VECTORS OF NEWLY ENDEMIC CANINE DIROFILARIASIS
IN STILLWATER, OKLAHOMA

By

JULIUS SUNDAY AFOLABI

Bachelor of Science
Bethany Nazarene College
Bethany, Oklahoma
1978

Master of Public Health
Oklahoma University Health Sciences Center
Oklahoma City, Oklahoma
1980

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Thesis Approved:

Thesis Adviser

Dean of the Graduate College

1233528

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CHAPTER I

INTRODUCTION

Dirofilaria immitis (Leidy, 1856) (*Nematoda*: *Filariidae*), commonly known as dog heartworm, is the most important of the two canine filariids known to occur in the United States. It is abundant and often produces severe clinical disease in affected dogs.

Mature males and females live mainly in the right ventricle of the heart, but when many adults are present some may be located in the pulmonary arteries. Adult females produce microfilariae viviparously which circulate freely in the peripheral circulatory system of infected dogs. Microfilariae (not sausage stage), the first-stage larvae, are ingested by adult female mosquitoes when they ingest a blood meal. These insects serve as obligatory intermediate hosts. From the gut lumen, microfilariae migrate to the cells of the distal end of malpighian tubules where further development continues for about 6-7 days. In other words, microfilariae are intracellular parasites in the malpighian tubules. These parasites feed in this location and subsequently break out of the cells into the lumen of malpighian tubules where they molt twice to complete development to become third-stage larvae. When infected mosquitoes feed, infective larvae that have migrated to the proboscis or labium enter the dog host through a wound made by the stylet.

In the dog the parasite molts twice as it migrates through different

types of tissues. Finally it penetrates a blood vessel and continues its journey to the heart. The period of migration from the time an infective larva enters the dog until it reaches the heart is about 70-90 days. Several workers have studied the developmental stages of *D. immitis* in the dog (Kume and Itagaki, 1955; Orihel, 1961; Kotani and Powers, 1982). They all agree that two additional molts occur in the dog but differ in opinion about the number of days between each molt.

Apart from the dog, the natural definitive host, many authors have reported *D. immitis* in red foxes, beavers, coyotes, wolves, dingoes, gibbons, cats, seals, tigers, jaguars, sea lions and man. No human deaths have been associated with *D. immitis* infections. However, physicians in endemic areas are becoming aware of the zoonotic potential of this nematode. The lesions produced may mimic pulmonary carcinoma.

Dirofilaria immitis has been reported from all parts of the world and is known to occur in most of the continental United States. Escalated numbers of severe clinical cases have been reported in recent years and rapid northward spread has generated awareness of this enzootic disease (Otto, 1972). In northcentral Oklahoma the prevalence of dog heartworm has been increasing steadily since it was first reported in Oklahoma dogs in the mid-1960's (Jordan, 1982).

The Research Problem

Within recent years heartworm disease has become endemic in parts of Oklahoma where the problem did not exist previously in native dogs. It is known, for example, that only "imported" dogs were treated for

Dirofilaria immitis infections at the Oklahoma State University Veterinary Medical Teaching Hospital for many years and not until the mid-1960's did local dogs begin to suffer from the infection. The problem has grown in importance to the point that dogs in Stillwater, Oklahoma are now routinely maintained on preventive therapy throughout the year. No one has determined which mosquitoes are vectors nor when they are capable of transmitting the parasite. Until such information is available, inadequate scientific basis will exist to determine appropriate periods of prophylactic treatment. Good veterinary medical practice demands that overtreatment be avoided. This is especially true in view of the fact that adverse side effects such as male sterility and resistance of nematodes to drugs may develop after prolonged treatment. In a more general sense, it is important to study vectors in a geographic location where the parasite has recently become endemic. It is recognized by parasitologists that the difficulty of detecting the important vectors of *D. immitis* is compounded by the occurrence of what has been called "genetically controlled geographic populations" with different potential for transmission, even in the same mosquito species.

The objectives for investigation were:

1) To determine what mosquito species is(are) serving as vectors for *Dirofilaria immitis* in Stillwater, Oklahoma.
2) To determine the potential for transmission by the vectors based on observations for one season.

CHAPTER II

LITERATURE REVIEW

Classification and Evolution of *Dirofilaria immitis* (Leidy, 1856)

Newmann and Maqueen (1905) reported that *Dirofilaria immitis* was first observed in the blood of dogs by Panthot in 1679. In 1850 Leidy described the adults and assigned the name *Filaria Canis cardis*. His description was made from two worms in the collection of the Philadelphia Academy of Natural Sciences. They apparently were collected by one Dr. Coates who obtained them, according to the label, from the heart of a dog. Leidy (1850) described the worms as:

> . . . body white, opaque, linear, nearly uniform throughout, posteriorly subulate, pointed; mouth simple, rounded. Length 10 - 12½ inches, greatest breadth 2-5th of a line, anteriorly 1-5 of a line; halve an inch from posterior end, 1-10th of a line (p. 117).

Leidy (1856a) dissected the heart of a dog in which the right ventricle and the pulmonary artery and its branches were completely filled with worms. Earlier, microfilariae had been observed in the blood and called haematozoa. Leidy (1856b) redescribed the parasite from specimens provided by a Mr. Joseph Jones, and assigned the name *Filaria immitis*. His descriptions of the worms were:

> . . . body cylindrical, obtusely rounded at the extremities. Mouth small, round, unarmed. Caudal

> extremity of male spiral, with a row of five tubercles and a narrow ala upon each side. Penis protruding a short distance above the anus. Length of female to 10 inches, breadth to ½ a line; length of male to 5 inches, breadth ¼ of a line (p. 58).

A new genus, Dirofilaria, was created by Raillet and Henry (1911) and D. immitis became the type species.

According to Hawking and Worms (1961) two main characteristics of filariid worms are production of larvae viviparously by the females and ingestion of the larvae by arthropods. These nematodes undergo metamorphosis and molt twice in the anthropod vector and infective larvae are introduced into the definitive host when female mosquitoes are taking a blood meal.

In discussing the evolution and life cycle of members of the family Dipetalomatidae of which D. immitis is a member, Anderson (1957) posited that members of the Filarioidea and Spiruroidea evolved from a common ancestor with a life cycle resembling those of modern-day Thelazia rhodensii, T. skrjabini and T. gulosa. This postulated ancestor is believed to have inhabited the orbital area of the definitive host where larvae were picked up by arthropods feeding on lachrymal fluids and then transmitted to the eyes of other hosts. Next in the evolutionary process the adults are thought to have become established in the subcutaneous tissue but to have returned to the eye to deposit their larvae. Later the adult worms probably evolved to acquire the ability to enter lesions to deposit their larvae, thus making them accessible to haematophagous arthropods. With this development, larvae could accumulate in subcutaneous tissue, thus restricting transmission to those arthropods capable of piercing the skin. Finally, Anderson (1957) hypothesized that larvae went into the circulatory system allowing

adults to migrate deeper into the host tissue. *Dirofilaria immitis* has evolved with the ability to penetrate blood vessels and travel to the heart where they develop to maturity.

Life Cycle and Vector Determination

Fleas As Vectors

Breinl (1921) hypothesized that *D. immitis* could probably develop in fleas as well as in mosquitoes. His hypothesis was based on the fact that *D. immitis* is an euryxenous parasite in its culicine intermediate hosts unlike the malaria parasite which is stenoxenous and development in the invertebrate host is restricted to *Anopheles* mosquitoes. Breinl collected and dissected fleas from a dog with microfilariae circulating in its peripheral blood stream and noted developmental stages of a nematode which was assumed to be *D. immitis*. Complete development was observed in *Ctenocephalides canis* and *C. felis*. Brown and Sheldon (1940) also reported that 28 fleas were collected from an adult female bulldog with a low level of circulating microfilariae. Twelve of the 26 fleas dissected harbored various intermediate stages of parasites thought to be *D. immitis*; this was interpreted to represent a complete developmental cycle in the insect. The authors concluded that *C. canis* and *C. felis* were vectors of *D. immitis*.

Similar studies were done by Summers (1940; 1942). Summers (1940) collected fleas from a clean dog and then transferred them to a dog with circulating microfilariae. Complete development was noticed in *C. canis*, *C. felis* and *Pulex irritans*. Steuben (1954) completed the

most extensive study using fleas as vectors of *D. immitis*. He found what he thought to be third-stage larvae of *D. immitis* in 446 of 1,203 *C. felis* collected from 21 dogs and also reported that *C. canis*, *C. felis*, *Xenopsylla cheopis*, *Pulex irritans*, *Echidnophaga gallinacea* and *Orchopeas wickhami* supported development of *D. immitis* to the "infective stage." Ironically, none of these studies (Breinl, 1921; Brown and Sheldon, 1940; Summers, 1940, 1942; Steuben, 1954) verified the presence of *D. immitis* by identification of mature heartworms in dogs used to infect fleas. Failure to give attention to this important detail led to the eventual rejection of these studies.

Mosquitoes As Vectors

Manson's discovery in 1878 that *Wuchereria bancrofti* developed in mosquitoes stimulated other researchers to an awareness that other filariids might be transmitted by mosquitoes. Grassi and Noe (1900) experimentally studied the development of *Dirofilaria immitis* in *Anopheles claviger*. They observed that microfilariae picked up during a blood meal migrate to malpighian tubules where they develop for a while, then enter the lumen of the tubules to continue their development. Following metamorphosis and two molts infective stages, leaving their casted sheaths behind, migrate to the proboscis or labia where they are introduced to a dog through a punctured wound produced by the feeding mosquito (Grassi and Noe, 1900). Grassi had noticed previously that the geographical distribution of *D. immitis* was similar to that of malaria. Following recognition that *Anopheles* was involved in spread of malaria, Grassi and Noe (1900) postulated that mosquitoes might be vectors of heartworms.

Their work confirmed mosquitoes' potential for doing so.

Grassi and Noe's study was further substantiated by Bancroft (1904). In his study Bancroft exposed a clean dog to 183 experimentally infected mosquitoes (Culex fatigans). Of these, 70 were engorged after the exposure, suggesting that they could have transmitted the filariids to the dog. Nine months later the dog, which had been protected against additional exposure, was killed and 32 adult worms, 16 males and 16 females, were recovered from the right ventricle and pulmonary arteries (Bancroft, 1904).

Since mosquitoes were first found to transmit D. immitis, numerous species of culiciids have been reported to serve as potential vectors of dog heartworm. Most studies of D. immitis vectors have been conducted under experimental conditions and techniques have varied widely. One technique commonly used involves exposing laboratory-reared mosquitoes to a dirofilaremic dog and then studying development of larvae in the arthropods. Another technique that has been used frequently to investigate vector potential involves use of a modified Magoon trap developed by Magoon (1935); an infected dog is placed in a cage as bait to attract feral mosquitoes seeking a blood meal. With this type of trap mosquitoes can fly in freely to feed on the infected dog but are unable to escape. Mosquitoes collected in this way are usually held in the laboratory under experimental conditions for 17-20 days to allow development of larvae to the infective stage.

In investigation of vector potential under natural conditions, mosquitoes are collected with light traps; these are usually baited with CO_2. Mosquitoes captured in this manner are speciated, dissected and examined for the presence of D. immitis larvae. Bemrick and

Sandholm (1966) and Ludlam et al. (1970) have summarized mosquito species that are known to support development of D. immitis. This summary, updated to include studies published from 1970 to 1984, follows (Table I).

Of about 75 species of mosquitoes that have been reported as potential vectors of D. immitis world-wide, including 42 in North America, only twelve have been found naturally infected with third-stage larvae. In many instances identification of the nematodes could not be confirmed beyond superfamily but were assumed to be D. immitis because the mosquitoes were trapped in highly endemic areas. In North America, the following ten species have been captured naturally infected with nematodes thought to be third-stage larvae of D. immitis: Aedes canadensis, Ae. stimulans, Ae. excrucians, Ae. vexans, Ae. trivittatus, Ae. sticticus, Anopheles punctipennis, An. quadrimaculatus, Culex pipiens quinquefasciatus and Psorophora ferox.

Arnott and Edman (1978), in their study in western Massachusetts, used Centers for Disease Control (CDC) light traps baited with CO_2 to trap 3,445 female mosquitoes representing 23 species; the mosquitoes were held in the laboratory for 10 days in order to allow any microfilariae in the trapped mosquitoes to develop to third-stage. Aedes canadensis and Ae. excrucians were found naturally infected with third-stage larvae and were incriminated as potential vectors in the area. In Connecticut, Magnarelli (1978) used a human bait to attract and capture 3,294 mosquitoes. Third-stage larvae were found in Ae. canadensis, Ae. excrucians, Ae. stimulans and Ps. ferox. Villavaso and Steelman (1970) in Louisiana used dog-baited traps to capture feral mosquitoes. Culex pipiens quinquefasciatus accounted

TABLE I

SUMMARY OF MOSQUITO SPECIES KNOWN TO SUPPORT DEVELOPMENT OF *DIROFILARIA IMMITIS* TO THIRD-STAGE LARVAE

Mosquito Species	E•I	N•I	Geographic Location	References
Aedes aegypti	X		Europe	Bancroft (1901)
		X	Africa	Nelson et al. (1959)
	X		U.S.	Hu (1931)
Ae. albopictus	X		Japan	Inoue (1970)
Ae. atropalpus	X		U.S.	Keegan et al. (1968)
Ae. canadensis	X		U.S.	Hu (1931)
		X	U.S.	Magnarelli (1978)
		X	U.S.	Crans and Feldauter (1974)
	X		U.S.	Bickley et al. (1976)
Ae. caspius	X		Europe	Grassi and Noe (1900)
Ae. campestus	X		Canada	Frimeth and Arai (1983)
Ae. cinereus	X		U.S.	Yen (1938)
Ae. cataphylla	X		Canada	Frimeth and Arai (1983)
Ae. dorsalis	X		U.S.	Weinmann and Garcia (1974)
Ae. flavescens	X		Canada	Frimeth and Arai (1983)
Ae. edgari	X		U.S.	Rosen (1954)
Ae. excrucians	X		U.S.	Phillips (1939)
		X	U.S.	Magnarelli (1978)
		X	U.S.	Arnott and Edman (1978)
Ae. fijiensis	X	X	Fiji	Symes (1960)
Ae. fitchii	X		U.S.	Bemrick and Sandholm (1966)
Ae. geniculatus	X		Europe	Rouband and Collas-Belcour (1937)
Ae. guamensis	X		Guam	Travis (1947)
Ae. hendersoni	X		U.S.	Rogers and Newson (1979)
Ae. infirmatus	X		U.S.	Summers (1942)
Ae. coreicus	X		Korea	Feng (1930)
Ae. notoscriptus	X		Australia	Bemrick and Moorehouse (1968)
Ae. pandani	X		Guam	Travis (1947)
Ae. pempaesis	X	X	Philippines	Estrada (1965)
Ae. polynesiensis	X	X	French Oceana	Rosen (1954)
		X	Fiji	Ramalingam (1968)
		X	Samoa	Symes (1960)
Ae. pseudoscutellaris	X	X	Fiji	Symes (1960)
Ae. punctor	X	X	Europe	Rouband and Collas-Belcour (1937)
Ae. samoanus		X	Samoa	Ramalingam (1968)
Ae. sierrensis	X		U.S.	Weinmann and Garcia (1974)
	X		U.S.	Acevedo (1982)
Ae. sollicitans	X		U.S.	Hu (1931)
Ae. sticticus	X		U.S.	Bemrick and Sandholm (1966)
Ae. stimulans	X		U.S.	Yen (1938)
Ae. taeniorhynchus	X		U.S.	Hu (1931)
Ae. togoi	X		Japan	Inoue (1937)
Ae. triseriatus	X		U.S.	Phillips (1939)
	X		U.S.	Rogers and Newson (1979)
Ae. trivittatus	X	X	U.S.	Christensen and Andrews (1976)
Ae. vexans	X		U.S.	Hu (1931)
		X	U.S.	Hendrix et al. (1980)
		X	U.S.	Buxton and Mullen (1981)
		X	U.S.	Pinger (1982)
	X		U.S.	Todaro et al. (1977)
Ae. vigilax	X		Australia	Bemrick and Moorehouse (1968)
Ae. zoosophus	X		U.S.	Keegan et al. (1968)

TABLE I (Continued)

Mosquito Species	E•I	N•I	Geographic Location	References
Armegeres subylbatus		X	Asia	Cheong et al. (1981)
Anopheles crucians	X		U.S.	Keegan et al. (1968)
An. earlei	X		U.S.	Yen (1938)
An. franciscoi		X	Philippines	Cabrera (1968)
An. freeborni	X		U.S.	Kartman (1953a)
An. hyrcanopseudopictus	X		Europe	Grassi and Noe (1900)
An. maculipennis	X		Europe	Grassi and Noe (1900)
An. minimus-flavirostus		X	Philippines	Cabrera (1968)
An. plumbeus	X		Europe	Rouband and Collas-Belcour (1937)
An. punctipennis	X		U.S.	Hu (1931)
		X	U.S.	Tolbert and Johnson (1982)
		X	U.S.	Courtney and Christensen (1983)
		X	U.S.	Buxton and Mullen (1981)
An. quandrimaculatus	X		U.S.	Phillips (1939)
An. sinensis	X		China	Feng (1930)
An. superpictus	X		U.S.	Rosario (1936)
An. tenebrosus		X	Africa	Mosha Magayuka (1979)
		X	Africa	East Africa Inst. Mal. and Vector Borne Dis. (1984)
An. walkeri	X		U.S.	Bemrick and Moorehouse (1968)
Coquillettidia perturbans	X		U.S.	Bemrick and Sandholm (1966)
Culex annilirostris	X	X	Guam	Travis (1947)
		X	French Oceana	Symes (1960)
Cx. gelidus	X		Philippines	Estrada (1965)
Cx. incidens	X		U.S.	Acevedo (1982)
Cx. nigripalpus	X		U.S.	Mayan and Saverman (1975)
Cx. particeps	X		U.S.	Acevedo (1982)
Cx. pipiens	X		U.S.	Hu (1938)
Cx. pipiens pipiens	X		England	Webber and Hawking (1955)
Cx. pipiens pallens	X		Japan	Inoue (1937)
Cx. pipiens quinque fasciatus	X		Europe	Bancroft (1901)
		X	U.S.	Villavaso and Steelman (1970)
	X	X	Fiji	Symes (1960)
Cx. restuans	X		U.S.	Bemrick and Sandholm (1966)
Cx. salinarius	X		U.S.	Seeley and Bickley (1974)
Cx. sitiens	X		U.S.	Travis (1947)
Cx. tarsalis	X		U.S.	Yen (1938)
	X		U.S.	Yen (1938)
Cx. territans	X		U.S.	Hu (1931)
Cx. tritaeniorhynchus	X		Japan	Inoue (1970)
Cx. tritaeniorhynchus summorosus	X	X	Philippines	Estrada (1965)
Mansonia annulata		X	Malaya	Wharton (1962)
M. africanus		X	Africa	Mosha and Magayuka (1979)
M. bonneae		X	Malaya	Wharton (1962)
M. dives		X	Malaya	Poynton and Hodkins (1938)
M. indiana		X	Malaya	Poynton and Hodkins (1938)
M. tittillans	X		Argentina	Bacigatupo as cited by Ludlam et al. (1970)
M. uniformis	X		Singapore	Estrada (1965)
Psorophora ferox	X		U.S.	Steuben (1954)
		X	U.S.	Magnarelli (1978)

E•I = Experimental infection.
N•I = Natural infection.

for 91% of 11,248 mosquitoes trapped over two mosquito seasons. About 1.5% of the captured specimens of this species were infected with D. immitis larvae but the authors did not specify the stages of parasites recovered. In Tehama County, California, Walters and Lavoipierre (1982) captured 256 Ae. vexans with light traps baited with CO_2 and found that eight (3.1%) were infected with D. immitis; one harbored third-stage larvae. Tolbert and Johnson (1982) reported a study in Macon County, Alabama in which 2,549 wild mosquitoes were trapped with CDC light traps baited with CO_2. The traps were set in residential areas with known infected dogs. Of 326 An. punctipennis captured, 34 were positive for third-stage larvae, and of 84 Ae. vexans, six were positive for third-stage; and 14 of 520 Culex quinquefasciatus were positive for second-stage larvae, but none harbored third-stage larvae. Infective larvae were recovered from mosquitoes captured from May to September; An. punctipennis and Ae. vexans were incriminated as potential vectors of D. immitis in their study area.

In a study conducted in central Iowa and reported by Christensen and Andrews (1976), CDC light traps baited with CO_2 were used to trap mosquitoes in residential areas of Ames, Iowa in July and August. Mosquitoes captured were speciated; some were dissected individually and others in pools of 25, aggregated according to species. Aedes trivittatus comprised 60% of the mosquitoes captured and third-stage larvae of D. immitis were recovered from 17 Ae. trivittatus dissected individually and from seven of 31 pools (911 mosquitoes). Aedes trivittatus was incriminated as a potential vector in the area. Recently in Kentucky, Courtney and Christensen (1983) reported recovery of second-stage filariid larvae, assumed to be D. immitis,

in An. punctipennis.

According to Ludlam et al. (1970), the mere finding of third-stage larvae or any developmental stage in a given species of mosquito does not mean that the larvae would be transmitted to a dog or that they would develop to maturity in the dog. In incriminating an arthropod as vector, the population and feeding habits of suspected arthropods must be investigated (Ludlam et al., 1970). Genetic factors may also account for the ability of certain strains of a given species to serve as vectors in different geographic locations. It appears from review of literature that some species of mosquitoes may support the development of D. immitis up to the infective-stage under experimental conditions but may not be capable of serving as vectors under feral conditions.

Rejection of Fleas As Vectors

Grassi (1888) discounted fleas as vectors of heartworms. He dissected several hundred fleas from a dog that had been confirmed at necropsy to have D. immitis infection but they were all negative. Kosuge (1924), Joyeux and Sauter (1938), Phillips (1939), Tuniguchi et al. (1944) and Rosen (1954) also discounted fleas as vectors of D. immitis. Grassi and Calandruccio (1890) described the developmental stage of another dog filariid Filaria recondita (Dipetalonema reconditum) in the hemocoele of fleas. It appears that many workers such as Breinl (1921), Brown and Sheldon (1940), and Summers (1940) who reported fleas as vectors of D. immitis were not aware of Grassi and Calandruccio's study or failed to consider that other filariids might be present concurrently with D. immitis. Although Steuben (1954) cited Grassi

and Calandruccio's study, he may not have been fully cognizant of its implications. Rosen (1954) dissected fleas from dogs that were shown at autopsy to harbor D. immitis and did not find development beyond the sausage stage. Because of confusing reports about the ability of D. immitis to develop in fleas, Rosen suggested that another filariid, possibly Dip. reconditum, was present in areas where complete development was reported to occur in fleas. Newton and Wright (1956) who were also aware that "several species of dog filariids have been described in other parts of the world" undertook a study of microfilarial morphology. Their important work led eventually to recognition that Dip. reconditum as well as D. immitis was present in the United States.

Development in the Mosquito

The development of D. immitis in mosquitoes has been studied extensively. Microfilariae are picked up by mosquitoes while taking blood from a microfilaremic dog. The microfilariae usually remain in the lumen of the mosquito gut for about 24 hours and then migrate to malpighian tubules where further development continues (Taylor, 1960; Intermill, 1973; Symes, 1960; Kartman, 1953a, b). According to Kershaw et al. (1955), the number of microfilariae ingested is small in relation to the amount of blood ingested. Gordon and Lumsden (1939) hypothesized that variation in microfilariae ingested at the time of feeding might be due to different concentrations of microfilariae in the blood stream. Their hypothesis was based on a study with a frog filariid, Foleyella dolichroptera. For example, a mosquito feeding in an artery would likely ingest more microfilariae than one feeding in a capillary.

Studies by Kartman (1953a, 1953b) and Christensen (1978) have also

shown that the number of microfilariae ingested by mosquitoes varies with the parasitemia level, and this also correlates with survival of mosquitoes. Kartman (1953a) exposed An. quadrimaculatus to two dogs (designated D and S) with different parasitemia levels. Dog D's dirofilaremia count ranged from 30,000 to 34,000/cm^3 of blood and dog S had a count of 16,000 to 18,000/cm^3; upon dissection 15 days later 40 mosquitoes that fed on dog S were found to have an average of 17.3 parasites each, whereas 30 females that fed on dog D averaged 99.3 filariids each. Of the total 105 females that fed on dog S originally, 50 (47.5%) survived for 15 days, whereas only 45 (5%) of 900 females that fed on dog D survived that long. The average number of third-stage larvae recovered from four groups of ten mosquitoes exposed to dog S ranged from 9.6 to 15.2 per group and 0.2 to 1.6 in three groups of ten mosquitoes. Kartman's (1953b) extensive study using the same dog ("D") with high dirofilaremia found that at 18 days post exposure, an average of 20.8 third-stage larvae were recovered from ten Aedes albopictus dissected.

According to Taylor (1960), the development of D. immitis in Ae. aegypti occurs inside the cells of the distal end of the malpighian tubules. Development occurs within the cells of the tubules for the first 6-7 days. Following this intracellular phase, they migrate to the lumen of the tubules for further development. The first-stage larva is similar to the microfilaria picked up from the blood. It is filiform in shape; within the next 6-8 days there is a marked development; changes occur especially in shape and in development of organs as the larva metamorphoses to the "sausage" stage. This stage, as the name implies, is sausage-shaped and most of the organs are developed. The

reduction in length and change from filiform to sausage stage is believed to be necessary for better body surface/volume ratio (Taylor, 1960). During this stage there is a pointed tail which persists until the parasite molts to second-stage. There are two molts during the course of development in the mosquito. The first molt occurs about the tenth day (9-13). In this second-stage larva, there is elaboration of organ systems. The gut is present but does not open to the outside; the anus is closed by a structure called the anal plug which disappears during the second molt. The second-stage larva is filiform in shape and this transformation from sausage-shape back to filiform is thought to be important for migration. The second molt occurs 13-17 days after the blood meal. All the organs are developed and functional, and the gut is open from stoma to the anus (Taylor, 1960). The esophagus and the nerve ring are developed. The filiform shape enables migration to the proboscis and escape to infect a susceptible vertebrate host. Burton (1963) has reported observing infective larvae of *D. immitis* and *Wuchereria bancrofti* emerge from the antennae and palpi of *Ae. taeniorrhynchus* and *Cx. pipiens quinquefasciatus*. McGreevy and Theis et al. (1974) also observed emergence of infective *D. immitis* larvae from the mouth parts of *Ae. aegypti*. This suggests that infective larvae must migrate to the proboscis before they can be transmitted to the definitive host.

Development in the Dog

Infective larvae escape from the intermediate host's proboscis to enter the vertebrate host during feeding. Entry into the tissue is possible only through broken skin, such as through a lesion produced

when the mosquito feeds. In the definitive host the larva molts two more times before maturity. In the early phase of infection, developing worms are found in submuscular membranes, subcutaneous tissue, adipose tissue, subserosa and muscles (Kume and Itagaki, 1955; Orihel, 1961; Kotani and Powers, 1982). The third molt (the first in the vertebrate host) occurs 9-12 days after exposure (Orihel, 1961). However, in _in vitro_ studies, the third molt has been observed earlier (Sawyer, 1965; Sawyer and Weinstein, 1965; Yeoli et al., 1964). According to Sawyer and Weinstein (1965), ecdysis depends on the type of media and conditions of incubation used. The third ecdysis has been observed as early as 48 hours after exposure. Orihel (1961) indicated that the fourth molt (second in the vertebrate host) occurs 60-68 days after exposure and Kotani and Powers (1982) observed the fourth molt as early as 50 days post exposure.

According to Kotani and Powers (1982), the specific route of migration to the heart is still ill-defined. It is not likely that immature worms penetrate the thoracic and pericardial wall to reach the right ventricle of the heart. However, they hypothesized that most worms migrate anteriorly to the upper abdomen and thorax and to the head, neck and fore-limb regions. These areas contain the jugular veins and other veins carrying blood directly to the heart. Once in these areas, fifth-stage larvae could penetrate the veins and travel to the heart and pulmonary arteries. Kume and Itagaki (1955) recorded observing a worm attached to the intercoastal vein and stated that it might have been in the process of entering the vein and migrating to the heart. Immature adult worms start to arrive at the heart 70 days post exposure or 20 days after the fourth molt (Orihel, 1961; Kume and

Itagaki, 1955; Kotani and Powers, 1982). Orihel (1961) indicated that migration of immature worms to the heart is always completed by about 90 days post exposure. This observation was inconsistent with that of Kume and Itagaki (1955) and Kotani and Powers (1982) who reported finding immature adults from other locations at 95, 98 and 111 days post exposure. Sexual maturity is attained in the heart and microfilariae can be observed in the blood in about six months (Kotani and Powers, 1982).

Microfilarial Periodicity

Fluctuations in the number of circulating microfilariae in peripheral blood of dogs infected with Dirofilaria immitis have been reported from different parts of the world (Hinman, 1935; Hawking, 1956; Hawking, 1962; Tongson and Romero, 1962; Ansari, 1970; Kume, 1974; Sawyer, 1974). The term periodicity was first applied to the nocturnal abundance of the microfilariae of Wuchereria bancrofti and their reduction or disappearance from peripheral circulation during the day. Ansari (1970) suggested that periodicity in microfilariae could be due to synchronized parturition in all adult worms; he postulated that this parturition takes place every 24 hours.

According to Ansari's speculation, nightly broods are destroyed during the following day by the action of macrophages. Sawyer's (1974) observation did not agree with Ansari's theory in that dogs infected with D. immitis in which the adult worms had been killed (or clean dogs transfused) with blood containing high concentration of D. immitis microfilariae showed periodicity patterns. The feeding habits of the blood-sucking intermediate host and the sleeping habits of the definitive host were thought to be important factors in microfilarial periodicity (Ansari, 1970). Ansari also indicated that there are two

phases of periodicity; in the active phase microfilariae concentrate more in small capillaries in the lungs and in the passive phase they are evenly distributed in the peripheral blood vessels. According to Hawking (1967) microfilarial periodicity might be related to the concentration of oxygen in the blood. When the oxygen concentration is low, microfilariae are stimulated to initiate undulating movement and find their way to small vessels including terminal capillaries where oxygen is abundant. Under normal oxygen concentration they circulate freely in the peripheral blood stream. Otto (1969) indicated that the spleen might play a role in filariid periodicity; large numbers are accumulated in this organ during the day time. However, an earlier study by Hawking (1967) had discredited the possibility of splenic involvement.

Seasonal fluctuations in levels of circulating microfilariae are also observed in *D. immitis* infections (Eyles, 1954; Kume, 1974; Sawyer, 1974; Hawking, 1967; Alls and Greve, 1974; Artwell and Carlisle, 1979). Higher concentrations of microfilariae were observed during the hot summer months than in colder months. Sawyer (1974) observed higher concentrations of microfilariae in a dog confined to a hot room than when the dog was moved to a room with low temperature. He thought that other factors such as relative humidity and light could be important in affecting periodicity of *D. immitis* microfilariae.

Unusual Hosts of *Dirofilaria immitis*

According to Otto (1974) the normal host of *D. immitis* is the dog, *Canis familiaris*. However, reports of finding this parasite in other mammalian hosts including man have been increasing. In these abnormal hosts worms were often not found in the heart but in unusual locations

such as the brain, lungs, muscles, etc. In many cases, the validity of identification is questionable. At least some of the reports were undoubtedly wrong, with Dracunculus insignis (not a filariid) being mistaken for D. immitis.

Besides dogs, D. immitis has been reliably reported in red foxes (Erickson, 1944; Stuht and Youatt, 1972; Schlotthauer, 1964; Monson et al., 1973); beavers (Foil and Orihel, 1975); coyotes (Gier and Ameel, 1959; Crowell et al., 1977; Graham, 1975); wolves (Coffin, 1944; Faust et al., 1941); dingoes (Otto, 1974); gibbons (Johnson et al., 1970); cats (Faries et al., 1974; Sharp, 1974; Abbott, 1966; Donahoe, 1975); seals (Medway and Wieland, 1975); and has been reported from tigers, jaguars and sea lions (Faust et al., 1941). Several human cases have been reported also (Abadie et al., 1965; Brine et al., 1971; Moorehouse et al., 1971, 1976; Feld, 1973; Martire et al., 1975). Recently D. immitis was reported in horses (Klein and Stoddard, 1977; Thurman et al, 1984). Questions arise as to whether these abnormal hosts constitute a reservoir. According to Otto (1974) canine filariasis in mammals other than the dog illustrates that man, his domesticated animals and wild animals are being constantly exposed to infection with parasites which have little capacity to complete normal development in any but one species or at most a group of closely related species. With heavy exposure in highly endemic or highly enzootic areas a few parasites will find it physiologically possible to initiate development and a few of these may even complete development and produce progeny. Only a few reports concerning D. immitis in abnormal hosts mentioned the presence of microfilariae in the blood (Forrester et al., 1973; Abbott, 1966; Greene, 1974; Faust, 1937 as quoted by Otto, 1974). Therefore,

it seems unlikely that any hosts other than dogs are important reservoirs for the dog heartworm.

Host-Parasite Interaction

Effect Upon Arthropod Host

Some filariid nematodes may be harmful to their mosquito host (Lavoipierre, 1958). There is very little specific information about the effects of filariids on mosquito functions such as fecundity and flight capability. Such information obviously will enhance our knowledge of filariasis epidemiology. Workers such as Taylor (1960), Yen (1938), Kartman (1953b) and Christensen (1978) have shown that there is histological damage associated with D. immitis infection in mosquitoes. High mortality is associated with the intracellular stage, especially when larvae disrupt cells to enter the lumen of malpighian tubules. High mortality in mosquitoes due to D. immitis has been reported by many workers in controlled studies using several different mosquito species, e.g., An. quadrimaculatus, Kartman (1953b), Kutz and Dobson (1974); Aedes polynesiensis, Rosen (1954); Ae. aegypti, Kershaw et al. (1955) and Ae. solliicitans, Beam (1966), Kartman (1953b), and Christensen (1978). All these workers have reported higher mortality rates among mosquitoes feeding on dogs with high microfilaremia than in controls that fed on dogs with low microfilaremia.

Scanty data are available on the effects of filariid nematodes on mosquito fecundity. Wulker (1964) has cited several examples of nematodes, the majority of which parasitized the hemocoele of inverte-

brate hosts, causing suppression and physical damage to the gonads. Hacker (1971) reported a reduced fecundity in Ae. aegypti infected with Plasmodium gallinaceum, a protozoan parasite which does not invade the gonads.

Javadian and Macdonald (1974) and recently, Christensen (1981) studied the effects of filariid nematodes on mosquito feduncity. Javadian and Macdonald (1974) studied Ae. aegypti infected with either Brugia pahangi or Dirofilaria repens and reported that egg production was not significantly different between control mosquitoes and those infected with B. pahangi. However, following a second blood meal (uninfective), there was a significant decrease in the quantity of eggs produced by mosquitoes infected earlier. In mosquitoes infected with D. repens there was a significant decrease in egg production after the infective and also after a subsequent uninfective blood meal. Christensen (1981) exposed Ae. trivittatus to dogs infected with D. immitis. Oviposition by engorged mosquitoes was monitored. The mean number of eggs oviposited by infected and noninfected (control) mosquitoes were essentially similar; 72.3% of exposed mosquitoes and 71.6% of the controls oviposited. The mean number of eggs oviposited was 48.9 in exposed and 52.4 in control mosquitoes. But when mosquitoes were dissected and eggs remaining in the ovaries were included, infected mosquitoes had lower egg production, 59.3 to 66.9. Infected mosquitoes that took a second blood meal from rabbits produced an average of only 32.9 eggs compared to 46.4 eggs produced by uninfected control mosquitoes that were fed on rabbits. The reduction in egg production was not significant until the level of infection reached 15 third-stage larvae per mosquito.

Despite the fact that the ability of mosquitoes to fly after taking an infective blood meal determines, in part, their vectorial capacity, very few studies have been done on this aspect of entomology (Bidlingmayer, 1970). Townson (1971) reported significantly greater numbers of nonflying individuals among those infected with *Br. pahangi* than among uninfected controls. He also reported higher parasite burdens in infected, nonflying mosquitoes than infected flying ones. A study by Hockmeyer et al. (1975), with the same mosquito species and parasite showed that infected mosquitoes flew significantly shorter distances and for markedly shorter periods during a 24-hour period than did uninfected mosquitoes. Total flight range and duration flown by infected mosquitoes remained relatively constant throughout the infection process, but control mosquitoes flew further and longer with increasing time after their blood meal. Dissection revealed that nonflying mosquitoes had heavier parasite burdens than did flying ones. *Brugia pahangi* develops in the flight muscles of mosquitoes. It is logical to assume that this parasite can cause histological damage in the muscle tissue and thus reduce flight capability. What effects, if any, *D. immitis* and other related filariids that develop in the malpighian tubules have on the ability of mosquitoes to fly has not been reported.

Effects of Mosquito Upon *Dirofilaria immitis*

The susceptibility of mosquitoes to *D. immitis* infection varies from one species to another, and even among strains of the same species. As indicated by Kartman (1953b), susceptibility varies from one

geographical location to another. For example, Ae. aegypti from the United States, South Africa and Anglo-Egyptian Sudan showed similar host and parasite reactions. The Hawaiian strain of the same species was susceptible but the Fugi strain was refractory to D. immitis infection.

Macdonald (1962a; 1962b; 1963) clearly proved that susceptibility of mosquitoes to Brugia malayi was an inherited character. Beginning with a population of Ae. aegypti that had a susceptibility rate of about 17-31% to B. malayi Macdonald (1962a) was able to obtain a strain with a susceptibility rate of 84.4%. This was done by familial selection through 15 generations. Cross breeding individuals from this highly susceptible strain with those from uniformly refractory strains led to information about heritability. By testing the offspring (F_1, F_2 and backcross) for susceptibility of B. malayi, he was able to show that susceptibility was controlled by a sex-linked gene and this characteristic was designated f^m for susceptibility to B. malayi. Macdonald (1963) tested refractory individuals from his highly susceptible strain and found that they were either homozygous (f^m/f^m) or heterozygous (F^m/f^m). This showed that f^m does not entirely control susceptibility. He concluded that there must also be modifying genes present, and that f^m was the major factor, but it did not entirely control susceptibility.

Macdonald and Ramachandran (1965) demonstrated that the f^m gene in Ae. aegypti also controled susceptibility to B. malayi, B. pahanga and Wuchereria bancrofti in which microfilariae develop in the flight muscles. However, this f^m gene does not influence susceptibility to D. immitis or to D. repens both of which develop in the malpighian

tubules.

According to Raghavan et al. (1967), susceptibility of Ae. aegypti to D. immitis is inheritable. McGreevy and McClelland et al. (1974), in their study through selection pressure on f^m stock of Ae. aegypti, established one substock that was susceptible to D. immitis infection and another that was refractory. By crossing these substocks and determining the susceptibility rate of the F_1 and backcross progeny, it was established that susceptibility of Ae. aegypti to D. immitis infection was controlled by a sex-linked recessive gene that is designated f^t (= filarial susceptibility malpighian tubules). In recent studies by Sulaiman and Townson (1980), genetics of susceptibility to D. immitis was studied by means of a series of crosses and backcrosses between susceptible and refractory stock of Ae. aegypti. The study confirmed that susceptibility to D. immitis infection is under the control of a single major, sex-linked gene and that susceptibility is recessive to refractoriness. The susceptibility gene was found to have a high penetrance and expressivity.

Although the mechanism of action of genes f^m and f^t is not clearly known, according to Macdonald (1976), reports from India suggest that gene f^t may influence alkaline phosphatase activity in Ae. aegypti. High levels of alkaline phosphatase activity were observed in f^t homozygotes but no significant differences were observed in alkaline phosphatase levels in susceptible and refractory Ae. aegypti.

Large numbers of microfilariae are often killed in the midgut of some mosquitoes. This phenomenon is more pronounced in certain species of mosquitoes than in others. Kartman (1953b) noticed that

microfilariae may stay alive in the midgut of Ae. aegypti and Ae. albopictus, but they are usually killed in Cx. quinquefasciatus and Cx. pipiens. Travis (1947) also observed that after two days microfilariae remaining in the midgut of Cx. species were dead but they were still alive in Ae. species. According to Kartman (1953b, 1953c), microfilariae are often killed by mosquitoes and only dead ones are digested. He thought that certain factors that are present in the salivary glands of Cx. pipiens and Cx. quinquefasciatus (not present in Ae. aegypti or Ae. albopictus) might be responsible for killing microfilariae before they were digested.

Buccopharyngeal armature, as described by Bryan et al. (1974), consists of cibarial armature, a row of well developed teeth projecting into the anterior lumen of the cibarial pump, and the pharngeal armature which is a ring of slender teeth at the posterior end of the pharynx. Bryan et al. (1974) hypothesized that possession of buccopharyngeal armature might be responsible for the destruction of microfilariae in certain mosquito species. These workers investigated the effect of the pharyngeal armature of mosquitoes on microfilariae of Brugia pahangi. They used An.gambiae and An. pahanti which have both pharyngeal and cibarial armature and Ae. aegypti and Ae. togoi, which have only a pharyngeal armature. They studied the importance of these structures in affecting parasites. Microfilariae recovered after an infective blood meal were found to have loss of motility and abraded cuticles; the proportion of damaged microfilariae was greater in Anopheles species possibly because they possess cibarial armature.

The role of the peritrophic membrane as a barrier which might prevent penetration of the midgut epithelium by parasites has been

mentioned by several workers, Orihel (1975); Esslinger (1962); Ewert (1965); Ramachandran (1966); Wharton (1962). According to Orihel (1975), after a blood meal, the midgut epithelium cells begin to lay down an amorphous layer of material which eventually solidifies to form a hard inelastic membrane separating the blood bolus from the epithelium. Filariids that develop in malpighian tubules do not penetrate the midgut; rather, they migrate to the openings of the tubules. Therefore, the peritrophic membranes may not be an effective defense mechanism against *D. immitis* or *D. repens*, both of which develop in malpighian tubules.

Another defense mechanism often observed in mosquitoes is encapsulation. Capsule formation occurs by aggregation and adhesion of hemocytes over foreign particles too large to be phagocytized by individual hemocytes (Salt, 1970; Nappi, 1975). This reaction is often accompanied by oxidation and polymerization of phenols (tyrosine and dopa) by phenoloxidases to form melanin (Nappi, 1975; Lipke, 1975). It was first thought that encapsulation only occurred around dead organisms (Hu, 1931). Later work by Kartman (1953b) and by Highby (1943) showed that encapsulation could occur around either living or dead parasites.

Geographical Distribution of *Dirofilaria immitis*

Dirofilariasis is cosmopolitan in distribution, having been reported from many countries of the world (Barriga, 1982). In the United States, according to Otto (1949, 1969, 1972, 1974; Otto and Bauman, 1959), dirofilariasis was once a disease of coastal areas, especially the

southeastern seaboard from New Jersey south along the Atlantic and Gulf coasts to Texas. Within the past decade, the infection has been recognized with increasing frequency in the Middle Atlantic states and the Midwest. In fact, *D. immitis* has been reported from all 50 states in the United States; however, actual transmission has not been reported in all the states (Otto, 1972).

The northern movement of an infection which is basically tropical and subtropical has led several workers to be concerned. According to Otto (1974) the two most common explanations for this rapid movement are: the increase in canine movement between southern enzootic areas and other areas of the country and the reduction in mosquito control because of widespread concern about pesticide safety. Another possible explanation offered by Otto is that *D. immitis* has undergone evolutionary change that led to ability to develop in mosquitoes at lower temperatures. Thus worms in infected dogs that are moved from, say, Florida to Minnesota where it is colder are thought to have undergone evolutionary adaptation permitting them to develop at lower temperatures in their mosquito hosts.

Reports of *D. immitis* in the United States are so numerous that it would not be productive to review all of them. For that reason only those that have been reported in Oklahoma will be reviewed.

In northcentral Oklahoma including the Stillwater area, Pennington et al. (1970) undertook a local survey of dogs for microfilariae in five cities (Stillwater, Enid, Ponca City, Edmond and Guthrie) during June and July of 1969 and indicated that 15% of the 100 dogs surveyed were positive for microfilariae. All proved to be *Dipetalonema reconditum* with not a single *D. immitis* observed. Kocan and Laubach

(1976) undertook a one-year survey of dogs 6 months old and older admitted to the Small Animal Clinic of Oklahoma State University Veterinary Teaching Hospital between April, 1974, and March, 1975. Of 286 blood samples tested, 13 (4.5%) were positive for D. immitis and 14 (4.9%) for Dip. reconditum; and of 150 dogs examined at necropsy 11 (7.3%) harbored adult D. immitis. A survey of veterinarians practicing in Oklahoma was conducted to determine the prevalence with which microfilaremia was detected by private practitioners. Hombs and Ewing (1980) reported a prevalence rate of 2.8% among 5,624 dogs tested within the first six months of the survey and 3.6% in 1,031 dogs in the second phase of the survey. Currently, according to Jordan (1982), the prevalence rate among dogs tested at the Oklahoma State University Veterinary Medical Teaching Hospital is about 10-13%.

Dirofilariasis: A Zoonotic Disease

Human dirofilariasis is an emerging zoonotic disease particularly in the areas where the disease is enzootic in the dog population. According to Ciferri (1982), 60 cases of human pulmonary dirofilariasis have been reported in the United States. Some workers believe that the figure is a gross underestimation. Thirteen of the cases were from Florida.

Apart from the United States, human pulmonary dirofilariasis has been reported in Japan, Yoshimura et al. (1980); Australia, Moorehouse et al. (1976), and Brine et al. (1971); Malaysia, Dissanaike et al. (1977); and Colombia, Pierson (1972).

According to Pierson (1972) infection by D. immitis in man is

characterized by the presence of solitary, well circumscribed nodules of 1-4 centimeters in the pulmonary parenchyma. These are called "coin lesions" because they are coin shaped. Usually there is only one lesion in the lung, but two cases have been reported in which there were two lesions, and one of these was bilateral (Awe et al., 1975). In these human infections there is usually a solitary dead parasite found within a branch of a pulmonary artery in the periphery of the lungs; but two cases have been reported with two worms in a single lesion. Histologically, the lesions include septal necrosis, erythrocyte accumulation and infiltration by polymorphonuclear and mononuclear cells. Early lesions are spherical, hemorrhagic infarcts which progress to caseating granulomas. In older lesions a granulomatous infiltrate encloses a caseous center and is surrounded by a fibrous capsule with histiocytes, lymphocytes, plasma cells and eosinophils on the periphery (Pierson, 1972).

The infection is asymptomatic in most cases, but in patients with clinical disease, the most common symptoms are chest pain, cough and hemoptysis (Gershwin et al., 1974). Clinical infection is uncommon in man and few cases have been reported with eosinophilia.

In human dirofilariasis the primary threat to human health does not come from the presence of D. immitis per se. Rather, it results from the drastic measures which sometimes must be taken to obtain a diagnosis. Most infarcts due to D. immitis resemble malignancy (Awe et al., 1975; Kahn et al., 1983; Norden News, 1983; Allbritton, 1982; Martire et al., 1975). Typically an infarct formed in the tissue around a pulmonary artery is detected on a routine chest roentgenograph. Because no other reliable procedures are currently available for identifying

heartworm infection in humans, diagnostic thoracotomy and lung biopsy must be performed to rule out pulmonary malignancy. Postoperative complication may result ranging from severe chest pain to death.

Besides human dirofilariasis caused by D. immitis, ocular and subcutaneous dirofilariasis of man in the United States are caused by D. tenuis, a raccoon parasite. Elsewhere, similar infections are caused by D. repens, another dog parasite. Dirofilaria immitis in man is usually in the lungs (Barriga, 1982). With dirofilariasis becoming enzootic in dog populations all over the United States, and with availability of mosquito vectors that feed indiscriminately on dogs and man, dirofilariasis may become an important zoonotic disease all over the country.

CHAPTER III

MATERIALS AND METHODS

Site Selection

Selection of a site for trapping mosquitoes was based on geographic location of known cases of heartworm disease. One particular patient (reference case) with a very complete history was diagnosed and treated at Oklahoma State University Veterinary Medicine Teaching Hospital. The dog had never travelled outside the neighborhood and was one of several known cases of heartworm disease in the immediate vicinity.

Permission was received to trap in the yard where the reference case resided and on nearby city-owned property. The area was in the north portion of Stillwater in northcentral Oklahoma, near the back-waters of Boomer Lake. The area was wooded and, being of low elevation, often had many stagnant pools of water suitable for mosquito breeding.

Trapping in a Screen Cage

A 1.8 x 1.8 x 1.8 meter (6' x 6' x 6') screen cage[1] was used to trap blood-seeking mosquitoes. A dirofilaremic dog was used as a bait, and carbon dioxide (as dry ice) was employed as an anciliary attractant. The screen cage was set up in the yard of the

[1]Purchased from Chicopee Company, Gainesville, Georgia.

owner of the reference case for trapping throughout the mosquito season from May to September, 1983. The cage was anchored to the ground with four poles and remained secured throughout the trapping season. It was equipped with a zipper on one side which facilitated placing the dog inside and made entry and collection of mosquitoes convenient. The screen came to within 30.5 cm (1 foot) of ground level which allowed mosquitoes to enter from the bottom. (After feeding mosquitoes tended to fly up and rest near the top of the cage.) Trapping was done almost daily at the beginning of the study but later was reduced to three times per week. Weather conditions influenced the schedule; the dog was not placed in the cage when rain was expected. On a few occasions the dog had to be removed when a storm developed unexpectedly, but there were never fewer than two trappings/week.

<u>Dog As A Bait</u>

A female English Setter about 6 years old with natural dirofilariasis was used in the screen cage. The dog was under the care of the Laboratory Animal Resources Unit, College of Veterinary Medicine. The microfilaremia was ascertained by Modified Knott's technique (Knott, 1930) on May 24, 1983, and was found to be about 3,716/cc of blood. Later, in July, it was rechecked and found to be about 25,000/cc of blood.

The dog was restrained in a sturdy wire cage within the screen cage. The wire cage was equipped with a sanitary pan at the bottom and the pan was cleaned after each use. The dog was provided with drinking water but was not fed during the dusk to dawn trapping period. The dog's hair was clipped regularly from the flanks to

facilitate feeding by the mosquitoes.

Collection of Mosquitoes from Screen Cage

Mosquitoes caught in the screen cage were collected at approximately 6:30 a.m. utilizing a suction device. The device was a modified household vacuum cleaner (Hoover Handivac) purchased locally. A speed-control mechanism was also purchased locally and attached to the vacuum cleaner to reduce its force; the weak vacuum was adequate to capture mosquitoes without damaging them. The vacuum cleaner hose was modified to receive an attachment piece in which the mosquitoes were collected. The attachment piece was a plastic tube about 10 cm (4 inches) long and 2.5 cm (1 inch) in diameter equipped with a screw-on lid covered with screen. The other end of the tube which fit into the appropriately-narrowed hose was covered with screen which was glued to it securely. Thus when mosquitoes were gently sucked in by the vacuum, they collected in the attachment piece. A maximum of 30-50 mosquitoes were collected in an attachment piece at a time; it was then closed securely with the screen-covered lid for transport to the laboratory.

Trapping with Light Traps

Solid State Army Miniature (S.S.A.M.) traps[2], a type of light trap, were set up in a wooded area along the shore of Boomer Lake near the home of the reference case. On some occasions the traps were set in

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[2]Purchased from John W. Hock Company, Gainesville, Florida.

shrubs in the residential area. Three traps were set most of the time, but on some occasions only two were available as a result of malfunction. The specific sites of trapping in the woods varied. Traps were always at least 46 meters (50 yards) apart to prevent repelling against each other. Light traps were set throughout the mosquito season, May to October. Carbon dioxide (as dry ice) was used as an ancillary attractant as it was in the dog-baited cage. The traps were set up from dusk to about 6:30 a.m. the following day. Trapping was done 2-3 times per week at onset and later reduced to once a week. The light trapping schedule was also influenced by the weather, and sometimes trapping was temporarily discontinued when few mosquitoes were emerging. Light trapping was resumed when mosquitoes emerged after a rainfall. As stated previously, trapping in the dog-baited screen-cage was done every week throughout the period with never fewer than two trappings per week.

Transportation of Mosquitoes to the Laboratory

Mosquitoes captured in light traps were transported to the laboratory alive in the traps in which they were caught. The flat bottom of the traps were positioned so that the nets extended, and the mosquitoes could fly freely.

Mosquitoes captured in the screen cage were transported in groups of 30-50 in the attachment pieces in which they were collected. To reduce their activity during transport to the laboratory, the attachment piece(s) with the mosquitoes inside were placed in a large styrofoam ice chest that contained frozen freezer packs. The temperature in the styrofoam chest was approximately 10°C.

Holding of Mosquitoes in Environmental Chamber

Mosquitoes collected in the dog-baited screen cage on a given day were transported to the laboratory within approximately 15 minutes. They were then held in an environmental chamber approximately 2 x 3 x 1.75 m (6' x 9 3/4' x 5 3/4'). The chamber was maintained at 25 to 27°C and 80 ± 10% relative humidity. These conditions have been shown to be suitable for laboratory-held mosquitoes and to enhance development of mcirofilariae (Taylor, 1960; Christensen, 1977a). The chamber was equipped with 2 rows of shelves on each side to accommodate cages. The chamber was well lighted and an automatic timer was set to provide 12 hours of light (8 a.m. to 8 p.m.) and 12 hours of darkness (8 p.m. to 8 a.m. the following morning).

Mosquitoes captured in the dog-baited screen cage were kept in the chamber in containers of one of two sizes depending upon the number of mosquitoes trapped on a given day. The larger containers were woodframe screen cages approximately 30.5 x 45.7 x 30.5 cm (1' x 1¼' x 1'). The bottom, back and front were made of plywood and the sides and top were covered with screen. The plywood front had an opening about 12.7 cm (5 inches) in diameter to which a cloth sleeve was glued to provide controlled access to the cage. When the cloth sleeve was loosely knotted, entrance into the cage was closed; and when untied, easy access to the enclosure for removal of dead or moribund specimens was possible without risk of escape by viable, flying mosquitoes.

The smaller cages were ice cream containers (½ U.S. gal.) and were 17.7 cm long and 12.7 cm in diameter (7" x 7"). They were modified for

use as cages by removing the bottom and replacing it with a lid from another carton. The then-removable bottom allowed easy access for collection of dead or moribund mosquitoes; viable mosquitoes tended to fly upward when containers were handled and thus escape was not usually a problem. Tops were modified by removing the central portion from the lid leaving the frame intact. The top of the container was covered with nylon net, and when the lid frame was applied it held the nylon net in place.

Mosquitoes collected in the dog-baited screen cage were held in appropriate-size cages (depending upon size of catch on a given day) within the environmental chamber for as long as they lived or up to 17-22 days. Mosquitoes were not speciated before being placed in the environmental chamber because handling would likely have caused increased mortality. They were fed _ad libitum_ during the holding period by being provided access to cotton balls soaked in 10% glucose solution. Mosquitoes that were still alive after 17-22 days in the environmental chamber were killed by freezing at -12.2^{o}C for 5 minutes, speciated and either dissected immediately or preserved for later dissection.

Speciation and Storage of Mosquitoes

Speciation of mosquitoes collected in light traps was done with the aid of a dissecting microscope at 10X magnification. When large numbers were captured, they were killed by freezing at -12.2^{o}C for 5 minutes and examined in small groups in a petri dish. Each mosquito was identified to species by using the mosquito key written by Stojanovich (1961) and a more recent one by Darsie and Ward (1981).

When large numbers of mosquitoes were captured, immediate dissection was impracticable. Thus mosquitoes were stored in 10 cc screw-cap vials in either 70% alcohol or an aqueous solution containing 1% Tween 80 and 10% glycerol (Bemrick and Sandholm, 1966).

When only few mosquitoes were collected in the light traps on a given day, they were anaesthetized on an anaesthetizing table and speciated. Mosquitoes speciated on the anaesthetizing table were dissected immediately. Dead or moribund mosquitoes removed from the cages in the environmental chamber were speciated under a dissecting microscope in the manner described above. Mosquitoes that could not be dissected immediately were stored in the aqueous freezing solution in groups of up to 7/vial. No more than seven were stored in one vial because, upon thawing, dissection had to be completed in a rather short time.

Anaesthetizing Table Used to Immobilize the Mosquitoes

An anaesthetizing table was used to reduce mosquitoes' activity during speciation and was used only when few mosquitoes were collected in light traps on a given day. The table was constructed by cutting a 3.6 liter (1 U.S. gal.) ice cream carton into two equal parts. The bottom piece was filled with sponge. The top was covered with two sheets of paper towels which were secured around the carton with tape. A hole was made on the side of the carton through which a tube carrying CO_2 gas from a tank was inserted. Perforations about the size of a pin were made in the paper towels to allow CO_2 gas to flow through to the

top. Mosquitoes speciated on the anaesthetizing table were dissected immediately.

Dissection of Mosquitoes

The dissection technique described by Ungureanu (1972) was used. Mosquitoes were dissected in either normal saline or Tween 80 solution using watch-maker's forcepts, micropin teasors and a dissecting microscope with 10X, 12X, 16X and 18X magnifications as necessary. The wings and legs were removed first and discarded. The body was separated into its three major parts; the abdomen, thorax and head were dissected separately on the same slide, each in a separate drop of fluid. After the three body parts were dissected thoroughly in the fluid, a separate coverslip was applied to each making certain that fluid did not flow from one coverslip to another. The forcepts and teasors were wiped clean with paper towels after each body part had been dissected, thus reducing the chance of contamination. A compound microscope was used to examine the entire area of all three coverslips for the presence of nematodes. Examination was made at 40X, 100X and, when necessary, at 400X to identify the developmental stages of the filariids observed. A mechanical counter was used to enumerate larvae of each stage present in each body section of the mosquito. All mosquitoes (5,810) collected in the dog-baited screen cage were dissected. Up to 30 of each species captured on a given day in the light traps were dissected. When more than 30 specimens of a particular species were captured on a given day, the 30 to be dissected were picked randomly. About 2,500 mosquitoes were dissected from those captured in the light traps.

Data Entry and Analysis

A separate data sheet was used to record information regarding each mosquito dissected (see Appendix A). Information recorded included species of mosquitoes, method of capture, date of collection, date of death (and whether killed or not) and presence or absence of nematodes. In mosquitoes positive for microfilariae or other stages, the number and locations (abdomen, thorax, head, proboscis) of the larval stages were recorded. The different morphologic forms were coded and data were prepared for analysis by the university main frame computer.

In mosquitoes captured in light traps, infectivity rates for naturally-occurring filiariasis in each month were determined. Rates in different species were compared. The period of communicability for each species was determined by observation of the seasonal abundance of those species that supported development of *Dirofilaria immitis* up to the infective stage.

CHAPTER IV

RESULTS

Mosquito Population

There were 23 species of mosquitoes representing eight genera trapped during the trapping season, May to October 1983. A summary of species and numbers of mosquitoes captured by the two trapping methods is presented in Table II. The average numbers of mosquitoes trapped weekly in the dog-baited screen cage and in light traps are shown in Tables III and IV, respectively. *Aedes canadensis*, *Ae. epactius* and *Ae. zoosophus* were trapped only in the dog-baited screen cage and *Uranotaenia sapphirina* was captured only in the light traps.

Aedes trivittatus and *Ae. vexans* were the most abundant species trapped by both trapping methods (Table II). The two species together constituted about 73% of mosquitoes trapped; *Ae. trivittatus* alone accounted for 50% and *Ae. vexans* for 23%. *Anopheles punctipennis* and *An. quadrimaculatus* were trapped in small numbers. *Culex salinarius* and *Cx. erraticus* were trapped in fairly large numbers, but *Cx. tarsalis* and *Cx. pipiens* were trapped in small numbers.

Over 7,000 specimens of *Psorophora* were captured. Of these, *Ps. horrida*, *Ps. ferox*, *Ps. longipalpus*, *Ps. cyanescens* and *Ps. mathesoni* were trapped in fairly large numbers, but *Ps. ciliata* and

TABLE II

NUMBERS OF VARIOUS MOSQUITO SPECIES CAPTURED IN LIGHT TRAPS AND IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG DURING THE TRAPPING SEASON, MAY-OCTOBER, 1983

Mosquito Species	Light Trap	Screen Cage	Total	
			Number	Percent
Aedes canadensis	0	1	1	<1
Ae. epactius	0	1	1	<1
Ae. nigromaculis	4	21	25	<1
Ae. trivittatus	16,915	2,855	19,770	50
Ae. vexans	7,734	1,173	8,907	23
Ae. zoosophus	0	1	1	<1
Anopheles punctipennis	107	14	121	<1
An. quadrimaculatus	113	274	387	1
Coquillettia perturbans	8	1	9	<1
Culex erraticus	627	387	1,014	3
Cx. pipiens	49	10	59	<1
Cx. salinarius	788	989	1,777	5
Cx. tarsalis	38	23	61	1
Culiseta inornata	61	8	69	<1
Othopodomyia signifera	2	1	3	<1
Psorophora ciliata	53	18	71	<1
Ps. columbiae	5	26	31	<1
Ps. cyanescens	495	2	497	1
Ps. ferox	1,457	1	1,458	4
Ps. horrida	2,035	2	2,037	5
Ps. longipalpus	1,090	1	1,091	3
Ps. mathesoni	1,892	1	1,893	5
Uranotaenia sapphirina	4	0	4	<1
Total	33,477	5,810	39,257	(100)

TABLE III

AVERAGE* NUMBER OF MOSQUITOES CAPTURED IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG DURING THE TRAPPING SEASON, MAY TO SEPTEMBER, 1983

Mosquito Species	5/23-5/29	5/30-6/5	6/6-6/12	6/13-6/19	6/20-6/26	6/27-7/3	7/4-7/10	7/11-7/17	7/18-7/24
Aedes canadensis	0	0	1	0	0	0	0	0	0
Ae. epactius	0	0	1	0	0	0	0	0	0
Ae. nigromaculis	2	2	1	0	0	0	0	0	0
Ae. trivittatus	7	73	143	195	177	17	59	81	15
Ae. vexans	35	6	16	42	49	7	5	21	12
Ae. zoosophus	0	0	0	1	0	0	0	0	0
Anopheles punctipennis	1	1	0	1	1	1	1	1	0
An. quadrimaculatus	0	0	0	0	0	1	1	1	5
Coquillettidia perturbans	0	0	0	0	0	0	0	0	0
Culex erraticus	0	0	0	0	0	0	0	0	2
Cx. pipiens	1	0	0	0	0	0	0	0	0
Cx. salinarius	39	6	2	8	6	2	7	17	46
Cx. tarsalis	1	0	1	0	1	0	1	0	1
Culiseta inornata	1	0	0	0	0	0	0	1	1
Othopodomyia signifera	0	0	1	0	0	0	0	0	0
Psorophora ciliata	0	0	0	0	0	0	0	2	2
Ps. columbiae	0	0	0	0	0	1	2	4	1
Ps cyanescens	1	0	1	0	0	0	0	0	0
Ps. ferox	0	0	0	0	1	0	0	0	0
Ps. horrida	0	0	0	0	1	0	0	0	0
Ps longipalpus	0	0	0	0	1	0	0	1	0
Ps mathesoni	0	1	0	0	0	0	1	0	0

TABLE III (Continued)

Mosquito Species	7/25-7/31	8/1-8/7	8/8-8/14	8/15-8/21	8/22-8/28	8/29-9/4	9/5-9/11	9/12-9/18	9/19-9/25
Aedes canadensis	0	0	0	0	0	0	0	0	0
Ae. epactius	0	0	0	0	0	0	0	0	0
Ae. nigromaculis	0	0	0	0	0	0	0	0	0
Ae. trivittatus	1	0	1	0	0	0	0	0	0
Ae. vexans	5	5	2	0	1	1	1	3	0
Ae. zoosophus	0	0	0	0	0	0	0	0	0
Anopheles punctipennis	0	0	0	0	0	0	0	0	0
An. quadrimaculatus	4	16	15	4	5	4	1	1	0
Coquillettidia perturbans	0	0	1	0	0	0	0	0	0
Culex erraticus	7	37	20	2	11	4	1	1	0
Cx. pipiens	0	0	0	0	0	0	0	0	0
Cx. salinarius	19	22	22	4	2	4	0	0	0
Cx. tarsalis	1	1	0	1	1	0	0	0	0
Culiseta inornata	0	0	0	0	0	0	0	0	0
Othopodomyia signifera	0	0	0	0	0	0	0	0	0
Psorophora ciliata	1	0	0	0	0	1	0	0	0
Ps. columbiae	0	1	0	0	0	0	0	0	0
Ps. cyanescens	0	0	0	0	0	0	0	0	0
Ps. ferox	0	0	0	0	0	0	0	0	0
Ps. horrida	0	0	0	0	0	0	0	0	0
Ps longipalpus	0	0	0	0	0	0	0	0	0
Ps. mathesoni	0	0	0	0	0	0	0	0	0

*Averages were computed by dividing total number of mosquitoes captured/week by the number of trappings/week; numbers rounded up to next whole.

TABLE IV

AVERAGE NUMBER OF MOSQUITOES CAPTURED IN LIGHT TRAPS DURING THE TRAPPING SEASON, MAY TO OCTOBER, 1983

Mosquito Species	5/9-5/15	5/16-5/22	5/23-5/29	5/30-6/5	6/6-6/12	6/13-6/19	6/20-6/26	6/27-7/3	7/4-7/10	7/11-7/17	7/18-7/24	7/25-7/31
Aedes trivittatus	8	4	709	1784	3053	2774	1302	X	67	417	X	17
Ae. vexans	100	115	78	563	1174	1508	417	X	36	146	X	82
Ae. nigromaculis	0	0	1	1	0	0	0	X	0	0	X	0
Anopheles punctipennis	1	6	2	4	14	18	6	X	8	7	X	0
An. quadrimaculatus	0	0	0	0	0	1	1	X	1	20	X	9
Cutex pipiens	0	13	7	3	0	0	0	X	0	0	X	0
Cx. salinarius	4	92	34	32	66	54	21	X	14	26	X	11
Cx. tarsalis	2	6	3	2	0	0	0	X	0	0	X	0
Cx. erraticus	0	0	0	0	0	0	0	X	0	0	X	202
Culiseta inornata	2	3	0	1	3	12	3	X	3	0	X	2
Orthopodomyia signifera	0	0	0	1	0	0	0	X	0	0	X	0
Psorophora ciliata	0	0	2	6	9	6	3	X	0	5	X	0
Ps. columbiae	0	0	0	6	0	1	0	X	0	0	X	1
Ps. cyanesens	0	0	0	48	163	18	9	X	1	0	X	0
Ps. ferox	0	0	2	33	255	452	210	X	13	145	X	25
Ps. longipalpus	0	0	0	156	214	144	33	X	6	12	X	0
Ps. mathesoni	0	0	12	296	263	120	19	X	0	9	X	0
Ps. horrida	0	0	0	35	597	374	312	X	29	20	X	5
Coquilletidia perturbans	0	0	0	0	0	0	0	X	0	0	X	0
Uranotaenia sapphirina	0	0	0	0	0	0	0	X	0	0	X	0

TABLE IV (Continued)

Mosquito Species	8/1-8/7	8/8-8/14	8/15-8/21	8/22-8/28	8/29-9/4	9/5-9/12	9/13-9/25	9/26-10/2	10/3-10/9	10/10-10/16	10/17-10/23
Aedes trivittatus	X	3	X	0	X	0	X	0	0	0	0
Ae. vexans	X	52	X	46	X	19	X	49	156	128	11
Ae. nigromaculis	X	0	X	0	X	0	X	0	0	0	0
Anopheles punctipennis	X	0	X	0	X	1	X	0	0	0	0
An. quadrimaculatus	X	55	X	13	X	10	X	1	0	0	0
Cutex pipiens	X	0	X	0	X	0	X	0	0	0	0
Cx. salinarius	Y	11	X	2	X	0	X	0	1	0	0
Cx. tarsalis	X	0	X	0	X	0	X	0	0	0	0
Cx. erraticus	X	233	X	52	X	39	X	0	0	0	0
Culiseta inornata	X	0	X	0	X	0	X	0	1	13	6
Orthopodomyia signifera	X	0	X	0	X	0	X	0	0	0	0
Psorophora ciliata	X	0	X	0	X	0	X	0	0	0	0
Ps. columbiae	X	0	X	0	X	0	X	0	0	0	0
Ps. cyanesens	X	0	X	0	X	0	X	0	0	0	0
Ps. ferox	X	0	X	0	X	0	X	0	0	0	0
Ps. longipalpus	X	0	X	0	X	0	X	0	0	0	0
Ps. mathesoni	X	0	X	0	X	0	X	0	0	0	0
Ps. horrida	X	0	X	0	X	0	X	0	1	0	0
Coquilletidia perturbans	X	4	X	3	X	1	X	0	0	0	0
Uranotaenia sapphirina	X	3	X	0	X	1	X	0	0	0	0

*Averages were computed by dividing total number of mosquitoes captured/week by the number of trappings/week; numbers rounded to next whole.

X = light traps were not set.

Ps. columbiae were trapped in small numbers; in fact, only 71 specimens of Ps. ciliata and 31 of Ps. columbiae were captured with both trapping methods combined (Table II). Other species caught in small numbers included Culiseta inornata, Orthopodomyia signifera and Coquillettidia perturbans.

Figure 1 shows the distribution of eight species of mosquitoes in which third-stage larvae were recovered. Aedes trivittatus and Ae. vexans were both abundant during late May, the entire month of June and early July. Aedes trivittatus was not trapped after late July, but Ae. vexans was trapped throughout the season. Anopheles quadrimaculatus was first captured about the middle of June and then persisted in small numbers throughout the rest of the season. Culex salinarius was captured throughout the summer and Cx. tarsalis was captured from May to August. Culex erraticus was first captured in late July and was trapped throughout August and early September. Psorophora columbiae and Ps. ciliata were trapped from late May to August, being most abundant in June and July.

Daily Temperature and Precipitation

Daily temperatures (^{o}F) and precipitation records (inches) were obtained from the Oklahoma Regional Weather Survey. These data were converted to Celsius scale and to metric and are presented in Figure 2 as weekly averages of high and low temperatures and as average precipitation in mm. Rain fell at least once a week during the first 8 weeks of trapping but this period was followed by 7 weeks of no rainfall. Rainfall resumed in August and it rained during 7 of 9 weeks in the latter part of the trapping season. Temperatures were

Figure 1. Relative Abundance and Distribution of Eight Screen Cage-captured Mosquito Species in Which *Dirofilaria immitis* Third-Stage Larvae Developed.

Population levels expressed are average numbers of specimens captured per week. The longest bar (for *Aedes trivittatus* in mid-June) represents an average of 195 mosquitoes/trapping for the week. Asterisks indicate that no specimens were trapped in the screen cage that week but that the species was captured in light traps set concurrently. Question marks indicate that no specimens were trapped in the screen cage that week and that light traps were not set concurrently.

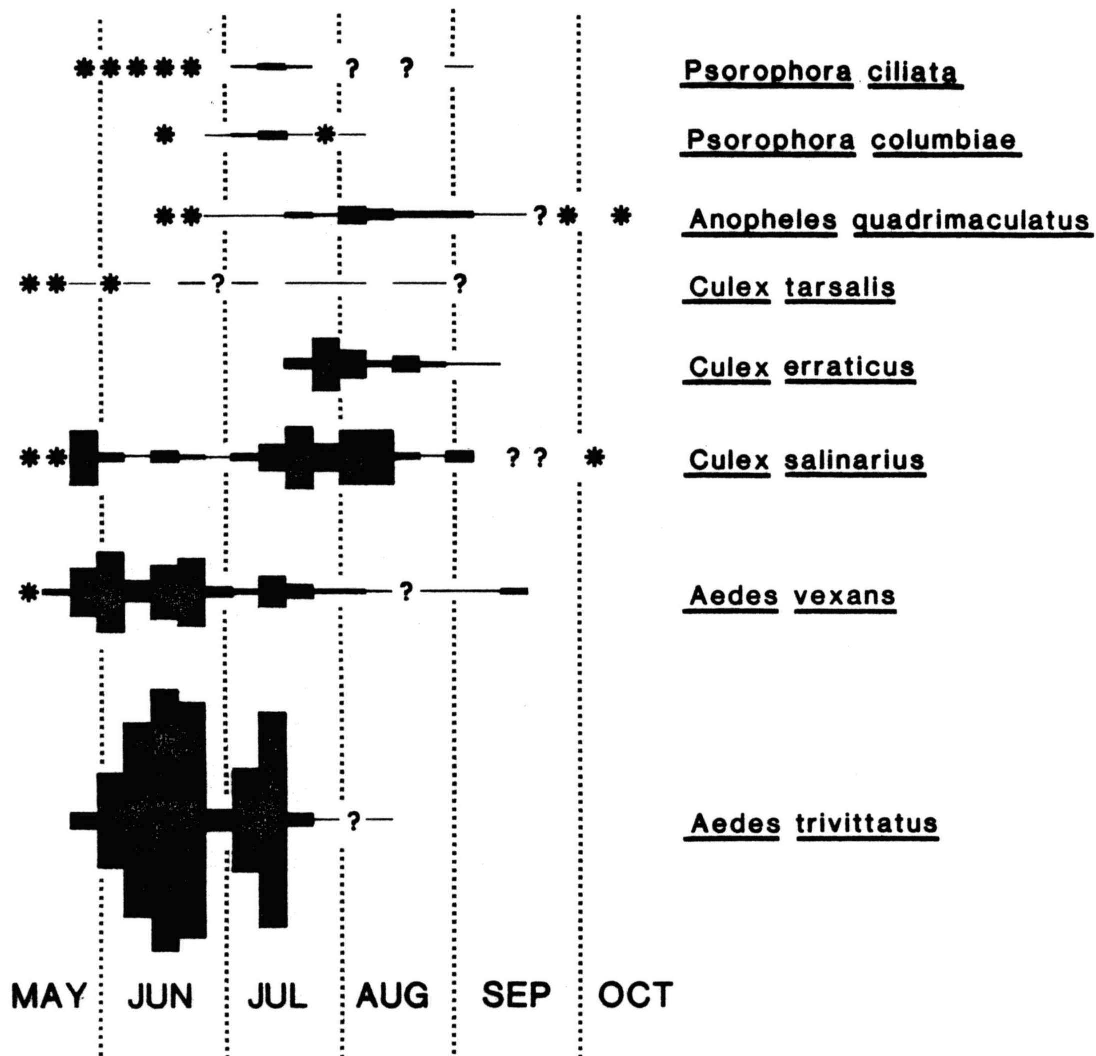
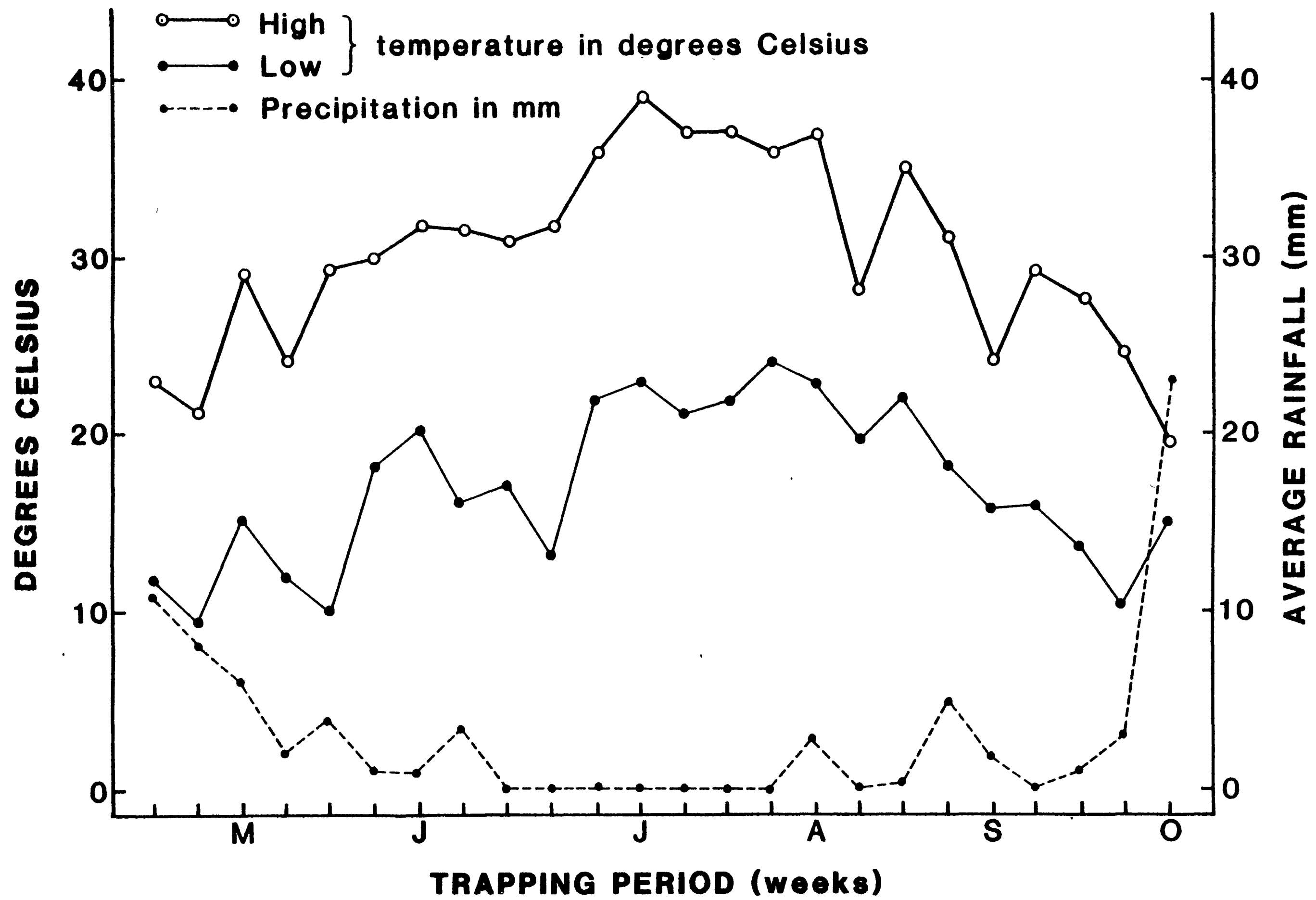

Psorophora ciliata
Psorophora columbiae
Anopheles quadrimaculatus
Culex tarsalis
Culex erraticus
Culex salinarius
Aedes vexans
Aedes trivittatus
MAY
JUN
JUL
AUG
SEP
OCT

Figure 2. Average Weekly High and Low Temperatures and Average Weekly Precipitation During Trapping Season May to October, 1983.

Temperature and precipitation data were obtained from the Oklahoma Regional Weather Survey for each day of the study period. Originally expressed in degrees Farenheit and inches, the data were converted to degrees Celsius and millimeters and weekly averages were computed.


High
Low
temperature in degrees Celsius
Precipitation in mm
DEGREES CELSIUS
AVERAGE RAINFALL (mm)
40
30
20
10
0
M
J
J
A
S
O
TRAPPING PERIOD (weeks)

generally mild in May and June with highs in the low thirties (80°F), but it was continuously hot in July and August with temperatures in the high thirties (90°F). Cooler temperatures, often accompanied by rain, were recorded in September and October; temperatures dropped to the low thirties (80°F) for a high and fifteen (50°F) for a low.

Recovery of Filariid Larvae from Mosquitoes Captured in Light Traps

About 33,450 mosquitoes representing 20 species were captured in light traps. Of these, 2,513 specimens representing 17 species were dissected. Two *Orthopodomyia signifera*, five *Psorophora columbiae* and four *Uranotaenia sapphirina* were not dissected but kept as samples. Up to 30 specimens of a given species were dissected from each trapping. When more than 30 specimens were captured on a given day, 30 were selected randomly to be dissected. Only ten specimens, representing two species, *Ae. trivittatus* and *Ae. vexans*, were found to harbor filariid larvae from among the samples of over 2,500 specimens (representing 17 species) dissected (Tables V and VI). These nematodes were assumed to be *Dirofilaria immitis* because of their morphological characteristics. Of about 16,900 *Ae. trivittatus* captured in light traps, 589 were dissected, eight were positive and one specimen contained one third-stage larva in its proboscis, and the remaining seven harbored microfilariae in their abdomens. No other stages were found in any of the eight specimens. Of 7,734 *Ae. vexans* captured in light traps, 674 were dissected. Only two of these specimens were positive; one had sausage stages in the abdomen and the other, microfilariae. *Aedes trivittatus* was captured in large

TABLE V

OCCURRENCE OF FILARIID LARVAE IN MOSQUITOES CAPTURED IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG AND IN A SAMPLE OF THOSE CAPTURED IN LIGHT TRAPS

Mosquito Species	Light Traps					Screen Cage		
	Number Trapped	Number Dissected	Percent Dissected	Number Positive	Percent Positive	Number Trapped and Dissected	Number Positive	Percent Positive
Aedes canadensis*	0	0		0		1	0	0
Ae. epactius*	0	0		0		1	0	0
Ae. nigromaculis	4	4	(100)	0		21	3	14
Ae. trivittatus	16,915	589	(3)	8	1.3+	2,855	2,404	84
Ae. vexans	7,734	674	(9)	2	0.3+	1,173	554	47
Ae. zoosophus*	0	0				1	0	0
Anopheles punctipennis	107	57	(53)	0		14	10	73
An. quadrimaculatus	113	85	(75)	0		274	250	91
Coquillettidia perturbans	8	4		0		1	0	0
Culex erraticus	627	144	(23)	0		387	29	7
Cx. pipiens	49	32	(65)	0		10	0	0
Cx. salinarius	788	358	(45)	0		989	171	17
Cx. tarsalis	38	28	(74)	0		23	9	39
Culisetta inornata	61	17	(28)	0		8	0	0
Orthopodomyia signifera	2	0		0		1	0	0
Psorophora ciliata	53	10	(19)	0		18	6	22
Ps. columbiae	5	0		0		26	13	50
Ps. cyanescens	495	142	(29)	0		2	1	50
Ps. ferox	1,457	186	(13)	0		1	1	100
Ps. horrida	2,035	109	(5)	0		2	0	0
Ps. longipalpus	1,090	127	(11)	0		1	0	0
Ps. mathesoni	1,892	138	(7)	0		1	0	0
Uranotaenia sapphirina**	4	0		0		0	0	0
Total	33,477	2,513		10		5,810	3,451	

*Not captured in light traps and not dissected.

**Not captured in screen cage.

+Percent positive of those dissected.

TABLE VI

OCCURRENCE OF FILARIID LARVAE IN SAMPLE OF *AEDES TRIVITTATUS* AND *AEDES VEXANS* CAPTURED IN LIGHT TRAPS THROUGHOUT TRAPPING SEASON

Month	*Aedes trivittatus*			*Aedes vexans*		
	Number Trapped	Number Dissected	Number Positive	Number Trapped	Number Dissected	Number Positive
May	2,163	94	0	1,171	315	1**
June	14,248	394	8*	5,834	141	1**
July	501	101	0	264	53	0
August	3	0	0	102	31	0
September	0	0	0	68	48	0
October	0	0	0	295	86	0
Total	16,915	589	8	7,734	674	2

*Of the 8 mosquitoes positive for filariid larvae, one had a third-stage larva in the proboscis; the other 7 harbored microfilariae.

**Of the 2 mosquitoes positive for filariid larvae, one had sausage stages in its abdomen, the other, microfilariae.

numbers during only three months (May, June, July); therefore, a smaller percentage of these specimens were dissected than was the case for some other species (Table VI). For example, *Ae. vexans* and *Cx. salinarius* were captured throughout the entire mosquito season, and thus a higher percentage of those trapped were dissected over the course of the summer. As stated earlier, up to 30 specimens of each species captured per trapping were dissected.

Unlike *Ae. trivittatus* and *Ae. vexans*, none of the remaining 14 species captured in light traps and dissected contained larvae. About 1,440 specimens were dissected among these species.

Recovery of Filariid Larvae from Mosquitoes Captured in Screen Cage Baited with Dirofilaremic Dog

About 5,800 mosquitoes representing 22 species were captured in the dog-baited screen cage and 19 species were dissected. Only one specimen each of *Ae. canadensis*, *Ae. epactius* and *Ae. zoosophus* were captured and they were not dissected but kept as samples. Of the 19 species dissected, microfilariae and developmental stages of *Dirofilaria immitis* were recovered from 3,440 specimens representing 13 species (Table VII). The average number of parasites/infected mosquito, that is, microfilarial, sausage-, second- and third-stage larvae, inclusive, varied among species. It ranged from one in *Ps. cyanescens*, *Ps. ferox* and *Ps. horrida* to 106 in *An. punctipennis*. The average number of parasite/infected *Ae. trivittatus* was 81 (Table VII).

TABLE VII

OCCURRENCE OF DEVELOPMENTAL STAGES OF DIROFILARIA IMMITIS IN MOSQUITOES CAPTURED IN DOG-BAITED SCREEN CAGE AND HELD IN ENVIRONMENTAL CHAMBER

Mosquito Species	Number Trapped and Dissected	Number of Mosquitoes Positive	Numbers of Various Filariid Larval Stages Recovered				Average Number of Parasites/ Positive Mosquito
			Microfilariae	Sausage	Second Stage	Third Stage	
Aedes nigromaculis	21	3	48	5	0	0	3
Ae. trivittatus	2,855	2,404	190,163	2,355	294	950	81
Ae. vexans	1,173	554	34,306	511	0	6	63
Anopheles punctipennis	14	10	1,063	0	0	0	106
An. quadrimaculatus	274	250	22,533	874	638	741	90
Culex erraticus	387	29	243	13	1	36	10
Cx. salinarius	989	171	7,331	96	0	36	44
Cx. tarsalis	23	9	80	7	0	4	10
Psorophora ciliata	18	6	1	0	0	38	6
Ps. columbiae	26	13	478	14	11	32	45
Ps. cyanescens	27	1	0	1	0	0	1
Ps. ferox	1	1	1	0	0	0	1
Ps. horrida	2	1	1	0	0	0	1

Microfilariae

Twelve species harbored microfilariae in their different body regions (abdomen, thorax and head). Aedes trivittatus harbored the greatest number of microfilariae with 2,220 (of 2,404 positive for any filariid stage) specimens harboring 190,163 microfilariae (Tables VII and VIII). Of 554 Ae. vexans positive for any filariid stage, 533 contained microfilariae. Similarly, of 250 positive An. quadrimaculatus, 195 harbored microfilariae. Only one Ps. ciliata, one Ps. ferox and four Ps. columbiae harbored microfilariae (Table VIII). A total of 243 microfilariae were found among five Cx. erraticus found to harbor this stage.

Sausage-Stage Larvae

Sausage-stage larvae were recovered from nine of the 19 species dissected (Tables VII and IX). About 2,355 sausage-stages were recovered from 134 Ae. trivittatus. Also, 46 Anopheles quadrimaculatus and 16 Ae. vexans harbored 874 and 511 sausage-stage larvae, respectively (Tables VII and IX). The average number of sausage-stage larvae/infected An. quadrimaculatus harboring sausage stages was 19. For the 20 Cx. salinarius and two Cx. erraticus that contained sausage-stage larvae, the average number/mosquito was five and six, respectively. Of 134 Ae. trivittatus positive for sausage-stage larvae, 99 died within the first 5 days after capture, 31 lived in the environmental chamber for 6-10 days and four lived for 11-15 days. All 16 Ae. vexans that harbored sausage-stage larvae died within 10 days after capture. Similarly, 35 An. quadrimaculatus died within the

TABLE VIII

NUMBER OF LABORATORY-HELD MOSQUITOES POSITIVE FOR MICROFILARIAE WHEN DISSECTED AT INTERVALS AFTER TRAPPING IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG

Mosquito Species	Days Post Trapping				Total
	0-5	6-10	11-15	≥16	
Aedes trivittatus	2,123	81	9	7	2,220
Ae. nigromaculis	2	0	0	0	2
Ae. vexans	533	2	1	0	536
Anopheles punctipennis	10	0	0	0	10
An. quadrimaculatus	190	4	1	0	195
Culex erraticus	5	0	0	0	5
Cx. salinarius	135	19	0	0	154
Cx. tarsalis	4	0	0	0	4
Psorophora ciliata	1	0	0	0	1
Ps. columbiae	4	0	0	0	4
Ps. ferox	1	0	0	0	1

TABLE IX

NUMBER OF LABORATORY-HELD MOSQUITOES POSITIVE FOR SAUSAGE-STAGE LARVAE WHEN DISSECTED AT INTERVALS AFTER TRAPPING IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG

Mosquito Species	Days Post Trapping				Total
	0-5	6-10	11-15	≥16	
Aedes trivittatus	99	31	4	0	134
Ae. nigromaculis	0	1	0	0	1
Ae. vexans	8	8	0	0	16
Anopheles quadrimaculatus	21	14	11	0	46
Culex erraticus	0	1	1	0	2
Cx. salinarius	6	14	0	0	20
Cx. tarsalis	1	0	0	0	1
Psorophora columbiae	1	2	0	0	3
Ps. cynascens	1	0	0	0	1

same period and 11 lived for 11 to 15 days. No developmental stages beyond sausage stage were recovered from Ae. nigromaculis and Ps. cyanescens.

Second-Stage Larvae

Second-stage larvae were recovered from Ae. trivittatus, An. quadrimaculatus, Cx. erraticus and Ps. columbiae (Table X). Of 41 Ae. trivittatus that harbored second-stage larvae, seven died within the first five days in the laboratory, 17 in 6-10 days and 17 in 11-15 days. No Ae. trivittatus from which second-stage larvae were recovered had been in the environmental chamber more than 15 days. Of 28 Anopheles quadrimaculatus that harbored second-stage larvae, eight died in the environmental chamber within 10 days after capture, 17 lived for 11-15 days and three for 16 days or longer. One Culex erraticus that harbored second-stage larvae had lived in the laboratory for at least 16 days; and one Psorophora columbiae with second-stage larvae died within 6-10 days after capture.

Third-Stage Larvae

Eight species of mosquitoes captured in the dog-baited screen cage were positive for third-stage larvae (Table XI). Of 2,855 Ae. trivittatus captured in the screen cage and dissected, 144 (5%) were positive for third-stage larvae. On the other hand, of 1,173 Ae. vexans captured and dissected, only six (0.5%) were positive for third-stage larvae. Twenty-seven (9.9%) of 274 An. quadrimaculatus dissected harbored third-stage larvae. Very small numbers of Psorophora columbiae and Ps. ciliata were trapped but both had a very

TABLE X

NUMBER OF LABORATORY-HELD MOSQUITOES POSITIVE FOR SECOND-STAGE FILARIID LARVAE WHEN DISSECTED AT INTERVALS AFTER TRAPPING IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG

Mosquito Species	Days Post Trapping				Total
	0-5	6-10	11-15	≥16	
Aedes trivittatus	7	17	17	0	41
Anopheles quadrimaculatus	2	6	17	3	28
Culex erraticus	0	0	0	1	1
Psorophora columbiae	0	1	0	0	1

TABLE XI

NUMBERS OF LABORATORY-HELD MOSQUITOES POSITIVE FOR THIRD-STAGE FILARIID LARVAE WHEN DISSECTED AT INTERVALS AFTER TRAPPING IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG

Mosquito Species	Days Post-Trapping					Total
	0	1-5	6-10	11-15	≥16	
Aedes trivittatus	6	18	1	63	56	144
Ae. vexans	0	0	4	0	2	6
Anopheles quadrimaculatus	0	1	1	16	9	27
Culex erraticus	0	0	0	5	17	22
Cx. salinarius	0	0	0	4	8	12
Cx. tarsalis	0	0	2	0	2	4
Psorophora ciliata	0	0	3	0	2	5
Ps. columbiae	0	0	0	4	2	6

high percentage with third-stage larvae (Table XII). Of 18 *Ps. ciliata* that were captured in the screen cage, five (28%) harbored third-stage larvae, and of 26 *Ps. columbiae* captured, six (23%) had third-stage larvae in them. Similarly, of 387 *Cx. erraticus* captured 22 (5.7%) harbored third-stage larvae.

The average number of third-stage larvae/mosquito infected with this stage varied widely among species. It ranged from as few as one in *Cx. tarsalis* to as many as 27 in *An. quadrimaculatus*. The average number of third-stage/infected specimen for *Ae. trivittatus* and *Ae. vexans* was 6.9 and 1.2, respectively (Table XII).

Of 144 *Ae. trivittatus* that harbored third stage larvae, 25 died within 10 days after capture, 2 of 27 *Ae. quadrimaculatus*, 2 of 4 *Cx. tarsalis* and 3 of 5 *Ps. ciliata* died within a similar short period. Laboratory-held mosquitoes that were positive for third-stage larvae were captured in May, June, July and August (Table XII). Of 144 *Ae. trivittatus* that were positive, 36 and 108 were captured in June and July, whereas positive *An. quadrimaculatus* specimens were captured only in July and August. There were no mosquitoes positive for third-stage larvae in September among the 79 specimens dissected. The majority of mosquitoes captured during this period were *Ae. vexans*, *Cx. erraticus*, *Cx. salinarius* and *An. quadrimaculatus*.

TABLE XII

OCCURRENCE OF THIRD-STAGE FILARIID LARVAE IN LABORATORY-HELD MOSQUITOES CAPTURED IN SCREEN CAGE BAITED WITH DIROFILAREMIC DOG

Mosquito Species	Month of Capture					Specimens Positive	Total Specimens Dissected	Percent Positive	Average* Number of 3rd Stage larvae/mosquito
	May	June	July	Aug	Sept				
Aedes trivittatus	0	36	108	0	0	144	2,855	5.0	6.9
Aedes vexans	4	0	2	0	0	6	1,173	0.5	1
Anopheles quadrimaculatus	0	0	3	24	0	27	274	9.9	27.4
Culex erraticus	0	0	6	16	0	22	387	5.7	1.6
Culex salinarius	0	0	4	8	0	12	989	1.2	3
Culex tarsalis	0	0	3	1	0	4	23	17.4	1
Psorophora ciliata	1	0	4	0	0	5	18	27.7	7.6
Psorophora columbiae	0	0	6	0	0	6	26	23.1	5.3

*Average was computed by dividing total number of third-stage larvae by number of mosquitoes positive for third-stage larvae.

CHAPTER V

DISCUSSION

General Discussion

In incriminating an arthropod as a vector, its total biology and vector effectiveness must be determined. Harwood and James (1979) have listed some criteria which constitute vector effectiveness. They are:

1) Pathogen receptivity - The vector must be able to support complete development of the pathogen under experimental and natural conditions. Also, the pathogen must be able to develop or multiply and concentrate in large numbers in the vector. This is necessary in order to improve chances that an infective dose will be introduced when the vector feeds on a susceptible vertebrate host.

2) Host specificity and feeding habits - The etiologic agents causing disease exclusive to (or nearly so) a specific vertebrate host are best transmitted by vectors that feed preferentially on such hosts.

3) Longevity - The arthropod vector must live long enough to allow the pathogens to develop to the infective stage. In the case of *D. immitis* this interval is about ≥ 12 days depending on geographic location, and it is probably a function of relative humidity and temperature (Ludlam et al., 1970.

4) Frequency of feeding - Frequent vector/host contact increases the effectiveness of a vector. The vector must take at least two blood meals in order to transfer the pathogens.

5) Mobility - The ability of a vector to fly and reach its host is important in determining its vector effectiveness. The ability of a vector to reach many hosts is important in rapid dissemination of pathogens over a wide area; otherwise, their associated diseases will be focal in nature. The vertebrate hosts also aid in dissemination of pathogens assuming that there are suitable arthropod hosts in different localities, e.g., interstate transportation of dogs infected with heartworms to non-endemic areas where suitable mosquitoes are already present.

6) Numbers/Population - High population density with enormous numbers is important for making contact with susceptible vertebrate hosts. Presence of very large populations may permit some vectors that are otherwise poor hosts for pathogens to be of significance in spread of that pathogen.

7) Ability to withstand harsh environment - The ideal vector must be able to withstand environmental extremes such as cold and drought.

Vectors of *Dirofilaria immitis*

Several species of mosquitoes have been reported to support development of *D. immitis* microfilariae to infective stage larvae; but relatively little information is available on isolation of infective larvae from feral mosquitoes. Likewise, little information is available on the ability of a given species to transmit

the parasite from dog to dog (Ludlam et al., 1970). The problem of isolating filariid larvae from feral mosquitoes is compounded by the fact that large numbers of mosquitoes must be dissected even in enzootic areas to accumulate meaningful data. For example, in a highly endemic area in French Oceania, Cx. quinquefasciatus was found to support complete development of D. immitis in the laboratory, but only one individual from over 1,000 specimens dissected harbored filariid larvae (Rosen, 1954). Ludlam et al. (1970) have posited theoretically that in a highly endemic area with an infection rate as high as 25% in the dogs, approximately 3 of 4 susceptible mosquitoes feeding on dogs would be negative.

Another problem often encountered in incriminating specific mosquito species as the principal vector of D. immitis is inability to mimic feral conditions in the laboratory. In a study reported by Lewandowski et al. (1980) Ae. vexans was incriminated as a potential vector because the species supported development of D. immitis larvae to the infective stage in the laboratory, and some captured feral specimens harbored infective filariid larvae. However, Lewandowski (1977) had reported earlier that experimentally infective Ae. vexans harboring third-stage larvae would not feed on a susceptible dog in the laboratory. Similar attempts to transmit D. immitis from dog to dog experimentally using Cx. salinarius failed in an area where it had been reported as a potential vector (Bickley, 1976).

The suitability of a given mosquito species to serve as a vector of D. immitis varies considerably among geographic regions. For example, Ae. vexans was reported to be a potential vector in Minnesota

(Hendrix et al., 1980), Michigan (Lewandowski et al., 1980) and New York (Todaro et al., 1977). On the other hand, the species was not a good vector in Iowa (Christensen, 1977a).

Kartman (1953b) reported variation in the susceptibility of Ae. aegypti from United States, South Africa, Anglo-Egyptian Sudan and Figi strains. Certainly in a newly endemic area the fact that some species support development of D. immitis in the laboratory is not sufficient basis to incriminate them as vectors.

The suitability of a given mosquito species to serve as a vector of D. immitis also varies with its genetic background which is probably a function of geographic isolation. The role of genetic susceptibility in filarial infection has been investigated by several authors (Macdonald, 1962a, 1962b; Macdonald, 1963; Macdonald and Ramachandran, 1965; McGreevy, McClelland et al. (1974). It is now generally accepted that susceptibility of mosquitoes to D. immitis is controlled by a sex-linked recessive gene. McGreevy and his colleagues mated highly susceptible Ae. aegypti (f^mf^t) from their stock with highly refractory Ae. aegypti from two different geographically isolated populations, Bwanda and Lanfiera (f^mf^t x Bwanda and f^mf^t x Lanfiera). They noted that the progeny of f^mf^t x Bwanda was completely refractory with development being arrested at the microfilarial stage, whereas development was quite variable in progeny of f^mf^t x Lanfiera parents with a susceptibility rate of 5%. They concluded that it is likely that microfilariae within a host constitute a population of individuals that vary in infectivity to a vector, and there is also a variation in the susceptibility of a vector to infection with D. immitis. Therefore, the development of a parasite depends on its

infectivity relative to its specific host's susceptibility. That is, a parasite of low infectivity would only develop normally in hosts of high susceptibility but a parasite of high infectivity might develop normally in hosts of either high or low susceptibility. They thought that the intergration of parasite infectivity and host susceptibility may account for the acquisition of new vector species by filarial worms and for the patterns of host specificity seen in field populations. According to Lawrence and Pester (1967), during an initial encounter with a filariid under feral conditions, only the more susceptible mosquitoes will support development of the more infective filariae. Subsequent adaption of the filariids to the new vector then occurs as the worms are successfully passed from the vector to the definitive host and back.

Mosquitoes may also be nonsusceptible for a variety of reasons. One factor that may account for their refractoriness is their defense mechanism. These have been studied extensively and probably vary among species. Travis (1947) and Kartman (1953b) reported observing death of microfilariae in the gut of Cx. species. Kartman (1953b, 1953c) also reported seeing encapsulation of microfilariae in An. quadrimaculatus and subsequent passage of undigested microfilariae in mosquitoes' feces. The effect of buccopharyngeal armature of mosquitoes upon microfilariae was investigated by Bryan et al. (1974). The authors concluded that these structures might be responsible for destruction of microfilariae in mosquitoes that possess them.

Specific Discussion for This Study

Infected Mosquitoes Captured in Dog-baited Screen Cage

There were 23 species of mosquitoes trapped during the trapping season May to October, 1983. Eight species trapped in a cage baited with a dirofilaremic dog supported development of *D. immitis* to the infective stage and two others supported partial development. Filariid larvae assumed to be those of *D. immitis* were recovered in two species captured in light traps. As stated earlier, before an arthropod can be incriminated as a vector, its biology and vectorial capacity must be examined carefully. For this reason, the eight species of mosquitoes from which third-stage larvae were recovered will be discussed individually. In addition, four other species that have been incriminated as vectors in other states but which were not found to harbor third-stage larvae in the present study will be discussed individually, too.

Aedes trivittatus. The first report concerning *Ae. trivittatus* and *D. immitis* was based upon work conducted in Minnesota by Yen (1938). He exposed 16 specimens to an infected dog. All 16 *Ae. trivittatus* fed on the dog, but upon dissection 15 days after exposure they were all negative. He concluded that *Ae. trivittatus* was refractory to *D. immitis*. However, Christensen (1977b) reported complete development of *D. immitis* in *Ae. trivittatus* under laboratory conditions in Iowa. Yen's negative findings could have resulted from the fact that he used a small sample size (16); Christensen's work was based upon

a much larger sample size (1,217).

Several different aspects of Ae. trivittatus biology have been studied. Tempelis (1975) reported that Aedes, including Ae. trivittatus, preferentially feed on mammals. In a survey of blood meal preference of Ae. trivittatus in central Iowa, Pinger and Rowley (1975) reported that almost 95% of 600 engorged specimens captured by C.D.C and Malaise traps reacted positively with anti-mammal serum. Only 3% reacted positively to anti-bird or with anti-amphibian serum. Wright and Knight (1966) also studied the feeding habits of Ae. trivittatus. They concluded that the peak biting activity occurs during the crespuscular period from ½-hour before until 45 minutes after sunset. According to Christensen (1978), under experimental conditions (27°C and 80% relative humidity), male and female Ae. trivittatus may live for as long as 27 or 60 days, respectively. Also, unpublished data gathered by Christensen indicated that Ae. trivittatus has a flight range of over 30,000 m. in a 24-hour period; these observations were based upon findings in a flight mill system in the laboratory (Christensen, 1977b). The species is also known to be multivoltine, i.e., it can produce two or more broods/ season.

In the present study over 19,700 Ae. trivittatus were captured. This represented about 50% of all mosquitoes trapped. Of 2,855 specimens captured in the dog-baited screen cage and dissected, 2,404 (84%) were infected with filariid larvae. Of these, 144 (5%) hosted third-stage larvae (Table XI). The average number of third-stage larvae/infected mosquitoes harboring third-stage larvae was seven (Table XII). Infective larvae were observed in the head region

and proboscis of infected mosquitoes in about 12 days. Data from the present study are consistent with those reported by Christensen (1977a); he observed a susceptibility rate of 76.4% among 1,217 Ae. trivittatus that he exposed to an infected dog.

Data from the present study suggests that Ae. trivittatus is a potential vector of heartworms in the Stillwater area. However, transmission by this flood-water species may be somewhat restricted seasonally. In 1983, for example, it was likely restricted to May, June and July, the period during which conditions existed that favored their abundance.

Aedes vexans. Hu (1931) reported that 45 (80.3%) of 56 Ae. vexans he exposed to an infected dog became infected with microfilariae, but relatively few larvae developed to third stage compared with An. punctipennis. The maximum number of parasites recovered from one Ae. vexans specimen was 19, the minimum was 4, and the average, 10.6. Yen (1938) also reported recovering third-stage larvae from 129 Ae. vexans exposed to an infected dog. Jankowski and Bickley (1976) reported an infection rate of 78.9% in 78 specimens that ingested an infective blood meal. The average number of larvae/infected mosquito recovered upon dissection in Jankowski and Bickley's study was 10.6. Bemrick and Sandholm (1966) reported that of 156 Ae. vexans that fed on an infected dog 113 (72.4%) were positive when dissected. Infective larvae were recovered from 50% of these 113 positive specimens. The average number of parasites/infected mosquito was 6.7. Other investigators that have reported Ae. vexans as a potential vector of D. immitis in the United States

are: in Michigan, Lewandowski et al. (1980); California, Walters and Lavoipierre (1982); Minnesota, Hendrix et al. (1980) and New York, Todaro et al. (1977).

Aedes vexans is zoosophilic and feeds on mammals readily according to Tempelis (1975). It is also known to be multivoltine throughout its range.

In the present study, of 1,173 Ae. vexans captured in the dog-baited screen cage less than 50%, 554 (47%), were found to be infected upon dissection. The vast majority of these died before development progressed beyond the microfilarial stage, and only 6 specimens contained third-stage larvae (Tables V and XI). There were 16 specimens with sausage stages but none was found with second-stage larvae (Tables IX and X). There were no third-stage larvae recovered from the head region. The average number of third-stage larbae/infected mosquitoes harboring third-stage was one, and the average number of parasites/infected Ae. vexans was 63 (Tables VII and XII). These parasite loads are somewhat lower than those observed in Ae. trivittatus in which their average number of third-stage larvae/infected mosquito harboring third-stage was seven and average parasite load (all stages inclusive) was 81/infected mosquito. It is pertinent to notice also that mortality was higher among Ae. vexans than Ae. trivittatus when they were maintained under the same laboratory conditions. It appears that most Ae. vexans died in the laboratory during the microfilarial stage; only 22 specimens showed development beyond the microfilarial stage and 16 of these were to sausage stage only. It may be that Ae. vexans cannot tolerate feeding on dogs with very high parasitemia such as that of the dog

used in the present study. The average number of parasites/infected Ae. vexans observed by Jankowski and Bickley (1976) and by Bemrick and Sandholm were much lower (10.6 and 6.7, respectively) than in the present study (63). The fact that there were no second-stage larvae recovered in Ae. vexans is somewhat puzzling. It could indicate that mosquito mortality is related to metamorphosis of microfilariae to the sausage stage and that the few that survive this period could support development to third-stage. Based on the 1983 season, it appears that Ae. vexans may not serve a significant role as a vector of heartworms in the Stillwater area; relatively few specimens (6 of 1,173) captured in the dog-baited cage and held in the laboratory contained infective larvae when they were dissected. The vast majority of positive specimens died very early after capture and before the parasites developed to the infective stage. Furthermore, no third-stage larvae were recovered in any of 674 light-trapped feral specimens dissected from among those caught in 27 trappings and only two of these contained filariid larvae, one with microfilariae and the other with sausage stage.

Anopheles quadrimaculatus. Phillips (1938) reported a 100% infection rate among 90 An. quadrimaculatus that he exposed to an infected dog and 72 of these harbored third-stage larvae 11-18 days post exposure. The average number of parasites/infected mosquito was 40. Kartman (1953a) reported that all 210 An. quadrimaculatus that took blood meals from an infected dog with 16,000-18,000 microfilariae/cm^3 of blood became infected. However, he noticed encapsulation in some specimens when mosquitoes were dissected. The average number of parasites/infected mosquito was 20. Newton (1957) reported experimental transmission of D. immitis to dogs with An. quadrimaculatus. Keegan et al. (1968),

Kutz and Dobson (1974) and Todaro et al. (1977) have reported that An. quadrimaculatus was a good vector of dog heartworms under experimental conditions. However, Kutz and Dobson (1974) reported a mortality rate of 75% in An. quadrimaculatus within 21 days post exposure among 100 specimens fed on an infected dog that had 25-135 microfilariae/20 mm^3 of blood. An. quadrimaculatus is multivoltine throughout its known habitat, producing two or more broods per season. They are anthropophilic but feed readily on other mammals (Tempelis, 1975).

In the present study, of 274 An. quadrimaculatus collected in the dog-baited screen cage, 250 (91%) were infected and were assumed to have taken their infective blood meal from the dog. Of these 250 specimens, 195 contained microfilariae, 46 sausage-stage larvae, and 27 harbored third-stage larvae (Table V, VIII, IX, X and XI). No filariid larvae were recovered from any of 85 specimens dissected from among 113 specimens captured in the light traps in ten of 27 trappings in which they were caught during the season.

It is probably significant in the present study, that mortality was very high in the laboratory-held An. quadrimaculatus, especially during the first five days following capture (Table VIII). The average number of parasites/infected mosquito was 91 and the large numbers ingested may have contributed to the high mortality. Among mosquitoes surviving long enough for third-stage larvae to develop, the average number of third-stage larvae/infected mosquito harboring the third-stage was 27 (Table XII). Third-stage larvae were found in the head region within 11-13 days after capture. One specimen that died on the first day in the laboratory was found to harbor 709

microfilariae in its abdomen. Although no quantitative data are available to substantiate the observation, An. quadrimaculatus was noted consistently to take heavy blood meals, and the mosquitoes often appeared fragile at the time of collection.

Although An. quadrimaculatus is an excellent intermediate host of heartworms under laboratory conditions, relatively few authors (Lewandowski et al., 1980) have reported recovery of third-stage larvae from the species under feral conditions. As stated previously, in the present study no filariid larvae were recovered from any of 85 species dissected from among 113 feral An. quadrimaculatus collected in light traps; even so, it could still serve as a vector. Its ability to tolerate large numbers of parasites, its anthropophilic/zoosophilic feeding habits, and its ability to survive the extreme summer temperatures when other mosquito species are likely absent argue that it could be a vector of heartworms in the Stillwater area.

Culex erraticus. There is no evidence in the literature that Cx. erraticus can support development of D. immitis to the infective stage. Tolbert and Johnson (1982) trapped and dissected ten feral Cx. erraticus but they were all negative. Tempelis (1975) indicated that Cx. species are generally ornithophilic but may also feed on other hosts including mammals and amphibians.

In the present study, of 627 Cx. erraticus captured in the light traps, 144 were dissected and all were negative (Table V). The 387 specimens captured in the dog-baited screen cage were all dissected; 29 (8%) of these were positive for filariid larvae and 22 of them harbored third-stage larvae. It is not known what portion of those

that were not infected did not feed on the dog. The large percentage of infected specimens that harbored third-stage larvae may indicate that, while not a good mammal feeder, Cx. erraticus, may be a vector. The present study shows that Cx. erraticus can live long enough for the parasite to develop to the infective stage. Furthermore, Cx. erraticus clearly has the ability to withstand extremely hot weather because it was captured during the drought when populations of other mosquito species were low. During this period (July, August and September), Cx. erraticus accounted for almost 50% of the mosquito population trapped.

Culex erraticus may serve an important role in the transmission of D. immitis in the Stillwater area because of its ability to persist in relatively large numbers during severe drought. Its ability to tolerate D. immitis infection with little mortality suggests that it could serve a role in transmission of D. immitis. Its abundance in the Stillwater area during the hot dry months when most other mosquito populations were low suggests that it should be studied in detail to assess its potential as a vector.

Culex salinarius. Culex salinarius has been reported to support development of D. immitis to the infective stage (Bickley et al., 1976). However, attempts by Bickley (1976) to use Cx. salinarius to transmit D. immitis from dog to dog failed. Hu (1931) observed partial development and Summers (1942) observed development up to sausage stages. Tolbert and Johnson (1982) dissected 553 feral specimens but did not find filariid larvae in any of them.

In the present study, of 788 Cx. salinarius captured in light

traps 358 were dissected, and they were all negative. Of 989 captured in the dog-baited screen cage, all were dissected and 171 (17%) were infected with D. immitis larvae (Table V). Twelve of these harbored third-stage larvae. The average number of third-stage larvae/infected mosquito harboring third-stage larvae was three (Table XII). Culex salinarius was captured throughout the mosquito season, May to October, 1983 and, like Cx. erraticus, persisted during hot dry weather. However, Cx. salinarius did not constitute a large percentage of total mosquitoes trapped in the hot, dry period. Conversely, Cx. salinarius did constitute a large proportion of specimens trapped by both methods in May. Unaccountably, Cx. salinarius trapped in the screen cage in May did not support development of third-stage larvae.

In the present study, it appeared that Cx. salinarius could serve a minor role in transmission of D. immitis. It occurred in large numbers, is multivoltine, persisted throughout the summer, and it supported development of D. immitis to the infective stage.

Culex tarsalis. Yen (1938) reported that 49 Cx. tarsalis were exposed to an infected dog; of these, third-stage larvae were recovered from 13 specimens. The average number of parasites/infected mosquito was three. The maximum number of larvae recovered from one specimen was nine and the minimum was one. He did not observe any encapsulation or arrested development. Yen concluded that Cx. tarsalis was not a good vector of D. immitis due to the low level of infection. Bemrick and Sandholm (1966), also under experimental conditions, reported similarly low levels of infection. Tempelis (1975) reported that Cx. tarsalis has a strong preference for avian

blood and tends to feed at night.

In the present study, 38 Cx. tarsalis were captured in light traps (11 of 27 trappings) and 28 of them were dissected with negative results (Table V). Only 23 specimens were captured in the dog-baited screen cage and all were dissected; nine (39%) of these were positive for filariids. Among the infected specimens, four (44%) were infected with third-stage larvae. That such a large percentage of infected specimens harbored third-stage larvae may indicate a tolerance of Cx. tarsalis for D. immitis. However, the average number of third-stage larvae/mosquito was one which is very low (Table XII).

Based upon data collected in the present study, it seems doubtful that Cx. tarsalis serves an important role in transmission of heartworms in the Stillwater area. It was captured in only small numbers, did not feed well on the dog, and had small numbers of parasites when they did develop.

Psorophora ciliata. Tempelis (1975) reported that Psorophora species feed mainly on large animals. There is no report in the literature concerning the ability of Ps. ciliata to support D. immitis development.

In the present study, 53 Ps. ciliata were captured in the light traps and ten of these were dissected with negative results (Table V). Eighteen specimens were captured in the dog-baited screen cage and all were dissected. Five (28%) were positive for third-stage larvae (Table XII). One specimen had one microfilaria. There were no specimens with either sausage- or second-stage larvae. The average number of infective larvae/infected mosquito harboring third-stage

larvae was eight. The large percentage of infected Ps. ciliata that harbored third-stage larvae is puzzling, especially since almost no other larval stages (except for one specimen with one microfilaria) were found. Likewise, it is very surprising that such large numbers of third-stage larvae were found in these specimens that supported development to the infective stage. It may be that Ps. ciliata is able to tolerate feeding on dogs with high parasitemia and that, with little mortality, the filariids are able to develop to third-stage.

Given that Ps. ciliata was captured in only small numbers, it is unlikely that it could serve even a minor role as a vector. It is interesting that a large percentage of the few specimens captured supported development of D. immitis to the infective stage and that the parasites developed in rather large numbers. It seems possible that under proper circumstances, Ps. ciliata could serve as a vector, but further study is required to support such conjecture.

Psorophora columbiae. Psorophora columbiae has not been reported in the literature to support development of Dirofilaria immitis to infective stage. Psorophora species feed preferentially on large mammals (Tempelis, 1975; Edman, 1971).

In the present study, only 31 Ps. columbiae were captured throughout the entire mosquito season, five in light traps and 26 in the screen cage. All 26 captured in the dog-baited screen cage were dissected, and 13 contained filariids. Four specimens were positive for microfilariae, three hosted sausage stages, and one contained second-stage larvae. Six specimens contained third-stage larvae. The average number of third-stage larvae/infected mosquito harboring the third-

stage was five (Tables VIII, IX, X, XI and XII).

Based upon data from a single season, it seems unlikely that Ps. columbiae could be of importance in transmission of dog heartworms in the specific study area of Stillwater because the population levels were so low. Nevertheless, it is very interesting to note that it has the ability to support development of D. immitis to the infective stage and that relatively large numbers of parasites developed in them.

Anopheles punctipennis. Anopheles punctipennis has been reported to be a good vector of D. immitis. Hu (1931) reported 100% infection rate among 36 specimens he exposed to an infected dog. A similar report by Yen (1938) indicated that ten specimens were exposed to an infected dog and all ten became infected; seven of these lived long enough for development of D. immitis to the infective stage. The highest number of parasites recovered in a single specimen was 37 and the minimum was 16 (Yen, 1938). Phillips (1939) also reported An. punctipennis to be a good vector of D. immitis. Bickley et al. (1976); Tolbert and Johnson (1982); Courtney and Christensen (1983) have all reported isolation of filariid larvae from feral An. punctipennis.

In the present study of 107 An. punctipennis captured in light traps 57 were dissected and all were negative. Of 14 specimens captured in the dog-baited screen cage, all were dissected and ten (73%) were positive for microfilariae. All of these died within five days of capture. No development beyond the microfilarial stage was observed and no An. punctipennis lived beyond five days in the laboratory. One specimen that died on the day it was captured had

502 microfilariae in its abdomen. The average number of parasites/ infected mosquito was 106 and this heavy parasite load may have contributed to the high mortality.

In the present study, one could not draw a definite conclusion about the potential of *An. punctipennis* to function as a vector because it was captured in such small numbers. Its potential to serve as a vector in the Stillwater area needs further investigation.

Other *Psorophora* Species. Steuben (1954) reported that he found third-stage larvae in *Ps. ferox*. There is no report in the literature confirming the ability of other *Psorophora* species to support development of *D. immitis*. As stated earlier, *Ps.* species preferentially feed on large animals (Tempelis, 1975). *Psorophora varipes* in the southeastern United States has been renamed *Ps. mathesoni* (Belkin and Heinemann, 1975). All the *Ps. varipes* captured in this study have been designated *Ps. mathesoni*.

In the present study, *Ps. horrida*, *Ps. longipalpus*, *Ps. cyanescens*, *Ps. ferox* and *Ps. mathesoni* were caught in large numbers in light traps (Table II), but relatively few specimens of each of them were caught in the dog-baited screen cage (Table II). One *Ps. ferox* contained microfilariae and one *Ps. cyanescens* contained a sausage stage (Tables VIII and IX). No larval stages were observed in *Ps. horrida*, *Ps. longipalpus* and *Ps. mathesoni*. It is puzzling to note that large numbers of *Psorophora* species were captured in light traps but relatively few in the dog-baited screen cage. It could be that these species do not readily leave their habitat to feed. According to Barr (1958), some mosquito species do not leave their breeding

area, e.g., *Ae. triseriatus*. According to Harwood and James (1975), *Psorophora* species have habitat similar to that of floodwater *Aedes*. They breed in water accumulations due to irrigation and seepage, and such habitat was available in the wooded area where light traps were set.

Based upon the data in the present study, it appears that these species, *Ps. ferox*, *Ps. longipalpus*, *Ps. cyanescens* and *Ps. mathesoni*, cannot serve as vectors because they were not attracted to the dog in large numbers. The type of trap utilized probably influenced the number caught. However, the few that fed on the dirofilaremic dog did not support development of *D. immitis* to infective stage. In addition, it appears that further investigation is necessary to see if, in fact, these *Ps.* species do not leave their breeding habitat to feed.

Other *Aedes* Species. *Aedes canadensis* has been reported by several workers to support development of *D. immitis* to the infective stage under experimental conditions (Hu, 1931; Yen, 1938; Jankowski and Bickley, 1976). Bickley et al. (1977) accomplished dog to dog transmission of *D. immitis* using *Ae. canadensis*. Keegan et al. (1968) reported complete development of *D. immitis* in *Ae. zoosophus* under laboratory conditions. There are no reports in the literature concerning the ability of *Ae. epactius* and *Ae. nigromaculis* to support development of *D. immitis* larvae.

In the present study one specimen each of *Ae. canadensis*, *Ae. epactius* and *Ae. zoosophus* were captured in the dog-baited screen cage, but they were not dissected. All four *Ae. nigromaculis* captured in light traps were dissected, and they were negative (Table V). In

addition, 21 Ae. nigromaculis were captured in the dog-baited screen cage and dissected; two were positive with microfilariae, and these two specimens died within the first five days after capture (Table VIII). It appears, at least based upon observation for the 1983 season and for the specific study site, that the population of these species, Ae. epactius, Ae. canadensis, Ae. nigromaculis and Ae. zoosophus, is so low that none are likely to serve an important role in the transmission of D. immitis in the Stillwater area.

The possible role of Ae. canadensis and Ae. zoosophus in transmission of D. immitis needs further investigation. Their potential to serve as vectors has been reported in other areas, but obviously the 1983 population levels in the Stillwater area were too low to be of significance.

Naturally Infected Mosquitoes. Christensen and Andrews (1976) reported capture of Ae. trivittatus naturally infected with Dirofilaria immitis larvae. Feral Ae. vexans have also been trapped naturally infected with D. immitis larvae (Bemrick and Sandholm, 1966; Hendrix et al., 1980; Todaro et al., 1977; Tolbert and Johnson, 1982; Walters and Lavoipierre, 1982).

In the present study, of 16,915 Ae. trivittatus captured in light traps, 589 were dissected; eight (1.3%) were positive for filariid larvae. One of these eight specimens harbored one third-stage larva in its proboscis; the remaining seven contained microfilariae in their abdomens (Table V). Although all the naturally infected specimens were captured in June, Ae. trivittatus were trapped in large numbers in May, June and July (Figure 1 and Table XII). It is unknown why no

naturally infected specimens were found in May or July.

Of 7,734 Ae. vexans captured in the light traps, 674 were dissected; two of these were positive for filariid larvae, one harboring microfilariae and the other sausage stages. In both cases, parasites were in the abdomen. Naturally infected Ae. vexans were captured only in May and June; however, Ae. vexans were present throughout the summer (Figure 1 and Table XII).

In addition to the light-trapped Ae. vexans and Ae. trivittatus that were captured naturally infected, at least 24 Ae. trivittatus and one An. quadrimaculatus captured in the dog-baited screen cage were probably naturally infected before entering the cage (Table XI). This assumption is based upon knowledge that 10-12 days are required for microfilariae to develop to third-stage larvae. Therefore, mosquitoes from which third-stage larvae were recovered after they were held in the laboratory for less than 10 days after capture are assumed to have been infected naturally (Table XI). One Ae. trivittatus that was captured on June 23, 1983, and died on June 25 had one third-stage larva in its abdomen. The mosquito's eggs were tanned and more fully developed than one would expect for a mosquito that had had a blood meal only two days earlier. It appears that this particular mosquito acquired its infection naturally or at least on the day of capture. One An. quadrimaculatus that was captured on September 7 and died the following day, was fully gravid and harbored both sausage-stage and third-stage larvae in its abdomen; this, too, was assumed to be a naturally infected specimen. There is a remote possibility that either of these two specimens could have been missed on a previous collection.

The filariid larvae recovered from these feral mosquitoes (Ae. trivittatus and Ae. vexans) were morphologically indistinguishable from D. immitis. Furthermore, the mosquitoes were captured in a residential area where D. immitis is endemic. Neither D. repens nor D. tenuis, both of which develop in the malpighian tubules of mosquitoes. has been reported in the Stillwater area. Recently in Oklahoma City, Simmons et al. (1984) reported a case of human filariasis that was caused by Brugia beaveri. The patient, who was immunodeficient and had traveled abroad, was thought to have become infected in Oklahoma. Larvae of Brugia species develop in the flight muscles of mosquitoes, and almost all filariid larvae recovered from feral mosquitoes in the present study were recovered from malpighian tubules, the normal site of development for D. immitis. The exception was a third-stage larva that had migrated to the proboscis of Ae. trivittatus. Therefore, it is likely that filariid larvae recovered from feral mosquitoes in the present study were D. immitis and not B. beaveri, D. repens or D. tenuis.

Unusual Findings and Recovery of Larvae from Unexpected Locations

Kartman (1953b) reported that as late as 15 days after exposure, he still observed microfilariae in the gut of An. quadrimaculatus. He referred to this phenomenon as arrested development. Buxton and Mullen (1981) also observed arrested development in Ae. aegypti. Similar observations were made in the present study with respect to some specimens of Ae. trivittatus, Ae. vexans and An. quadrimaculatus (Table VIII). Microfilariae were found in Ae. trivittatus as late as 16 days after trapping, and in Ae. vexans and An. quadrimaculatus as

late as 11 days after exposure (Table VIII).

Four Ae. trivittatus, 11 An. quadrimaculatus and one Cx. erraticus contained sausage-stage larvae 11 or more days after exposure (Table IX). Also, three An. quadrimaculatus and one Cx. erraticus contained second-stage larvae as late as 16 days after exposure (Table X). Christensen (1977a) reported similar observations in Ae. trivittatus. He noticed that as late as 15 days after feeding sausage- and second-stage larvae were still present in Ae. trivittatus.

Aside from unexpected recovery of immature larvae from some mosquitoes in the present study, microfilariae, sausage-stage and second-stage larvae were sometimes observed in unexpected locations, e.g., in the thorax rather than the abdomen. These latter findings probably resulted from technical difficulties that were hard to avoid until experience was gained in storage of specimens. Specifically, after mosquitoes thawed in the freezing solution in which they were stored, it was sometimes difficult to separate the body parts clearly. The problem was noted especially in An. quadrimaculatus which often were very fragile. This technical difficulty was corrected after it was realized that all specimens should be dissected very quickly after thawing. Thereafter, not more than seven specimens were stored in a vial, and all specimens could be dissected soon after thawing. It seems unlikely that sausage-stage and second-stage larvae were found in the thorax as a result of contamination from dissecting instruments because this was consciously avoided by wiping the instrument after dissecting each body part. It is likely that minimal contamination occurred in some vials in which mosquitoes were stored because occasionally mosquitoes were noted to have ruptured.

CHAPTER VI

CONCLUSIONS

This study suggests that *Aedes trivittatus* is the major potential vector of *Dirofilaria immitis* in the Stillwater area. Other mosquito species that may serve ancillary roles as vectors, in possible order of importance, are: *Anopheles quadrimaculatus*, *Culex erraticus* and *Cx. salinarius*. It is unlikely that *Ae. vexans*, *Cx. tarsalis*, *Ps. ciliata* or *Ps. columbiae*, all of which were found to harbor third-stage larvae, serve an important role in transmission of *D. immitis* in the Stillwater area.

Based upon observation for a single season, it appears that *Ae. trivittatus* meets the criteria of Harwood and James (1979) for incriminating arthropods as vectors. Specifically, *Ae. trivittatus* occurs in large numbers, is zoosophilic, multivoltine, long-lived and takes two or more blood meals during its lifetime. The species fed readily on the dog; 84% of *Ae. trivittatus* that were trapped in the dog-baited screen cage were infected, apparently having fed on the dirofilaremic dog. Sausage- and second-stage larvae were also recovered from many specimens. Approximately 5% of all infected *Ae. trivittatus* hosted third-stage larvae at the time of dissection. The species appeared to tolerate feeding on blood with a high parasitemia because only moderate mortality occurred during the holding period in the laboratory. Third-stage larvae were observed in the head region and proboscis as early as

12 days after capture. Finally, a third-stage filariid larva was recovered from the proboscis of one feral Ae. trivittatus captured in a light trap, suggesting that natural infection was occurring in the vicinity.

Dirofilaria immitis larvae also developed to the infective stage in An. quadrimaculatus. About 91% of 274 specimens that were trapped in the screen cage apparently fed on the dog and became infected. Almost 10% of the infected specimens hosted third-stage larvae at the time of dissection. The average number of infective larvae/mosquito harboring third-stage larvae was 27. Third-stage larvae were observed in the head region and proboscis in 11-13 days. Thus the parasite seems to have been remarkably compatible with the species. Anopheles quadrimaculatus is multivoltine, takes two or more blood meals and feeds readily on mammals. It persisted throughout most of the summer, though in rather small numbers (387 specimens, summing both trapping methods) during the 1983 trapping season. Therefore, An. quadrimaculatus seems to be a potential vector of D. immitis in the Stillwater area.

Culex erraticus and Cx. salinarius may each serve a minor role as vectors. They both supported development of D. immitis larvae to the infective stage. Relatively few (8%) Cx. erraticus that were captured in the screen cage harbored filariid larvae of any stage; however, 6% of those that were positive harbored third-stage larvae. The species was captured during hot summer months when most other mosquito populations were very low. During this period (July, August and September) Cx. erraticus accounted for almost 50% of all mosquitoes trapped. This tolerance of hot, dry weather may be an important factor; it could result in opportunity for transmission of D. immitis among dogs when

prime vectors are not abundant. No previous reports have been found indicating that Cx. erraticus supports development of D. immitis to the infective stage.

Culex salinarius was also captured throughout the summer in fairly large numbers, but unlike Cx. erraticus, it never constituted a large percentage of mosquitoes captured on a given day during the hot summer months. Less than 20% (171 of 989) of Cx. salinarius captured in the screen cage were infected with filariid larvae of any stage and only about 1% of those that were infected hosted third-stage larvae. However, its ability to persist throughout the summer, notably during hot, dry weather when the putative prime vectors were not abundant, or were absent, suggests that it might serve a minor role in transmission of D. immitis in the study area.

It is not likely that Ae. vexans is an important vector of D. immitis in the Stillwater area. Although it was trapped in large numbers by both trapping methods, less than 50% of those captured in the screen cage appear to have fed on the dirofilaremic dog. Among the 53% that were negative for filariids, the vast majority lived ≥ 15 days in the laboratory, but very few specimens (6 of 554) that were positive for filariids harbored infective larvae. The majority of laboratory-held Ae. vexans specimens died when larvae were at the microfilarial or sausage-stage of development. Furthermore, only one parasite was recovered from each of the six specimens that harbored third-stage larvae. Finally, of the two light-trapped Ae. vexans found to be infected, one harbored microfilariae and the other sausage stages.

Similarly, it is unlikely that Ps. ciliata and Ps. columbiae are

vectors of D. immitis in the Stillwater area. Although the two species supported development of D. immitis larvae to the infective stage and the parasites became established in fairly large numbers, the population levels of these mosquitoes were seemingly too low during the 1983 trapping season for them to play a role in transmission. It is interesting to note that, like Cx. erraticus, Ps. ciliata and Ps. columbiae have not been reported previously to support development of D. immitis larvae to the infective stage.

It is also unlikely that Cx. tarsalis serves as a vector of D. immitis in the study area. As observed by others, it supports development of D. immitis larvae to the infective stage, but its population levels were too low in the 1983 trapping season to justify incriminating this largely ornithophilic species as a vector.

Anopheles punctipennis is also unlikely to serve as a vector of D. immitis in the Stillwater area. The species is reported to be a good vector in some geographic areas, but in the present study no development was observed in it beyond the microfilarial stage. Only 121 specimens were captured, 14 of these in the screen cage. Of the 14 specimens, only 10 were positive, all harboring microfilariae.

Although Ae. canadensis, Ae. zoosophus and Coquillettidia perturbans have been reported to support development of D. immitis larvae to the infective stage, they were trapped in such small numbers in the present study that conclusions of any kind are hardly warranted. Aedes canadensis and Ae. zoosophus were not caught in the light traps, and only one specimen of each was captured in the dog-baited screen cage. These were not dissected.

It is unlikely that Ps. longipalpus, Ps. ferox, Ps. cyanescens

and Ps. mathesoni are vectors of heartworms in the Stillwater area. They were trapped in large numbers in the light traps but were rarely attracted to the dog. Furthermore, very few specimens harbored filariids, and no third-stage larvae were recovered. Of these three species, only Ps. ferox has been reported to support D. immitis to the infective stage.

It is unlikely that Ae. epactius, Ae. nigromaculis, Uranotaenia sapphirina, and Orthopodomyia signifera are vectors. All four species were trapped in small numbers. Aedes epactius and Uranotaenia sapphirina were not dissected because the few specimens were retained for reference. Three of 21 Ae. nigromaculis harbored microfilariae. One O. signifera dissected was negative.

Results of this single-season study thus suggested that at least four species of mosquitoes (among 23 species captured) are capable of serving as vectors of D. immitis in the Stillwater area. They are: Ae. trivittatus, An. quadrimaculatus, Cx. erraticus and Cx. salinarius. Transmission by Ae. trivittatus, probably principal among the putative vectors in the study area, may be seasonally restricted. It was captured in large numbers in May, June and July, but not later when environmental conditions apparently precluded its reproduction. Similarly, Cx. erraticus may be seasonally restricted to July, August and September. Anopheles quadrimaculatus and Cx. salinarius may serve ancillary roles as vectors throughout most of the summer. Culex salinarius was trapped in fairly large numbers from May to October, and An. quadrimaculatus from June to October.

LITERATURE CITED

Abadie, J. H., Swartzwelder, J. C. and Holman, R. L. 1965. A human case of _Dirofilaria immitis_ infection. Amer. J. Trop. Med. and Hyg. 14:117-119.

Abbott, P. K. 1966. Feline Dirofilariasis in Papua, New Guinea. Aust. Vet.J. 42(7):247-249.

Acevedo, R. A. 1982. Potential vectors of _Dirofilaria immitis_ in San Mateo Co. California. Mosq. News 42(2):272-274.

Allbritton, A. R. 1982. Human infection underscores importance of canine heartworm control. Norden News 57(1):41-42.

Alls, M. E. and Greve, J. H. 1974. Canine dirofilariasis in Iowa. J. Amer. Vet. Med. Assoc. 165:532-533.

Anderson, R. C. 1957. The life cycle of dipetalonematid nematodes (Filarioidea: Dipetalonematidae): the problem of their evolution. J. Helm. 31:203-224.

Ansari, J. A. 1970. A review of the mechanisms of microfilarial periodicity. Zool. Anz. 185:387-392.

Arnott, J. J. and Edman, J. D. 1978. Mosquito vectors of dog heartworm, _Dirofilaria immitis_ in western Massachusetts. Mosq. News 38:222-230.

Artwell, R. B. and Carlisle, C. 1979. Canine dirofilariasis in the metropolitan area of Brisbane. Aus. Vet J. 55(8):399.

Awe, R. J., Matok, K. L., Alvarez, A., Stork, W. J., Estrada, R. and Greenburg, S. D. 1975. Solitary and bilateral pulmonary nodules due to _Dirofilaria immitis_. Am. Rev. Resp. Dis. 112:445-451.

Bacigatupo as cited by Ludlam et al., 1970.

Bancroft, T. L. 1901. Preliminary notes on the intermediary host of _Filaria immitis_. J. Trop. Med. 4:347-349.

Bancroft, T. L. 1904. Some further observations on the life history of _Filaria immitis_ Leidy. British Med. J. 1:822-823.

Barr, A. R. 1958. The mosquitoes of Minnesota (Diptera:Culicidae: Culicinae). Univ. Minn. Agr. Exp. Sta. Tech. Bull. 228. 154 pp.

Barriga, O. O. 1982. Dirofilariasis, In C. R. C. Handbook Series in Zoonoses, James H. Steele ed. C. R. C. Press Inc. Boca Raton, Florida. 350 pp.

Beam, F. D. 1966. Mortality of Aedes sollicitans Walker due to a high microfilaremia of dog heartworm, Dirofilaria immitis (Leidy). Proc. N. J. Mosq. Exterm. Ass. 54:128-136.

Belkin, J. N. and Heinemann, S. J. 1975. Psorophora (Janthinosoma) mathesoni, sp. nov. for "varipes" of the Southeastern U.S.A. Mosq. Syst. 7:363-365.

Bemrick, W. J. and Sandholm, H. A. 1966. Aedes vexans and other potential mosquito vectors of Dirofilaria immitis in Minnesota. J. Para. 52:762-765.

Bemrick, W. J. and Moorehouse, D. E. 1968. Potential vectors of Dirofilaria immitis in the Brisbane area of Queensland, Australia. J. Med. Ento. 5:269-272.

Bickley, W. E. 1976. Failure of Culex salinarius to transmit Dirofilaria immitis from dog to dog. Mosq. News 36:366-367.

Bickley, W. E., Mallack, J. and Seeley, D. C. 1976. Filaroid nematodes in field-collected mosquitoes in Maryland. Mosq. News 36:92.

Bickley, W. E., Lawrence, R. S., Ward, G. M. and Shillinger 1977. Dog to dog transmission of heartworm by Aedes canadensis. Mosq. News 37:367-372.

Bidlingmayer, W. L. 1970. Mosquito flight paths in relation to the environment. 1. Illumination levels, orientation and resting areas. Ann. Ento. Soc. Am. 64:1121-1123.

Breinl, A. 1921. Preliminary note on the development of the larvae of Dirofilaria immitis in dog fleas Ctenocephalus felix and canis. Ann. Trop. Med. and Parasitol. 14:390-391.

Brine, J. S., Finlay-Jones, L. R. and Charters, A. D. 1971. Pulmonary dirofilariasis - a case in western Australia. Aus. Med. Journal 2:1238-1241.

Brown, H. W. and Sheldon, A. J. 1940. Natural infection of fleas with dog heartworm (Dirofilaria immitis). N. Amer. Vet. 21:230-231.

Bryan, J. H., Oothman, P., Andrews, B. J. and McGreevy, P. B. 1974. Effect of pharyngeal armature of mosquitoes on microfilariae of Brugia pahangi. Trans. Roy. Soc. Trop. Med. Hyg. 68:14.

Burton, G. T. 1963. Wuchereria bancrofti and Dirofilaria immitis larvae in palpi and antennae of vector mosquitoes. J. Parasitol. 49:771-772.

Buxton, B. A. and Mullen, G. R. 1980. Field isolations of Dirofilaria from mosquitoes in Alabama. J. Parasitol. 66:140-144.

Buxton, B. A. and Mullen, G. R. 1981. Comparative susceptibility of four strains of Aedes aegypti (Diptera: Culicidae) to infection with Dirofilaria immitis. J. Med. Ento. 18:434-440.

Cabrera, B. D. 1968. Determination of mosquito vector of Filariasis in Southern Sulu. J. Phillip. Med. Ass. 44:117-124.

Chellappah, W. T. and Chellappah, G. R., Jr. 1968. Susceptibility of four common Singapore mosquitoes to Dirofilaria immitis. J. Med. Ento. 5:358-361.

Cheong, W. H., Mak, J. W., Naibu, S. and Mohadevan, S. 1981. Armigeres subalbatus incriminated as an important vector of the dog heartworm Dirofilaria immitis and the bird Cardiofilaria in urban Kuala Lumpur. Southeast Asian J. of Trop. Med. and Public Health 12(4):611.

Christensen, B. M. and Andrews, W. N. 1976. Infection of Aedes trivittatus (COQ) with Dirofilaria immitis in Central Iowa. J. Parasitol. 62:276-279.

Christensen, B. M. 1977a. Laboratory studies on the development and transmission of Dirofilaria immitis by Aedes trivittatus. Mosq. News 37(3):367-372.

Christensen, B. M. 1977b. Biology of Dirofilaria immitis (Leidy 1856) (Nematoda:Onchocercidae) and Aedes trivittatus (Coguillett 1902) in Central Iowa. PhD Thesis, Iowa State University, Ames, Iowa.

Christensen, B. M. 1978. Dirofilaria immitis: Effect on the longevity of Aedes trivittatus. Exp. Parasitol. 44:116-123.

Christensen, B. M. 1981. Effect of Dirofilaria immitis on the fecundity of Aedes trivittatus. Mosq. News 41(1):78-81.

Ciferri, F. 1982. Human pulmonary dirofilariasis in the West (California USA). California Vet. 35:23-26.

Coffin, D. L. 1944. A case of Dirofilaria immitis infection in a captive-bred timber wolf (Canis occidentalis, Richardson). N. Amer. Vet. 25:611-612.

Courtney, C. C. and Christensen, B. M. 1983. Field isolation of filarial worms presumed to be Dirofilaris immitis. Mosq. News 43:366-367.

Crans, W. J. and Feldlaufer, M. F. 1974. The likely mosquito vectors of dog heartworm in a coastal area of southern New Jersey. Annu. Conf. Calif. Mosq. Cont. Ass. 42:168.

Crowell, W. A., Klei, T. R., Hall, D. I., Smith, N. K. and Newson, J. D. 1977. Occurrence of Dirofilaria immitis and associated pathology in coyotes and foxes from Louisiana. In Proceedings of the Heartworm Symposium March 18-20, 1977. Atlanta, Georgia U.S.A. Edited by G. F. Otto, Bonner Springs, Kansas. Vet. Med. Publ. Co.

Darsie, R. F. and Ward, R. A. 1981. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. Amer. Mosq. Control Assoc. 313 pp.

Dissanaike, A. S., Ramalinham, S., Fong, A., Pathmayokan, S., Thomas, V. and Kan, S. P. 1977. Filaria in the vitreous of the eye of man in Peninsular Malaysia. Am. J. Trop. Med. and Hyg. 26(6):1143-1147.

East African Institute of Malaria and Vector-Borne Diseases. 1984. Development of two filarial parasites in the mosquitoes Anopheles tenebrosus, Taeniorynchus (Mansonioides) africanus and Taeniorynchus (Mansonioides) uniformis. pp. 150-157.

Edman, J. D. 1971. Host feeding patterns of Florida mosquitoes: Aedes, Anopheles, Coquillettidia, Mansonia and Psorophora. J. Med. Ento. 8:687-695.

Erickson, A. B. 1944. Helminths of Minnesota Canidae in relation to food habits, and a host list and key to the species reported from North America. Amer. Midl. Natural. 32:358-372.

Esslinger, J. H. 1962. Behavior of microfilariae of Brugia pahangi in Anopheles quadrimaculatus. Amer. J. Trop. Med. Hyg. 11:749-758.

Estrada, J. P. 1965. A report of Dirofilaria immitis in Sorsogon Province, Philippines. J. Phillip. Med. Ass. 41:191-196.

Ewert, A. 1965. Comparative migration of microfilariae and development of Brugia pahangi in various mosquitoes. Amer. J. Trop. Med. Hyg. 14:254-259.

Eyles, D. E. 1954. Prevalence of Dirofilaria immitis in Memphis, Tennessee. J. Parasitol. 40:216-221.

Faries, F. C., Mainster, M. E. and Martin, P. W. 1974. Incidental findings of Dirofilaria immitis in domestic cats. Vet. Med. and Small Animal Clinician 69:599-600.

Faust, E. C., Thomas, E. P. and Jones, J. 1941. Discovery of human heartworm infection in New Orleans. J. Parasitol. 27:115-122.

Feld, H. 1973. Dirofilaria immitis (dog heartworm) as a cause of pulmonary lesion in man. Radiology 108:311-312.

Feng, L. C. 1930. Experiments with Dirofilaria immitis and local species of mosquitoes in Peiping, North China, with a note on Lankesteria culicis found in Aedes koreicus. Ann. Trop. Med. 24:347-366.

Foil, L. and Orihel, T. C. 1975. Dirofilaria immitis (Leidy 1856) in the beaver Castor canadensis. J. Parasitol. 61:433.

Forrester, D. J., Jackson, R. F., Miller, J. F. and Townsend, B. C. 1973. Heartworm in Captive California Sea Lions. J. Amer. Vet. Med. Assoc. 163(6):568-570.

Frimeth, J. P. and Arai, H. P. 1983. Some potential mosquito vectors of the canine heartworm, Dirofilaria immitis, in the Calgari region of southern Alberta. Can. J. of Zoology 61(5):1156-1158.

Gershwin, L. J., Gershwin, M. E. and Kritzman, J. 1974. Human pulmonary dirofilariasis. Chest 66:92-97.

Gier, H. T. and Ameel, D. J. 1959. Parasites and diseases of Kansas coyotes. Kansas State Univ. Agr. Expt. Sta. Tech. Bull. 91, 34 pp.

Gordon, R. M. and Lumsden, W. H. R. 1939. A study of the behavior of the mouthparts of mosquitoes when taking up blood from living tissue: together with some observations on the ingestion of microfilariae. Ann. Trop. Med. Parasitol. 32:259-278.

Graham, J. M. 1975. Filariasis in coyotes from Kansas and Colorado. J. Parasitol. 61:513-516.

Grassi, B. 1888. Beitrage zur Kenntniss des Entwicklungscyslus von funf Parasiten des Hundes (Taenia cucumerina Goeze; Ascaris marginata Rud.; Spirometra sanguinolenta Rud.; Filaria immitis Leidy und Haematozoon Lewis) Centralbl. f. Bakteriol. Parasitenk. 4:609-620.

Grassi, B. and Calandruccio, S. 1890. Uber Haematozoon Lewis. Entwicklungscyklus einer Filaria (Filaria recondita Grassi) des Hundes. Centralbl. f. Bakteriol. Parasitenk. 7:18-26.

Grassi, B. and Noe, G. 1900. The propagation of the filaria of the blood exclusively by means of the puncture of peculiar mosquitoes. Brit. Med. J. 2079:1306-1307.

Greene, B. M. 1974. Microfilaremia in a patient with Systemic Lupus Erythematosus: case report. In Proceedings of the heartworm symposium '74. Edited by Morgan, H. C., Otto, G., Jackson, R. F. and Jackson, W. F., V. M. Publ. Inc. Bonner Springs, Kansas. 162 pp.

Hacker, C. S. 1971. The differential effect of Plasmodium gallinaceum on the fecundity of several strains of Aedes aegypti. J. Invert. Pathol. 18:373-377.

Harwood, R. F. and James, M. T. 1979. Entomology in Human and Animal Health. 2nd Edition, Macmillan Publ. Co., Inc. New York. 548 pp.

Hawking, F. 1956. The periodicity of microfilaria. IV. Stimuli affecting the migration of the microfilaria of Dirofilaria aethiops, D. immitis, D. repens, Dipetalonema blanci and Litomosoides carinii. Trans. Roy. Soc. Trop. Med. and Hyg. 50:397-417.

Hawking, F. 1962. The role of the spleen in controlling the number of microfilaria Dirofilaria immitis, D. repens, Litomosoides carinii and Dipetalonema witei in the blood. Ann. Trop. Med. Parasitol. 56:168-172.

Hawking, F. 1967. The 24 hour periodicity of microfilariae: biological mechanisms responsible for its production and control. Roy. Soc. (London) Proc., Ser. B., Biol. Sci. 169:59-76.

Hawking, F. and Worms, M. 1961. Transmission of filarid nematodes. Ann. Rev. Ento. 6:413-429.

Hendrix, C. M., Bemrick, W. J. and Schlotthauer, J. C. 1980. Natural transmission of Dirofilaria immitis by Aedes vexans. Am. J. Vet. Res. 41:1253-1254.

Highby, P. R. 1943. Mosquito vectors and larval development of Dipetalonema arbuta Highby (Nematoda) from the porcupine, Erethizon dorsatum. J. Parasitol. 29:243-252.

Hinman, E. H. 1935. Studies in dog heartworm, Dirofilaria immitis with special reference to filarial periodicity. Amer. J. Trop. Med. 15:371-383.

Hockmeyer, W. T., Schiefer, B. A., Redington, B. C. and Eldrigde, B. F. 1975. Brugia pahangi: Effects upon the flight capability of Aedes aegypti. Exp. Parasitol. 38:1-5.

Hombs, K. V. and Ewing, S. A. 1980. An update on heartworm prevalence in Oklahoma. Okla. Vet. 32:124-125.

Hu, S. M. K. 1931. Studies on host-parasite relationships of Dirofilaria immitis Leidy and its culicine intermediate hosts. Amer. J. Hyg. 14:614-629.

Inoue, T. 1937. Development of Dirofilaria immitis in nine species of mosquitoes in Tokyo. Keio Igaku 17:1097-1128.

Intermill, R. W. 1973. Development of Dirofilaria immitis in Aedes triseriatus. Mosq. News 33:176-181.

Jankowski, T. J. and Bickley, W. E. 1976. The mosquitoes Aedes canadensis and Aedes vexans, as potential vectors of Dirofilaria immitis in Maryland. Ann. Ento. Soc. Amer. 69:781-783.

Javadian, E. and Macdonald, W. W. 1974. The effect of infection with Brugia pahangi and Dirofilaria repens on the egg production of Aedes aegypti. Ann. Trop. Med. and Parasitol. 68:477-481.

Johnson, D. O., Gould, D. J., Tanlicharoenyos, P., Diggs, C. L. and Wooding, W. L. 1970. Experimental infections of gibbons with Dirofilaria immitis. Trans. Roy. Soc. Trop. Med. and Hyg. 64:937-938.

Jordan, H. E. 1982. Dept. Vet. Parasitol. Micro. Publ. Health. Okla. State Univ. Personal Communication.

Joyeux, C. and Sauter, J. 1938. Nouvelles recherches sur Dirofilaria immitis (Leidy). Bull. Soc. Pathol. Exot. 31:122-123.

Kahn, F. W., Western, S. M. and Agger, W. A. 1983. Pulmonary dirofilariasis and transitional cell carcinoma. Arch. of Int. Med. 143(6):1259-1260.

Kartman, L. 1953a. Effect of feeding mosquitoes upon dogs with differential microfilaraemias. J. Parasitol. 39:572.

Kartman, L. 1953b. Factors influencing infection of the mosquito with Dirofilaria immitis (Leidy, 1856). Exp. Parasitol. 2:27-78.

Kartman, L. 1953c. An observation on the loss of microfilaria from the mosquito host during its infective blood meal. J. Parasitol. 39:571-572.

Keegan, H. L., Fitzgerald, C. M., McCrary, T. L. and Doyles, M. D. 1968. Studies on dog heartworm in south Texas. Texas Reports on Biology and Medicine 26:321-330.

Kershaw, W. E., Laviopierre, M. F. and Breesley, W. N. 1955. Studies on the intake of microfilariae by their insect vectors, their survival and their effect on the survival of their vectors. VII. Further observations of the intake of the microfilariae of Dirofilaria immitis by Aedes aegypti in laboratory conditions: The pattern of the uptake of a group of flies. Ann. Trop. Med. and Parasitol. 49:203-211.

Klein, J. B. and Stoddard, E. D. 1977. Dirofilaria immitis recovered from a horse. J. Amer. Vet. Med. Assoc. 171(4):354-355.

Knott, J. 1939. A method for making microfilaria surveys on dog blood. Tr. Roy. Soc. Trop. Med. 33:191-196.

Kocan, A. A. and Laubach, H. E. 1976. Dirofilaria immitis and Dipetalonema reconditum infections in Oklahoma dogs. J. Amer. Vet. Med. Assoc. 168:419-420.

Kotani, T. and Powers, K. G. 1982. Developmental stages of Dirofilaria immitis in the dog. Amer. J. Vet. Res. 43:2199-2206.

Kosuge, I. 1924. Beitrage zur Biologie der Mikrofilaria immitis. Arch. Schiffs-u Tropenhyb. 28:340-351.

Kume, S. and Itagaki, S. 1955. On the life cycle of Dirofilaria immitis in the dog as the final host. Brit. Vet. J. 111:16-24.

Kume, S. 1974. Experimental observations of seasonal periodicity of microfilariae. In Proceedings of the heartworm symposium '74. Edited by H. C. Morgan, G. Otto, R. F. Jackson and W. F. Jackson. V. M. Publ., Inc. Bonner Springs, Kansas. 162 pp.

Kutz, F. W. and Dobson, R. C. 1974. Effect of temperature on the development of Dirofilaria immitis (Leidy) in Anopheles quadri maculatus Say and on vector mortality resulting from this development. Ann. Ento. Soc. Amer. 67:325-331.

Laurence, B. R. and Pester, F. R. N. 1967. Adaptation of filarial worms Brugia patei to a new mosquito host, Aedes togoi. J. Helm. 41:365-392.

Lavoipierre, M. M. J. 1958. Studies on the host-parasite relationships of filarial nematodes and their arthropod hosts. II. The arthropod as a host to the nematode: A brief appraisal of our present knowledge based on a study of the more important literature from 1878-1955. Ann. Trop. Med. and Parasitol. 52:326-345.

Leidy, J. 1850. Description of three Filariinae. Proc. Acad. Nat. Sci., Phila. 5:117-118.

Leidy, J. 1856a. Worms in the heart of a dog. Proc. Acad. Nat. Sci., Phila. 8:2.

Leidy, J. 1856b. A description of Entozoa and some of their ectocongeners observed by the author. Proc. Acad. Nat. Sci., Phila. 8:55.

Lewandowski, H. B. 1977. Determination of the important vectors of dog heartworm in Michigan. PhD Thesis, Michigan State Univ.

Lewandowski, H. B., Hooper, G. R. and Newson, H. D. 1980. Determination of some important natural potential vectors of dog heartworm in Central Michigan. Mosq. News 40:73-78.

Lipke, H. 1975. Melanin in host-parasite interaction. In Invertebrate Immunity. Edited by K. Maramorosch and R. E. Shope. Academic Press, New York. 365 pp.

Ludlam, K. W., Jachowski, L. A. and Otto, G. F. 1970. Potential Vectors of *Dirofilaria immitis*. J. Amer. Vet. Med. Assoc. 157:1354-1358.

Macdonald, W. W. 1962a. The selection of a strain of *Aedes aegypti* susceptible to infection with semi-periodic *Brugia malayi*. Ann. Trop. Med. Parasitol. 56:368-372.

Macdonald, W. W. 1962b. The genetic basis of susceptibility to infection with semi-periodic *Brugia malayi* in *Aedes aegypti*. Ann. Trop. Med. Parasitol. 56:373-382.

Macdonald, W. W. 1963. Further studies on a strain of *Aedes aegypti* susceptible to infection with sub-periodic *Brugia malayi*. Ann. Trop. Med. Parasitol. 57:452-460.

Macdonald, W. W. and Ramachandran, C. P. 1965. The influence of the gene f^m (filarial susceptibility, *Brugia malayi*) on the susceptibility of *Aedes aegypti* to seven strains of *Brugia*, *Wuchereria* and *Dirofilaria*. Ann. Trop. Med. Parasitol. 59:64-73.

Macdonald, W. W. 1976. Mosquito genetics in relation to filarial infections. Symp. Brit. Soc. Parasitol. 14:1-24.

Magnarelli, L. A. 1978. *Dirofilaria immitis* infection in mosquitoes. J. Med. Ento. 15:84-85.

Magoon, E. H. 1935. A portable stable trap for capturing mosquitoes. Bull. Ento. Res. 26:363-373.

Manson, P. 1878. On the development of *Filaria sanguinis hominis*, and on the mosquito considered as a nurse. J. Linn. Soc. Zool. London 14:304-311.

McGreevy, P. B., Theis, J. H., Lavoipierre, M. M. J. and Clark, J. 1974. Studies on filariasis. III. *Dirofilaria immitis*: emergence of infective larvae from mouth parts of *Aedes aegypti*. J. Helminth. 48:221-228.

McGreevy, P. B., McClelland, G. A. H. and Lavoipierre, M. M. J. 1974. Inheritance of susceptibility to *Dirofilaria immitis* infection in *Aedes aegypti*. Ann. Trop. Med. and Parasitol. 68(1):97-109.

Medway, W. and Wieland, T. C. 1975. *Dirofilaria immitis* in a harbor seal. J. Amer. Vet. Med. Assoc. 167:549-550.

Moorehouse, D. E., Abrahams, E. W. and Stephens, B. J. 1971. Human pulmonary dirofilariasis in Queensland. Aus. Med. J. 2:1230-1233.

Moorehouse, D. E., Abrahams, E. W., Stephens, B. J. 1976. Human pulmonary dirofilariasis associated with pleural effusion. Aus. Med. J. 2(24):902-903.

Monson, R. A., Stone, W. B. and Weber, B. L. 1973. Heartworm in foxes and wild canids in New York. N. Y. Fish Game J. 20:48-53.

Mosha, F. W. and Magayuka, S. A. 1979. Potential vectors and non-vectors of bancroftian filariasis in East Africa. East African Med. Journal 56(5):197-202.

Nappi, A. J. 1975. Parasite encapsulation in insects. In Invertebrate Immunity. Edited by K. Maramorosch and R. E. Shope. Academic Press, New York. 365 pp.

Nayar, J. K. and Sauerman, D. M. 1975. Physiological basis of susceptibility of Florida mosquitoes to Dirofilaria immitis. J. Insect Physiology 21:1965-1975.

Nelson, G. S. 1959. The identification of infective filarial larvae in mosquitoes: with a note on the species found in "wild" mosquitoes on the Kenya Coast. J. Helm. 33:233-256.

Newmann, L. G. and Maqueen, J. 1905. A treatise on the parasites and parasitic diseases of domesticated animals. Bailliere, Tindall, and Cox, London. 637 pp.

Newton, W. L. and Wright, W. H. 1956. The occurrence of a dog filariid other than Dirofilaria immitis in the United States. J. Parasitol. 42:246-258.

Newton, W. L. 1957. Experimental transmission of the dog heartworm, Dirofilaria immitis, by Anopheles quadrimaculatus. J. Parasitol. 43:589.

Norden News. 1983. Human dirofilariasis, an emerging zoonosis. 58(1):4-8.

Orihel, T. C. 1961. Morphology of the larval stages of Dirofilaria immitis in the dog. J. Parasitol. 47:251-262.

Orihel, T. C. 1975. The peritrophic membrane: Its role as barrier to infection of the arthropod host. In Invertebrate Immunity. Edited by K. Maramorosch and R. E. Shope. Academic Press, New York. 365 pp.

Otto, G. F. 1949. Heartworm in dogs. N. Amer. Vet. 30:181-189.

Otto, G. F. 1969. Geographic distribution, vectors, and life cycle of Dirofilaria immitis. J. Amer. Vet. Med. Assoc. 154:370-373.

Otto, G. F. 1972. Epizootiology of canine heartworm disease. In Canine Heartworm Disease. The Current Knowledge. R. E. Bradley ed. University of Florida, Dept. Vet. Sci. Gainesville, Florida. 148 pp.

Otto, G. F. 1974. Occurrence of heartworm in unusual locations and in unusual hosts. In Proceedings of the heartworm symposium '74. V. M. Publ. Inc. Bonner Springs, Kansas. 162 pp.

Pennington, N. E., Parish, D. W. and Ewing, S. A. 1970. Canine microfilaria survey in northcentral Oklahoma. Okla. Vet. 22:12-13.

Phillips, J. H. 1939. Studies on the transmission of Dirofilaria immitis in Massachusetts. Amer. J. Hyg. 29:121-129.

Pierson, K. K. 1972. Gumma-like pulmonary granulomas due to Dirofilaria spp. in man. In Canine Heartworm Disease. The Current Knowledge, Institute of Food Agric. Science, University of Florida, Gainesville. 148 pp.

Pinger, R. R. and Rowley, W. A. 1975. Host preferences of Aedes trivittatus (Diptera:Culicidae) in central Iowa. Amer. J. Trop. Med. Hyg. 24:889-893.

Pinger, R. R. 1982. Presumed Dirofilaria immitis infections in mosquitoes in Indiana, U.S.A. J. Med. Ento. 19(5):553-555.

Poynton, J. D. and Hodgkin. 1938. Endemic filariasis in the Federated Malaya States. Bull. Inst. Med. Res., F.M.S., 1:1-67.

Raghavan, N. G. S., Das, M., Mammen, M. L., Singh, N. N. and Wattal, B. L. 1967. Genetic basis of differential susceptibility of Aedes aegypti to dirofilarial infection. Part 1: Preliminary observation on selection of Aedes aegypti strains susceptible and refractory to Dirofilaria immitis infection. Bull. Ind. Soc. Malaria Comm. Dis. 4:318-323.

Raillet, A. and Henry, A. 1911. Sur une filaire peritoneale des porcins. Bull. Soc. Path. Exot. 4:386-389.

Ramachandran, C. P. 1966. Biological aspects in the transmission of Brugia malayi by Aedes aegypti in the laboratory. J. Med. Ento. 3:239-252.

Ramalingam, S. 1968. The epidemiology of filarial transmission in Samo and Tonga. Ann. Trop. Med. 62:305-324.

Rogers, J. S. and Newson, H. D. 1979. Comparisons of Aedes hendersonii and Aedes triseriatus as potential vectors of Dirofilaria immitis. Mosq. News 39(3):463-466.

Rosario, F. del. 1936. Dirofilaria immitis and its culicine intermediate hosts in Manila island. Philippine J. of Science 60:45-55.

Rosen, L. 1954. Observations on Dirofilaria immitis in French Oceania. Ann. Trop. Med. Parasitol. 48:318-328.

Rouband, E. and Collas-Belcour, J. 1937. Nouvelles recherches sur le' volution experimentale de Dirofilaria immitis chez quelques culicides indigenes. Bull. Soc. Path. Exot. 30:480-484.

Salt, G. 1970. The cellular defense reactions of insects. Cambridge Univ. Press. London. 111 pp.

Sawyer, T. K. 1965. Molting and exsheathment in vitro of third-stage larvae of Dirofilaria immitis. J. Parasitol. 51:1016-1017.

Sawyer, T. K. and Weinstein, P. P. 1965. Third molt of Dirofilaria immitis in vitro and in vivo. J. Parasitol. 51:48.

Sawyer, T. K. 1974. Seasonal fluctuations of microfilariae in two dogs naturally infected with Dirofilaria immitis. In Proceedings of the heartworm symposium '74. Edited by H. C. Morgan, G. Otto, R. F. Jackson, and W. F. Jackson. V. M. Publishing, Inc., Bonner Springs, Kansas. 162 pp.

Schlotthauer, J. C. 1864. Dirofilaria immitis in the red fox Vulpes fulva in Minnesota. J. Parasitol. 50:801-802.

Seeley, D. C. and Bickley, W. E. 1974. Culex salinarius as a potential vector of Dirofilaria immitis (Leidy). Calif. Mosq. Control Assoc. Proc. 42:87-92.

Simmons, C. F., Winter, H. S., Berde, C., Schrater, F., Humphrey, G. B., Rosen, F. S., Beaver, P. L. and Weller, P. B. 1984. Zoonotic filariasis with Lymphedema in an immunodeficient infant. New Eng. J. Med. 310:1243-1245.

Stojanovich, C. J. 1961. Illustrated key to common mosquitoes of southeastern United States. Atlanta, Ga., 49 pp.

Steuben, E. B. 1954. Larval development of Dirofilaria immitis (Leidy) in fleas. J. Parasitol. 40:580-589.

Stuht, J. N. and Youatt, W. G. 1972. Heartworms and lung flukes from red foxes in Michigan. J. Wildlife Management 36:166-170.

Sulaiman, I. and Townson, H. 1980. The genetic basis of susceptibility to infection with Dirofilaria immitis in Aedes aegypti. Ann. Trop. Med. and Parasitol. 74(6):635-644.

Summers, W. A. 1940. Fleas as acceptable intermediate hosts of the dog heartworm, Dirofilaria immitis. Proc. Soc. Exp. Biol. Med. 43:448-450.

Summers, W. A. 1942. Experimental studies on the larval development of Dirofilaria immitis in certain insects. Amer. J. Hyg. 37:173-178.

Symes, C. B. 1960. A note on Dirofilaria immitis and its vectors in Fiji. J. Helminth. 34:39-42.

Taylor, A. E. R. 1960. The development of Dirofilaria immitis in the mosquito Aedes aegypti. J. Helminth. 34:27-38.

Tempelis, C. H. 1975. Host-feeding patterns of mosquitoes with a review of advances in analysis of blood meals by serology. J. Med. Ento. 11:635-653.

Thurman, J. D., Johnson, B. J. and Lichtenfels, J. R. 1984. Dirofilariasis with arteriosclerosis in a horse. J. Amer. Vet. Med. Assoc. 185(5):532-533.

Todaro, W. S., Morris, C. D. and Heacock, N. A. 1977. Dirofilaria immitis and its potential mosquito vectors in central New York State. Am. J. Vet. Res. 38(8):1197-1200.

Tolbert, R. H. and Johnson, W. E. 1982. Potential vectors of Dirofilaria immitis in Macon County, Alabama. Amer. J. Vet. Res. 43:2054-2056.

Tongson, M. S. and Romero, F. R. 1962. Observations on the periodicity of Dirofilaria immitis in the peripheral circulation of the dog. Brit. Vet. J. 118:299-304.

Townson, H. 1971. The effect of infection with Brugia pahangi on the flight of Aedes aegypti. Ann. Trop. Med. Parasitol. 64:411-420.

Travis, B. V. 1947. Relative efficiency of six species of mosquitoes from Guam as developmental hosts of Dirofilaria immitis. J. Parasitol. 33:142-145.

Tuniguchi, M., Kume, S., Mitsuteru, K. and Itagaki, S. 1944. Studies on Dirofilaria immitis. Report No. 7. Ticks, lice and fleas as possible intermediate hosts of Dirofilaria immitis. Oyo Juigaku Zasshi 17:85-112.

Ungureanu, E. M. 1972. Methods for dissecting dry insects and insects preserved in fixative solutions or by refrigeration. Bull. W. H. O. 47:239-244.

Villavaso, E. J. and Steelman, C. D. 1970. Laboratory and field studies of the southern house mosquito Culex pipiens quinquefasciatus Say, infected with dog heartworm Dirofilaria immitis (Leidy) in Louisiana. J. Med. Ento. 7:471-476.

Walters, L. L. and Lavoipierre, M. M. J. 1982. Aedes vexans and Aedes sierrensis (Diptera: Culicidae): potential vectors of Dirofilaria immitis in Tehama County, Northern California, U.S.A. J. Med. Ento. 19:15-23.

Webber, W. A. F. and Hawking, F. 1955. Experimental maintenance of Dirofilaria immitis and D. repens in dogs. Exp. Parasitol. 4:143-164.

Weinmann, C. J. and Garcia, R. 1974. Canine heartworm in California, with observations on *Aedes sierrensis* as potential vector. Calif. Vector News 21:45-50.

Wharton, R. H. 1962. The biology of *Mansonia* mosquitoes in relation to the transmission of filariasis in Malaya. Bull. Inst. Med. Res. Fed. Mal. 11:1-114.

Wright, R. E. and Knight, K. L. 1966. Effect of environmental factors on biting activity of *Aedes vexans* Meigen and *Ae. trivittatus* Coquillett. Mosq. News 26:465-478.

Wulker, W. 1964. Parasite-induced changes of internal and external sex characters in insects. Exp. Parasitol. 15:561-597.

Yen, C. 1938. Studies on *Dirofilaria immitis* Leidy with special reference to the susceptibility of some Minnesota species of mosquitoes to the infection. J. Parasitol. 24:189-205.

Yeoli, M., Upmanis, R. S. and Most, H. 1964. Studies on filariasis. III. Partial growth of the mammalian stages of *Dirofilaria immitis* in vitro. Exp. Parasitol. 58:325-334.

Yoshimura, H., Akao, N., Kondo, K. and Ohnishi, Y. 1980. Human Dirofilariasis in Japan - case report and review of literature. Int. J. of Zoonosis 7:143-147.

APPENDIXES

APPENDIX A

FORM USED TO RECORD INFORMATION REGARDING EACH MOSQUITO DISSECTED

Date of Collection: ________________ Date of Mosquito Death: ________________

Mosquito species: ____________________________________

Gravid: Yes ___1 No _____2

Positive __1 Negative __2

Larval stage in the mosquito:

Microfilaria Sausage stage 2nd stage 3rd stage

___1 ___2 ___3 ___4

Location of microfilaria in the mosquito:

Abdomen Thorax Head Region Proboscis

___1 ___2 ___3 ___4

Number of Parasites: ________________

Source of mosquito:

___1 light trap ___2 magoon trap

Data were recorded with this standard format to facilitate transfer to punch cards and storage in Oklahoma State University I.B.M. Main Frame Computer.

APPENDIX B

PHOTOGRAPHS OF *DIROFILARIA IMMITIS* LARVAE RECOVERED FROM SELECTED MOSQUITOES CAPTURED IN SCREEN CAGE BAITED WITH A DIROFILAREMIC DOG

Figure 3. Microfilaria of Dirofilaria immitis Recovered from the Gut of a Mosquito Captured in Screen Cage Baited with a Dirofilaremic Dog.

Figure 4. Sausage-stage Larvae of Dirofilaria immitis Recovered from the Gut of a Mosquito Captured in Screen Cage Baited with a Dirofilaremic Dog.

Figure 5. Second-stage Larva of *Dirofilaria immitis* Recovered from the Gut of a Mosquito Captured in Screen Cage Baited with a Dirofilaremic Dog.

Notice eggs of *Anopheles quadrimaculatus*.

Figure 6. Third-stage Larva of *Dirofilaria immitis* Recovered from *Anopheles quadrimaculatus* Captured in Screen Cage Baited with a Dirofilaremic Dog.

Notice egg of *Anopheles quadrimaculatus*.

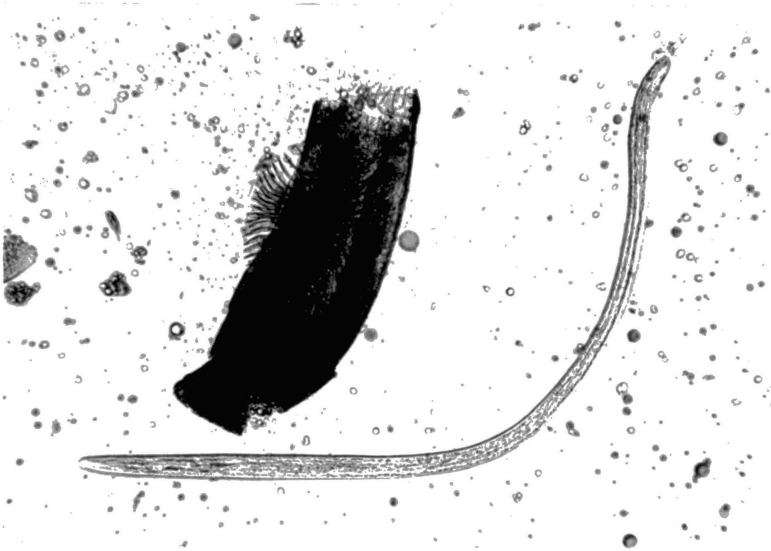
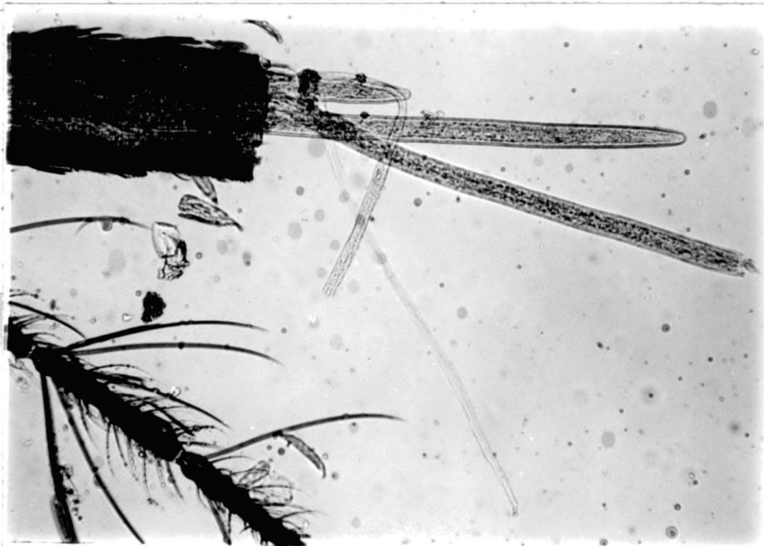


Figure 7. Third-stage Larvae of *Dirofilaria immitis* Protruding from the Dissected Proboscis of a Mosquito Captured in Screen Cage Baited with a Dirofilaremic Dog.

Notice also a portion of an antenna of the host mosquito.



VITA 2

Julius Sunday Afolabi

Candidate for the Degree of

Doctor of Philosophy

Thesis: VECTORS OF NEWLY ENDEMIC CANINE DIROFILARIASIS IN STILLWATER, OKLAHOMA

Major Field: Veterinary Parasitology and Public Health

Biographical:

Personal Data: Born in Egbe, Kwara State, Nigeria, January 10, 1948, the son of the late Mr. Orisagbemi Afolabi and Mrs. Ige Afolabi.

Education: Graduated from E. C. W. A. Primary School, Odo-Ere, Kwara State, Nigeria in 1961. Received Dispensary Assistant (Physician Associate) certificate from S. I. M. Medical Auxiliary Training School in 1967. Attended Advanced Course for Dispensary Assistants at S. I. M. Medical Auxiliary Training School from June 1972 to May 1973. Passed London University General Certificate of Education O' Level in Biology, Human Anatomy and Physiology, Chemistry, Economics of West Africa, West African Government and Religious Knowledge; received Bachelor of Science degree from Bethany Nazarene College, Bethany, Oklahoma, in 1978 with a major in Biological Sciences; received the Master of Public Health degree from the University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, in 1980 with a major in Epidemiology; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in May, 1985.

Professional Experience: Dispensary Assistant (Physician Assistant) 1968-1972; Teaching Assistant at S. I. M. Medical Auxiliary Training School, 1972-1973; Emergency, Intensive Care Unit and Operating Room technician at Deaconess Hospital, Oklahoma City, Oklahoma, 1975-1981.

Professional Associations: Nigerian Public Health Association, Northern Nigeria Rural Health Workers Association; Oklahoma Academy of Sciences, Southwestern Association of Parasitologists and American Society of Parasitologists.