ARTICLES

COMPARISONS OF *AEDES HENDERSONI* AND *AE. TRISERIATUS* AS POTENTIAL VECTORS OF *DIROFILARIA IMMITIS*[1]

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ABSTRACT. The tree-hole mosquitoes *Aedes hendersoni* and *Ae. triseriatus* were compared as intermediate hosts of *Dirofilaria immitis* (dog heartworm). Both species of mosquitoes supported the development of *D. immitis* to the infective (L_3) stage. On post infection day 16 *Ae. hendersoni* had greater mortality than *Ae. triseriatus*, and the mean number of infective *D. immitis* larvae in the heads and mouthparts of *Ae. hendersoni* was greater than in *Ae. triseriatus*. The behavior of *Ae. triseriatus* in nature makes it highly suspect as a vector of *D. immitis*, but little is known about the natural behavior of *Ae. hendersoni* as it relates to its potential for transmitting this parasite.

INTRODUCTION

In recent years dog heartworm, *Dirofilaria immitis* (Leidy) has become a major problem in the upper mid-western portion of the United States. The species of mosquitoes that may act as vectors of *D. immitis* in this area are not known with certainty, but 2 species, *Aedes triseriatus* (Say) and *Ae. hendersoni* Cockerell, appear to be likely suspects. These 2 tree-hole mosquitoes are sympatric throughout the eastern United States (Zavortink 1972) and are common in hardwood forests and woodlots of the Great Lakes region. Until Breland (1963) elevated *Ae. hendersoni* to full specific rank, it was considered to be a variety of *Ae. triseriatus*. The close similarity of both the immature and adult forms of these 2 species presents problems in any study concerning their role as disease vectors. Although controlled experiments have not yet proven conclusively that *Ae. triseriatus* is a vector of *D. immitis*, there is no question that it has the potential for serving in this capacity. It has been demonstrated that it can support the development of *D. immitis* from the microfilarial to the infective (L_3) larval stage (Keegan et al. 1968, Intermill 1973) and in nature it does feed on dogs (Phillips 1939, Lewandowski 1977). To this time, however, there have been no reported studies on the possible role of *Ae. hendersoni* as a vector of *D. immitis*. This study compares the development of *D. immitis* in *Ae. hendersoni* and *Ae. triseriatus*, and is the 1st report on the possible role of *Ae. hendersoni* as a vector of *D. immitis*.

MATERIALS AND METHODS

The *Ae. hendersoni* and *Ae. triseriatus* adults used in this study were from stock colonies that had been maintained in the laboratory for approximately 1 year and had progressed through several generations before the study was started. Both colonies were started from eggs collected in ovitraps located in a woodlot on the campus of Michigan State University, East Lansing, Michigan. In starting these colonies, special precautions were taken to insure that the 2 species were kept separate and the colonies remained unmixed. After the field collected eggs were

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brought to the laboratory they were allowed to maturate, and then the eggs from each ovitrap were placed in a separate pan containing deoxygenated water and allowed to hatch. After hatching, all the egg cases were identified to species by the method of Zaim et al. (1977). Larvae from egg batches that contained only 1 species, either *Ae. hendersoni* or *Ae. triseriatus*, were reared and added to the appropriate colony while those that originated from mixed egg batches were discarded. After the initial colonies were established both larvae and adults were periodically checked according to the species criteria of Grimstad et al. (1974) to insure that the colonies remained unmixed. The *Ae. triseriatus* colony was free breeding and the *Ae. hendersoni* colony was propagated by the forced copulation technique of McDaniel and Horsfall (1957). Otherwise, the colonies were maintained by identical methods. Larvae were fed Tetramin® fish food and the adults were provided with a 7% sucrose solution in a reservoir with a cotton wick. For egg production females were allowed to feed on an unanesthetized guinea pig placed in a restraining device inside the mosquito cages. During the experiments the adults were 4–7 days old when given the infective blood meal and subsequently were kept in an insectary where the temperature ranged from 21–25.6°C and the relative humidity from 80–100%.

The experiments reported here each consisted of 10 replicates performed over a period of approximately 15 weeks. The same *D. immitis* infected dog was used as the source of all the infective blood meals and the microfilaremia level was determined periodically during the study period. For these determinations, blood samples were obtained at 9 am, the time at which infective feeding for each replicate was started. Blood was taken from the cephalic vein of the infected dog with a syringe containing a pinch of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, and 20 mm^3 of the sample was transferred from the syringe and divided between 2 slides. A 20 × 60 mm coverslip was then placed on each slide, the slides were examined under a compound microscope, and the microfilariae on both slides were counted.

To infect the mosquitoes with *D. immitis* a shaved leg of the infected dog was placed in a cage containing a colony of either *Ae. hendersoni* or *Ae. triseriatus*, and the mosquitoes were allowed to feed for approximately 10 min (both species fed aggressively on the dog in these studies). The dog's leg was then removed from the first cage and placed in the cage containing the other mosquito species and kept there for a 10 min period. The order in which *Ae. hendersoni* and *Ae. triseriatus* were allowed to feed in each replicate was determined by flipping a coin. After each feeding, 300 fully engorged females of each species were transferred to separate cages and held for 16 days. (Under conditions of this experiment *D. immitis* larvae developed to the infective stage and migrated to the mosquito's head and mouthparts ca. 14 days after an infective meal.) At the end of the holding period the number of survivors in each cage were counted, 12 survivors of each species were dissected in a drop of saline, and the number of infective stage larvae found in the head and mouthparts of each was recorded.

RESULTS AND DISCUSSION

Circulating microfilariae were present in the donor dog's blood throughout the study period with the observed densities ranging from a low of 259 to a high of 400 per 20 mm^3 of blood. The mortality rate in the infected mosquitoes of both species was very high (Table 1) with that of *Ae. hendersoni* being significantly greater than *Ae. triseriatus* ($t = 3.01$, 9 df). Dissection results (Table 2) clearly demonstrate that *Ae. hendersoni* is biologically capable of supporting the development of *D. immitis* from the microfiliarial to the infective larval stage and is, therefore, a potential vector of dog heartworm. These data also indicate, with 99% confidence, that *Ae. hendersoni* can support the devel-

Table 1. Sixteen day survival rate of *Dirofilaria immitis* infected mosquitoes.

Species	No. survivors/300 infected adults									
	Replicate No.									
	1	2	3	4	5	6	7	8	9	10
Aedes hendersoni	33	16	15	17	12	13	37	26	45	16
Ae. triseriatus	50	73	21	16	40	24	55	134	116	31

Table 2. Mean number of third stage *Dirofilaria immitis* larvae in mosquito heads and mouthparts on post-infection day 16.

Species	Replicate No.									
	1	2	3	4	5	6	7	8	9	10
Aedes hendersoni	6.7	4.7	3.7	8.4	3.7	8.1	10.2	8.5	13.0	5.4
Ae. triseriatus	4.4	6.4	3.0	4.7	2.7	4.9	4.3	6.1	4.8	1.8

opment of a greater number of *D. immitis* than *Ae. triseriatus* (t = 3.36, 9 df).

Even though both of these species of mosquitoes are suitable intermediate hosts of *D. immitis*, whether or not either of them actually functions as vectors of dog heartworm under natural conditions still is a matter of speculation. *Ae. triseriatus* appears to have no marked host preferences and will feed on a wide variety of vertebrates, both warm and cold blooded (Wright and DeFoliart 1970, Benach et al. 1971). On the other hand, virtually nothing is known about the host selections of *Ae. hendersoni*. The known behavioral characteristics of the 2 species also may play an important part in whether or not they transmit *D. immitis* to any significant degree in nature. As measured by oviposition, *Ae. triseriatus* reportedly exhibits a strong preference for ground level activity while *Ae. hendersoni* appears to be more arboreal (Scholl and DeFoliart 1977, Sinsko and Grimstad 1977). There is some indication however that this reported behavior of *Ae. triseriatus* may not be consistent throughout its entire range (J. S. Rogers, unpublished data).

Given its propensity for opportunistic feeding and activity near ground level, and its known concentration in hardwood forested areas, *Ae. triseriatus* appears to be a prime suspect as a *D. immitis* vector in many parts of the mid-west, especially in developed campgrounds and housing subdivisions where concentrated dog populations frequent the natural habitat of this species. Although *Ae. hendersoni* is now known to be biologically capable of supporting the development of *D. immitis* larvae to the infective stage, the other factors needed to evaluate its true vector potential remain to be studied.

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