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MOSQUITO VECTORS OF DOG HEARTWORM, *DIROFILARIA IMMITIS*, IN WESTERN MASSACHUSETTS[1]

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ABSTRACT. A total of 3445 female mosquitoes representing 23 species were collected in the field, held for 10 days and dissected for the presence of infective stage larvae of *Dirofilaria immitis*. Eleven naturally infected individuals of 3 species of *Aedes* mosquitoes were found. In the laboratory, 1451 females representing 19 species were fed on a heartworm infected dog and subsequently dissected for evidence of larval development. Ten species were found to support development of at least some filariae to the infective stage.

Based on these data and available biologic information, *Aedes canadensis* and *Ae. excrucians* appear to have the greatest potential as vectors of dog heartworm in western Massachusetts.

INTRODUCTION

Dirofilaria immitis was first isolated from Massachusetts dogs in 1937 (Augustine 1938) but has not been considered a general problem in New England until very recently. Although no systematic records exist, voluntary case reporting by veterinarians in western Massachusetts from 1973 to 1975 showed that about 10% of dogs tested were positive for *D. immitis* microfilaria (Downhill, Edmonds, Hilt, O'Conner, Ruder, and Roy, 1975, per commun.). Recognizing the common, widespread occurrence of heartworm within the last 5 yrs, area veterinarians now routinely test for infection and recommend prophylactic drugs for all dogs during the warm season.

It is not clear whether the current picture reflects new geographic and numeric spread or if greater awareness and better diagnosis have merely brought into true focus a long extant situation.

At least 36 nearctic mosquitoes have been indicated as possible vectors of dog heartworm (Ludlam et al. 1970) and the primary vector, when known, appears to vary greatly with geography (Schlotthauer et al. 1969). Research reported here was directed toward identifying potential vectors in western Massachusetts since effective vector control appears to be an important ingredient in arresting local transmission of this disease. Two approaches were employed: 1) attempted isolation of naturally infected mosquitoes, and 2) comparison of laboratory infection

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rates among different field-derived populations.

MATERIALS AND METHODS

ISOLATION OF NATURAL INFECTIONS. Collection sites were selected on the basis of having at least 2 known recent cases of heartworm in the neighborhood. All sites were in or near suitable, i.e. damp and wooded, mosquito resting habitat. Four sites were in the Town of Amherst (Lawrence Swamp and Podick Conservation Areas, Salem Street, and Potwine Road), one in Northampton (Pinchon Meadows) and one in South Hadley (Route 116).

Daytime power-aspirator sweeps, human biting collections at dusk, and standard New Jersey and CDC light traps, often baited with dry ice, all were used to obtain female mosquitoes for filaria isolation attempts. Fabricated from aluminum sheeting and clear plastic, the cylindrical aspirator had an 11-inch diameter opening through which insects were drawn into a collection bag by an 8-inch DC fan, powered by a 12-V motorcycle battery.

Dead and moribund specimens were identified and dissected soon after the collections reached the lab. Most live females were sorted by species and retained at 27°C in pint ice cream containers and provided with 5% sucrose for 10 days before dissection. Specimens dying during this holding period were immediately dissected. When collections were large, some live females were dissected immediately as well. The rationale for holding specimens 10 days was to increase the probability of finding infective stage larvae. These larvae (stage 3) are at least tentatively recognizable to species and their presence can be taken as proof of complete development within the mosquito host observed. In contrast, sausage stage larvae (stage 2) in the malpighian tubules are impossible to differentiate, and they do not necessarily continue to develop to stage 3 in every mosquito in which they are found.

All dissections were done in a drop of saline on glass microscope slides. The head was first removed and examined under a dissecting microscope, then the malpighian tubules. The whole preps were then squashed and examined at 100x and 430x under a compound scope. When species were too numerous for dissection of each individual, a modification of the pool method of Muller and Denham (1974) was used. No more than 50 specimens were placed in any one pool.

All infective larvae found were fixed in 70% alcohol with 5% glycerin, mounted in glycerin jelly, and measured with an ocular micrometer. No comprehensive key to 3rd stage larvae of nearctic filariae is available. Identification of *D. immitis* was accomplished using a combination of the descriptions and measurements shown in Table 1. *Setaria equina* was easily eliminated as a source of confusion because of its much larger size. *Foleyella* spp. were discounted because they have no anal papillae and are transmitted by amphibian feeding mosquitoes. *Dirofilaria scapiceps* and *Dipetalonema arbuta* can be eliminated because they are narrower than *D. immitis* and have 3 anal papillae. *Dirofilaria tenuis* and *D. striata* are very similar to *D. immitis* in size; however, *D. tenuis* has no anal papillae while *D. immitis* has one. Further differentiating characteristics are unavailable for *D. striata* other than the fact that it has only been reported from bobcats in the South. Hence, *Dirofilaria* which met all the characteristics of *D. immitis* were considered to be this species.

During the first year of this study, members of the *Ae. stimulans* groups, which are difficult to separate, were not identified to species. During the second year, *Ae. excrucians* were separate from other members of the group : e. *Ae. fitchii* and *stimulans*.

LABORATORY INFECTION EXPERIMENTS. Mosquitoes used in laboratory studies were either field collected as biting adults or as eggs/larvae/pupae and reared to adults. Reared adults were held at 27°C in pint containers with a 5% sucrose for 3–5 days before being offered a bloodmeal on a *D. immitis* infected Brittany Spaniel ob-

Table 1. Some key characteristics of infective (3rd) stage larvae of known mosquito-borne filaria potentially occurring in N.E. U.S.

Filaria	Vertebrate host	Width (μ)	Length (μ) Total	Length (μ) Anus-Tail	No. Anal papillae	Reference
Setaria equina	Equines	21–28	1280–1720	40–52	1 lg/2 sm	Becklund & Walker 1969
Foleyella						
brachyoptera	Amphibia	13–17	650–900	50	0	Benach & Crans 1975;
dolichoptera	Amphibia	13–17	650–900	50	0	Kotcher 1941; Witenberg
ranae	Amphibia	20–25	702–1066	50	0	& Gerichter 1944
Dirofilaria						
immitis	Canids & Felids	18–26	800–1040	26–40	1	Kartman 1953; Nelson 1959;
tenuis	Raccoons	21–28	781–1157	—	0	Pistey 1958
striata	Felids (wild?)	24–26	950–1140	—	-	Orihel & Ash 1964
scapiceps	Rabbits	14	789	—	3	Highby 1938, 1943b; Tuff 1975
Dipetalonema						
arbuta	Rodents	16–18	880–1160	22–36	3	Highby 1943a

tained through a local veterinarian (Dr. J. W. Hilt). Females captured biting in the field were held overnight and fed the following evening. All post-fed females were maintained on 5% sucrose at 27°C for 14–18 days. Samples were removed at various intervals during this holding period, dissected and examined for developing *D. immitis* larvae.

RESULTS

ISOLATION OF NATURAL INFECTIONS. Results of individuals dissected and pools examined are presented in Tables 2 and 3. Five additional species, *Ae. aurifer* and *trivittatus, Anopheles walkeri, Culiseta inornata* and *impatiens*, representing a total of only 9 specimens, also were dissected with negative results.

Of the 13 mosquitoes found to contain filariae by dissection (Table 2), 6 mosquitoes (1 *Ae. canadensis*, 4 *Ae. excrucians*, 1 *Ae. sticticus*) were positive for infective larvae of *Dirofilaria immitis*. Four others contained *Dirofilaria* that may have been *D. immitis* but positive identifications were not made. Also, one of 3 pools (Table 3) containing filariae was positive for infective larvae of *D. immitis*. The infection rate for each positive species was derived by dividing the total number of mosquitoes examined (individual dissections plus pools) by the number of mosquitoes with known or presumptive infective larvae of *D. immitis*. The following rates were obtained: *Ae. canadensis*—0.8% (2 of 254); *Ae. stimulans* group, including *Ae. excrucians*—2.2% (8 of 369); *Ae. excrucians* alone (1976)—3.3% (5 of 154) and *Ae. sticticus*—1.3% (1 of 80). In view of the laboratory infection results and the 1976 field results, the 4 unidentified *Dirofilaria* infections found in 1975 (1 in *Ae. canadensis* and 3 in *Ae. stimulans* group) were most likely *D. immitis* and were therefore included in these calculations.

LABORATORY INFECTION EXPERIMENTS. With the exception of *Psorophora ferox* and *Ae. cinereus*, at least 50% of dog-fed females of species listed in Table 4 became infected, i.e., filarial larvae ap-

Table 2. Mosquitoes individually dissected for natural filarial infections

Species	1975		1976	
	Total Dissec.	No. Pos.[a]	Total Dissec.	No. Pos.[a]
Ae. aberratus	–	–	36	0
Ae. canadensis	46	(1)[b]	158	1 (2)[c]
Ae. cinereus	102	0	146	0
Ae. excrucians	–[d]	–[d]	134	4
Ae. sticticus	33	0	47	1
Ae. stimulans group[e]	50	(3)[b]	88	0
Ae. triseriatus	8	0	83	0
Ae. vexans	548	0	171	0 (1)[f]
An. punctipennis	12	0	45	0
Cx. spp.	620	0	130	0
Cq. perturbans	24	0	76	0
Cs. morsitans	1	0	12	0
Ur. sapphirina	14	0	–	–
Ps. ferox	21	0	1	0

[a] *Dirofilaria immitis* unless otherwise indicated.
[b] Unidentified *Dirofilaria* spp.
[c] Unidentified but not *Dirofilaria* spp.
[d] Included in *stimulans* group in 1975.
[e] Includes *stimulans, fitchii* and *excrucians* in 1975; *excrucians* not included in 1976.
[f] *Setaria equina.*

peared in their malpighian tubules. A low percentage of both *Ae. vexans* and *Ae. stimulans* group (mostly *fitchii*) females were able to support continued filarial development to infective stage larvae. At least 20% of all others supported complete development to the infective stage although the small numbers of certain species (e.g. *An. punctipennis* and *Ae. trivittatus*) render percentages rather meaningless.

A total of 250 *Culex pipiens, restuans* and *salinarius* were offered blood meals on the infected dog but none engorged except 1 of 4 *salinarius* which died before development could occur. Five other species, *Ae. provocans* (Walker) [=*trichurus* (Dyar) auct.] *An. quadrimaculatus, Coquillettidia perturbans, Cs. morsitans* and *Ps. ciliata*, also were collected and offered a blood meal but like *Cx. salinarius*, the numbers involved were insufficient for meaningful results.

Some melanized larvae were found in nearly every species tested (varying from 3–15%), but the significance of these host defensive reactions in terms of vector potential is not readily apparent. Although melanized larvae die, healthy non-melanized larvae were generally found in the same tubules as were melanized forms.

DISCUSSION

Mosquitoes may support the development of parasites in the laboratory that normally will not develop under field conditions. Also, infective mosquitoes may be unable to efficiently transmit infective stages under either laboratory or field conditions (Bickley 1976). Moreover, species able to transmit *D. immitis* still must be ecologically and behaviorally suited to the task. For example, the species must be sufficiently numerous and have a range and synchrony of flight that frequently bring it into close contact with the definitive canine host. Also, the mosquito must readily feed on dogs and ideally do so when the microfilaremia is highest. After feeding, the mosquito must

Table 3. Field collected females pooled for natural filarial infections (1976).

Species	Number pooled	Number of pools	Positive[a] pools
Ae. abserratus	16	1	0
Ae. canadensis	54	2	0
Ae. cinereus	149	5	0
Ae. excrucians	20	1	1
Ae. stimulans group[b]	97	4	0 (1)[c]
Ae. triseriatus	20	1	0
Ae. vexans	410	16	0 (1)[c]
Cq. perturbans	113	3	0

[a] *Dirofilaria immitis* unless otherwise noted.
[b] Includes *stimulans* and *fitchii* but not *excrucians*.
[c] Filaria other than *D. immitis*.

survive long enough (11+ days) to allow the microfilariae to develop and reach the head of the insect and then feed again. Finally, a good vector should be present throughout much of the warm season when conditions for transmission are favorable. Of course, the latter circumstance also could be met by multiple vectors, with seasonally divergent distributions acting in concert.

Though often an elusive goal, the finding of naturally infected females that meet all necessary biological criteria is consequently the strongest single body of evidence for the initial incrimination of vectors. Demonstration of successful dog to dog transmission must then follow.

Twenty-five species were dissected in this study and many were the same species considered as possible vectors in other areas of the United States. Through interpretation of these data, along with other available biologic data, it was possible to greatly reduce this list and to identify species with the greatest vector potential in western Massachusetts (see Table 5).

Cx. p. pipiens, Cx. restuans, Cx. territans, Cs. morsitans and *Ur. sapphirinia* were eliminated as potential vectors because these species do not normally feed on mammals and the latter 3 occur in insufficient numbers to be reliable vectors in any event.

Ae. aurifer, Ae. abserratus, Ae. provocans, Cq. perturbans and several others have low or erratic populations, short seasonal distributions or poor laboratory/field infection rates. *Cq. perturbans* is relatively widespread and common during most of the summer but its distribution is erratic—depending on the availability of suitable larval habitats. Our negative laboratory/field infection results with this species

Table 4. Dissection results of mosquitoes fed on *Dirofilaria immitis* infected dog.

Species	Total Fed	Days 1–10 post-fed			% Mosq. Infected	Days 11+ post-fed			% Mosq. Infective
		Pos.[a]	(#Mel.)[c]	Neg.		Pos.[b]	(#Mel.[c])	Neg.	
Ae. canadensis	142	44	(8)	34	56	15	(1)	49	23
Ae. cinereus	140	34	(1)	59	37	4		43	9
Ae. excrucians	27	18	(1)	0	100	2		7	22
Ae. sticticus	80	51	(2)	12	86	12		4	75
Ae. stimulans group[d]	169	119	(5)	13	90	2		35	5
Ae. triseriatus	84	29	(1)	14	67	14		27	34
Ae. trivittatus	4	1		0	100	1		2	33
Ae. vexans	515	191	(11)	99	66	28	(3)	197	12
An. punctipennis	11	2		2	50	2		5	29
Ps. ferox	45	6		32	16	1		6	14

[a] Microfilaria or sausage stage larvae in malpighian tubules.
[b] Infective stage larvae present (usually in the head).
[c] Number of dissected mosquitoes in which melanized nematodes were found.
[d] Includes *stimulans, fitchii* and *excrucians*.

Table 5. Summary of vector potential based on current biological and infectivity data for all species collected in Western Massachusetts

Species	Biological Characteristics: Mammal Feeder	Relatively Abundant	Wide Distribution Geograph./Season.	Multiple Generat.	Vector Characteristics: Lab Infect.	Field Isola.	Vector Potential
Ur. sapph., Cs. mors. Cx. terr.	NO[1]	NO	YES	YES	—	NO	None
Cx. p. pip. & rest. Cs. impat., Ps. cil.	NO	YES	YES	YES	—[5]	NO	None
Ae. prov., Ae. aur. Cs. inor., An. walk. An. quad	YES	NO	NO	NO[3]	—[6]	NO	Low
	YES	NO	YES[2]	YES	—[6]	NO	Low
Cq. pert., Ae. abser.	YES	YES	NO	NO	—[6]	NO	Low
Ae. triv., Ps. ferox	YES	NO	NO	YES[2]	YES	NO[8]	Low/Moderate
Ae. triseriatus	YES	YES	NO	YES	YES	NO	Low/Moderate
Cx. salinarius	YES	YES	YES[2]	YES	±7	NO	Low/Moderate
An. punctipennis	YES	YES	YES[2]	YES	YES	NO[8]	Moderate
Ae. ciner. & vex.	YES	YES	YES	YES	YES	NO	Moderate
Ae. stim./fitchii	YES	YES	NO	NO	YES	NO (?)	Moderate
Ae. sticticus	YES	NO	YES	YES[3]	YES	YES	Moderate/High
Ae. excrucians	YES	YES	YES	NO[4]	YES	YES	High
Ae. canadensis	YES	YES	YES	YES	YES	YES	High

[1] Key negative factors in reducing vector potential are underlined.
[2] *Anopheles* & *Culex* are present throughout the season but are not abundant until mid-summer.
[3] Although *Psorophora* & *Ae. triv.* & *stic.* can have mult. gen. they rarely do in this area.
[4] A single generation species but adults are unusually long-lived.
[5] Would not feed on infected dog.
[6] Insufficient numbers collected for lab infection studies.
[7] Lab infection results mixed but Bickley (1976) failed to obtain dog-to-dog transmission.
[8] Field isolations form *Ae. triv.* & *An. punt. in* Iowa (Christensen & Andrews 1976).

agree with those of Yen (1938) and Bemrick and Sandholm (1966). *Ae. abserratus* is often abundant in western Mass. but only very early in the season.

Ae. cinereus, Ae. triseriatus, Ae. trivittatus, Ae. vexans, An. punctipennis, Cx. salinarius and *Ps. ferox* could possibly serve as vectors since all supported some filarial development in the lab. However, the absence of natural infections together with unfulfilled biological requirements suggest only modest vector potential for any of these species. In experiments by Seeley and Bickley (1974) on strains of *Cx. salinarius* from Connecticut, Louisiana and Maryland, only the Conn. strain supported development to the infective stage. Later, Bickley (1976) failed to demonstrate dog to dog transmission with this strain. *Ps. ferox* became infective at a relatively low rate (14%), and it is not common in Mass.—usually only occurring sporadically later in the season. *Ae. triseriatus* successfully supported filarial development (34% infective) in our study as well as in those by Phillips (1939) and Intermill (1973). This tree-hole and container breeder may be locally common but its distribution in western Mass. is even more pocketed than *Cq. perturbans*. Yen (1938) found no trace of infection among *Ae. trivittatus* fed on an infected dog while Christensen and Andrews (1976) found naturally infected females in Iowa, and Christensen (1977) obtained dog to dog transmission by *Ae. trivittatus* in the laboratory. Our limited lab results support the view that this species can sustain development, but it is too uncommon in western Mass. to be an important vector here.

Early reports suggest successful filarial development to the infective stage in both *Ae. cinereus* (Phillips 1939, Yen 1938) and *Ae. vexans* (Bemrick & Sandholm 1966, Hu 1931, Yen 1938). In our laboratory trials, rather low infection rates were obtained (8.5 & 12% respectively). Summers (1943) obtained no development in *Ae. vexans* and Crans and Feldlaufer (1974) reported low vector potential based on field collections. In contrast, Jankowski and Bickley (1976) recently found 68% of *Ae. vexans* fed on an infected dog to develop infective stage larvae and consider this species to have high vector potential. We found no naturally infected *Ae. cinereus* or *vexans* even though the largest number of dissected females were of these two common species. This would be of less significance were it not for the fact that infected females of other species were collected from the same areas. Several reports suggest that *Anopheles* (including *punctipennis*) can successfully support *D. immitis* development (Bemrick & Sandholm 1966, Hu 1931, Kartman 1953, Nayer & Sauerman 1975, Phillips 1939, Summers 1943, Yen 1938). Christensen and Andrews (1976) found 1 of 468 field collected *An. punctipennis* to contain filaria larvae. *Anopheles* are generally more abundant than most trap collections indicate; however, *punctipennis* is usually the only abundant form in this area. Still, its late seasonal build-up seems to relegate its potential to a minor role in this area.

Thus, the list of species with maximum vector potential in this area has been tentatively reduced to the 3 species (or 4 if *Ae. excrucians* and the *stimulans* group are considered separately) in which we found natural infections. Although lab trials with *Ae. sticticus* were highly successful (75% infection) this species appears to have the least vector potential of the 3 since it is normally not common. After considering the 1976 data it appears in retrospect as though the 1975 positives from the *stimulans* group were in all probability *Ae. excrucians*. For the sake of discussion at least, they will be considered as such. Both *Ae. excrucians* and *canadensis* seem to meet all the biological requirement of an efficient vector and both had similar infection rates in the lab (22 & 23% respectively). Seeley and Bickley (1974) obtained a 75% infection rate and Bickley et al. (1977) a 50% rate in their trials with *Ae. canadensis*. Natural infections were found in 1% of *Ae. canadensis* and in 3% of *Ae. excrucians*.

Aedes excrucians has a single spring brood and may therefore appear biologi-

cally to have less vector potential than *Ae. canadensis* which has 2 to 3 broods each year. Still, *Ae. excrucians* is usually long lived—adults first appearing in April-May and persisting well into August. As pointed out by Jankowski and Bickley (1976), *Ae. canadensis* is more active in wooded habitats while *Ae. excrucians*, like *vexans*, tends to disperse more widely. After this study was completed, Bickley et al. (1977) demonstrated that infected *Ae. canadensis* can readily transmit *D. immitis* to susceptible dogs. Dog to dog transmission experiments with *Ae. excrucians* still are needed, as well as the dissection of more field collected mosquitoes, before the precise vector role of these species can be fully assessed for this area.

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HEAD CAPSULE GROWTH IN *CULEX TERRITANS* WALKER

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ABSTRACT. The rate of growth of the head capsule in *Culex territans* Walker has been ascertained in 435 larvae from the population of a permanent marsh south of the Richelieu River, Quebec. The mean and range of head capsule widths have been determined for each larval instar. The rate of growth of the head capsule with larval instar follows Dyar's law. The exponential function $Y = 0.1939\text{–}1.5655^x$ was accorded with the data better than a linear function.

INTRODUCTION

As part of a bio-ecological study of *Culicidae* on the sector south of the Richelieu River, Quebec (Durand 1977) a natural population of *Culex territans* was sampled regularly. It was necessary to develop a quick and precise method for determining larval instars.

Head capsule width seems to have a constant relationship with larval stage, more so than any other variable. Dyar (1890) stated that the ratio of the head widths of 2 successive instars of caterpillars tended to be constant and that the rate of growth of this variable followed a geometric progression. Danks and Corbet (1973) also used this variable to distinguish *Aedes impiger* (Walker) larvae from those of *Ae. nigripes* (Zetterstedt).

MATERIALS AND METHODS

A total of 435 larvae were sampled from a permanent marsh at Ile-aux-Cendres on the sector south of the Richelieu River (45°10'N, 73°16'W). The specimens were fixed and preserved in