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DETERMINATION OF SOME IMPORTANT NATURAL POTENTIAL VECTORS OF DOG HEARTWORM IN CENTRAL MICHIGAN

H. B. LEWANDOWSKI, JR.,[1] G. R. HOOPER AND H. D. NEWSON
Department of Entomology, Michigan State University, East Lansing, Michigan 48823

ABSTRACT. Adult female mosquitoes were collected in the Lansing, Michigan area during the summers of 1974 and 1975 in dog-baited traps and CDC miniature light traps. Mosquitoes were identified and placed in groups of 25 or 30, crushed in 0.9% saline, and examined for the presence of *Dirofilaria immitis* (Leidy) larvae. Presumed *D. immitis* infective larvae were isolated from field-captured *Aedes vexans* (Meigen) on 3 occasions and *Anopheles quadrimaculatus* Say on 2 occasions. *D. immitis* developmental trials were conducted in the laboratory using Michigan strains of *Ae. stimulans* (Walker, *Ae. vexans, An. quadrimaculatus, Coquillettidia perturbans* (Walker) and *Culex pipiens* Linnaeus. Complete development of *D. immitis* to the infective stage was noted in *Ae. vexans, An. quadrimaculatus*, and *Cx. pipiens. Ae. vexans* and *An. quadrimaculatus* are considered to be the primary vectors of *D. immitis* in the study area. *Cx. pipiens* is a possible natural vector.

INTRODUCTION

Credit is generally given to Grassi and Noe (1900) for experimentally demonstrating the development of *Dirofilaria immitis* (Leidy) in the mosquito, but Feng (1930) was the first to report that all mosquito species are not efficient hosts of this parasite. Reviews by Bemrick and Sandholm (1966), Ludlam et al. (1970), and Kutz (1972) indicate that there are now at least 80 species of mosquitoes which have been experimentally infected with *D. immitis*, although complete development to the infective stage has not always been observed. In spite of the numerous reports of laboratory investigations, little is known about the natural vectors of this nematode because third stage filarid larvae isolated from field-captured mosquitoes cannot always be specifically identified. Those which develop in the Malpighian tubules of a mosquito host are considered to be in the genus *Dirofilaria* (Christensen and Andrews 1976), but species other than *D. immitis* may be isolated from the Malpighian tubules of field-captured mosquitoes. Because filarids in this genus lack distinguishing anatomical features, the results of field investigations have been difficult to evaluate, and relatively few reports of these investigations have been published. Some authors (Arnott and Edman 1978) have used morphological descriptions in the literature to differentiate several common, 3rd stage, filarid nematodes, including *D. immitis* and *D.*

[1] Present Address: Navy Environmental and Preventive Medicine Unit No. 5, Naval Station, Box 143, San Diego, CA 92136.

tenuis Chandler. Orihel (1959), however, after a detailed examination of these 2 species concluded that they could not be distinguished and many others concur with this conclusion. Obviously, further study will help clarify the problem of identifying filarid larvae isolated from field-captured mosquitoes.

Presently, a renewed interest in dog heartworm has been stimulated by an alarming increase in its reported incidence. As a result, researchers are now attempting to identify the specific mosquito vectors of *D. immitis* in various areas of the U. S. The reports of field investigations to date include those of Bemrick and Sandholm (1966) in Minnesota, Villavaso and Steelman (1970) in Louisiana, Bickley et al. (1976) in Maryland, Christensen and Andrews (1976) in Iowa, Arnott and Edman (1978) in Massachusetts and Magnarelli (1978) in Connecticut. This is a report of field research to determine the natural vectors of *D. immitis* in the metropolitan area of Lansing, Michigan.

MATERIALS AND METHODS

Field research was conducted during the summers of 1974 and 1975. Adult female mosquitoes were collected at 5 sites where dogs had recently developed heartworm infections and taken to the laboratory for examination. Collecting periods, which were approximately 12 hours in duration, began at dusk and ended at dawn. Biweekly collections using CO_2 baited CDC miniature light traps and dog-baited traps were made at 3 of the 5 sites. Weekly collections were made at the remaining 2 sites utilizing only the CO_2 baited CDC light traps, modified (Fig. 1) to increase the longevity of captured mosquitoes. Light weight dog-baited traps (Fig. 2) were especially constructed for this project. The louvers of the collecting boxes were designed as suggested by Bates (1949).

In the laboratory, captured mosquitoes were anesthetized with CO_2 and identified. Each species then was placed in pools of 25 or 30 mosquitoes (or less if fewer had been captured) and examined for the presence of infective filarid larvae using the mass separation method of Crans (personal communication). This technique was selective for extracting the larvae present in the hemocoel of mosquitoes and was preferred to individual dissection because large numbers of mosquitoes could be examined in a relatively short time. Pooled mosquitoes were crushed in 0.9% saline then placed in a plastic container from which the bottom had been removed and replaced with a fine mesh gauze. This container was set in a crystallization dish and additional saline was added to a level which just covered

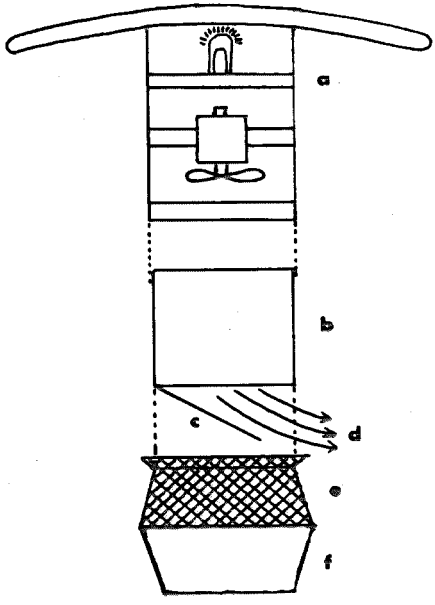

Fig. 1. Modified CDC trap with hardware cloth removed at (a), pint ice cream container inserted at (b) with bottom (c) partially cut out to form a baffle to direct air flow (d) through the stockinette (e). Mosquitoes are held in the collecting chamber (f) in which moist paper toweling is placed on the bottom.

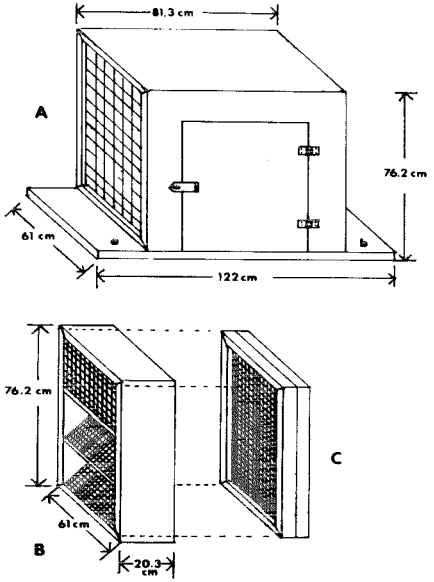

Fig. 2. Dog-baited trap: A) Main compartment where dog is held; the wire screen is 1" hardware cloth; B) Mosquito collecting chambers which are set on the platform of the dog chamber at points (a) and (b); C) Removable screen frame which is taken off to remove mosquitoes.

the gauze. Living, third stage nematode larvae left the mosquito debris and dropped through the gauze into the crystallization dish. After 1 hr, the contents of the crystallization dish were placed in a 60 ml separatory funnel and concentrated by gravitation for 30 minutes. Then, 5–6 ml of the saline was drawn off from the bottom of the funnel and examined for 3rd stage larvae.

Nematodes belonging to several genera were isolated from field-captured mosquitoes and many were readily distinguishable from *Dirofilaria* sp. larvae. In this study, all 3rd stage filarid larvae within the normal size range for *D. immitis* and whose activity matched that of *D. immitis* were presumed to be that species.

In 1976, *D. immitis* developmental studies were conducted in the laboratory to determine the host suitability of those mosquito species suspected of being natural vectors. The species selected for these experiments had been: 1) consistently attracted to the dog-baited traps; 2) most numerous in CDC trap collections; 3) incriminated in the literature as potential vectors of dog heartworm, and; 4) 3 of the 5 species tested were found to be harboring natural infections indistinguishable from *D. immitis*. The mosquitoes were collected as larvae or adults in the study area. Larvae were raised to the adult stage in the laboratory. Adult females were allowed to feed directly on a *D. immitis* infected dog or through cow-gut membranes stretched over glass containers that held blood taken from an infected dog. Blood was maintained at room temperature during membrane feeding trials. After the infective blood meal, mosquitoes were maintained at 26.7°C and 80% relative humudity and dissected at various intervals to determine the developmental prograss of *D. immitis*.

RESULTS AND DISCUSSION

During the 2 field seasons 2,166 mosquitoes belonging to 14 species were collected in dog-baited traps and 90,907 mosquitoes belonging to 30 species were collected in CO_2 baited CDC light traps. The composition of the most abundant species captured in dog-baited/CDC light traps, respectively, were as follows: *Cq. perturbans* (Walker), 44%/2%; *Cx. pipiens* Linnaeus, 17%/2%; *Ae. vexans* (Meigen), 12%/76%; *An. walkeri* Theobald, 11%/7%; *An. quadrimaculatus* Say, 7%/3%; and *Ae. stimulans* (Walker) and *Ae. fitchii* (Felt and Young), collectively, 3%/2%. The CDC trap collections indicated that those species attracted to the dog-baited traps were also those of great abundance in the study area. Except for *Cx. pipiens*, the species collected in the dog-baited traps are known to prefer mammalian hosts (Edman 1971 and 1974, and Tempelis 1975) so were considered initially as likely

vectors of dog heartworm in the study area.

Of the mosquitoes collected, 43,627, belonging to 25 species, were examined for the presence of *D. immitis* larvae. Third stage larvae indistinguishable from *D. immitis* were found only in *Cx. pipiens, An. quadrimaculatus* and *Ae. vexans*. Thirty-three larvae were isolated from a pool of 12 *Cx. pipiens*; 4 and 20 larvae from pools of 4 and 25 *An. quadrimaculatus*; and 12, 3, and 20 larvae from 3 individual pools of 25 *Ae. vexans*.

There is additional, indirect, evidence that nematodes obtained from one of the pools of *Ae. vexans* may have been *D. immitis*. After these infective larvae were found, 7 dogs living at the capture site were examined for microfilariae, and one of these, thought to be cured of heartworm the previous year, again exhibited a low level microfilaremia. This infected dog was at the study site preceding and during the time that mosquitoes were collected and examined for *D. immitis* larvae.

Cq. perturbans also seemed likely to be a vector because it was by far the most abundant mosquito collected in the dog-baited traps. *Ae. stimulans* also was abundant in dog-baited traps and was found to be exceptionally long-lived. During this study, adult females of this univoltine species which emerged in late May or early June were collected as late as mid-August. Assuming it was a suitable host, its longevity could greatly enhance its potential for transmitting *D. immitis* under natural conditions.

On the basis of the field collections and laboratory examinations 5 species were selected for testing as biological hosts of *D. immitis: Ae. stimulans, Ae. vexans, Cx. pipiens, Cq. perturbans* and *An. quadrimaculatus*. Results of these laboratory development trials are summarized in Table 1. *Ae. stimulans* proved to be a poor host for *D. immitis*. Microfilaria did not become established in the Malpighian tubules of 16 of 19 mosquitoes dissected and encapsulation was observed in 3 mosquitoes in which larvae did reach the Malpighian tubules. (Encapsulation is a mechanism that prevents full development of filarid larvae in the mosquito host.) Development also appeared to be retarded because 3rd stage larvae were still present in the Malpighian tubules as

Table 1. Summary of *Dirofilaria immitis* developmental trials.

Species	Total Taking/No. Blood Meal Dissected	No. Uninfected	No. with Encapsulated Larvae	Minimum Days Required for Development		
				L_2[a]	L_3[b] M.T.[c]	L_3[b] Head
Aedes stimulans	57/19	16	3	[d]	18	[e]
Coquillettidia perturbans	244/87	58	4	8	[e]	[e]
Aedes vexans	481/29	10	7	7	9	12
Anopheles quadrimaculatus	83/15	0	0	9	10	13
Culex pipiens	121/72	63	0	9	14	15

[a] Second stage larvae.
[b] Third stage larvae.
[c] Malpighian tubules.
[d] Development to this stage obviously occurred but was not observed.
[e] Development to this stage was not observed.

were 2nd stage larvae 18 and 26 days respectively, after the infective blood meal. All 3rd stage larvae in the Malpighian tubules were encapsulated and never were they found in the head of this species. Yen (1938) reported similar results with *Ae. stimulans* but still felt that this species was a likely vector of dog heartworm in Minnesota. We believe that our data indicate that Michigan strains of *Ae. stimulans* are not natural vectors of this parasite.

Cq. perturbans also proved to be an unacceptable host for *D. immitis*. Microfilariae did not become established in 58 of 87 individuals examined and encapsulation of larvae was observed in 4 individuals in which microfilariae did reach the Malpighian tubules. Second stage larvae were seen only once and infective, 3rd stage, larvae were never observed during the developmental studies. Yen (1938) had similar results with this species, and Bemrick and Sandholm (1966) reported development of *D. immitis* to the infective stage but never found larvae in the head. Our results indicate that Michigan strains of *Cq. perturbans* are not vectors of dog heartworm.

Ae. vexans proved to be a suitable host for *D. immitis*. Microfilariae became established in the Malpighian tubules of 19 of 29 dissected individuals. Encapsulation of larvae at various stages of development was common but some larvae in every infected mosquito examined remained unencapsulated. Infective larvae reached the mouthparts of infected mosquitoes in as little as 12 days after the infective blood meal. These results closely parallel those of other investigators. Hu (1931) found higher rates of infection but believed that relatively few larvae became established in the Malpighian tubules of this species. Complete development to the infective stage has also been reported by Yen (1938), Bemrick and Sandholm (1966), Jankowski and Bickley (1976), and Arnott and Edman (1978). There is general agreement that *Ae. vexans* is a suitable host for *D. immitis* and our results indicate that this species is probably involved in the natural transmission of this parasite in Michigan.

An. quadrimaculatus was found to be an excellent host for *D. immitis*. All individuals taking an infective blood meal became infected. Infective larvae reached the mouthparts of this mosquito in as little as 13 days after the infective blood meal and no encapsulated larvae were observed. Phillips (1939) and Kartman (1953) also reported high rates of infection and successful development of *D. immitis* in *An. quadrimaculatus*. This species is an efficient host for *D. immitis* and is probably one of the most important species involved in the natural transmission of this parasite in Michigan.

Cx. pipiens was found to be an inefficient host for *D. immitis*. Microfilariae did not become established in the Malpighian tubules of 63 of 72 dissected mosquitoes and never was more than a single larvae, at any stage of development, observed in an individual mosquito. However, larval development did not appear to be retarded and was completed in only 15 days. No encapsulated larvae were found. A single infective larva was dissected from the proboscis of one *Cx. pipiens* during these experiments. Our data seem to indicate that the 33 larvae isolated, presumably from a single mosquito (because of the scarcity of larval isolates) in a pool of 12 field-captured *Cx. pipiens* during the field studies were not *D. immitis* because our laboratory experiments have revealed that *Cx. pipiens* could not support such a heavy parasite load. Hu (1931) also reported low infectivity rates for this species. Even recognizing that *Cx. pipiens* is a poor host for *D. immitis* and a species which prefers avian hosts, however, its importance as a vector of dog heartworm should not be discounted. No doubt it is not a major vector of this parasite, but *Cx. pipiens* is a very domestic mosquito which often utilizes man-made breeding sites. In urban areas, it may be one of only a few species able to utilize these habitats and under such circumstances it might play a role in *D. immitis* transmission. Considering that *Cx. pipiens* can support complete

development of *D. immitis*, this species should be regarded as a potential vector of dog heartworm in Michigan. Determination of its relative importance as a vector deserves further study.

To conclude, *An. quadrimaculatus* and *Ae. vexans* appear to be the primary vectors of dog heartworm in central Michigan. Infective *D. immitis* larvae were isolated from field-captured specimens and Michigan strains of both species supported complete development of this parasite. Although *An. quadrimaculatus* is the more efficient host for *D. immitis*, *Ae. vexans* may be the more important vector because of its overwhelming abundance in the study area. As discussed earlier, *Cx. pipiens* also shows some potential to transmit *D. immitis*. In addition to the species considered in this study, other species of mosquitoes in central Michigan appear to have secondary importance as natural vectors of dog heartworm. Their importance, however, is limited primarily by habitat requirements, seasonal distribution, local abundance, and host suitability. Therefore the maintenance of *D. immitis* infections depends on a complex set of factors, each of which must be considered in determining the relative importance of local vectors.

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INHERITANCE OF WHITE-BODY AND BROWN-EYE IN *AEDES ALBOPICTUS*

TAKEO TADANO
Dept. of Medical Zoology, St. Marianna University School of Medicine, Sugao, Kawasaki City 213, Kanagawa, Japan
AKIO MORI AND YOSHITO WADA
Dept. of Medical Zoology, Nagasaki University School of Medicine, Nagasaki City 852, Japan

ABSTRACT. A recessive mutant, *brown-eye* (*b*), and a dominant mutant, *White-body* (*Wb*), were isolated from a strain of *Aedes* (*Stegomyia*) *albopictus* a vector of dengue. The *b* phenotype can be best distinguished in the pupal eyes, and the expressivity is rather variable but with complete penetrance. The whole abdomen, scutum and vertex of the *Wb* mutant are covered with white scales; *Wb* homozygotes are lethal most probably in their egg stage, although they may rarely survive to the adult stage. Backcrosses involving *Wb* and *b* alleles revealed absence of genetic linkage among *Wb*, *b* and the sex allele (*M*); the 3 alleles might be markers of all 3 linkage groups in this mosquito.

Aedes albopictus (Skuse), subgenus *Stegomyia*, occurs widely in the Oriental Region, Oceania and Australia, and is a vector of viral diseases such as dengue. Formal genetics of this species have been reported in only a few papers; Bat-Miriam and Craig (1966) described several mutants including a homeotic mutant *proboscipedia*, which is located about 20 recombination units far from the sex locus (Quinn and Craig 1971). No further linkage study has been made in this mosquito.

A new dominant mutant *White-body* (*Wb*) was recovered from the Nagasaki strain of this species by two of us (Mori and Wada). We had selected for the *Wb* trait for 6 generations of this mutant strain before they supplied the eggs to the senior author. Selection for the trait was continued by the senior author who could later isolate another new mutant *brown-eyed* pupae (*b*) from the *Wb* strain. But it appeared that homozygosity for *Wb* was not made in this strain, since many wild-type individuals came out even after 8 generations of selection. As will be seen below, homozygotes for *Wb* are lethal most probably in the egg stage. This paper gives a description of the two mutants *Wb* and *b*, and their mode of inheritance.

MATERIALS AND METHODS

The rearing methods and facilities employed for this study were similar to those mentioned by Tadano (1977) for *Aedes* (*Finlaya*) *togoi*. Tap water was used for rearing. Eggs were dried 3 days after deposition before storage. Mass crosses