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A SURVEY FOR NATURAL POTENTIAL VECTORS OF *DIROFILARIA IMMITIS* IN VERO BEACH, FLORIDA[1]

D. M. SAUERMAN, JR. AND J. K. NAYAR

Florida Medical Entomology Laboratory, Institute of Food and Agricultural Sciences, University of Florida, 200 9th Street, S.E., Vero Beach, FL 32960

ABSTRACT. A survey to identify natural potential vectors of canine heartworm in the enzootic area of Vero Beach, Florida, revealed three species of wild-caught mosquitoes harboring presumed *Dirofilaria immitis* larvae. Two of these, *Aedes taeniorhynchus* and *Culex nigripalpus*, have not been previously described as vectors of this parasite, while the third, *Culex quinquefasciatus*, has been implicated in other studies.

INTRODUCTION

More than 30 years ago, Otto (1949) expressed his conviction that brackish water mosquitoes occurring along the eastern seaboard were important vectors of canine heartworm. Otto was aware of the heavy mortality suffered by one such species, *Aedes sollicitans* (Walker), when infected with *Dirofilaria immitis* (Leidy), the etiologic agent; his survey records nevertheless strongly suggested an association between salt marsh species and the geographic distribution of the disease. Nayar and Sauerman (1975) confirmed the occurrence of high mortality in infected *Ae. sollicitans* but at the same time demonstrated high experimental vector potential in another salt marsh species, *Ae. taeniorhynchus* (Wiedemann), which was capable of surviving and refeeding while heavily infected. It was the purpose of the survey reported herein to determine the natural potential vectors of canine heartworm in the enzootic region of Vero Beach, Florida, where substantial *Ae. taeniorhynchus* populations occur in association with an abundance of fresh water mosquito species.

MATERIALS AND METHODS

The basic strategy of epidemiological surveys to determine the natural potential vectors of *D. immitis* is to collect infected, wild mosquitoes from suspect areas. In this study, 3 residential yard sites in Vero Beach, Florida, were selected for the collection of indigenous mosquitoes. Site 1, in northeast Vero Beach, was most proximal (1.5 km) of the 3 sites to the Indian River salt marsh, while Site 2 was near the center of the city, about 2 km further west of the Indian River. Site 3 was southwest of the city, about 4 km from the river and considerably closer to the rural agricultural (citrus grove and cattle ranches) region west of the city. Each site was within a residential subdivision.

Mosquito populations were sampled using one CDC-type light trap and one lard-can, chick-baited trap (each with dry-ice adjuvant) suspended by wire from available vegetation, not more than a meter from the ground. Traps were set out from 1700 hr to 0800 hr twice weekly from April to August and yielded 210 samples (35 collections × 3 sites × 2 traps/site). In both traps, captured mosquitoes had access to 10% sucrose solution.

At the laboratory, dissections were initiated immediately after anesthetizing and sorting to species. Midguts lacking blood or blood-meal remnants were discarded, while a midgut with a blood meal was examined at 100X for the presence of microfilariae, which were removed via drawn capillary pipette to a droplet of 2% formalin and measured. Malpighian tubules lacking obvious pathology (swollen or cleared areas with reticular discontinuities) were subjected to a mild coverslip compression to reveal the presence of prelarvae or early sausage stages, both

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of which are difficult to detect in the non-compressed tubule as the parasites are intracellular (Taylor 1960). If larvae were partially visible or there was obvious tubule pathology, more delicate compression gently squeezed these forms from the tubules for examination without disruption of the parasite.

Presumptive identification of potentially infecting filarioids was based on the definitive host fauna present, morphological characteristics of the parasites, and the site of development of the parasite in the vector. Morphological parameters included length, number of caudal papillae and the presence of cuticular wrinkles or striations. The definitions of each stage used herein and parameters used to make a presumptive identification were as follows:

Microfilariae—herein defined as the state in association with vertebrate blood, with no potential for further development until they obtain lodging in the specific target tissue (in this case, the Malpighian tubule cells) prior to initiation of development. A microfilaria was rejected as being *D. immitis* if it was less than 280μ in length, characteristic of *Dipetalonema reconditum* (Grassi) (Newton and Wright 1956), or greater than 327μ in length, as with *Dirofilaria striata* (Molin) from the bobcat (Orihel and Ash 1964), *Dirofilaria lutrae* Orihel from the otter (Orihel 1965) or *Dirofilaria tenuis* Chandler from the raccoon (Pistey 1958). The presence of microfilariae in the blood meal does not constitute a basis for designating the mosquito as "infected," since not all the Onchocercidae use mosquitoes as vectors, in which case ingestion by the mosquito terminates the life-cycle.

Prelarvae—identical to microfilariae in size and morphology, prelarvae have succeeded in reaching the Malpighian tubule target tissue, in which, amongst Onchocercidae, only *Dirofilaria* develops. Other than *D. immitis,* only *D. tenuis, D. striata* and probably *D. lutrae* develop in the Malpighian tubules and could occur in the study area (based on the presence of the definitive host). The presence of prelarvae constitutes a basis for designating the mosquito as infected, although barriers that prohibit development beyond this stage in several mosquito species (Nayar and Sauerman 1975) were evaluated.

Developing Forms—the absence of information regarding the comparative morphology of the trophic stages of different species precluded other than a presumptive identification, based mainly on the station of development. However, their presence is epidemiologically relevant, since it constitutes the basis for designating the mosquito as infected, and there are no further barriers to development at this stage. The most probable parasites other than *D. immitis* were *D. tenuis,* which can have characteristic cuticular wrinkles (Pistey 1958), and *D. striata* and *D. lutrae,* for which there are no known discriminating characters at this stage. Stage III Larvae (L_3)—when found in the tubules, the potential filarioid nematodes are those described above for prelarvae and developing forms. Cuticular striations characteristic of *D. tenuis* L_3 (Pistey 1958) can be used to reject *D. immitis,* but *D. striata* L_3 from bobcats are of comparable length or slightly longer (Orihel and Ash 1964), and no data are available for *D. lutrae* L_3.

With filarioid larvae recovered from the head/proboscis, the mosquito-vectored Onchocercidae with definitive hosts in the study area were considered, using criteria tabulated by Arnott and Edman (1978), with the following additions. *Brugia beaveri* Ash and Little: the L_3 of this parasite of raccoons has not been described. *Dirofilaria uniformis* Price: the length of this parasite of rabbits, as described by Duxbury et al. (1961), encompasses that of *D. immitis;* other morphological parameters, mainly the number of caudal papillae, have not been described. Based on its similarity in some respects to *Dirofilaria scapiceps* (Leidy), it is probable that the arrangement of caudal papillae is dissimilar to that of *D. immitis.* The capability of discriminating the L_3 of *D. immitis* and *D. tenuis* is in controversy, and the crowding of parasites in the arthropod host can reduce length compared with that attained in light infections (T. C. Orihel, personal communication).

RESULTS AND DISCUSSION

A yield of 1030 mosquitoes, representing 13 species in 5 genera, was obtained from 210 trap samples (Table 1). Three species, *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, and *Ae. taeniorhynchus,* accounted for 84% of the total collection with no other species contributing more than 5% of the total.

The same 3 species accounted for 100% of the 16 nematode-parasitized mosquitoes (Table 2). Two of these (*Cx. quinquefasciatus* #2 and *Cx. nigripalpus* #7) were not considered infected, although they had presumed-*D. immitis* microfilariae in their midgut blood meals, while 1 infected *Ae. taeniorhynchus* (#10) harbored a non-identifiable L_3. Thirteen mosquitoes, or 1.3% of the total of all mosquitoes collected, harbored presumed-*D. immitis* larvae (Table 2).

The presence of presumed-*D. immitis* in *Ae. taeniorhynchus* confirmed the prediction of Otto (1949) regarding the probable involvement of salt marsh mosquitoes in the transmission of canine heartworm disease. The survey also implicated *Cx. nigripalpus* as a natural potential

Table 1. Combined (35) collections by species, trap site and species found positive for *Dirofilaria immitis*.

	No. of mosquitoes*								
	Site			Total	% each	No.	%	No.	%
	1	2	3	no.	species	positive	positive	infected	infected
Culex nigripalpus	87	72	244	403	39.1	3**	0.7	2	0.5
Culex quinquefasciatus	49	29	122	200	19.4	4**	2.0	3	1.5
Culex (Melanoconion) sp.	12	3	29	44	4.3	0	0	0	0
Culex salinarius	4	3	5	12	1.2	0	0	0	0
Culex restuans	0	0	2	2	0.2	0	0	0	0
Aedes taeniorhynchus	152	33	79	264	25.6	9***	3.4	8	3.0
Aedes vexans	2	5	20	27	2.6	0	0	0	0
Aedes aegypti	0	0	4	4	0.4	0	0	0	0
Aedes sollicitans	0	1	1	2	0.2	0	0	0	0
Anopheles quadrimaculatus	2	5	4	11	1.1	0	0	0	0
Anopheles crucians	0	3	0	3	0.3	0	0	0	0
Coquillettidia perturbans	2	0	6	8	0.8	0	0	0	0
Psorophora columbiae	29	11	10	50	4.8	0	0	0	0
TOTALS	339	165	526	1030		16	1.6	13	1.3

* Combined light trap and bait trap yields.

** Dead microfilariae found in blood meals from 1 specimen each of these 2 species were presumed to be *D. immitis* based on length (average was 315μ) and width (all were 6–8μ); these mosquitoes were not designated "infected" (see text).

*** One specimen from site 1 had 1 L_3 in proboscis that was damaged in processing.

vector of dog heartworm; this species has already been linked to the epidemiology of a variety of diseases, as reviewed by Nayar (1982). *Culex quinquefasciatus* has been linked to the transmission of canine heartworm disease in the U.S. by Villavaso and Steelman (1970), but previous laboratory experiments (Nayar and Sauerman 1975) indicated a low vector potential for this species.

No assessment of vector potential could be made for mosquitoes representing the 10 other mosquito species captured and found not harboring worms. The absence of infected mosquitoes would be expected from the small sample sizes if the actual incidence was similar to that found for the 3 infected species. This may explain why *Ae. vexans* (Meigen), a species incriminated by others as a potential vector of canine heartworm outside of Florida (Bemrick and Sandholm 1966, Todaro et al. 1977, Walters and Lavoipierre 1982) was not found positive for *D. immitis* in this study.

Table 2. Analysis of 16 females from 3 species found positive for *Dirofilaria immitis*.

Species	Mosquito no.	Trap site	Trap type	Larval location	Larval type	Larval number
Culex quinquefasciatus	1	1	BT[a]	MT[c]	L1	2
	2	1	BT	MG:BM[d]	MF[f]	25 (all dead)
	3	3	BT	MT	PL[g]	5
	4	1	BT	H:P[e]	L3	3
Culex nigripalpus	5	1	BT	H:P	L3	2
	6	3	LT[b]	MT	L2	2
	7	2	LT	MG:BM	MF[f]	10 (all dead)
Aedes taeniorhynchus	8	1	BT	H:P	L3	2
	9	1	BT	MT	L1/L2	2/2
	10	1	LT	H:P	L3	1
	11	2	BT	MT/H:P	L3	1/3
	12	1	BT	MT	L2	16
	13	1	LT	MT	L1	50[h]
	14	1	LT	MT	L1	8[i]
	15	1	LT	H:P	L3	1
	16	1	BT	MT	L2	7

[a] BT—bait trap; [b] LT—light trap; [c] MT—Malpighian tubules; [d] MG:BM—midgut:bloodmeal; [e] H:P—head:proboscis; [f] MF—Microfilariae (bloodmeal form; does not constitute infectious agents); [g] PL—prelarvae (identical to MF but constitutes infectious agents); [h] with 15 melanized L1; [i] with one melanized L1.

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