided predilection for warm-blooded vertebrates. There is a slight preference for mammals over birds. Among the latter, herons are by far the hosts of choice. In the absence of many of the large domestic animals, rodents are decidedly preferred over other mammals. However, when the sample is biased by the presence of large numbers of cattle, the latter become the preferred hosts.

C. aikenii is definitely a ground-feeding mosquito. In one year of simultaneous ground and canopy catches, the species was seldom taken even at maximum height of 8 meters above the ground. In areas where *C. aikenii* is the main vector of endemic VE, this habit is reflected in the greater frequency with which VE antibodies are found in terrestrial as against arboreal vertebrates, as well as in the much higher rate of virus isolations from sentinel hamsters exposed on the ground as against those placed in the canopy (*14*).

The species has been taken in numbers attacking pigs inside rather open enclosures, but it has not been observed to enter human dwellings. *C. aikenii*, when biting man, prefers to do so around the lower extremities and over 85 per cent of bites counted were restricted to the bare feet. Rodents and marsupials, either exposed as sentinels or recently captured in live-baited traps, are usually bitten on the paws or around the ears. Sentinel hamsters have been frequently noted to swallow specimens approaching to bite.

No exact figures have been obtained as yet on the mean longevity of *C. aikenii* in the insectary. However, it has been noted that the species is very sensitive to sudden drops in relative humidity and rises in temperature. There is rapid die-off in adults if the temperature rises above 27.7°C unless there is a cooling device where adults may rest, such as the leaves of *Pisita* plants or a wet, raw clay surface. At a mean temperature of 26.6°C (±1.7°) and a relative humidity above 90 per cent, females have been kept alive for over three months.

No carefully computed data are available as yet on the different aspects of the gonotrophic cycle. However, the following observations have been made. Most females begin laying eggs about seven days after their first blood meal, but they may refeed three days after having fed on blood for the first time and several days before they are ready to oviposit. They have been observed to refeed on blood three times during a period of four weeks following the initial blood meal. Eggs are deposited singly on both sides of the *Pistia* leaves, usually close to surface of the water. In the insectary large numbers of eggs are accumulated on a single leaf, but in nature oviposition is widely scattered so that few eggs are deposited on one leaf. Hatching takes place from two to six days after oviposition, apparently depending on the contact of the eggs with the surface of the water. No prolonged delay in hatching or resistance to dessication has been noted.

The water-lettuce plant, on which *C. aikenii* is greatly dependent for its existence, has very exacting growth requirements. It needs large daily amounts of direct sunlight. It reproduces by seeds and by shooting off stolons, and prefers the slowly running, sunlit waters of open meandering streams carrying large amounts of silt in suspension. During quiet water periods, *Pistia* communities in such places proliferate rapidly from the banks of the streams, where they are loosely anchored, toward the center of the current. At such times, populations of *C. aikenii* reach very high peaks. During heavy rains, when surface run-off speeds up the current of the stream, large patches of *Pistia* plants, varying in size from a few plants to whole communities, are dislodged from their anchorage and transported downstream, for greater or lesser distances, until they become entangled in inlets or oxbows to give rise to new communities. These patches of *Pistia* carry with them large numbers of larvae and pupae of *C. aikenii* which remain attached to the leaves and seed the new anchorage with a fresh population of mosquitoes. When the dislodgement of the plants takes place at night, gravid females in the act of laying may also be transported downstream. These movements of *Pistia* serve a triple purpose: (1) they act as a restraining mechanism on the local population growth of *C. aikenii* in areas where permanent communities of water lettuce occur; (2) they serve as a method of dispersal of this mosquito species; and (3) they probably help to introduce VE virus from endemic, highly localized centers of intense virus activity to clean localities downstream by transporting infected gravid females.

In the middle Chagres River basin of Panama, permanent communities of *Pistia* and intense, year-round VE activity occur only in the Chilibre River, a tributary of the Chagres. Movements of water lettuce from the tributary to the main channel constitute the controlling factor responsible for the periodic appearance and disappearance of VE virus along the banks of the Chagres, downstream from the mouth of the Chilibre River. *Pistia* communities along the main channel are short-lived, since periodic spillage from Madden Dam above has a flushing effect along the channel that removes the water-lettuce communities to the area of Gamboa, where they are gathered and burnt to prevent littering the main channel of the Panama Canal.

The preliminary observations on the bionomics and VE transmission ability of *C. aikenii* noted above would seem to indicate that this species has the potential to become a most formidable vector of arboviruses. This presumption, coupled with the wide distribution of the species and its common occurrence near human communities, warrants further studies in depth on the systematics, ecology, and disease transmission potential of this mosquito.

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