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Mosquito vectors of dog heartworm, Dirofilaria immitis (Nematoda: Filariodea) in western Massachusetts.

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MOSQUITO VECTORS OF DOG HEARTWORM, *Dirofilaria immitis* (NEMATODA:FILARIOIDEA) IN WESTERN MASSACHUSETTS

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INTRODUCTION

Dog heartworm has long been recognized as a disease of dogs in the southeastern area of the United States. In Massachusetts, dog heartworm was first isolated from native dogs in 1937 and 1938 by Augustine (1938). Research animals that had never been out of the state were found to be infected with heartworms. Interest in the disease in Massachusetts diminished and it was not considered a general problem until 2 to 3 years ago when veterinarians started to test dogs in earnest. There are no extensive records for this area but voluntary reporting of cases by veterinarians around Springfield, Massachusetts from 1973 to 1975 showed that approximately 10% of the dog population tested was positive for the microfilaria of Dirofilaria immitis (Downhill, Edmonds, Hilt, O'Connor, Ruder and Roy, 1975 personal communications). As a result of recent recognition of the widespread occurrence of dog heartworm, many area dogs are now on a prophylactic drug regimen.

At least sixty species of mosquitoes have been indicated as possible vectors of dog heartworm throughout the world (Ludlam et al., 1970). Of these, thirty-six are known to occur in the U.S. D. immitis has been shown to have different species of mosquitoes serving as its vector depending on the geographic area (Schlotthauer et al., 1969). Mosquito

control must therefore be considered as a possible solution to halt the advance of this disease especially because many positive cases may exist in the form of stray dogs or yard hounds which receive little or no veterinary care.

OBJECTIVES

This research was directed toward identifying the potential vectors of Dirofilaria immitis in western Massachusetts. The objective was partially achieved by narrowing the list of possible vectors, which have been identified in other parts of the United States. Two approaches were employed to reach this goal: first, field isolation of natural infections and second, testing the potential for infection in the laboratory.

In the first approach, field collections of female mosquitoes were dissected and examined for natural infection by the immature nematodes. In the second approach, some field collections of mosquitoes were fed in the laboratory on a heartworm infected dog. These mosquitoes were held in the lab and later dissected to determine whether or not development of the nematode to infective stage occurred. The latter was an attempt to elucidate which mosquitoes of western Massachusetts are physiologically capable of transmitting dog heartworm. This information, together with other known biological data, might then be used as an aid in determining target mosquito species for control from a public health

standpoint; as well as protecting dogs in areas where heartworm and vector mosquitoes are present.

THE DISEASE IN DOGS

The causative agent of canine heartworm disease is the filarial worm, Dirofilaria immitis, Leidy. Early workers first suspected that the vectors of this filarial worm were mosquitoes and fleas. In 1943 Summers reported on the successful development of Dirofilaria immitis in four species of mosquitoes and found three species of fleas to be naturally infected. Because of the closer physical relationship between canines and fleas, they were thought to be the more likely vector. Years later, Newton and Wright (1956, 1957) demonstrated that there were actually two different filarial worms in dogs in the United States. Each filarial worm was studied and the mosquito-flea vector system was elucidated. It was demonstrated that fleas were vectors of the innocuous filarial worm of dogs, Dipetalonema reconditum, and mosquitoes were vectors of dog heartworm, Dirofilaria immitis. The microfilariae of the former are much more active swimmers in canine blood samples, have a cephalic hook and usually occur in relatively low numbers compared to the latter microfilariae which are erratic swimmers and have no cephalic hook. Adults of D. reconditum are apparently harmless subcutaneous worm (Jackson, 1969b; Otto and Bauman, 1959); however, their distribution in the U.S. is quite simi-

lar to that of D. immitis. Therefore, it is necessary to differentiate the microfilariae of the two species.

In many areas of the United States D. immitis has been increasingly recognized as a major health hazard for dogs, often decreasing their usefulness and shortening their lives. Likewise, there has been an increase in the number of reported infections in humans, though relatively benign, by this same filarial worm (Gershwin et al., 1974).

Dogs are not the only natural hosts of the heartworm; mature adult worms have been recovered from coyotes, foxes, wolves, wolverine, beaver, black bear and numerous times from domestic cats (Donahoe, 1975; Foil and Orihel, 1975; Fraries et al., 1974; Hirth and Nielson, 1966; Johnson, 1975; Levine, 1968; Lillis, 1964; Schlotthauer, 1964; Williams and Dade, 1976). Though there are no reported cases of heartworm in cats and foxes in Massachusetts, the fact that they occur elsewhere in the U.S. suggests that these animals may also serve as reservoirs in the Northeast.

Canines may harbor infections of heartworm and yet display no clinical symptoms of the disease. Most heartworm infected dogs seen by veterinarians are asymptomatic cases. Even in asymptomatic dogs some pathologic changes generally have occurred in varying degrees in the arteries, lungs and liver (Jackson, 1972). Thromboemboli may form

and block the vessels in the lungs as well as causing chronic congestion of the liver. More serious involvement with enlargement of the heart and increased blood pressure may bring on congestive heart failure and/or congestion of the liver and kidneys. Related symptoms may be a persistent cough with rapid tiring and collapse after exercise.

Heartworm infection in an asymptomatic dog is usually treatable but involves a toxic drug. Thiacetarsamide is used to kill adult worms followed by dithiazinine iodide or Fenthion to kill any remaining microfilariae. Although not without potential risks (Jackson, 1969), most infected dogs can be treated successfully. Dogs with congestive heart failure pose much greater therapeutic risks and recovery to useful activity is not great. Surgical removal of heartworms also has been developed but remains of questionable value. Presently, the best treatment in canines is preventive medication (diethylcarbamazine daily at a dosage of $1\frac{1}{4}$ mg/lb) to inhibit development of filaria to the adult stage (Jackson, 1972; Pacheco, 1972).

As the reservoir of infected dogs increases so also may the involvement of human cases because of the close association between man and canines as pets. The fact that mosquitoes that normally feed on canines generally readily feed on man is another contributing factor. Therefore, physicians, veterinarians, and the pet owning public all should be aware of the increased incidence of dog heartworm.

MEDICAL IMPLICATIONS IN MAN

Infection by Dirofilaria immitis in man is neither as large a problem or as dangerous a disease as with dogs. However, an increasing number of Dirofilaria sp. infections have been reported in humans----a number of these being morphologically indistinguishable from adult Dirofilaria immitis (Dashiell, 1961).

There are two clinical forms of dirofilariasis that occur in man: subcutaneous and pulmonary. The former is usually caused by D. repens in Europe, Asia and Africa and by D. tenuis, a nematode parasite of raccoons, in the U.S. (often referred to as D. conjectivae in humans----Orihel and Beaver, 1965; Schlotthauer et al., 1969).

The pulmonary cases are caused by D. immitis which also has been found in cardiovascular cases. There have been at least twenty-four cases of human infection reported in the U.S. (Beaver and Orihel, 1965; Gershwin et al., 1974; Hoch et al., 1974; Navarrette, 1972). Humans are considered to be a dead-end host because the larvae do not normally develop through to the adult stage (Faust, 1961). Cardiovascular cases have been reported only from autopsies and none were considered to have been the cause of death (Abadie et al., 1965; Schlotthauer et al., 1969).

The infection in the lungs, an infarct caused by the nematode's presence, may manifest itself on an x-ray as a

coin lesion. It may appear as a neoplasm or a cyst in the lung. Diagnosis is difficult and may require surgery to differentiate. With resection of the involved area of the lung the patient's recovery is normally unremarkable and satisfactory. Identification of the nematode in resected lung tissue is necessary for definitive identification.

REVIEW OF LITERATURE

Filarioid parasite life cycles were reviewed by Schacher (1973), who listed 89 known filaria with life cycles dependent on hematophagous vectors. Of those, nine were listed as parasites of dogs in various regions of the world. Lindsey (1961) reported at least 11 species of filarial parasites from dogs. Only two of these, Dirofilaria immitis (Leidy, 1856) and Dipetalonema reconditum (Grassi, 1890), are known to occur in the United States.

BIOLOGY OF Dirofilaria immitis

Heartworm disease was recognized as a serious disease of dogs in the United States by Augustine (1938), Phillips (1939), Mundhenk and Greene in 1939 (Otto, 1972), Ward and Franklin (1953), Eyles et al. (1954), Groves and Koutz (1964) and even as early as 1899 by French (Pennington, 1971). Development of D. immitis in the mosquito was first demonstrated by Grassi and Noè in 1900. According to Kartman (1953a), Manson's observations in 1878 on Culex fatigans and Wuchereria bancrofti provided the first indication of the mosquito's role as an intermediate host for filarial worms. An extensive review of the literature on arthropod transmission of filarial worms was published by Hawking and Worms (1961) and more recently, by Schacher (1973).

Adults of D. immitis normally are found in the right ventricle of the heart, pulmonary artery, other adjacent large vessels, and occasionally in other locations, e.g. cerebral arteries. Descriptions of various loci of adult worms, associated pathological changes, and accompanying disease symptoms have been reported by Bradley (1971), Jackson (1972), Kotani et al. (1975a, 1975b), Krull (1969), Kume and Itagaki (1955), Levine (1968), Liu et al. (1966), Otto (1974b), Otto and Bauman (1959), Patton and Garner (1970), Schacher (1973) and Soulsby (1965). Dogs must harbor at least one male and one female adult worm to produce microfilaria and to be infective to mosquitoes. Microfilariae are liberated by the fertile adult female directly into the blood stream of the host where they circulate with the blood. The number of microfilaria (mf) present in the peripheral circulation varies greatly depending on: (1) the number of fertile adult females present and (2) the daily and seasonal periodicity of the microfilariae. With an increase in the number of adult worms in an infected dog there is a rapid decrease in the number of microfilaria per adult worm. The average number of mf/ml of blood does not reflect either the number of adult worms present or the severity of the disease (Otto et al., 1976). Pacheco (1974) demonstrated with transfusions of blood containing mf into clean dogs that maintenance of mf in circulation does not depend on constant production of mf by adult females. The seasonal

periodicity of mf in the circulating blood is correlated to atmospheric temperature; as the temperature increases so does the number of circulating mf (Aoki, 1971; Gubler, 1966; Jackson, 1969a; Katamine et al., 1970). Daily periodicity, though not as marked as in Wuchereria bancrofti, is present with the greatest numbers of mf/ml occurring between 6 P.M. and 2 A.M. and the lowest number between 6 A.M. and 12 noon (Hawking, 1953, 1956, 1967; Hawking and Thurston, 1951a, 1951b; Jackson, 1969b; Kartman, 1953a; Tongson and Romero, 1962). Other factors, such as activity of the dog, anoxia, chemicals, ambient temperature range and whether or not microfilariae are sequestered in capillaries or tissues, have been reported to affect the number of circulating microfilariae (Beam, 1967; Bemrick et al., 1965; Hawking, 1956; Pacheco, 1974).

Circulating microfilariae are taken into the digestive tract of the mosquito while engorging on the blood of an infected dog. The intake of microfilariae is directly proportional to the microfilaremia in the host and the volume of blood taken in by the mosquito (Ho and Ewert, 1967; Hu, 1931; Nelson, 1964; Zielke, 1973b). Microfilariae enter the malpighian tubules within 24 hrs (usually within 12 hrs) after uptake in order to continue development. This movement may be inhibited by chemical or physical barriers, mechanical blockage, and defecation, or it may be enhanced by anticoagulins (Clements, 1963; Coluzzi and

Trabucchi, 1968; Kartman, 1953a, 1953c; Nayer and Sauerman, 1975; Nelson, 1964; Taylor, 1960b). After entering the malpighian tubules, the microfilariae may be encapsulated (usually in refractory mosquito hosts) or, in direct contrast, they may be lethal to the mosquito (Beam, 1966; Kartman, 1953a; Poinar, 1969; Soulsby, 1965). Typically, microfilaria enter the malpighian tubules to develop through three stages and two molts to the infective larvae (third stage) in 10 to 17 days, dependent upon mosquito species and environmental conditions (Intermill, 1973; Kartman, 1953a, 1953b; Kutz and Dobson, 1974; Nelson, 1959; Newton and Wright, 1956, 1957; Orihel, 1959; Phillips, 1939; Sawyer and Weinstein, 1963; Villavaso and Steelman, 1970; Yen, 1938). The 1st larval stage (sausage stage) is spent inside the distal cells of the malpighian tubules (6 to 7 days); the 2nd more elongate stage develops in the lumen of the malpighian tubules (6 days); the mobile, infective 3rd stage larvae normally migrate from the malpighian tubules to the head and enter the labium (Ho et al., 1974a, 1974b; Taylor, 1960b). When the mosquito takes another blood meal, infective larvae penetrate the membranes at the tips of the labium or the labella into a small drop of hemolymph that apparently exuded onto the host from the lumen of the labium or labella as the larvae emerge. The larvae then enter through the puncture wound in the host's skin when the mouthparts are withdrawn (Ewert, 1967; Lavoipierre and Ho,

1973; McGreevy et al., 1974; Zielke, 1973a, 1973b). Once in the dog, larvae undergo further development and migrate to the heart where they mature to the adult stage. The prepatent period (from larval entry into dog to microfilarial production by adult worm) may vary from 5 to 8 months (Kume and Itagaki, 1955; Krull, 1969; Newton, 1957; Summers, 1943; Webber and Hawking, 1955).

DISTRIBUTION AND PREVALENCE OF DOG HEARTWORM IN THE U.S.

Traditionally heartworm has been thought of as a disease of the southeastern area of the U.S. The increase in the number of cases being discovered in the north may be a result of the northward movement of the disease, carried by a mobile population, or be an increase in surveillance by practicing veterinarians. Many northern area veterinarians now carry on heartworm testing clinics. It is not known whether this resulted from an increase in infected dogs brought into an area or an increased awareness of a disease that was already present, but largely unrecognized. Results of the clinics definitely show that heartworm is present in western Massachusetts (Downhill, Edmonds, Hilt, O'Connor, Roy, Ruder, personal communications). Prevalence data for the Amherst-Northampton area is presented in the Appendix.

The prevalence of filaria in dogs is most often determined by examining blood samples for microfilaria (Bauman and Otto, 1974; Knott, 1939; Jackson, 1969b; Taylor, 1960a).

However, screening tests using precipitin tests and fluorescent antibody technique have been used (Ellsworth and Johnson, 1973). Occasionally, surveys have been conducted by examination for adult worms at necropsy. Many surveys of heartworm occurrence were conducted in the United States prior to the recognition of the second filarial parasite of dogs, Dipetalonema reconditum (Newton and Wright, 1956, 1957) and must be viewed with this in mind (Augustine, 1938; Eyles et al., 1954; Phillips, 1939; Stueben, 1954; Ward and Franklin, 1953; Yen, 1938).

Since 1957 many researchers have reevaluated the distribution and prevalence of filarial worms of dogs. Appendix B contains a review of this literature. The data reviewed by Otto and Bauman (1959) indicated that heartworm was rare in inland areas of southeastern United States and more prevalent along the eastern and southern seaboards from central New Jersey south and west to Texas (Otto, 1969, 1972). Healy and Kagan (1961) surveyed 116 dogs for microfilaria and found 44% positive (mostly Dipetalonema spp.). Adult heartworms were found in 9.3% of 550 dogs and 0.95% of 317 cats from central New Jersey (Lillis, 1964). Over a period of nine years Ward (1965) examined 6,660 dogs from pounds between Memphis, Tennessee and New Orleans, Louisiana and found 760 (11%) positive for adult heartworms. Anastos (1965) found heartworm in 2 dogs after finding

filarial larvae in mosquitoes at Patuxent Wildlife Research Center, Maryland. A review by Otto (1974a) of D. immitis distribution increase through Middle Atlantic and North Central states revealed a unique high infection area in Hennepin County, Minnesota. Georgi and Cupp (1975) surveyed veterinarians in New York state and found 14% of 235 veterinarians reporting D. immitis and 18% of 232 reporting D. reconditum. These figures are a little deceiving since most veterinarians reporting positives also indicated much higher rates of D. immitis. Lindsey (1961) stated that the prevalence of D. immitis did not vary significantly according to sex or hair length of the animals, but others indicated it was higher in outside dogs (Alls and Greve, 1974; Hirth et al., 1966; Thrasher and Clanton, 1968; Thrasher et al., 1963).

The distribution of D. immitis in animals other than dogs has been reported by Schlotthauer (1964) with 4 positives out of 92 foxes tested in Minnesota. Stuht and Youatt (1972) also reported on red foxes; 11 positives of 79 tested in Michigan. In 1975 Graham surveyed 133 coyotes from Kansas and Colorado and demonstrated 11 positives. Wild mammals probably are not a major reservoir for D. immitis because of the relatively low positive infection rates encountered (Schlotthauer, 1964) and their sparse distribution, especially in and near populated areas.

However, the presence of infected wild animals could well act as a local nidus for infecting or reinfecting the dog population.

In 1957 Faust reviewed the world-wide status of human Dirofilaria spp. infections and reported 37 cases but only 4 in the United States (Beaver and Orihel, 1965). Beaver and Orihel (1965) reported 25 cases of human dirofilariasis, most presumably D. conjunctivae (=D. tenuis). Abadie et al. (1965) reported finding an adult, non-gravid female D. immitis in the right heart on autopsy. Filariae morphologically indistinguishable from D. immitis have been reported in 14 cases of pulmonary and cardiovascular dirofilariasis (Schlotthauer et al., 1969). Navarrette (1972) reported two new cases which expanded the list of United States human dirofilariasis to 21. Nayar and Sauerman (1973) reported that from 1960 to 1970 there were between 30 to 40 cases of D. immitis in humans. The number of human cases was listed as at least 35 cases by Gershwin et al. (1974). The first two cases from Pennsylvania were recorded by Hoch et al. (1974). The first reported human case in New England occurred in 1964 (Goodman and Gore, 1964). Two immature adult nematodes were removed from a woman's lung by resection. The list of reported human dirofilariasis varies between authors as a result of confusion or disagreement in nematode identification. Though the number of human

cases reported (35 to 40) has not been great, a potential hazard does exist, especially where there is a close association between humans, dogs, and mammal feeding mosquitoes.

MOSQUITO VECTORS IN U.S.

Identification of mosquito vectors of D. immitis has been a piecemeal effort with several workers providing small bits of information (Kartman, 1953a; Phillips, 1939; Summers, 1943; Yen, 1938). Two extensive reviews of potential vectors of D. immitis have been published by Bemrick and Sandholm (1966) and by Ludlam, Jachowski and Otto (1970). These lists include 27 species of U.S. mosquitoes which had been reported as possible vectors. The results of these reviews are summarized in Appendix C. Appendix D lists those species of mosquitoes which have been reported with natural infections.

More recent research has added additional support for some of the mosquitoes labelled as possible vectors (Bickley et al., 1976; Crans and Feldlaufer, 1974; Intermill, 1973; Nayar and Sauerman, 1975; Villavaso and Steelman, 1970; Weiner and Bradley, 1970). In 1974 Crans and Feldlaufer added Aedes cantator and Culex salinarius to the list from New Jersey. Seeley and Bickley (1974) added a Connecticut strain of Culex salinarius. Aedes

sierrensis and A. dorsalis were shown to support D. immitis development in California (Weinmann and Garcia, 1974). Nayar and Sauerman (1975) demonstrated development to infective larvae in Mansonia titillans and Culex nigripalpus in Florida. Culex salinarius from Maryland were added by Bickley et al. (1976). Christensen and Andrews (1976) demonstrated natural infections in Aedes trivittatus in Iowa. With these additions to the list there are now 34 mosquito species indicated as possible D. immitis vectors in the U.S.

MATERIALS AND METHODS

COLLECTION METHODS AND SITES

Standard New Jersey light traps and CDC light traps were used to collect adult mosquitoes during the 1975 and 1976 mosquito seasons. Dry ice was added as a CO_2 bait during 1976. All light traps ran from at least one hour before sunset until one hour after sunrise. A power aspirator was fabricated and used to make sweep collections of mosquitoes resting on ground vegetation and litter. The aspirator had an 11-inch diameter opening through which mosquitoes were drawn into a collecting bag by a motor powered by a 12-volt motorcycle battery. Aspirating collections were made between 1000 hrs and 1600 hrs. In addition, biting collections were made around dusk using human subjects. Larvae were collected using a white enamel dipper and an ADCAS (Automatic Device for Collecting Aquatic Specimens, Earle, 1956) to concentrate the collections. Eight sites in Hampshire County and one site in Franklin County were used for adult and/or larval collections.

SITE 1. Lawrence Swamp, Amherst. A wooded, marsh watershed co-located with farmland and residential areas. This is a town conservation area with trails used by both humans and canines. The area is drained by one main creek.

SITE 2. Potwine Road, Amherst. Located along a wooded creek with standing pools in a lightly populated residential

area.

SITE 3. Podick Conservation Area, Amherst. An area of woods and wooded marsh with trails throughout. In a rural area but within one half mile of residential areas.

SITE 4. South Hadley #1. A treehole breeding site located in an oak in a residential area.

SITE 5. South Hadley #2. A wooded marsh area off Route 116 near a residential area.

SITE 6. Pinchon Meadows, Northampton. A flood prone area of woods and farm land between residential properties and the Connecticut River oxbow.

SITE 7. Salem Street, Amherst. A small wooded, marsh area within a residential district.

SITE 8. Apiary, University of Massachusetts. An old excavation that was frequently a temporary pool of water.

SITE 9. Lake Wyola, Shutesbury. Temporary pools near summer cabins and year-round homes.

FIELD SURVEY FOR NATURAL INFECTIONS

All sites used for natural infection collections were in an area where more than one recent case of dog heartworm had been reported. Adult female mosquitoes were

collected at sites 1, 6 and 7 using light traps, power aspirator and biting methods. Biting collections were made at sites 2 and 3. At site 5 light trap collections were made. All mosquitoes collected were removed to the laboratory where all dead mosquitoes were identified using appropriate keys and dissected. Some live mosquitoes were lightly anaesthetized with chloroform, identified and dissected. Most live specimens were retained for 10 days in a rearing chamber at 27°C. before dissection. Any specimens that died during the holding period were removed, identified and dissected as soon as possible.

All live mosquitoes collected in the field were transferred to pint ice cream containers for holding. The container tops were replaced with clear plastic wrap for ease of observation. A small hole was cut in the side of the containers to allow insertion of a 2-dram vial containing a 5% sugar solution and a cotton wick. To insure sufficient humidity a small plastic dish filled with water was placed in the bottom of each container. Cartons containing specimens were then retained in the rearing chamber.

All dissections were done in a drop of insect saline (Taylor, 1960b) on a glass slide. The head was first removed into the drop, followed by the malpighian tubules. The preps were then squashed and examined at 100x or 430x

with a compound microscope. Those mosquitoes not individually dissected were combined in pools with one species to a pool. No more than 50 mosquitoes were placed in any one pool. A modification of the method used by Muller and Denham (1974) was employed for the pooled specimens. Mosquitoes were immobilized by chloroform, lightly crushed with a mortar and pestle containing a few drops of saline. The mosquitoes were then transferred to a small plastic cup which had its bottom removed and replaced by standard window screening. This cup was suspended in a glass funnel, with a short piece of clamped plastic tubing at the bottom, filled with insect saline. At 1-hour intervals for 3 hours, 2 to 3 ml samples were drawn off into small plastic petri dishes and were examined at 9x and 36x under a dissecting microscope. 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 infective larvae (3rd stage) in the U.S. has been published. Identification of D. immitis was accomplished through the use of descriptions and measurements reported by Kartman (1953b) and Nelson (1959). Kartman (1953b) reported measurements of infective larvae from experimentally infected hosts, Anopheles quadrimaculatus, Aedes albopictus and Culex p. quinquefasciatus as follows: length, 700u to 1100u (mean 850u); breadth, 23u

to 26 u (mean 25u). Nelson (1959) found the measurements in A. pembaensis, A. aegypti and C. fatigans as follows: length, 800u to 1040u; breadth, 18u to 26u; anus to caudal extremity, 26u to 40u. Comparisons were made to descriptions of other mosquito-vectored species of nematode larvae and their reported distribution. The other described species are: Setaria equina (Becklund and Walker, 1969; Nelson, 1959); Dirofilaria tenuis (Pistey, 1958); D. scapiceps (Highby, 1938, 1943b; Tuff, 1975); D. striata (Orihel and Ash, 1964); Dipetalonema arbuta (Highby, 1943a).

Foleyella spp. were not considered to be a point of confusion because the amphibiophilic mosquito vector is not likely to be feeding on mammals (Benach and Crans, 1975; Kotcher, 1941; Witenberg and Gerichter, 1944). The key characteristics of infective larvae are compared in Table I.

LABORATORY INFECTION EXPERIMENTS

Collections of adult female mosquitoes used in laboratory infection experiments were made at sites 1, 2, 3, 5, 6 and 7. These collections were made using humans as bait for biting female mosquitoes. Larval mosquitoes were collected at sites 1, 2, 4, 6, 8 and 9. Larvae were taken to the lab, put into trays half filled with distilled water and reared to adults on a diet of ground rabbit chow.

TABLE I

SOME KEY CHARACTERISTICS OF INFECTIVE STAGE LARVAE OF MOSQUITO-BORNE FILARIA IN NORTHEASTERN U.S.

Filaria spp.	Length	Width	Anus to Tail	#Anal Papillae	Host
Setaria equina	1280-1720	21-28	40-52	1 lg/2 sm	Equidae
Foleyella brachyoptera	650-900	13-17	50	0	Amphibia:Anura
F. dolichoptera	650-900	13-17	50	0	Amphibia:Anura
F. ranae	702-1066	20-25	50	0	Amphibia:Anura
Dirofilaria immitis	800-1040	18-26	26-40	1	Canidae:Felidae
D. tenuis	781-1157	21-28	----	0	Procyonidae
D. striata	950-1140	24-26	----	----	Felidae
D. scapiceps	780	14	----	3	Lagomorpha:Leporidae
Dipetalonema arbuta	880-1160	16-18	22-36	3	Rodentia:Erethizontidae

Adults collected in the field were transferred to half-pint ice cream containers which had the bottom removed and replaced by nylon mesh screening. These mosquitoes then were offered a blood meal on an infected dog between 2000 and 2200 hrs on the day collected. The feeding container was held on the dog's underbelly area until the mosquitoes had fed. Adult females that had been reared in the lab were maintained on 5% glucose solution until they were 3 to 5 days old. The sugar solution was withdrawn in the morning, mosquitoes were transferred to the feeding cartons in the late afternoon, and offered a blood meal on the infected dog at 2000 to 2200 hrs (normally for 15 min). After feeding, all mosquitoes were transferred to the one pint ice cream containers with 5% glucose and held in the rearing chamber. All collections were identified by date and site collected, and date fed.

Any dead mosquitoes were removed each morning, identified and then the head and malpighian tubules were dissected. Sample specimens were removed from each container at intervals during the 14 to 18 day holding period. Between days 14 to 18 all remaining mosquitoes in an individual collection were dissected. All dissections were examined with a compound microscope at 100x and 430x and the stage of larval nematode development recorded.

CARE OF LABORATORY DOG

The infected dog used in the feeding experiments was a pedigree Brittany Spaniel obtained through Dr. J. W. Hilt (a local practicing veterinarian). The dog was first treated to remove intestinal worm infections. Once healthy, except for heartworm, it was maintained on a diet of dog chow and canned dog food. It was confined to prevent its becoming overexerted. The underbelly area had little hair so shaving was unnecessary and the dog remained calm during the mosquito feeding sessions.

RESULTS

During the summers of 1975 and 1976 field collections of adult mosquitoes were made in an attempt to recover female mosquitoes that were naturally infected with D. immitis. Also, during the summer of 1975 laboratory infection trials were undertaken using adult mosquitoes most of which were reared from field collected larvae. Most of the 1975 field season was spent in identifying suitable areas to trap for infected mosquitoes and in determining the best methods for collecting specimens as well as in familiarization with mosquito identification. The summer of 1976 was relatively dry resulting in abnormally low mosquito population throughout the state. During both years, a total of 3445 mosquitoes, representing 23 species, were collected. Of these, 859 were pooled by species for examination and the remainder were individually dissected. Feedings on an infected dog were attempted with 1451 mosquitoes representing 19 species.

During the 1975 field collections and early laboratory infection tests the author was unable to separate Aedes excrucians females from other Aedes stimulans group females. With further experience, the aid of other adult identifying characteristics (McDaniel and Webb, 1974), and larval identifications, A. excrucians was separately identified in the later laboratory tests (as indicated under A. stimulans in Table IV) and in the 1976 field collections (Tables II and III).

TABLE II

FIELD COLLECTED FEMALES INDIVIDUALLY DISSECTED FOR NATURAL INFECTIONS IN 1975 AND 1976

Species	1975 Total Dissected	1975 Number Positive[a]	1976 Total Dissected	1976 Number Positive[a]	
Aedes abserratus	-	-	36	0	
A. canadensis	46	(1)[b]	158	1	(2)[c]
A. cinereus	102	0	146	0	
A. excrucians	---[d]	---[d]	134	4	
A. sticticus	33	0	47	1	
A. stimulans group[e]	50	(3)[b]	88	0	
A. triseriatus	8	0	83	0	
A. vexans	548	0	171	0	(1)[f]
Anopheles punctipennis	12	0	45	0	
Culex species	620	0	130	0	

[a] *Dirofilaria immitis* unless otherwise indicated.

[b] Unidentified *Dirofilaria* spp.

[c] Unidentified but not *Dirofilaria* spp.

[d] *Aedes excrucians* included under *A. stimulans* group in 1975 field identifications.

[e] Includes *A. stimulans*, *A. fitchii* and *A. excrucians* which are difficult to differentiate to species by male genitalia, larvae or female tarsal claws.

[f] *Setaria equina*.

TABLE II (Continued)

Species	1975 Total Dissected	1975 Number Positive[a]	1976 Total Dissected	1976 Number Positive[a]
Coquillettidia perturbans	24	0	76	0
Culiseta morsitans	1	0	12	0
Uranotaenia sapphrina	14	0	-	-
Psorophora ferox	21	0	1	0

TABLE III

FIELD COLLECTED FEMALES POOLED FOR NATURAL INFECTIONS
IN 1976

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

[a]Dirofilaria immitis unless otherwise noted.

[b]Includes Aedes stimulans and A. fitchii but not A. excrucians.

[c]Filaria other than D. immitis.

TABLE IV

DISSECTION RESULTS OF MOSQUITOES FED ON INFECTED DOG

Species	Total Fed	Positive Day 1-10 (#Mel.)[a]	Negative Day 1-10	% Mosq. Infected	Positive Day 11+ (#Mel.)[a]	Negative Day 11+	% Mosq. Infective
Aedes vexans	515	191 (11)	99	65.9	28 (3)	197	12.4
A. stimulans group[b]	169	119 (5)	13	90.1	2	35	5.4
(A. excrucians)	(27)	(18) (1)	(0)	(100.0)	(2)	(7)	(22.2)
A. canadensis	142	44 (8)	34	56.1	15 (1)	49	23.4
A. triseriatus	84	29 (1)	14	67.4	14	27	34.2
A. cinereus	140	34 (1)	59	36.6	4	43	8.5
A. sticticus	80	51 (2)	12	85.7	12	4	75.0
A. trivittatus	4	1	0	100.0	1	2	33.3
Anopheles punctipennis	11	2	2	50.0	2	5	28.6
Psorophora ferox	45	6	32	15.8	1	6	14.3

[a]The number of mosquitoes in which some of the larval nematodes were melanized.

[b]Includes Aedes stimulans, A. fitchii and A. excrucians which are difficult to differentiate to species except by male genitalia, larvae, or female tarsal claws.

FIELD SURVEY FOR NATURAL INFECTIONS

Results of the individuals dissected and the pools examined are presented in Tables II and III. Five additional species, Aedes aurifer, A. trivittatus, Anopheles walkeri, Culiseta inornata and C. impatiens, representing a total of 9 specimens, also were dissected with negative results.

Of the 13 mosquitoes found to contain filaria by dissection (Table II), 6 mosquitoes (1 Aedes canadensis, 4 A. excrucians, 1 A. sticticus) were positive for infective larvae of Dirofilaria immitis. Four other infections were Dirofilaria and may have been D. immitis but positive identifications were not made. One of the 3 pools (Table III) containing filarial larvae also was positive for infective larvae of D. immitis. The infective 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; Aedes canadensis - 0.8% (2 in 254); A. stimulans group, including A. excrucians - 2.2% (8 in 369); A. excrucians alone (1976) - 3.3% (5 in 154) and A. sticticus - 1.3% (1 in 80). In view of the laboratory infection results and the 1976 field results, the 4 field isolations (1 in A. canadensis and 3 in A. stimulans group) were probably D. immitis and were therefore included in these calculations.

LABORATORY INFECTION EXPERIMENTS

Table IV presents the results of the laboratory infection tests. A total of 250 individuals of Culex p. pipiens, C. restuans and C. salinarius were offered blood meals on the infected dog but no blood meals were taken except by 1 of 4 C. salinarius which died before the larvae of D. immitis could develop beyond the first stage. Five other species, Aedes trichurus, Anopheles quadrimaculatus, Coquillettidia perturbans, Culiseta morsitans and Psorophora ciliata, also were collected and offered blood meals but in insufficient numbers for meaningful results.

Of the 19 species of mosquitoes offered a blood meal on the infected dog only 9 species contained larvae that developed to the infective stage (3rd stage larvae in malpighian tubules or head). In Aedes vexans 65.9% of the individuals that took a blood meal became infected (sausage stage in malpighian tubules), but only 12.44% of the adults contained larvae that completed development to the infective stage. Melanization of the filarial larvae-resulting from a physiological defense mechanism on the part of the mosquito-occurred in 6.4% (14 of 219) of the female A. vexans which contained filarial larvae. A much larger nematode parasite of A. vexans, tentatively identified as Perutelimermis sp. by W. R. Nickel (personal communication), was found in 11.3% (58 in 515) of the laboratory infected mosquitoes and in 8.8% (48 in 548) of the 1975 field collected A. vexans.

(Specimens containing these nematodes were not included in the 1976 field results.) The larvae of these nematodes apparently enter the mosquito larvae and continue their development in the mosquito adult.

While 90.1% of the blood fed A. stimulans group became infected only 5.4% contained larvae that developed to the infective stage. If the identifiable A. excrucians are considered alone then the infected rate is 100% and the infective rate 22.2%. Melanization occurred in A. stimulans group in 4.1% (5 in 121) and in 5.0% (1 in 18) in A. excrucians. While melanization was fatal to the larvae involved there were other larvae in the same mosquito that continued to develop, apparently unaffected. Both A. vexans and A. stimulans group had a high mortality during the first 3 days after an infected blood meal.

The rates of infected adults in A. canadensis (56.4%), A. triseriatus (67.4%), A. cinereus (36.6%), A. sticticus (85.7%), A. trivittatus (100%) and Anopheles punctipennis (50%) were promising. The infective rate for A. cinereus was low (8.5%) but was much higher for A. canadensis (23.4%), A. triseriatus (34.2%), A. sticticus (75%), A. trivittatus (33.3%) and Anopheles punctipennis (28.6%). Psorophora ferox had a low infected rate (15.8%), however, the infective rate was quite similar (14.3%). Melanization also occurred in A. canadensis (15.3%), A. triseriatus (2.3%), A. cinereus

(2.6%) and A. sticticus (1.6%).

FILARIAL LARVAL IDENTIFICATION

The infective larvae were identified using the information in Table I. 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 amphibiophilic 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.

DISCUSSION

Under controlled laboratory conditions mosquitoes may physiologically support development of parasites that would not normally develop under field conditions. Also, the infective mosquito may be unable to efficiently transfer (=transmit) the infective stages of the parasite under either laboratory or natural conditions. Moreover, species that can both support and transmit the filarial parasite efficiently still must be ecologically and behaviorally suited to the life cycle of the parasite and its definitive host in order to be a good natural vector. For example, the mosquito must be present in sufficient numbers and have a range and synchrony of flight that frequently bring it into close contact with both infective and susceptible dogs. Also, the mosquito must naturally feed on dogs and preferably do so during the early evening hours when the animals are less active and the microfilaremia is highest. After feeding on an infective dog the mosquito must survive long enough to allow the microfilariae to develop and then feed a second time. In addition to the abundance requirement, a good vector species also should be present throughout the entire summer season when microfilariae are present in the peripheral circulation of the infected dog. Of course, if there proves to be more than one suitable vector in a given area then this requirement may be met by pooling the distributions of species that are seasonally divergent. Though

often an elusive goal, the finding of naturally infected females of species that meet all the necessary biological criteria is consequently the strongest single body of evidence for incriminating a vector.

Twenty-five species of mosquitoes were collected and dissected (Tables II, III, and IV). Not all of these were expected to be able to function as possible vectors. Many of the same species considered as possible vectors in other areas of the United States were collected. Through the use of data obtained from the field collections and the laboratory trials as well as other available biologic data it was possible to greatly reduce this list and to identify those species with the greatest vector potential in this area (see summary in Table V).

Culex territans and Uranotaenia sapphrina, which feed mainly on poikilotherms (Crans, 1970; Edman, personal communication), lack the necessary host-feeding behavior to fulfill the requirements of a vector of dog heartworm. Culex pipiens, Culex restuans and Culiseta morsitans feed primarily on birds (Tempelis, 1975) and therefore, also could be eliminated as possible vectors. This was further supported by the laboratory results where 246 specimens of Culex spp. (excluding Culex salinarius) were offered a blood meal and not one fed. One of 4 C. salinarius fed

TABLE V

SUMMARY OF VECTOR POTENTIAL OF WESTERN MASSACHUSETTS MOSQUITOES — CLASSIFICATION BASED ON CURRENT BIOLOGICAL AND INFECTIVITY DATA

Species	Mammal Feeders	Relatively Abundant	Multiple Generation	Laboratory Isolation of Infective Larvae	Field Isolation of Naturally Infected Females	Vector Potential
Aedes abserratus	———	———	———			Low
A. aurifer	———	———				Low
A. canadensis	———	———	———	———	———	Highest
A. cinereus	———	———	———	———		Moderate
A. excrucians	———	———	———	———	———	Highest
A. sticticus	———	———	———	———	———	High
A. stimulans	———	———	———			Low
A. trichurus	———	———				Low
A. triseriatus	———	———	———	———		Moderate
A. trivittatus	———	———				Moderate*
A. vexans	———	———	———	———		Moderate
Anopheles walkeri	———	———	———			Low
An. punctipennis	———	———	———	———		Moderate
An. quadrimaculatus	———	———	———	———		Moderate

*Naturally infected females reported in Iowa; Christensen and Andrews, 1976.

TABLE V (Continued)

Species	Mammal Feeders	Relatively Abundant	Multiple Generation	Laboratory Isolation of Infective Larvae	Field Isolation of Naturally Infected Females	Vector Potential
Culex pipiens pipiens	———					None
C. restuans	———					None
C. salinarius	———	———				Moderate**
C. territans	———					None
Coquillettidia perturbans	———	———	———			Low
Psorophora ferox	———	———	----------	----------		Moderate
Culiseta morsitans	———					None
Uranotaenia sapphrina	———					None

**Some development in small laboratory trials, no infective stage larvae; Seeley and Bickley (1974) had laboratory success with Connecticut strain.

but it died before the filarial larvae could develop beyond the sausage stage. Hu (1931) and Summers (1942) reported similar unsuccessful results with this species. Experiments by Seeley and Bickley (1974) on strains of C. salinarius from Connecticut, Louisiana and Maryland successfully supported development to the infective stage only in the Connecticut strain. Results such as these indicate that a species may be a vector in one geographic location but not another, thereby justifying experimental research on the same mosquito species in different regions in the United States. Studies in Maryland (Bickley et al., 1971) have shown this mosquito is most abundant in coastal areas becoming reasonable common in western Massachusetts only in late summer. Additionally, its catholic feeding habits include many non-mammalian hosts (Edman, 1974; Murphey et al., 1967; Wright and DeFoliart, 1970). Consequently, it appears to have low potential as a vector, at least in this area.

All of the species which remain to be considered are essentially mammalian feeders (Carpenter and LaCasse, 1955; Edman, 1971; Tempelis, 1975; Wright and DeFoliart, 1970) and as such cannot be excluded on the basis of their natural host-feeding patterns.

Mosquito species that were collected in extremely low numbers and thus are very unlikely to serve as vectors

are: Aedes aurifer, Aedes trichurus, Culiseta impatiens, Culiseta inornata and Psorophora ciliata. The low populations observed for these species agrees with the results reported by Fellton et al. (1950). In laboratory trials, filarial larvae did not develop beyond the sausage stage in the 4 infected A. trichurus examined.

Relatively few specimens (52) of Aedes abserratus, a single generation, spring species (Carpenter and LaCasse, 1955), were collected and all dissections were negative (Tables II and III). The modest population levels and short seasonal occurrence of this species coupled with no reported filaria-infected specimens combine to eliminate it as a good potential vector.

Psorophora ferox only supported development of D. immitis to the infective stage at a low rate (14%) in the laboratory (Table IV). Moreover, it is not a common mosquito in western Massachusetts and is reportedly a short-lived species. It usually only occurs late in the season, and has a strong tendency toward daytime feeding, especially within its woodland breeding areas which the adults are reluctant to leave (Edman, 1971; Fellton et al., 1950; Matheson, 1945). All of these features of Psorophora ferox combine to indicate poor vector potential.

Coquillettidia perturbans is a relatively widespread and common mosquito during the entire summer and fall;

however, its distribution often is erratic, with localized population pockets, due to the specialized larval habitat requirements of this species (Downe, 1962). No infective females were collected in the field here (Tables II and III) or elsewhere and none were found to support development of filaria larvae beyond the 1st stage in a small laboratory sample. These laboratory results are supported by those of Bemrick and Sandholm (1966) and Yen (1938) in Minnesota. Though often present in large numbers with a fairly long season, its erratic distribution and the lack of laboratory or field infections all suggest low to modest potential as a vector of heartworm.

Yen (1938) found no trace of infection in Aedes trivittatus that had been fed on an infected dog. In contrast, laboratory trials with just 4 specimens in 1975 resulted in 1 female supporting development of D. immitis to the infective stage (Table IV). Christensen and Andrews (1976) also reported natural infections in A. trivittatus in Iowa. Nonetheless, this is a rare species in western Massachusetts (Fellton et al., 1950) and as such is not likely to be an important vector here, though it likely may be elsewhere.

Laboratory trials with Aedes triseriatus demonstrated that 34% of the adults surviving long enough, supported development to the infective stage (Table IV). Phillips

(1939) and Intermill (1973) also demonstrated successful filarial development experimentally. No naturally infected females were collected. This species is a tree-hole breeder and as such requires extensive suitable deciduous woods for any significant mosquito population to occur (Benach et al, 1971; Sudia et al., 1971). Like P. ferox, this species will bite during the daytime within the wooded habitats to which it normally is restricted (Carpenter and LaCasse, 1955; Pinger and Rowley, 1972). It may be locally abundant at times (Fellton et al., 1950) and a moderate number of females were collected (Tables II and III). In laboratory trials (Table IV), 34% of the surviving females contained infective larvae. While A. triseriatus may experimentally support parasite development, no naturally infected females were collected (Tables II and III) and it is not consistently present in the numbers and locations that would be required of an efficient vector of D. immitis.

Some of the earlier workers have reported experimental success in the development of infective larvae in Aedes cinereus (Phillips, 1939; Yen, 1938) and in Aedes vexans (Bemrick and Sandholm, 1966; Hu, 1931; Yen, 1938) and indicated these 2 species to be promising vectors of D. immitis. In the laboratory trials in Table IV low infective rates were obtained for A. cinereus (8.5%) and A. vexans (12%). With A. vexans, Summers (1943) reported no development experimentally while Crans and Feldlaufer

(1974) reported a low vector potential based on field collected specimens. Females of A. cinereus were the second most abundant while A. vexans was collected in the largest numbers (1129 specimens) with neither species having any natural infections. Both of these rainpool and floodwater mosquitoes are normally present and abundant from late spring to fall, with population reductions occurring during the often drier mid-summer months in this area. Even though A. cinereus and A. vexans meet many of the biological requirements, the low experimental success and the negative field results obtained with these species are not suggestive of a primary role in the natural vectoring of D. immitis.

The laboratory trials with Anopheles punctipennis yielded successful development of infective larvae in 29% of those mosquitoes surviving long enough to support complete development (Table IV). Yen (1938) and Phillips (1939) also reported successful experimental development of D. immitis to the infective stage in An. punctipennis. Bemrick and Sandholm (1966), Hu (1931), Kartman (1953b), Nayar and Sauerman (1975) and Summers (1943) also reported experimental success with several Anopheles species. Christensen and Andrews (1976) reported finding 1 (of 468) An. punctipennis naturally infected with 1st larval stage. No naturally infected An. punctipennis were found among the

1975 and 1976 field collections (Tables II and III). Normal collecting methods seldom yield large numbers of most Anopheles species because of their special resting habits and limited attraction to light (Bemrick and Sandholm, 1966; Bidlingmayer, 1971; Edman, 1971). Fifty-seven specimens of An. punctipennis (Table II) and 3 specimens of An. walkeri were collected in the field. In the laboratory filaria larvae developed to the infective stage in the malpighian tubules of the 1 An. quadrimaculatus and in 28.6% of the experimentally infected An. punctipennis (Table IV). None of the Anopheles are particularly abundant in the northeastern United States—with An. quadrimaculatus and An. walkeri the least abundant—and significant populations are seldom encountered until late summer (Carpenter and LaCasse, 1955; Edman, 1971; Tempelis, 1975; Fellton et al., 1950). Though the potential of the Anopheles, especially An. punctipennis in this area, as a vector cannot be dismissed, it appears that they could only serve in a minor late season role in this area.

Thus the list of vectors with maximum potential has been reduced to the 3 (or 4 if Aedes excrucians and the Aedes stimulans group are considered separate) in which naturally infected females were collected during this research (Tables II and III). After careful consideration of the laboratory results for A. excrucians and the A. stimulans

group (Table IV) and the 1976 field data in which A. excrucians were separated from the rest of the A. stimulans group (Tables II and III), it appears as though the 1975 positives from the A. stimulans group were in all probability unidentified A. excrucians specimens. The laboratory trials with Aedes sticticus (Table IV) were highly successful (75% infective) and 1 naturally infective female was found. Nevertheless, this species appears to have the least vector potential of these 3 Aedes species since it is normally not common in western Massachusetts (Table II and Fellton, 1950).

The remaining species are common throughout the mosquito season and seem to meet the biological requirements of an efficient vector with equipollence. Although Aedes excrucians has a single generation per year, the females are long-lived, first appearing in April-May and some surviving until September. Aedes canadensis, a fresh water flood pool mosquito, also is a long-lived adult but with additional production occurring throughout the summer during reflooding (Carpenter and LaCasse, 1955). As a result of its being both long-lived and having additional production, biologically it may be the more suitable vector. Both A. canadensis and A. excrucians had similar infective rates in laboratory trials (23% and 22% respectively). Natural infections were found in 1% of the A. canadensis

females collected in the field and in 4% of the A. excrucians females. Although additional data from both field collections and laboratory infection and transmission experiments would be helpful in further assessing the vector potential of these and certain other species (especially those collected in small numbers), some guarded conclusions can be drawn from these data.

SUMMARY AND CONCLUSION

In 1975 and 1976 a study was carried out to determine the potential vector mosquitoes of Dirofilaria immitis in western Massachusetts. Two approaches were utilized: first, field isolation of naturally infected females and second, testing for experimental potential in the laboratory. Adults were collected using light traps, a power aspirator and human bait. Larvae were collected and reared to adults in the laboratory. All adults were maintained in a rearing chamber at 27°C.

A total of 3445 female mosquitoes, representing 23 species, were collected and examined for filaria. A total of 10 mosquitoes (3 species) containing natural infections of D. immitis were found. In the laboratory 1451 mosquitoes, representing 19 species, were fed on an infected dog. Ten species were represented among the 79 females that successfully supported filarial development to the infective stage. A total of 26 different species of mosquitoes were examined in the combined field and laboratory experiments.

For a mosquito to be an efficient vector it must be reliable. That is, it must be present in sufficient numbers each season, must maintain a fairly stable population, feed on both reservoir and host readily, support successful development of the parasite to the infective stage without

undue damage to itself and live long enough and be able to transmit the developing parasite to another host. The available data and the evidence gathered in this research support the following conclusions:

1. Aedes excrucians, Aedes canadensis and Aedes sticticus have the most potential as vectors of Dirofilaria immitis in western Massachusetts. Both A. excrucians, with highest percentage of natural infections, and A. canadensis fulfill the biological requirements, are reliable and would appear to be the prime vectors. Of these 3 species, A. sticticus least fulfills the qualities of a reliable vector because of its low densities. However, the isolation of 1 naturally infected female indicates a high potential as a vector of D. immitis.

2. Aedes cinereus, Aedes triseriatus, Aedes trivittatus, Aedes vexans, Anopheles punctipennis, Culex salinarius and Psorophora ferox may possibly act as vectors because all supported some filarial development in laboratory trials. However, because no naturally infected females were found and these species do not fulfill all the biological requirements, they only have moderate potential as vectors.

3. Aedes aurifer, Aedes abserratus, Aedes trichurus, Coquilletidia perturbans and other species collected (excluding those in 4 below) appear to have low potential as vectors due to either low or erratic populations, short seasonal distributions or low to no laboratory and field infection rates.

4. Culex pipiens, Culex restuans, Culex territans, Culiseta morsitans and Uranotaenia sapphrina were eliminated as vectors in western Massachusetts. These species normally do not feed on mammals and the last 3 do not occur in sufficient numbers to be reliable vectors in any event.

APPENDIX A

PREVALENCE OF *Dirofilaria immitis* IN DOGS IN WESTERN MASSACHUSETTS[a]

	Year	Number of dogs tested	Number positive[b]	Percent positive
Northampton	1973	505	38	7.5
	1974	597	58	9.7
	1975	715	25	3.4
	1976[c]	514	22	4.3
Holyoke	1973	510	29	5.7
	1974	586	30	5.1
	1975	612	19	3.1
	1976[c]	507	7	1.4
Amherst	1973	756	31	4.1
	1974[d]	706	20	2.8
	1975[d]	753	13	1.7
	1976[c,d]	759	12	1.6

[a]Source: Unpublished data from area veterinary clinics (Amherst Animal Clinic, Holyoke Animal Hospital, Northampton Animal Clinic).
[b]Corrected to remove repeated tests.
[c]Total through August 1976.
[d]Total number of dogs tested not corrected; number retested unavailable.

APPENDIX B

GEOGRAPHIC DISTRIBUTION AND INCIDENCE OF CANINE FILARIASIS IN THE U.S.

Geographic Area	Investigator	No. Dogs	% D.i.	% D.r.	% Mixed	% Total
Alabama - Mobile	Lindsey (1961)					
Pound Dogs		50	42	14	10	66
Clinic Dogs		50	28	18	4	50
Total of Above		100	35	16	7	58
Alabama - Auburn	Lindsey (1961)					
Clinic Dogs		100	2	31	1	34
Alabama - Montgomery	Rothstein et al. (1961)	555	37.8	1.1	0	38.9
California - Northern	McGreevy et al. (1970)					
Pound Dogs		515	0	5	0	5
Beagle Colony		800	0.12	0	0	0.12
Connecticut - Coast	Hirth et al. (1966)	299	7.7	1.7	-	9.4
Connecticut - Southwestern	Tritch et al. (1973)					
Random Sample		503	3.2	0.4	-	3.6
Clinic Sample		36	47.2	5.6	-	52.8

APPENDIX B (Continued)

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.i.	% D.r.	% Mixed	% Total
Florida - Jacksonville	Lindsey (1961)					
Pound Dogs		50	8	44	6	58
Clinic Dogs		50	26	18	6	50
Total of Above		100	17	31	6	54
Georgia - Columbus	Lindsey (1961)					
Pound Dogs		110	2	50	2	54
Georgia - North and South	Thrasher and Clanton (1968)					
Well-Cared-For-Dogs		672	19.6	4.7	0	24
Georgia - Atlanta	Thrasher *et al.* (1968)					
Pound Dogs		40	12.5	37.5	7.5	57.5
Private Dogs		273	5.4	14.6	0.7	20.9
Hawaii	Gubler (1966)					
Pound and Private Dogs		666	32.2	10.8	2.3	45.2
Hawaii	Ash (1962)	96	19	16	-	35
Illinois - Champaign, Co.	McKinney (1962)					
Clinic Dogs		212	1.4	1.4	0	2.8

APPENDIX B (Continued)

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.i.	% D.r.	% Mixed	% Total
Illinois -	Marquardt and					
Northern	Fabian (1966)	163	10.4	2.5	0	13
Central		73	21.9	16.4	0	38
Southern		110	34.6	8.2	0	43
Total of Above		346	20.5	7.2	0	28
Indiana - Indianapolis	Eshenour (1958)					
Research Farm Dogs		333	0.6	9	0	10
Iowa	Alls and Greve					
Random, Native Dogs	(1974)	385	6.5	-	-	6.5
Kansas - Northeastern	Graham (1974)	288	16.7	7.6	0	24.3
Louisiana - New Orleans	Orihel (1959)	137	42	28	6.6	63
Louisiana - New Orleans	Thrasher et al.					
Private Dogs	(1963)	543	44	2	0.4	46.2
Maryland - Hyattsville	Wallinstein and					
Clinic Dogs	Tibola (1960)	528	6.6	5.3	1.14	13.1
Maryland - Marlborough	Mallack et al. (1971)	102	44.1	0	0	44.1

APPENDIX B (Continued)

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.i.	% D.r.	% Mixed	% Total
Michigan - MSU Pound Dogs	Leash and Hanson (1961)	192	2	4	0	6
Michigan - Detroit Pound Dogs	Zydeck et al. (1970)	248	1.6	2.8	0	4.4
Michigan - Southeastern	Prouty (1972)					
Belleville		880	22	-	-	22
Detroit		399	6	-	-	6
Farmington		698	6	-	-	6
Minnesota	Schlotthauer and Griffiths (1964)	409	36	0	0	36
New York - Western Buffalo Pound	Sengbusch et al. (1975)	100	2	0	0	2
Northeast U.S. - Including Pennsylvania, Maryland and New Jersey	Newton and Wright (1956)	250	1	6	-	7
Ohio - Many Parts	Groves and Koutz (1964)	340	2.1	6.8	0	8.8

APPENDIX B (Continued)

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.i.	% D.r.	% Mixed	% Total
Oklahoma - North-Central Pound Dogs	Pennington et al. (1970)	100	0	15	0	15
Pennsylvania - Pittsburg	Rothstein et al. (1961)	841	2.5	2.4	0	5
Rhode Island - Providence	Rothstein et al. (1961)	69	1.5	1.5	0	3
Texas - South	Keegan et al. (1968)	522	19.7	0	-	19.7
Texas - Taylor County Pound Dogs	Joiner and Jardine (1970)	700	5	14	0.7	19.7
Virginia - Fairfax County Pound Dogs	Kimbell (1976)	213	18	0	0	18
Virginia - Norfolk	Newton and Wright (1956)	7	57	43	-	-
Many Areas of U.S.						
Military Dogs	Butts (1970)	3475	2.4	1.9	0.09	4.39
U.S.Army Sentry Dogs	Rothstein (1963)	1026	12.2	0.2	0	12.4

Source: Adapted from Pennington, Ph.D. Thesis, pp. 7-9, 1971.

APPENDIX C

POSSIBLE VECTORS OF *Dirofilaria immitis* IN THE U.S.
IN WHICH COMPLETE LARVAL DEVELOPMENT HAS BEEN REPORTED

Species	Additional References
Aedes aegypti	Nayar & Sauerman, 1975; Weiner & Bradley, 1970
*A. atropalpus	
*A. canadensis	Crans & Feldlaufer, 1974
*A. cantator	Crans & Feldlaufer, 1974
*A. cinereus	
*A. dorsalis	Weinmann & Garcia, 1974
*A. excrucians	
*A. fitchii	
A. infirmatus	
A. sierrensis	Weinmann & Garcia, 1974
*A. sollicitans	Beam, 1966; Crans & Feldlaufer, 1974; Nayar & Sauerman, 1975
*A. sticticus	
*A. stimulans	
*A. taeniorhynchus	Nayar & Sauerman, 1975; Weiner & Bradley, 1970
*A. triseriatus	Intermill, 1973
*A. trivittatus	Christensen & Andrews, 1976
*A. vexans	Crans & Feldlaufer, 1974
A. zoosophus	
*Anopheles crucians	
*A. earlei	
A. freeborni	
*A. punctipennis	
*A. quadrimaculatus	Nayar & Sauerman, 1975; Weiner & Bradley, 1970

APPENDIX C (Continued)

Species	Additional References
*Anopheles walkeri	
*Coquillettidia perturbans	
Culex nigripalpus	Nayar & Sauerman, 1975
*C. pipiens pipiens	
C. p. quinquefasciatus	Gubler, 1966; Nayar & Sauerman, 1975; Villavaso & Steelman, 1970; Weiner & Bradley, 1970
*C. restuans	
*C. salinarius	Crans & Feldlaufer, 1974; Seeley & Bickley, 1974
C. tarsalis	
*C. territans	
Mansonia titillans	Nayar & Sauerman, 1975
*Psorophora ferox	

*Mosquitoes found in Massachusetts.

Source: adapted from Bemrick and Sandholm, J. PARASIT. 52:763, 1966 and Ludlam, Jachowski and Otto, J.A.V.M.A. 157:1354-55, 1970.

APPENDIX D

MOSQUITOES IN WHICH NATURAL INFECTIONS OF
Dirofilaria immitis HAVE BEEN REPORTED

Species	Additional References
Aedes canadensis	Crans & Feldlaufer, 1974
A. cantator	Crans & Feldlaufer, 1974
A. excrucians	Phillips, 1939
A. sollicitans	Crans & Feldlaufer, 1974
A. trivittatus	Christensen & Andrews, 1976
A. vexans	Bickley et al., 1976; Crans & Feldlaufer, 1974
Anopheles punctipennis	Bickley et al., 1976; Christensen & Andrews, 1976; Phillips, 1939
A. quadrimaculatus	Anastos, 1965; Phillips, 1939
*Culex pipiens quinquefasciatus	Villavaso & Steelman, 1970
C. salinarius	Bickley et al., 1976; Crans & Feldlaufer, 1974

*Only species on list not reported from Massachusetts.

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