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Incidence of Dirofilaria immitis and Dipetalonema reconditum in Illinois and a Study of Potential Mosquito Vectors of Dirofilaria immitis

Bolaji Nelson Akande
Loyola University Chicago

INCIDENCE OF *DIROFILARIA IMMITIS* AND *DIPETALONEMA RECONDITUM* IN ILLINOIS AND A STUDY OF POTENTIAL MOSQUITO VECTORS OF *DIROFILARIA IMMITIS*

by

Bolaji Nelson Akande

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VITA

Bolaji Nelson Akande, son of Afolabi Akande and Olayinka Akande, was born on April 9, 1950.

After graduation from United Nations African School in 1964, he attended Ibadan Boys High School from 1965-1969. He received a Bachelor's Degree in Biology in 1975 from Loyola University. From 1969-72 he worked at Standard Oil Company; 1973-74 at St. Francis Hospital; and 1974-77 at AMCord Corporation.

In 1977, he joined American Heartworm Society which is an organization for veterinarians, parasitologists and other scientists who have an active interest in disease associated with the dog heartworm.

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INTRODUCTION

Dirofilaria immitis (Leidy, 1856) and Dipetalonema reconditum (Grassi, 1890), are parasite nematodes. The D. immitis is transmitted to the definitive host by the mosquito, and D. reconditum by the flea. The definitive host for both parasites is primarily the dog.

The developmental cycle of D. immitis was worked out by Taylor (1960). He found that the parasite develops in the Malpighian tubule of the mosquito. There have been some controversy as to where the developmental cycle of D. reconditum really takes place. According to Breinl (1921), Brown and Sheldon (1940), the developmental cycle took place in the Malpighian tubule. Summer (1940) reported both prelarva and developing larvae in the abdominal haemocoeles of the flea.

Many species of mosquitoes have been reported as vectors of the dog heartworm. As cited in Ludlam et al. (1970), it has been shown that 63 species of mosquitoes can become infected with D. immitis larvae.

The dog heartworm was at one time thought to have been confined to the southern region of the United States. The infection has also been recognized in the Middle Atlantic States and in the Midwest. Lillis (1964) reported an incidence of dirofilariasis of 9.3% in dogs from South-Central New Jersey, and Jaskoski (1974) also reported an

incidence of canine dirofilariasis of over 10% in the northern suburbs of Chicago.

The objectives of the present investigation are:

1. To determine the incidence of Dirofilaria immitis and Dipetalonema reconditum in Illinois based on the identification of the blood microfilariae of the parasite in the peripheral blood.

2. To collect, identify and dissect various species of mosquitoes for microfilariae of D. immitis and D. reconditum.

3. To feed mosquitoes on an infected dog in order to find out the species of mosquitoes that are responsible for the transmission of the dog heartworm infection.

LITERATURE REVIEW

Dirofilaria immitis (Leidy, 1856) and Dipetalonema reconditum (Grassi, 1890), are in the phylum Aschelminthes, class Nematoda. The adult female worm of D. immitis is 20-30 cm. long and 1-2 mm. in diameter, white in color, with a smooth integument. The male is slightly smaller and measures 10-16 cm. long, is proportionately narrower and the caudal end is spirally coiled. The adult female of D. reconditum is 2.27 cm. long and 0.15 mm. in diameter. The male is about half the size of the female and measures 1.24 cm. long.

Augustine (1938) reported microfilaremia, attributed to D. immitis in 8 out of 94 dogs from Eastern Massachusetts, although 28 dogs from Wonalacet, New Hampshire were tested and found negative for microfilariae. Thrasher et al. (1963) reported an incidence of 44% Dirofilaria immitis, 2% Dipetalonema reconditum and 0.4% mixed microfilariae infection in 543 New Orleans' dogs. A second survey by Thrasher and Clanton (1968) on 672 Georgia dogs revealed 19.6% infected with D. immitis and 4.7% with D. reconditum microfilariae. Lindsey (1961) examined 410 dogs from Alabama, Georgia and Florida and found 72 (17.5%) infected with D. immitis and 149 (36.3%) with D. reconditum microfilariae. In a later study on Alabama, Georgia and Florida dogs, Lindsey (1962) was able to confirm microfilarial findings by recovery of

the corresponding adults in 98% of 71 dogs examined for D. immitis and 65% of 20 dogs examined for D. reconditum. Rothstein et al. (1961) reported only 1.08% D. immitis and 37.8% with D. reconditum microfilariae in 555 Alabama dogs. Gubler (1966) surveyed 666 dogs in Hawaii and found 32.1% infected with D. immitis and 10.8% with D. reconditum microfilariae. Rabalais and VotoVa (1972) reported an incidence of 5.1% D. immitis, 4.4% D. reconditum and 0.4% mixed microfilariae in 274 Ohio dogs. Zydeck et al. (1970) examined 248 dogs from Detroit, Michigan and found 4 (1.6%) infected with D. immitis and 7 (2.8%) with D. reconditum microfilariae. Marquardt and Fabian (1966) reported 26 (61.9%) of the dogs sampled in Illinois to be infected with D. immitis and 17 (40.5%) with D. reconditum. It should be noted that this incidence for D. reconditum reported by Marquardt and Fabian (1966) is more than anyone has ever cited. The report was not based on statewide incidence since the investigators selectively chose 3 areas in Illinois to observe dog heartworm infection. McKinney (1962) found that 1.4% of the 212 dogs examined in Champaign County, Illinois were infected with both D. immitis and D. reconditum. Noyes (1971) surveyed the changing geographical distribution of heartworm disease in the State of Illinois, and found that heartworm disease had been appearing as a new endemic in some areas of Illinois.

The knowledge of the mosquito's role as host for filarial began with Manson's (1878) observations on the infection of Culex fatigans by Wuchereria bancrofti. Grassi and Noe (1900) first reported that the mosquito might serve as intermediate host for D. immitis. Bradley (1953) reported the complete larva development of D. immitis in Culex pipiens and Culex quinquefasciatus. Bemrick and Moorhouse (1968) observed the complete larva development of D. immitis in Aedes notoscriptus. Bickley (1976) reported the inability of Culex salinarius to transmit D. immitis. Most of these studies have involved experimental infection of mosquitoes in a controlled laboratory environment. Little work has been concerned with the capture of naturally infected mosquitoes. Christensen and Andrews (1976) recently reported natural infection of Aedes trivittatus (COQ) with D. immitis in Central Iowa. Hawkings and Worms (1961) prepared an excellent review of the transmission of filaroid nematodes which includes ingestion of microfilariae by the arthropod vector, development in the body of the arthropod and passage from the arthropod to the definitive host.

Taylor (1960) reported the development of dog heartworm (D. immitis) in the mosquito, Aedes egypti and found that the life cycle of the heartworm requires about 6 to 8 months for completion. Its significant phases are a 15-30 day developmental period in the mosquito, an 80-140 day migratory or intermediate phase in the dog, and the adult

phase in the right side of the heart with accompanying microfilaremia (Fig.1). According to Taylor (1960), ingested microfilariae (Fig.2) migrate from the midgut of the mosquito into the body cavity where they undergo a 3-stage metamorphosis. After about 12-15 days, depending upon environmental factors and the mosquito species involved, the infective third stage larva migrate through the thorax to the labium where they await transmission to the next host upon which the insect feeds. The infective larvae migrate down the proboscis through the labium while the mosquito is taking a blood meal and enters the vertebrate host through the bite wound. It has been shown by Newton and Pratt (1945) and Wharton (1957) that the third stage larva from the abdomen move rapidly to the proboscis when the mosquito starts feeding.

Kume and Itagaki (1949) reported that in the dog, the infective larva migrate through the subcutaneous tissues, connective tissues, muscles, adipose tissues, and reach the right ventricle of the heart in 85-120 days after infection. During this intermediate migratory phase, the worms increase in size from 1.0 mm. to 3.2-11.0 mm. Orihel (1961) infected dogs experimentally and sacrificed them at intervals of 5 to 278 days after inoculation. He recovered developing worms from muscle and other tissue during only the first 67 days, both tissues and heart from 67-80 days, and in heart alone after 90 days. This is a more rapid tissue migration

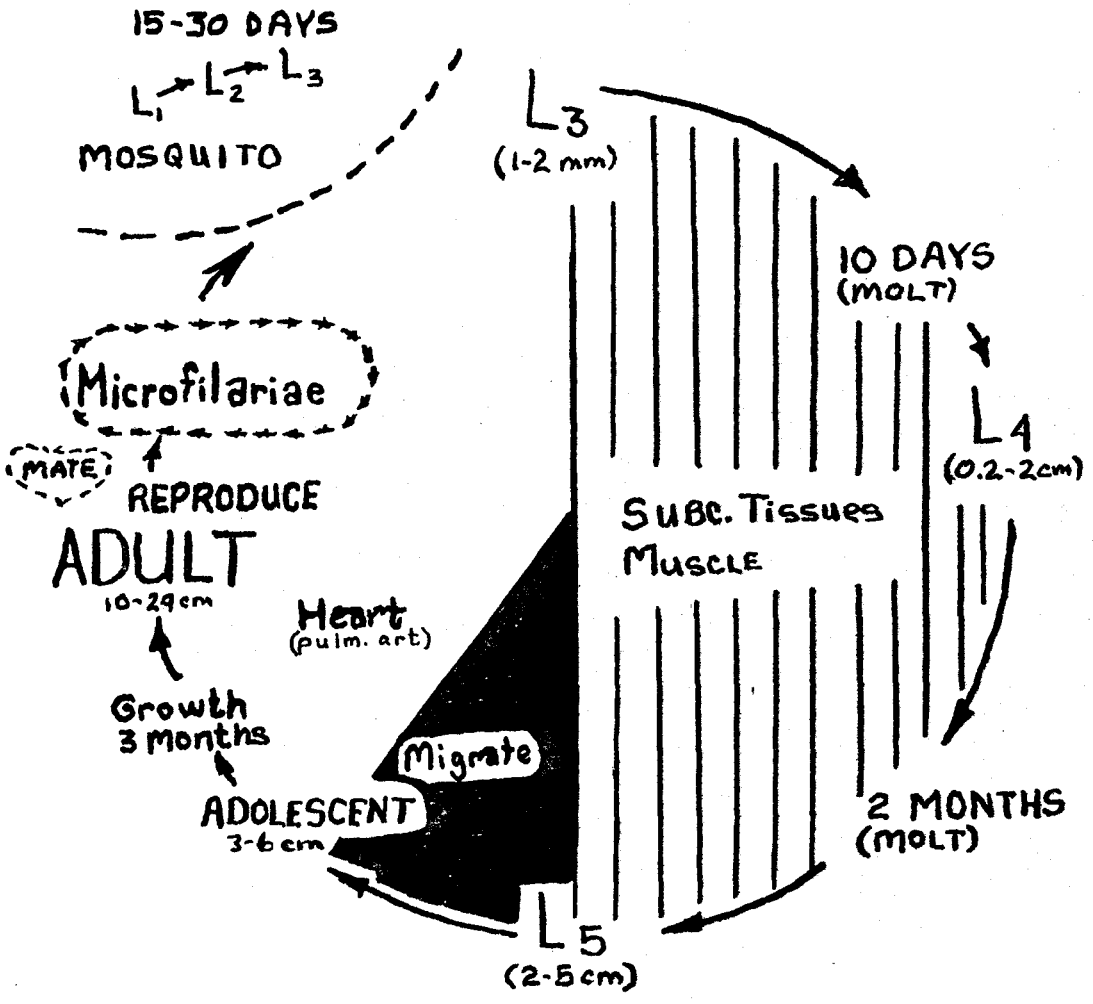

Figure 1

Life Cycle of Dirofilaria immitis.

L_{1-5} = Larva Stages

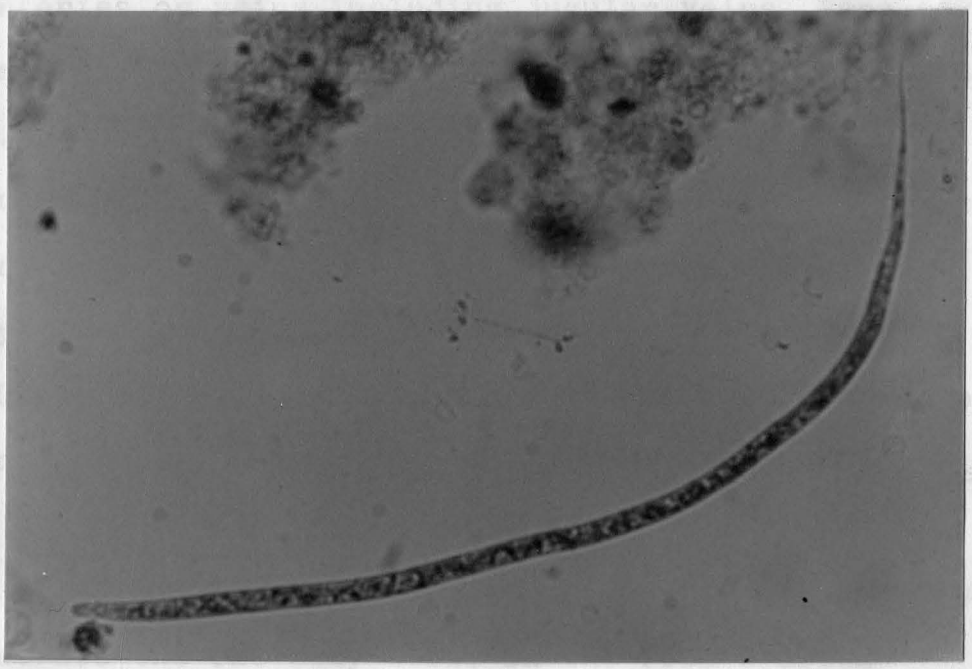

Figure 2

Microfilaria Stained with Methylene Blue

than reported by Kume and Itagaki (1949). Newton and Wright (1956) estimated the prepatent period to be between 7-9 months.

The adult heartworm of D. immitis have a strong affinity for the right heart and pulmonary artery, as was reported by Adcock (1961). They are also found in other areas within the host's body. Liu et al. (1966) reported major arteries or veins including jugular veins, vena cavae and femoral arteries as the occasional location for adult D. immitis. Hongo and Kurokawa (1967) and Donahoe (1974) reported adults in the brains of a dog, and Hayasaki et al. (1970) reported adults in the feces. The worms have a tendency to migrate to any area within the body.

Grassi and Calandruccio (1890) first traced the developmental cycle of D. reconditum in the flea, Ctenophalides canis. They found that after ingestion of microfilariae in a blood meal, the first state larva escape from the midgut and penetrate the fat body cells in the flea's abdomen. According to Steuben (1954), this happens as early as 1 hour after ingestion. The second stage larva develops inside the first cuticle. Completing its development in the cell lumen, the infective third stage larva is ready to be transmitted to the final host. Other species that serve as intermediate host for D. reconditum are Pulex irritans and Heterodoxus spininger.

Dirofilariasis in Man

Although the canine heartworm, Dirofilaria immitis, is known as a common parasite of dogs, it is also a zoonosis, since several human involvements have been reported. According to DeCarneri and Sacchi (1973), almost 100 cases of subcutaneous dirofilariasis in man have been reported throughout the world. Forty were verified as D. immitis infections. In most cases, the parasite affects the subconjunctivae, subcutaneous or soft tissues of the body, they also are found in vital body organs and may be of some importance in causing human illness. D. immitis is an unusual cause of pulmonary "coin" lesion in man. The parasite typically lodges in a small pulmonary artery and becomes surrounded by a small zone of infarction that appears as a "coin" lesion on chest radiographs. Dayal and Neafie (1975) have reviewed reported cases of human pulmonary dirofilariasis, most of which have occurred in the United States. The report lists 41 cases, 39 of which appeared as a "coin" on chest radiographs, and two were unidentified. Most of the reported cases occurred in Florida, but one or more cases occurred in Michigan, Connecticut, Wisconsin and New York. Dashiell (1961) reported the first case of human pulmonary dirofilariasis. Beskins et al. (1966) describes a case where degenerate specimen of the genus Dirofilaria, presumed to be D. immitis were removed from the upper lobe of the left lung of a 48-year-old Louisiana woman. Neafie

et al. (1971) reported 8 cases of human pulmonary dirofilariasis; Lau and Pierson (1972) reported 5 cases. Hoch et al. (1974) also reported two cases of human infection with D. immitis in Philadelphia. The investigators claimed that both patients had frequently visited mosquito-infected coastal areas of New Jersey. In more than 70 percent of the reported cases, there were no significant respiratory complaints, and the lesions were discovered incidentally during radiographic examination of the chest. Among those who had clinically significant complaints, chest pain and cough were most frequent, followed by hemoptysis, fever, and malais. According to Hoch et al. (1974), the infection is believed to be more common in the Eastern Atlantic states than the literature suggests.

Even though one might expect children to be bitten more often by mosquitoes than adults, no cases of D. immitis have been reported in children in the United States (Harrison and Thompson, 1965).

Dirofilariasis in Animals

Dirofilaria immitis is also known to exist in a variety of hosts in addition to the dog. It has been reported in two species of American wolves: in the Florida wolf (Canis floridanus) by Faust (1937), in the buffalo wolf (Canis nubilis) by Klemper and Moschkowitz (1938), in

coyotes (Canis latrans) by Graham (1975) and in the beaver (Castor canadensis) by Foil and Orihel (1975).

Faust (1937) in his review has also noted the occurrence of filariasis in Canis dingo (Australia), Canis brachyurus (Brazil), Canis sp. (Japan), Vulpes vulpes (China), Felis catus domestica (Virginia, U.S.A., Dutch Guiana, China), Felis tigris (French Indo-China).

Records of D. immitis in cats are rare. Tornes and Sambol (1959) reported several adults of D. immitis in the right ventricles of the heart and pulmonary artery of cats in Florida. A case of dirofilariasis in cats in Hawaii was reported by Ash (1962). Since adult worms of D. immitis and microfilariae have been found in cats, the cats may be an acceptable definitive host. If the peripheral blood of cats were examined more often for microfilariae, the heart and pulmonary artery for adult worms, the incidence of dirofilariasis in cats may be found to be higher than is generally accepted.

Differentiation of the Microfilariae of D. Immitis and D. Reconditum

Morphological and physiological characteristics are used to differentiate D. immitis and D. reconditum microfilariae. Newton and Wright (1956) reported an average length and width for D. reconditum to be 276μ and 4.6μ respectively. Lindsey (1961) reported an average length of D. reconditum

as 271μ and average width as 5.2μ, with a much broader range of values found. Notable differences in these values are also found for the microfilariae of D. immitis, with ranges of width values up to .1μ by Cheng (1964). Lindsey (1961), in his research, offered no explanation as to why so little homogeneity was found among the length and width measurement for microfilaria of D. reconditum. Lindsey (1962) also reported the length of D. immitis to range from 298μ to 314μ and D. reconditum from 258μ to 298μ; width of D. immitis ranged from 6.7μ to 6.9μ and D. reconditum from 4.7μ to 5.8μ. According to Sawyer et al. (1963), different methods of handling the blood specimens from dogs may account for the diversifying values. A frequently overlooked fact is that the width measurements should be made consistently at a given location on the microfilariae. Sawyer et al. (1963) reported the level of the excretory pore and Lindsey (1961) also reported a distance of 50μ to 60μ from the anterior extremity as being the preferred location for the width measurements.

Otto and Bauman (1959) believed that microfilariae are most easily differentiated when they are killed and stained in an extended position, as they are said to be in the Modified Knott method. Newton and Wright (1956) found that the tail in over 90 percent of the specimens of Dipetalonema microfilariae ended in a buttonhook while the tails of the microfilariae of D. immitis ended in a straight line.

Sawyer et al. (1965) reported that staining fresh (i.e., undried) blood smears in a 1:50 dilution of 1% brilliant cresol blue will reveal a cephalic hook on microfilariae of D. reconditum and the arrangement of the R-cells in both species. The R-cells of D. immitis are grouped in a 1-2-1 pattern, and those of D. reconditum in a 1-3 pattern.

Schalm and Jain (1966) observed the motility of D. reconditum microfilariae. They found that microfilariae of Dipetalonema are actively motile and tend to move across the field of the microscope. Stein and Lawton (1974) also found that in fresh blood smears, the microfilariae of D. immitis are sluggish and exhibit a slow undulating movement.

Chalifoux and Hunt (1971) observed differences in acid phosphatase activity between the microfilariae of D. immitis and D. reconditum. They concluded that acid phosphatase activity was restricted to two distinct zones in microfilariae of D. immitis (i.e., around the excretory pore and anal pore) while enzyme activity in Dipetalonema was never localized into two distinct zones, but spread uniformly throughout the body of the microfilariae. The difference in acid phosphatase activity between D. immitis and Dipetalonema species appears to offer the most accurate method of differentiation.

MATERIALS AND METHODS

Veterinary Survey

Letters were sent to nine hundred and ninety-eight practicing veterinarians in Illinois counties. Names of veterinarians were chosen at random from a list of Illinois veterinarians supplied by the American Veterinary Medical Association. Each veterinarian was asked the number of dogs tested in his area and the number of positive cases detected. They were also asked to give general information regarding the county of orgin of the infected dogs. The practitioners who agreed to help further were provided with mailing tubes and stamps. They were asked to place 1-2 ml. of blood sample from infected dogs diagnosed positive for D. immitis into the tubes and give general information such as dog's breed, sex, weight and appearance. The tubes were then mailed back to the laboratory for re-examination of the microfilariae. The length and width of at least three microfilariae were measured in each prepared slide.

Random Blood Samples[1]

Forty-four random blood samples from dogs were

[1]Blood samples obtained from dogs without any regard to breed, sex or whether suspected of having heartworm infection.

obtained from Save-A-Pet, 2019 North Rand Road, Palatine, Illinois; fifty-four from Anti-Cruelty Animal Welfare, 157 West Grand Avenue, Chicago, Illinois, and thirteen from visited veterinarians. General information on the dogs was obtained and recorded (Fig.3). All of the dogs were more than 1 year old and represented various breeds and mixture of breeds.

Diagnosis of the Dog Heartworm

The diagnosis of the blood samples from dogs obtained from the veterinarians and dog pounds were made on the basis of finding microfilariae of the parasite in the peripheral blood. The Modified Knott method reported by Kelley (1973) and Stein and Lawton (1974) as being the most sensitive diagnostic method for heartworms was used. 1.0 ml. of blood was added to 9 ml. of 2% formalin and centrifuged for five minutes at 1,500 r.p.m., the supernatant fluid was decanted, and 0.5 ml. of 2% formalin and two drops of methylene blue (1:1,000) were added. After mixing, two 0.1 ml. samples were placed on a glass slide, and the entire area under 22 by 22 mm. coverslips were examined at 10 and 43 magnifications for microfilariae.

This technique combines a number of important advantages. Low density infections can be detected because any microfilariae present are first concentrated in a small

DATE_____

AREA WHERE OBTAINED_____

BREED_____

AGE_____

SEX_____

ORIGIN_____

APPEARANCE (State of Health)_____

APPROXIMATE SIZE_____

DIAGNOSIS_____

COMMENTS:

Figure 3

Sample form for Heartworm Survey

amount of sediment composed mainly of leukocytes and debris of hemolysed red blood cells. This method is simple to perform, inexpensive and highly reliable. According to Stein and Lawton (1974), the method has an average detection rate of 87.5% for both microfilariae and a detection and differentiation rate of 83.5% for D. immitis and 89.3% for D. reconditum. In fact, after rapidly scanning blood specimens at low power (100X) and finding no microfilariae, one can be reasonably sure that the dog is free of filariae.

The wet smear, capillary sedimentation technique, filter technique and histochemical differentiation are some of the methods that could be used for diagnosing and differentiating microfilariae. The wet smear, although rapid and inexpensive, does not differentiate between various species of microfilariae. The capillary sedimentation technique also does not differentiate between various species of microfilariae. The filter technique is rapid insofar as no centrifugation is involved, and less time is required to scan the slide as compared with the Modified Knott technique. The histochemical differentiation in which the acid phosphatase activity between D. immitis and D. reconditum are observed offered the most accurate method of differentiation.

Calibration of an Ocular Stage Micrometer

With the lowest objective in position (i.e. 16X), the

stage micrometer was placed on the stage and focused on its scale. The ruling was in 0.01 mm. units at one end with the remainder divided into 0.1 mm units. The eyepiece was rotated until its scale was superimposed on the stage scale. The stage micrometer was moved so that its zero line was exactly even with that of the eyepiece scale. Another point at which the eyepiece scales and the stage micrometer scales coincide was determined. The spaces on each scale between these points were counted and the actual distance divided by the number of eyepiece scales gives the actual length measured by one space on the eyepiece scale. All other objectives of the microscope (i.e. 44X and 97X) were also calibrated in the same manner.

Measurements were made with an ocular stage micrometer at 44 magnifications. Microfilariae of D. immitis were differentiated from those of D. reconditum, using morphological criteria that was reported by Newton and Wright (1956) and Lindsey (1965).

Mosquito Collection

Different species of mosquitoes were collected with a CDC light trap at 1105 South Hough Street, Barrington, Illinois. This area was known to contain dogs with microfilaremia of the heartworm. The trap was set at 7:30 p.m. and mosquitoes were collected at 5:30 a.m. on August 25,

1976. These mosquitoes were taken to the laboratory and identified according to species.[1]

Different species of hibernating female mosquitoes were collected on December 29, 1977 at Willow and Graceland Road, Des Plaines, Illinois. The temperature at the time of collection was -2°C. (Fig.4). Mosquitoes were kept for a ten-day period of continuous fluourescent illumination in the laboratory to bring them out of hibernation (Fig.5).

Dissection of the Mosquitoes Collected with CDC Light Trap

Eighty-two mosquitoes were collected with CDC light trap and dissected in the summer of '76. The dissection was performed in insect saline at 20X magnification under a dissecting microscope. Malpighian tubules were removed and examined at 240X magnification on a compound microscope. The head, thorax and abdomen of each mosquito were also separated and examined for larva stages.

The following procedures were used for mosquitoes collected in the tunnel.

A. Feeding Method

The mosquitoes were placed in a funnel with a cheese cloth covering the opening to allow mosquitoes to obtain a

[1]The information on the collection and identification of species of mosquitoes was provided by South Cook County Mosquito Abatement District, 155th Street and Dixie Highway, Harvey, Illinois, and Northwest Mosquito Abatement District, 147th and Hinz Road, Wheeling, Illinois.

Figure 4

Tunnel where Mosquitoes were Collected

Figure 5

Fluorescent Illumination of Hibernating Mosquitoes

blood meal. They were infected by exposing twenty of them at a time to the shaved area of the infected dog's abdomen[1] (Fig.6). The dog was anesthetized using 1 grain/5x the body weight of Sodium Penthabarbital. The infection of the Anopheles punctipennis took place at 12:30-1:00 p.m. and Culex pipiens at 1:30-2:00 p.m., 2:30-3:00 p.m., 3:30-4:00 p.m. and 4:30-5:00 p.m. on Jan. 11, 1977. Blood samples were collected from the dog before each successive feeding, in order to obtain an indication of the microfilaremia present in the dog at the time of the mosquitoes' infection.

B. Maintenance

After engorging upon an infected dog, the mosquitoes were placed into a specially prepared cage covered with a transparent plastic. The cage contained cheese cloth and approximately one teaspoonful of raisins placed in a plastic dish. The cloth was saturated with 2 ml of 15% sucrose solution daily, through an opening on the side of the cage, to provide an additional supply of food for the mosquitoes. Infected mosquitoes were maintained at a constant room temperature before dissection. The constancy of the room environment and suitable food (raisin and sucrose solution)

[1]The infected dog with D. immitis was provided by Dr. M. Larson of Animal Research Facility, Loyola University of Chicago, Stritch School of Medicine, 2160 South First Avenue, Maywood, Illinois.

Figure 6

Funnel Containing Mosquitoes Placed on the Shaved Abdomen of the Anesthetized Dog Infected with *D. immitis*

were important factors responsible for survival of the mosquitoes after the blood meal. The uninfected mosquitoes survived for four weeks.

C. Dissection

Mosquitoes were placed in a test tube and stunned by immersing them in an ice bucket. Three parts of each mosquito were examined: the head and proboscis, the thorax and the Malpighian tubules. Each mosquito was dissected under low power (240X) magnification with micropins which were inserted into the ends of (14.5 cm.) wooden applicator sticks. Each of the previously mentioned parts was then examined under higher magnification (800X) on the phase contrast microscope for the larva stages of *D. immitis*. The larvae were identified using the three larval stages described by Iyengar (1957) and Taylor (1960), designating each of these as either first-, second-, or third-stage larvae. Warm insect saline (36C) was placed on the mouthpart of the mosquito during the observation of the infective larva stages in the proboscis. This helps to concentrate the larva in the head region, according to Menon and Ramamurti (1941).

RESULTS

Veterinary Survey

Eighty-eight blood samples from dogs diagnosed for heartworm were obtained through the cooperation of 67 veterinarians in 44 counties of Illinois. Of the 88 blood samples re-examined for microfilariae, 81 (92%) were positive for *D. immitis*, 1 (1.1%) with *D. reconditum* microfilariae and 6 (6.8%) with double infections. Microfilariae of *D. immitis* averaged 318.9μ in length, with a range of 311.0μ to 331.2μ; *D. reconditum* averaged 271.6μ in length, with a range of 264.2μ to 274.0μ. The width of *D. immitis* averaged 6.4μ, with a range of 5.9μ to 6.8μ; *D. reconditum* had a mean width of 4.7μ with a range of 4.4μ to 5.0μ. The length and width measurements of *D. immitis* and *D. reconditum* fall within the parameter of what most investigators found (Table 1).

Six dogs (Cases 50, 54, 58, 61, 64, 68) were found to be positive for both *D. immitis* and *D. reconditum*, based on morphological characteristics. No information as to the sex, age, breed or origin were given on cases 54, 58 and 68.

Case 50 - This 4 year-old german shepherd lived in Madison County. The dog had some symptoms of heartworm infection. A blood sample from the dog was found positive

Table 1. Average length and width of *D. immitis* and *D. reconditum*.

	D. immitis		*D. reconditum*	
	Average	Range	Average	Range
A. 69 canine blood samples (Present Investigation)				
Length (μ)	318.9	311-331.2	271.6	264.2-274.0
Width (μ)	6.4	5.9-6.8	4.7	4.4-5.0
B. 30 canine blood samples (Groves and Koutz, 1964)				
Length (μ)	307.3	264.9-334.7	253.5	179.2-279.1
Width (μ)	6.5	6.1-7.7	4.8	3.8-5.7
C. 299 canine blood samples (Hirch and Huizinga, 1966)				
Length (μ)	299.6	271-327	243.7	333-264
Width (μ)	5.8	5.5-8.3	3.9	3.7-4.6

for microfilariae of D. immitis averaging 309.0μ long, 6.4μ wide, and D. reconditum averaging 274.0μ long and 5.0μ wide.

Case 61 - In this 4 year-old male beagle, a blood examination was found positive for D. immitis microfilariae, averaging 314.6μ long, 5.9μ wide and D. reconditum microfilariae, averaging 264.2μ long, 4.4μ wide. The dog had lived in Arkansas before being transported to Chicago. Examination of the heart removed at autopsy revealed 11 adult worms found in the right side of the heart.

Case 64 - This 4½ year-old female collie lived in Evanston, Illinois. The dog was healthy with no apparent symptons of heartworm. A blood sample from the dog was found positive for microfilariae of D. immitis averaging 314.8μ long, 6.3μ wide and of D. reconditum, averaging 271.2μ long and 5.1μ wide. The dog died 14 days later after being admitted to the hospital.

Case 21 - This 2 year-old female of unknown breed had lived in the southern United States in the past years. The dog was found positive for D. reconditum microfilariae averaging 269.4μ long and 4.6μ wide. The remainder (62 dogs) were infected with D. immitis since the morphological characteristics and sizes were the same as described prevously for this species by Lindsey (1962).

The majority of dogs with Dirofilaria positive blood had no readily recognizable sign of heartworm infection. Only a few had typical clinical signs, such as weight loss

and fatigue when exercised. Thirty-two (46.4%) males and 12 (17.4%) females were positive for heartworm. Twenty-five (36.2%) others were unidentified as males or females, but were also found positive for heartworm.

The geographical location of heartworm infected dogs appeared to be in 44 of the 102 counties of Illinois (Fig.7). These results were similar to the work of Noyes (Unpublished data). One veterinarian reported D. reconditum. The rest of the reported cases were all D. immitis. Northeast Illinois has the highest number of practicing veterinarians and was also the area of greatest response to the survey of canine filariasis incidence. Twenty-five of the 49 counties that did not have veterinarians listed in the American Veterinary Medical Association Directory were contacted. Of the 25 counties contacted, 4 reported the incidence of heartworm infection, and 21 did not respond.

The report obtained from the veterinarians in this heartworm survey showed that the incidence of dirofilariasis in Illinois is varied within the county. The reported incidences range from 0.2% in Wilmette (Cook County) to 83.3% in San Jose (Tazewell County). It should be noted that these reports from the veterinarians are not necessarily true values for the entire area, since all veterinarians in each area did not respond to the survey. The majority of the reported cases had an increase in the infection in one year

RAND McNALLY
STATE COUNTY OUTLINE MAP

ILLINOIS
SIZE 8½ x 11

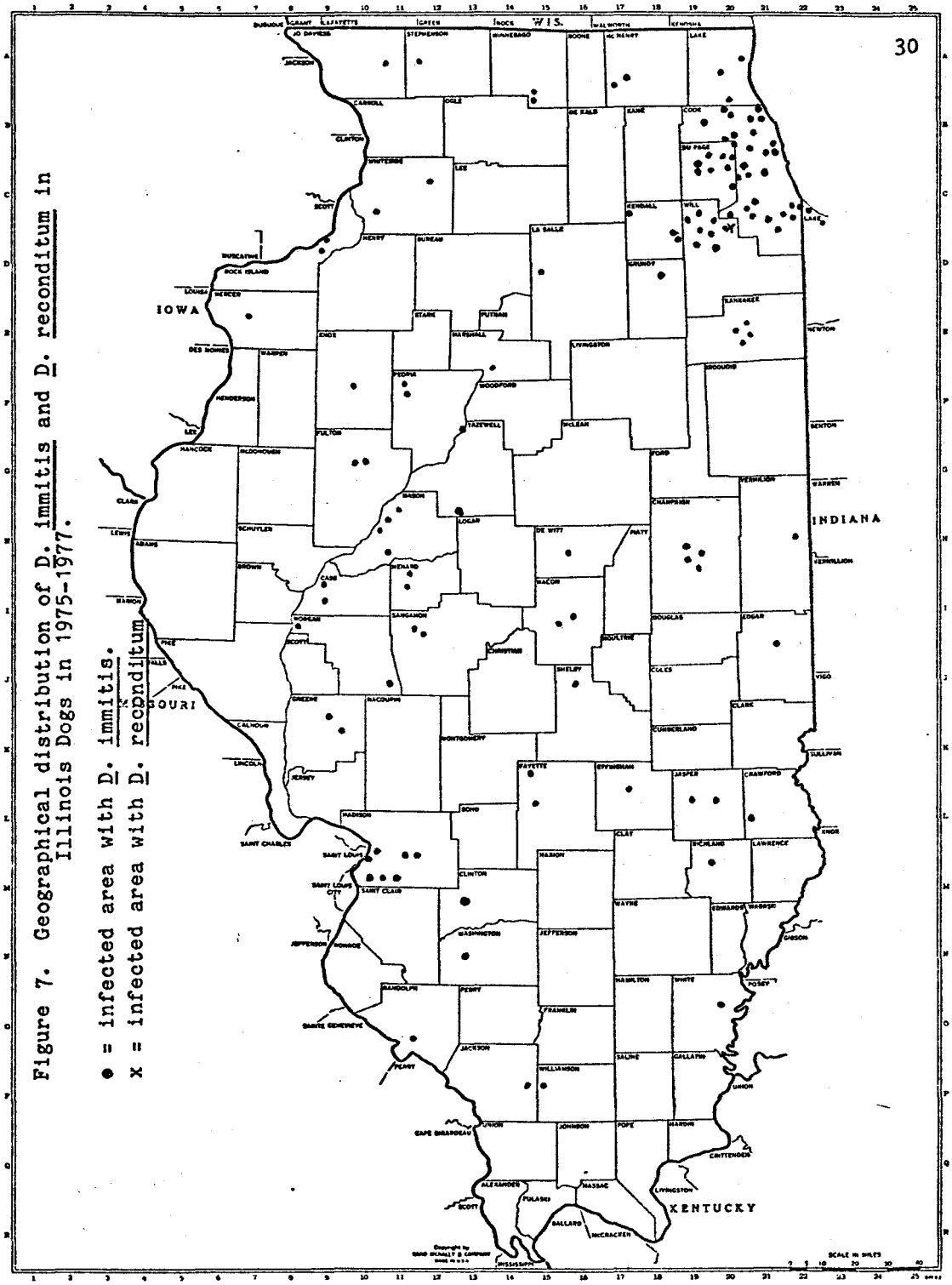

Figure 7. Geographical distribution of D. immitis and D. reconditum in Illinois Dogs in 1975-1977.

● = infected area with D. immitis.

x = infected area with D. reconditum

which decreased the following year. As an example, the percentage of infection in Erie, Illinois, was 5.9 in 1974. The following year, 1975, the infection rate decreased to 4.1%. Schaumburg, Illinois had an increase of 9.4% in 1975 which decreased to 3.8% in 1976. The reported incidence of infection of dog heartworm decreases but the number tested increases. The reported incidence of dog heartworm is shown in Table 2. Two veterinarians reported cases of adult infestation with no microfilariae.

Random Samples

Among the 101 random blood samples obtained from dogs at Save-A-Pet, Anti-Cruelty Animal Welfare and veterinarians 6 (5.9%) were found to be positive for *D. immitis* microfilariae, but no *D. reconditum* were present. Of the 44 dogs examined at Save-A-Pet, results were positive for 1 (2.3%); 54 dogs examined at the Anti-Cruelty Animal Welfare were positive for 5 (9.3%) and 13 samples from dogs obtained from the veterinarians were all negative.

Anopheles punctipennis and *Culiseta inornanta*

Twenty-eight *Anopheles punctipennis* and ten *Culiseta inornanta* were collected in the tunnel. These mosquitoes were allowed to feed on a dog infected with *D. immitis*, but the infections were unsuccessful.

Table 2. Reported Incidences of Dog Heartworm from Veterinarians.

City	County	# Examined	# Positive	Year	Percentage (%)
Greenwood	Kankakee	1284	26	'75	2.0
"	"	1260	24	'76	1.9
Bradley	"	?	?	'76	18.0
Aroma Park	"	1300	65	'75	5.0
San Jose	Tazewell	18	15	'75	83.3
Clinton	Dewitt	?	2	'75	?
"	"	26	1	'76	3.9
Moline	Rock Island	230	9	'75	3.9
"	"	263	5	'76	1.9
Markham	Cook	702	24	'75	3.4
"	"	634	18	'76	2.8
Evanston	"	756	7	'76	0.9
Wilmette	"	2618	6	'76	0.2
Park Ridge	"	450	2	'76	0.4
La Grange Park	"	284	2	'76	0.7
Maywood	"	127	34	'76	27.0
Palatine	"	600	6	'76	1.0
Oaklawn	"	?	?	'76	1.0
Western Springs	"	1375	13	'76	0.9
Libertyville	Lake	150	0	'76	0.0
Wheeling	"	206	15	'76	7.3
Danville	Vermilion	126	13	'76	10.3
Woodstock	McHenry	391	4	'76	1.0
Rockford	Winnebago	193	6	'75	3.1
"	"	147	4	'76	2.7
Champaign	Champaign	200	6	'76	3.0
Urbana	"	685	77	'76	11.3
Greenfield	Greene	?	?	'76	10.0
Decatur	Macon	?	?	'76	3.0

Table 2 (continued)

City	County	# Examined	# Positive	Year	Percentage (%)
Lockport*	Will	587	23	'74	3.9
"	"	1218	15	'75	1.2
"	"	1050	34	'76	3.2
Erie	Whiteside	272	15	'74	5.5
"	"	271	11	'75	4.1
Sterling	"	?	1	'74	?
"	"	?	1	'75	?
"	"	550	3	'76	0.6
Beardstown	Cass	109	26	'74	23.9
Oblong	Crawford	?	?	'76	10.0
Elk Grove Village	Du Page	400	?	'75	1.0
"	"	400	?	'76	1.0
Glen Ellyn	"	1000	5	'76	0.5
Lombard	"	653	5	'75	0.8
"	"	626	2	'76	0.3
Hinsdale	"	844	8	'75	1.0
"	"	?	6	'76	?
Stockton	Jo Daviess	50	2	'76	4.0
Malta	De Kalb	70	4	'76	5.7
Peoria	Peoria	6	?	'76	?
Geneva	Kane	700	11	'76	1.6
Schaumburg		128	12	'75	9.4
"		319	12	'76	3.8
Effingham	Effingham	?	40	'76	?
Petersburg	Menard	?	12	'67-74	?
"	"	108	27	'75	8.3
"	"	318	20	'76	6.3
Edwardsville	Madison	?	?	'76	10.0

? = Unprovided information
* = Reported 1 case of D. reconditum

Culex pipiens

Three hundred and seventy-five Culex pipiens were collected in the tunnels. Of the 80 mosquitoes offered the dog's blood, 61 fed. Percent feeding ranged from 65 to 90 with an overall average of 76.3 (Table 3). The percent that died immediately after feeding ranged from 5.6 to 50.0 with an overall average of 29.5. The microfilariae of the dog at the time of feeding ranged from 29,052-41,416/ml. of blood (Table 4). Of the 53 that fed and survived, 36 were dissected. The remaining 32% died before dissection and were not examined. Mosquito mortality was minimal through the first six days after the blood meal, but never rising above 1% each day. However, deaths increased on day 8 to between 2-2.5%, and this level of mortality remained constant until day 15, at which time another slight increase of 3% was observed.

An average of 17.5 microfilariae were located in the midgut of 4 out of 4 mosquitoes dissected 2 hours after feeding. The larvae appeared to be almost identical to the microfilariae from the blood (Fig.8). No microfilariae were observed in the Malpighian tubule on day 1. On day 2, an average of 16 microfilariae were found in the midgut, 8 were found in the tubule. An average of 3 larvae were also found in the tubule. The larvae was still idential to those from the blood but slightly wider (Fig.9). Of the total

Table 3. Feeding Response of *Culex pipiens* when Presented to an Anesthetized Dog for 30 Minutes.

Date of blood meal	Hours of blood feeding	Total females offered/taking blood meal	Total females died after feeding	% females died	% of females taking blood meal
January 11, 1977					
C. pipiens	1:30-2:00PM	20/18	1	5.6	90
	2:30-3:00PM	20/13	3	23.1	65
	3:30-4:00PM	20/14	6	42.9	70
	4:30-5:00PM	20/16	8	50.0	80

Table 4. Microfilariae at the Time of Feeding

Time	# of Microfilaria per ml. of Blood
12:23 p.m.	29,052
1:25 p.m.	31,721
2:25 p.m.	32,604
3:25 p.m.	37,813
4:24 p.m.	41,416

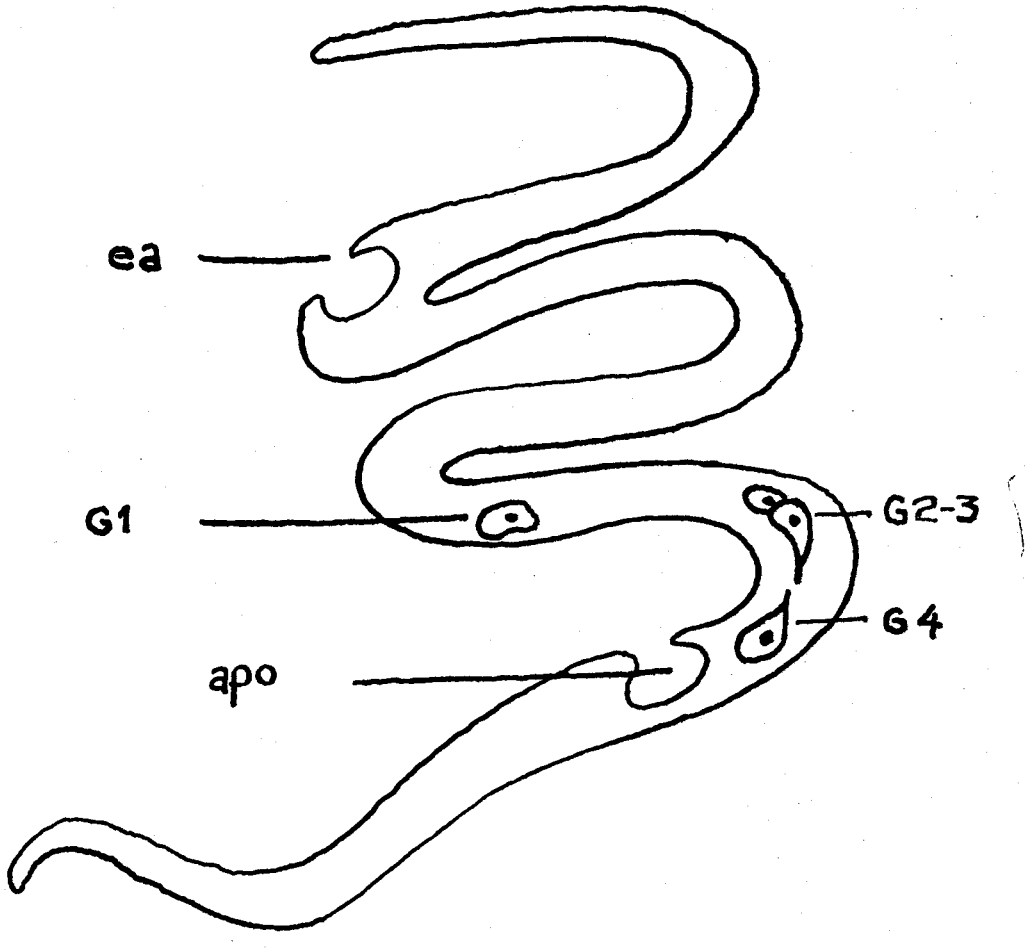

Figure 8

First Day Larvae of D. immitis

apo = Anal pore; ea = Excretory apparatus;
G_{1-4} = Germ cells; nr = Nerve ring.

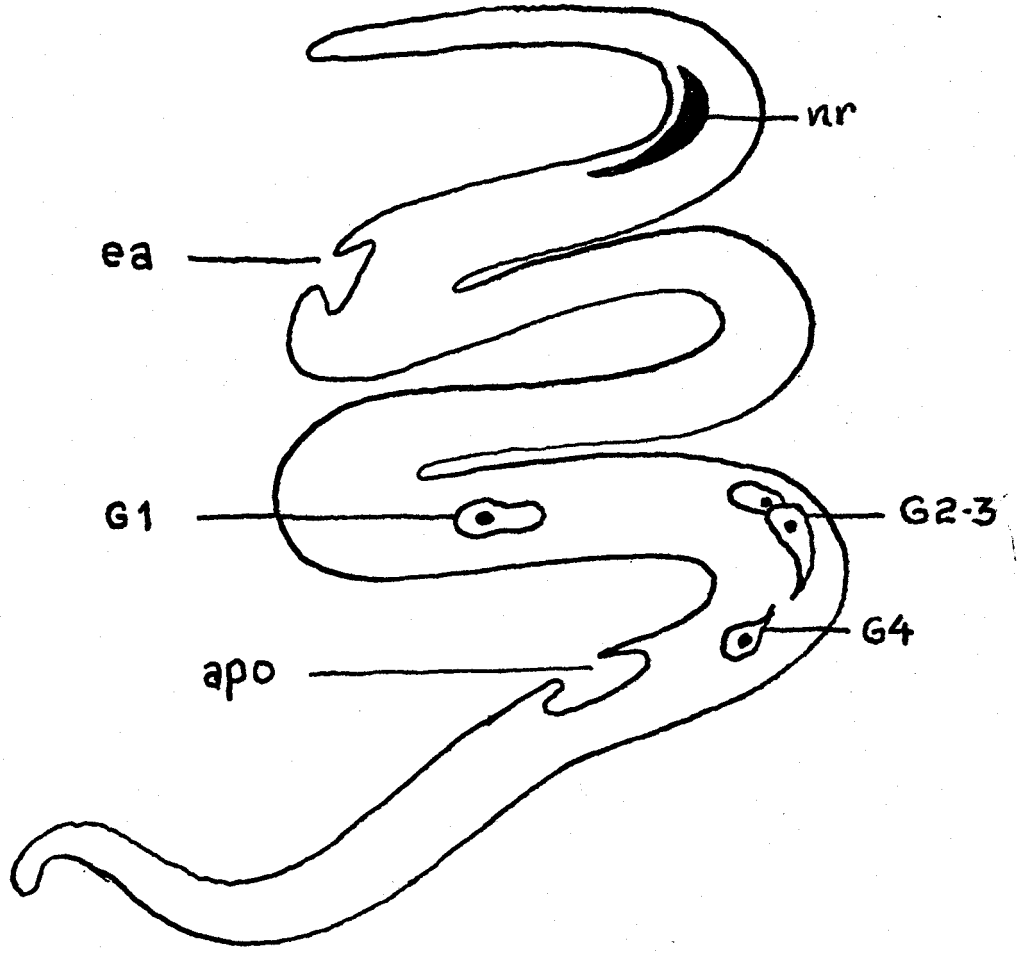

Figure 9

Second Day Larvae of D. immitis

apo = Anal pore; ea = Excretory apparatus;
G_{1-4} = Germ cells; nr = Nerve ring.

microfilariae observed in the midgut on days 1-4, approximately one-half this number were seen in the tubules. From day 6 through 11, no parasites were observed. Infective larvae first appeared in the tubules 12 days, and in the labium 13 days after the blood meal (Fig.10). These findings agree with the reports of Kartman (1953) Symes (1960) and Chellappah and Chellappah (1968). The overall dissection and development of D. immitis in Culex pipiens is shown in Table 5.

Efficiency of Culex pipiens was found to be 13.1%. It is calculated from the original number of microfilariae and the number of parasites that mature to infective stage. As an example, there was an average of 17.5 microfilariae observed on day 1 (Table 5). Of these microfilariae, 5.5 stage one and two larvae and 2.3 stage three larvae per mosquito developed. In other words, approximately 31.4% of the microfilariae ingested continued development to stage one and two larvae, and 13.1% reached the infective stage.

Uninfected Mosquitoes

Of the 82 Mosquitoes collected with CDC light trap (Table 6), an early stage of D. immitis larvae was found in one Culex pipiens. The other species of mosquitoes dissected were all negative for microfilariae.

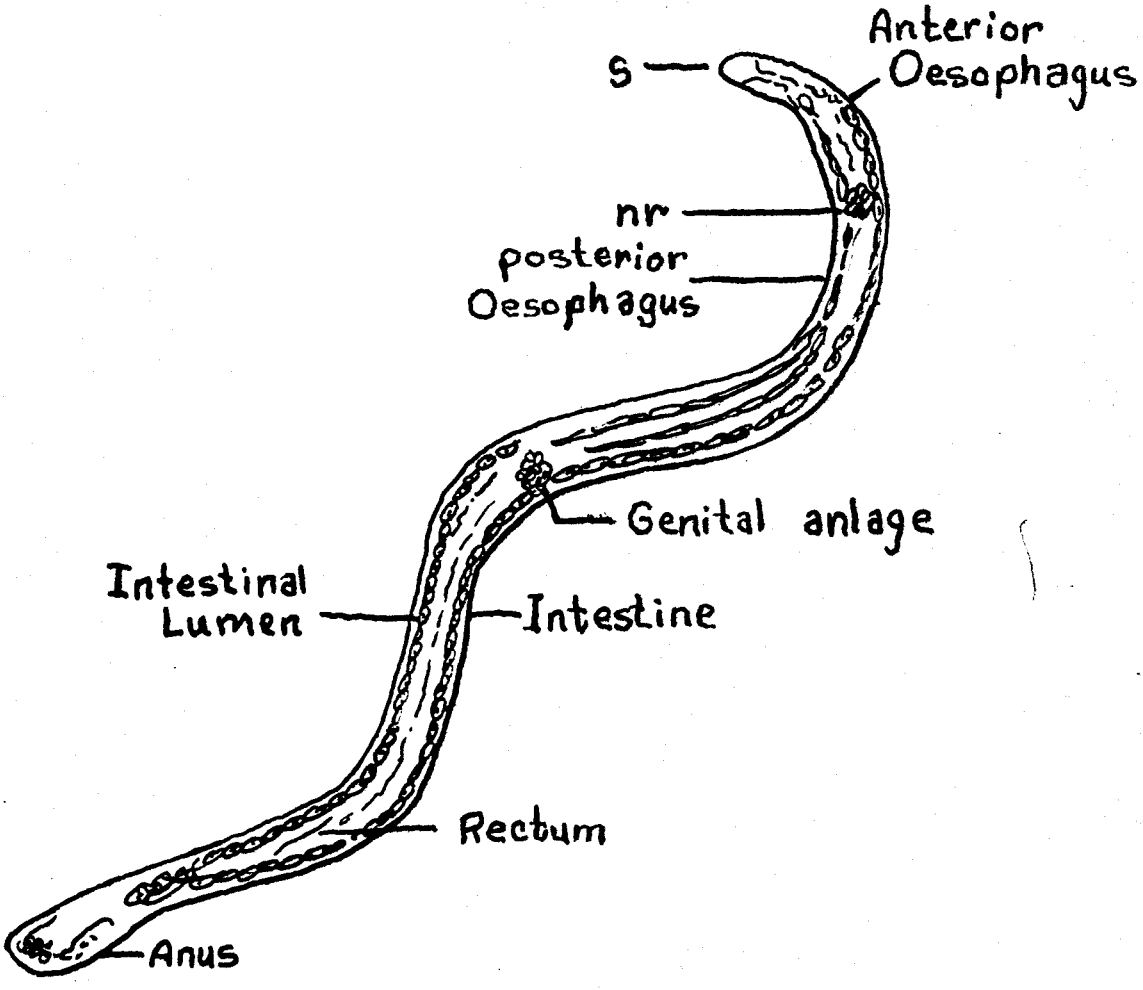

Figure 10

Thirteenth Day Larvae of D. immitis

nr = Nerve ring; s = Stoma.

Table 5. Development of *D. immitis* in *Culex pipiens*.

Day	Dissected	Positive	Mf.* in midgut	Av. Length (μ)	Av. Width (μ)	Mf.* in tubules	Larvae in tubule	Haemo-coele	Labium
1	4	4	17.5	321.5	6.0	9.2	-	-	-
2	3	2	16.0	321.5	6.3	8.0	3.0	-	-
3	3	2	11.0	298.1	7.6	5.0	12.0	-	-
4	3	2	6.0	276.6	8.1	3.5	8.5	-	-
5	3	2	-	-	-	-	5.5	-	-
6)									
7)									
8)	No parasites observed								
9)									
10)									
11)									
12	3	2	-	301.0	6.6	-	-	2.5	-
13	3	1	-	319.4	6.4	-	-	2.0	3.0
14	3	2	-	319.4	6.2	-	-	4.5	3.0
15	3	3	-	320.1	6.3	-	-	3.3	3.6
16	3	1	-	320.0	6.2	-	-	2.0	2.0
17	5	3	-	320.4	6.4	-	-	4.6	2.3

*Mf. = Microfilariae

Table 6. Mosquitoes Collected with CDC Light Trap.

Species	# Collected
Culex pipiens	48
Culex restuans	7
Aedes vexans	20
Aedes trivittatus	2
Aedes triseriatus	1
Anopheles punctipennis	2
Anopheles quadrimaculatus	1
Uranotaenia sapphirinia	1

DISCUSSION

I. In connection with the first objective of the present investigation, the results indicate that D. immitis and D. reconditum are present in much of Illinois. The incidence of Dirofilaria immitis is higher than that of Dipetalonema reconditum. D. reconditum is nonpathogenic, and often ignored by the veterinarians. It should be mentioned that the majority of the veterinarians did not cooperate in this present investigation. Approximately 18% of the veterinarians to whom letters were sent responded to the heartworm survey. A study to locate the unreported incidences of the infection is in progress in this laboratory. Heartworm is suspected to be in all counties of Illinois.

Generally, the incidence of dirofilariasis in Illinois is decreasing, but the number being tested is increasing, according to Noyes (unpublished data). This information was obtained from Noyes during Heartworm Symposium '77, held in Atlanta, Georgia, where he presented these findings. The former increase in the incidence and spread of dog heartworm infection in Illinois was due to the transportation of dogs for hunting, breeding, dog shows, and the movement of dogs belonging to army personnel during the war.

The results of the random blood examinations from dogs obtained at Save-A-Pet, Anti-Cruelty Animal Welfare and veterinarians indicate that the rate of heartworm infection

is low among those dogs. All of the dogs except those that were obtained from the veterinarians were stray dogs. One would expect a higher incidence among stray dogs since they are more exposed and receive less care. But as long as these dogs do not come in contact with the appropriate mosquito vectors for this disease, they will be free of heartworm infection

In this and other studies (Wallenstein and Tibola, (1960), male dogs showed higher infestation than female dogs. The reason for this possible sex difference is unknown, but it may be due to either the roaming habits of the male, who might therefore run more risk of exposure to the intermediate hosts, or to hormonal differences between the sexes according to Dobson (1964). The investigator claimed that estrogen appeared to offer some protection against parasite invasion.

As mentioned earlier, the report obtained from the veterinarians shows that the incidence of dirofilariasis in Illinois is varied within the counties. It is noteworthy, however, that the reported incidences represent examinations of dogs brought to the veterinarians. Many of the dogs may have shown typical clinical signs of heartworm infection before they were brought in for check-up. If this was the case, the actual incidence of the disease would be smaller than that reported by the veterinarians. As was mentioned, the majority of the reported cases had an increase in one

year which decreased the following year. This decrease is very significant because it shows that people are becoming more aware of the problem of dirofilariasis and are taking more measures to eradicate the disease in the counties. The dog owners are either checking their dogs every year or putting their dogs on preventive medication. An establishment of a mosquito control program within the infected area might also have led to the reduction in the incidences of the dirofilariasis.

II. In relation to the second objective of this investigation, it should be noted that, in Illinois, Culex pipiens, Anopheles punctipennis and Culiseta inornanta hibernate during the winter. Approximately 90% of the mosquitoes collected in the tunnel and 58.5% of those collected with CDC light trap constitute Culex pipiens. This study as well as another study (Oemick - unpublished data) indicates that larger numbers of this species of mosquitoes hibernate in winter than any other species of mosquitoes.

The results from the mosquitoes collected with CDC light trap as well as another study (Christensen and Andrews, 1976) indicate that natural infection is found in low proportion among the mosquitoes.

III. Pertaining to the third objective of this investigation the highest percentage in mortality observed immediately after the mosquitoes had the blood meal (Table 3) was probably due to a large number of microfilariae being ingested

during the blood meal. Kartman (1953) reported that some species of mosquitoes are killed by the number of microfilariae which they acquire from feeding on a heavily infected dog.

The mortality of the mosquitoes after the blood meal was minimal through the first six days. The rise in deaths of infected mosquitoes observed from days 8-15 corresponds to the movement of late stage two and development of active stage three larvae in the tubule. The increase in daily mortality on day 15 parallels the migration of infective larvae within the haemocoele. Kershaw et al. (1955) observed this pattern of mortality within Aedes egypti. Pistey (1959) also observed increased mortality in mosquitoes harboring Dirofilaria tenuis on the second and eighth days. These peaks were believed to result from larval migration to the tubules and emergence of third stage larvae from the Malpighian tubule.

The vector efficiency formula, employed by Kartman (1953) to measure the host's ability to support development of microfilariae to infective stage shows that Culex pipiens compares favorably with Aedes sp. and Anopheles sp. which serve as vectors of D. immitis.

SUMMARY

I. The first objective of the present investigation relates to the following:

A. Eighty-eight blood samples from dogs previously diagnosed positive for heartworm were obtained from 67 veterinarians in 44 of the 102 counties of Illinois. Re-examination revealed an incidence of 92% *D. immitis*, 1.1% *D. reconditum* and 6.8% double infection.

B. A total of 101 random dog blood samples were examined for canine filariae by a Modified Knott technique. An incidence of 2.3% *D. immitis* was found in 44 samples examined at Save-A-Pet and 9.3% in 54 samples examined at Anti-Cruelty Animal Welfare. None of the 13 samples obtained from veterinarians were found positive for *D. immitis*.

II. In relation to the second objective of this investigation, 8 different species of mosquitoes were collected, identified and dissected. *D. immitis* was found only in *Culex pipiens*.

III. In connection with the third objective of this investigation, 3 different species of mosquitoes were allowed to feed on a dog infected with *D. immitis*. The development of microfilariae to an infective stage was observed only in *Culex pipiens*. The infection in *Anopheles punctipennis* and *Culiseta inornanta* were unsuccessful.

CONCLUSION

A survey of Illinois counties indicated once again that D. immitis and D. reconditum are present in much of Illinois. Generally, the incidence of dog heartworm in Illinois is decreasing, but the number being tested is increasing. This is encouraging. But the fact that the infection still exists merits the attention of public health officials since D. immitis can cause infection in both the dog and man. The filariae involved are those whose normal hosts are domestic animals, often living in close association with man. There is a need for eradication of mosquito vectors responsible for the transmission of this infection. This is often difficult as it is impossible to completely erradicate the mosquito population in a given area. However, dog owners can make an important contribution to the control of this disease by taking their dogs to the veterinarians for check-ups at least twice a year or by putting the dogs on preventive medication.

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APPROVAL SHEET

The thesis submitted by Bolaji Nelson Akande has been read and approved by the director of the thesis.

Furthermore, the final copies have been examined by the director and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

7-13-1977

B D Dashoki

Signature of Advisor