ARTHROPOD VECTORS, CYCLODEVELOPMENT AND PRE-PATENT PERIOD OF *DIPETALONEMA RECONDITUM* (GRASSI) AND THE INCIDENCE OF CANINE FILARIASIS AND ECTOPARASITES IN NORTH-CENTRAL OKLAHOMA

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PREFACE

As a United States Army medical entomologist I became involved with canine filariasis in 1965, while I was serving as Chief, Medical Entomology Branch, U.S. Army Medical Center, Ryukyu Islands. During this period I was asked to provide recommendations to control the mosquito vectors of the dog heartworm which were causing dirofilariasis among sentry dogs on Okinawa. This request led to a study of canine filariasis and the first report of Dipetalonema reconditum (Grassi) occurrence on Okinawa. I became keenly interested in this filarial worm and it seemed natural for me to continue my work when I came to Oklahoma State University, particularly when I learned that D. reconditum had not previously been reported to occur in Oklahoma.

I wish to express my appreciation to: the U.S. Army for making this research possible; Dr. D. E. Howell, Dr. S. A. Ewing and Dr. R. D. Eikenbary for their guidance throughout this study; Major D. W. Parrish for his cooperation and assistance in conducting the surveys; Cpt. T. L. Biery for assistance in constructing animal housing facilities; Mr. J. C. Pennington for assistance in recording data; Mr. Curtis Bush for the many hours of devoted assistance he provided and to my wife, Violet, who gave encouragement and understanding throughout the research project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ILLUSTRATIONS

INTRODUCTION

Dirofilaria immitis (Leidy), the dog heartworm, was the only filarial worm recognized to occur in dogs in the United States until 1956 when it was found that Dipetalonema reconditum (Grassi), a subcutaneous filarial worm, also occurred (Newton and Wright, 1956). This meant that many of the reports dealing with the occurrence, incidence, distribution, vectors and life cycle of D. immitis published prior to 1956 probably dealt with D. reconditum either alone or together with D. immitis. The recognition of D. reconditum helped to explain many of the inconsistencies and contradictions in the literature and pointed to the need for continued re-evaluation of the filariasis problem of dogs. Although extensive work has been done on filariasis of dogs, many questions remain unanswered especially as concerns the arthropod vectors and cyclodevelopment of D. reconditum; therefore, the elucidation of these factors could make substantial contributions in the general area of canine filariasis.

REVIEW OF THE LITERATURE

At least eleven species of filarial parasites have been reported from dogs in various regions of the world (Lindsey, 1961). Only two of these are known to occur in the United States, viz., Dirofilaria immitis (Leidy, 1856) and Dipetalonema reconditum (Grassi, 1890).

Biology of D. immitis

Dirofilariasis, heartworm disease, has been recognized as a serious disease of dogs in the United States for many years. French (1899) reported finding worms in the heart of a dog in Florida. According to Kartman (1953a) our knowledge of the mosquito's role as host to filarial worms began with Manson's observations on the infection of Culex fatigans, Wiedemann by Wuchereria bancrofti (Cobbold) in 1878 and Calandruccio's observation in 1892 of filarial larvae in the gut of a mosquito which he presumed were D. immitis, but Grassi and Noe in 1900 were the first to demonstrate experimentally the development of D. immitis in the mosquito. Lavoipierre (1958) and Hawking and Worms (1961) have reviewed the literature on arthropods in the transmission of filarioid nematodes. Hawking and Worms (1961) present a table in which they list the filarial worms known to develop in arthropods. The literature is extensive in the area of D. immitis development in various mosquitoes. Bemrick and Sandholm (1966) make reference to 48 species of mosquitoes in which complete larval development of D. immitis has been reported and more recently Ludlam, et al. (1970) list 62 species

and cite 56 references.

The adults of D. immitis are generally found in the right ventricle of the heart and pulmonary artery, and occasionally in other areas. The location of adult worms, pathology, and symptoms accompanying the disease are described by Kume and Itagaki, 1955; Balch, 1957; Hennigar and Ferguson, 1957; Winter, 1959; Jackson, et al., 1962; Jaques, 1962; Liu, et al., 1966; Watson, 1968; Patton and Garner, 1970, and others. The adult fertile female liberates microfilariae into the blood stream which circulate in the dog's blood. The number of microfilariae in the peripheral circulation has been found to vary greatly during a 24 hour period. Many workers have demonstrated this phenomenon and considerable work has been conducted in an attempt to elucidate the cause of this periodicity (Harley, 1932; Underwood and Wright, 1933; Underwood and Harwood, 1939; Hinman, 1935a, 1935b, 1937; Schnelle and Young, 1944; Hawking and Thurston, 1951; Burch, 1952; Hawking, 1953, 1956, 1965, 1967; Hawking, et al., 1964, 1967; Tongson and Romero, 1962; Gubler, 1966; and Hawking and Clark, 1967).

The circulating microfilariae are ingested by the mosquito while taking a blood meal. The larvae enter the Malphigian tubules where they develop through a series of two molts into infective larvae which require about 10 to 15 days depending on the species and environmental conditions (Hu, 1931; Yen, 1938; Phillips, 1939; Travis, 1947; Bradley, 1953; Kartman, 1953a, 1953b, 1953c, 1956; Kershaw, et al., 1953; Kershaw, et al., 1955; Webber and Hawking, 1955; Newton and Wright, 1956, 1957; Newton, 1957; Orihel, 1959, 1961; Williams, 1959; Nelson, 1959; Taylor, 1960a, 1960b; Sawyer and Weinstein, 1963a, 1963b; Burton, 1963; Beam, 1965, 1966, 1967; Warne, et al., 1967; Keegan, et al.,

1967, 1968; Intermill and Frederick, 1970; and Villavaso and Steelman, 1970). The infective larvae leave the mosquito and enter the dog while the mosquito is taking another blood meal. The larvae, now in the dog, grow through a series of molts to maturity and migrate to the heart (Kume and Itagaki, 1955; Orihel, 1961). The prepatent period may vary from 6 to 8 months (Kume and Itagaki, 1955; Webber and Hawking, 1955; Newton, 1957; Orihel, 1959, 1961; Sawyer, et al., 1963a; and Warne, et al., 1967).

Biology of D. reconditum

This species was first differentiated from D. immitis by Grassi and Calandruccio (1890) in Italy, but it was not recognized to occur in the United States until the work of Newton and Wright (1956, 1957). Several earlier workers had suggested the presence of another filariid in dogs prior to this time (Mundhenk and Greene, 1939; Armistead, 1950; and Rosen, 1954). Rosen even suggested it could be D. reconditum.

Grassi and Calandruccio (1890) first described the development of D. reconditum in Ctenocephalides canis (Curtis) and considered Pulex irritans L. as a good intermediate host. Newton and Wright (1956) reported development to the infective stage larva in Ctenocephalides felis (Bouché) and C. canis. Nelson (1962) working in Kenya found development to occur in C. felis, and Heterodoxus spiniger (Enderlein) from the dog and in C. felis, and Echidnophaga larina, Jordon and Rothschild, from infected hyenas and jackals. Pennington and Phelps (1969) found development to occur in C. canis, C. felis, P. irritans, and H. spiniger and were the first to report development in Linognathus setosus (van Olfers) collected from infected dogs on Okinawa, Ryukyu Islands. With the discovery of D. reconditum occurrence in the United

States, and in light of our present knowledge, it appears that several workers who reported the development of larval stages in fleas as D. immitis were actually working with D. reconditum. Based on this premise one should also consider the work by the following as probably pertaining to development of D. reconditum: Breinl (1921) with C. felis and C. canis; Brown (1939) with C. felis; Brown and Sheldon (1940) with C. felis; Summers (1940, 1943) with C. felis, C. canis and P. irritans; Bradley (1952) with C. felis and Stueben (1954a, 1954b) with C. felis, C. canis, P. irritans, Xenopsylla cheopis (Rothschild), Echidnophaga gallinacea (Westwood), and Orchopeas wickhami (Baker).

The adults of D. reconditum are reported to inhabit the skin and subcutaneous tissues of the dog without causing any recognized lesions (Newton and Wright, 1957; McKinney, 1962; Lindsey, 1965; and Jackson, 1969). The fertile female liberates microfilariae which circulate in the dog's blood. The number of circulating microfilariae per volume of blood is almost always considerably less than with D. immitis. This difference has been suggested as one of the gross signs useful in separating the two species (Newton and Wright, 1957; Wallinstein and Tibola, 1960; Rothstein, et al., 1961; Nelson, 1962; and Lindsey, 1965). Although the number of microfilariae in the circulating blood may vary from time to time, the period of greatest numbers does not appear to occur at any given time in all dogs (Newton and Wright, 1956; Gubler, 1966; and Pennington and Phelps, 1969). The circulating microfilariae are ingested with the blood meal by the insect vector and the larvae develop to the infective stage in about 6 days. The infective larvae then leave the insect while it is feeding and enter the dog (Stueben, 1954a).

Geographic Distribution and Incidence of Canine Filariasis in the U.S.

Surveys to determine the prevalence of filariids in dogs are most often conducted by examining blood samples for microfilariae. Only occasionally are surveys conducted where examination for adult worms are made. Considerable work has been done to re-evaluate the distribution and incidence of dog filariasis since the recognition of the presence of D. reconditum in the United States. A review of this literature is presented in Table I. Worley (1964) examined 123 dogs from Southeastern Michigan and found 5.7% to contain adult heartworms. Lillis (1964) examined 550 dogs from Central New Jersey for adult D. immitis and found 9.3% positive. Schlotthauer (1962) examined microfilariae from 133 Minnesota dogs and found only D. immitis; although he later reported that both species are known to occur in Minnesota (Schlotthauer and Griffith, 1964). Ward (1965) examined the hearts of 6,660 dogs obtained from city pounds in an area between Memphis, Tenessee, and New Orleans, Louisiana, over a 9-year period, and adult D. immitis were recovered from 760 (11%); however, prevalence rates by locality could not be determined because the exact origin of the dogs frequently was unknown. Thrasher and Ash (1962) examined 255 microfilariae positive samples from clinic dogs in the New Orleans, Louisiana, area and found 98% were D. immitis, 1.6% were D. reconditum, and 0.4% contained both filariids.

Some of the more interesting surveys and reviews conducted prior to recognition of D. reconditum occurrence in the United States include those by the following: Hays, 1933; Hinman and Baker, 1936; Augustine, 1938; Brown, 1939; Mundhenk and Greene, 1939; Nelson and Morris, 1939; Phillips, 1939; Title, 1942; Roberts and Roberts, 1946; Ward and

TABLE I

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

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.r.	% D.i.	% Mixed	% Total
Alabama - Mobile						
Pound Dogs	Lindsey (1961)	50	14	42	10	66
Clinic Dogs	Lindsey (1961)	50	18	28	4	50
Total of Above		100	16	35	7	58
Alabama - Alburn						
Clinic Dogs	Lindsey (1961)	100	31	2	1	34
Alabama - Montgomery	Rothstein, et al. (1961)	555	1.1	37.8	0	38.9
California - Northern						
Pound Dogs	McGreevy, et al. (1970)	515	5	0	0	5
Beagle Colony	McGreevy, et al. (1970)	800	0	.12	0	.12
Florida - Jacksonville						
Pound Dogs	Lindsey (1961)	50	44	8	6	58
Clinic Dogs	Lindsey (1961)	50	18	26	6	50
Total of Above		100	31	17	6	54
Georgia - Columbus						
Pound Dogs	Lindsey (1961)	110	50	2	2	54
Georgia - North and South						
Well-Cared-For-Dogs	Thrasher and Clanton (1968)	672	4.7	19.6	0	24.0
Georgia - Atlanta						
Pound Dogs	Thrasher, et al. (1968)	40	37.5	12.5	7.5	57.5
Private Dogs	Thrasher, et al. (1968)	273	14.6	5.4	0.7	20.9

TABLE I (Continued)

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.r.	% D.i.	% Mixed	% Total
Hawaii Pound and Private Dogs	Gubler (1966)	666	10.8	32.2	2.3	45.2
Hawaii	Ash (1962)	96	16.0	19.0		35
Illinois - Champaign, Co. Clinic Dogs	McKinney (1962)	212	1.4	1.4	0	2.8
Illinois -						
Northern	Marquardt and Fabian (1966)	163	2.5	10.4	0	13
Central	Marquardt and Fabian (1966)	73	16.4	21.9	0	38
Southern	Marquardt and Fabian (1966)	110	8.2	34.6	0	43
Total of Above		346	7.2	20.5	0	28
Indiana - Indianopolis Research Farm Dogs	Eshenour (1958)	333	9.0	0.6	0	10
Louisiana - New Orleans	Orihel (1959)	137	28	42	6.6	63
Louisiana - New Orleans Private Dogs	Thrasher, et al. (1963)	543	2.0	44	.4	46.2
Maryland - Hyattsville Clinic Dogs	Wallinstein and Tibola (1960)	528	5.3	6.6	1.14	13.1
Michigan - MSU Pound Dogs	Leash and Hanson (1961)	192	4	2	0	6
Michigan - Detroit Pound Dogs	Zydeck, et al. (1970)	248	2.8	1.6	0	4.4

TABLE I (Continued)

Geographic Area	Investigator	Incidence				
		No. Dogs	% D.r.	% D.i.	% Mixed	% Total
Northeast U.S. - Including Pennsylvania, Maryland, and New Jersey	Newton and Wright (1956)	250	6	1	-	7
Ohio - Many Parts	Groves and Koutz (1964)	340	6.7	2.1	0	8.8
Oklahoma - North-Central Pound Dogs	Pennington, et al. (1970)	100	15	0	0	15
Pennsylvania - Pittsburg	Rothstein, et al. (1961)	841	2.4	2.5	0	5
Rhode Island - Providence	Rothstein, et al. (1961)	69	1.5	1.5	0	3
Texas - South	Keegan, et al. (1968)	522	0	19.7	-	19.7
Virginia - Norfolk	Newton and Wright (1956)	7	43	57	-	-
Many Areas of U.S. U.S. Army Sentry Dogs	Rothstein (1963)	1,026	0.2	12.2	0	12.4

Reeder, 1948; Otto, 1949; Armistead, 1950; Rubin, 1952; Ward and Franklin, 1953; Stueben, 1954b; Eyles, *et al.*, 1954; and Young, 1955.

METHODS AND MATERIALS

Canine Microfilaria Survey

Dogs included in this survey were from those available at city animal shelters in Stillwater, Guthrie, Edmond, Enid and Ponca City, Oklahoma, at the time each facility was visited. This survey was conducted during June and July, 1969 and May, 1970, and each location with the exceptions of Edmond and Guthrie was visited two or more times. Dogs were put on a leash, muzzled and held by one person who also held the foreleg, closing off the blood return in the cephalic vein. Blood samples were taken by drawing from 2 to 5 cc of blood from the cephalic vein using disposable 6 cc syringes and 20 gauge needles. Each sample was placed in a 7 ml test tube containing the anticoagulant E.D.T.A. (ethylenedinitrilo disodium tetraacetate). Blood samples were chilled on ice until returned to the laboratory and processed. The blood samples were processed by the modified Knott's technique (Newton and Wright, 1956). One ml of blood was added to 10 ml of 2% formalin in a 15 ml conical centrifuge tube and mixed well by inverting the tube several times. The sample was centrifuged at 1500 rpm for 5 minutes and the supernatant fluid decanted. A drop of sediment, approximately .02 ml, was placed on a clean slide, an equal amount of 1:1000 aqueous methylene blue was added and covered with a 22 x 22 mm coverslip. The slides were examined for the presence of microfilariae through a compound microscope at 100x. When microfilariae were found, the number on each slide was determined and when possible at least 5 microfilariae

were measured by use of a calibrated eyepiece micrometer at 430x. Blood samples were also examined as wet whole blood preparations. A drop of fresh citrated whole blood (.02 ml) was placed on a clean slide, covered with a 22 x 22 mm coverslip and examined for microfilariae at 100x. When microfilariae were found the number on each slide was determined by a systematic search of the slide. These methods were used to determine the presence and numbers of microfilariae in dogs throughout this study.

Canine Ectoparasite Survey

Ectoparasites were collected from dogs obtained at the same time and place as in the microfilaria survey. Fleas and lice were inactivated on the dog's body by placing each dog, after muzzling, in a 90 x 60 cm men's suit protective bag made of 0.3 mm plastic containing a 5 x 5 x 1.3 cm piece of synthetic sponge saturated with 90% dichlorvos. The bag, equipped with a full length zipper, was closed up to the dog's neck with the head extended outside the bag. Only occasionally, after placing the dog in the bag, was it necessary to exert any physical restraint to keep the dog docile. At first the dog's feet were tied together, but later it was found that this was unnecessary, as the strength of the plastic bag was sufficient to prevent any tearing or piercing. While the dog was in the bag, parasites found on the head were removed with forceps and placed in a vial of 70% alcohol. After 8 minutes, the time required to inactivate the fleas and lice with dichlorvos, the dog was removed from the bag and thoroughly combed with a fine-toothed comb over a white sheet. The parasites were collected from the sheet and inside the plastic bag and placed in 70% ethyl alcohol. Ticks were removed from the dog with forceps and similarly placed in 70% ethyl alcohol. The plastic bag was thoroughly cleaned with a

sponge moistened with 95% ethyl alcohol and wiped dry with gauze after each use. All parasites were counted and identified in the laboratory. Fleas were cleared in a 10% cold KOH solution for 24 hours, rinsed in tap water and processed through a series of 30, 50, 70, and 95% ethyl alcohol for 3 to 4 hours at each concentration and then placed in a saturated carbolxylol solution for 10 to 12 hours. Specimens were taken from the carbolxylol solution and mounted on slides in Canada balsam. The slides were then placed in a drying oven until the balsam hardened. Lice were cleared in a 10% KOH solution, neutralized with 1% acetic acid and processed through the alcohol series as with fleas and mounted. Ticks were examined and identified as alcohol preserved specimens.

Housing and Care of Test Dogs

Since no facilities were available for housing the dogs needed for this study it was necessary to construct outdoor facilities. Four concrete slabs, 2.44 m x 3.05 m, each equipped with a drain connected to a central disposal system with run off into a ditch, were constructed. The slabs were placed in a line with 1.5 m intervals between each slab. The slabs were provided with steel poles placed in such a manner as to allow the slabs to be fenced, divided into two 1.5 x 2.44 m pens and each pen equipped with a hinged gate. Each pen was provided with a dog house which was set off the slab with its opening into the pen. One slab was later divided into three pens and enclosed with screen wire to provide an arthropod proof facility to house experimental dogs which had not previously been exposed to disease vectors (Figure 1). A water line was installed and the pens were washed clean daily. All dogs were fed a commercial dog chow once a day and containers of water were

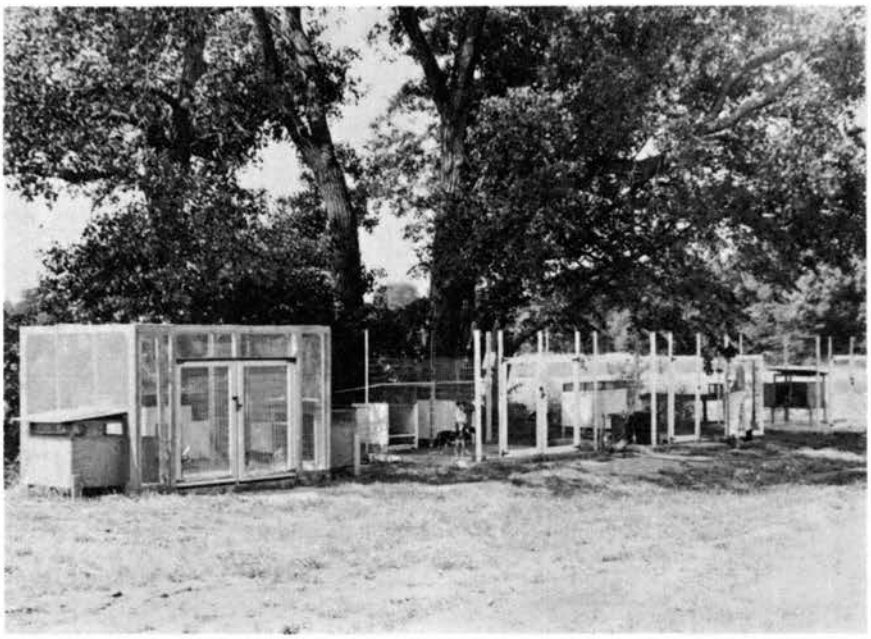


Figure 1. Facilities Used for Housing Test Dogs

available at all times. The non-exposed "clean" dogs used in this study were obtained from the College of Veterinary Medicine, Oklahoma State University and were known to be free of disease and/or exposure to arthropod vectors of disease. All other dogs, both infected and non-infected, were stray dogs obtained from the Stillwater Animal Shelter, Stillwater, Oklahoma.

Flea Rearing

Fleas were reared similar to the methods used by Linduska and Cochran (1946), Smith and Eddy (1954), and Hudson and Prince (1958). Two dog houses were constructed with hardware screen floors (1.27 cm mesh) and removable plywood panels underneath (Figure 2). These houses were located off the concrete slab with openings into the pen. The base of each house was enclosed and the space under the panel was filled with sand (Figure 3). The flea eggs from the flea infested dogs dropped off the dogs and fell onto the panels. The panels were removed every other day when eggs were needed and the eggs were swept off the panels into a container. When eggs were not needed for rearing the panels were not placed under the screen floors and the eggs were allowed to fall to the sand below where the fleas completed development and provided for continual reinfestation of the dogs. The eggs which were collected from the floor panels were taken to the laboratory, sifted through a 16 mesh screen to remove large debris and placed into 3.78 ℓ cylindrical jars, with stockinet sleeves attached around the tops. The jars contained a flea larval rearing media consisting of 100 cmm of powdered beef blood mixed with about 1.5 ℓ of dry sand (Figure 4). The jars were kept at a temperature of about 27^{o} C and a relative humidity of 80%. After about 10-12 days, when most of the larvae had pupated, the contents of the

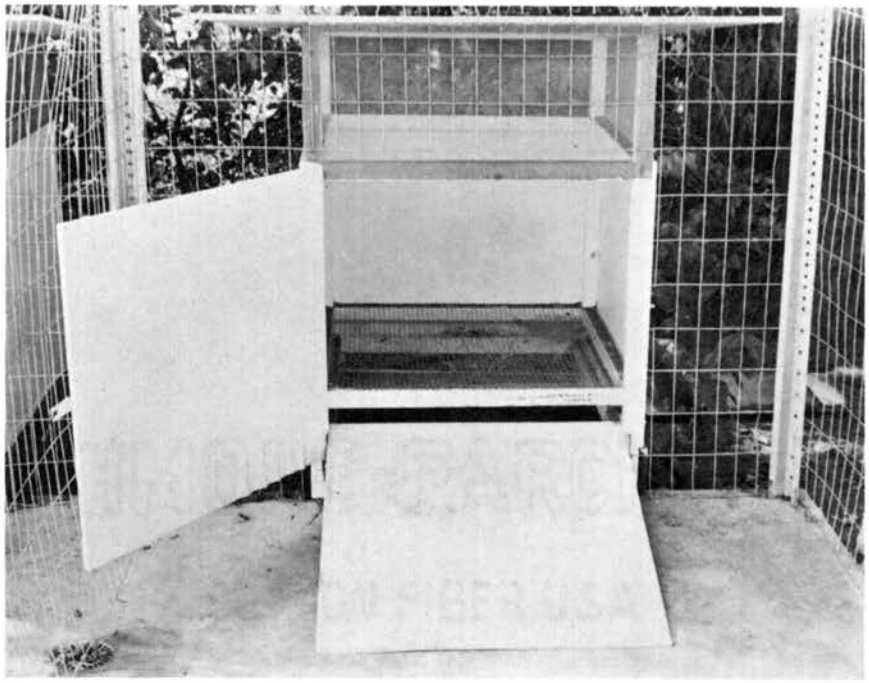


Figure 2. Dog House Showing Removable Panel for Flea Egg Collection

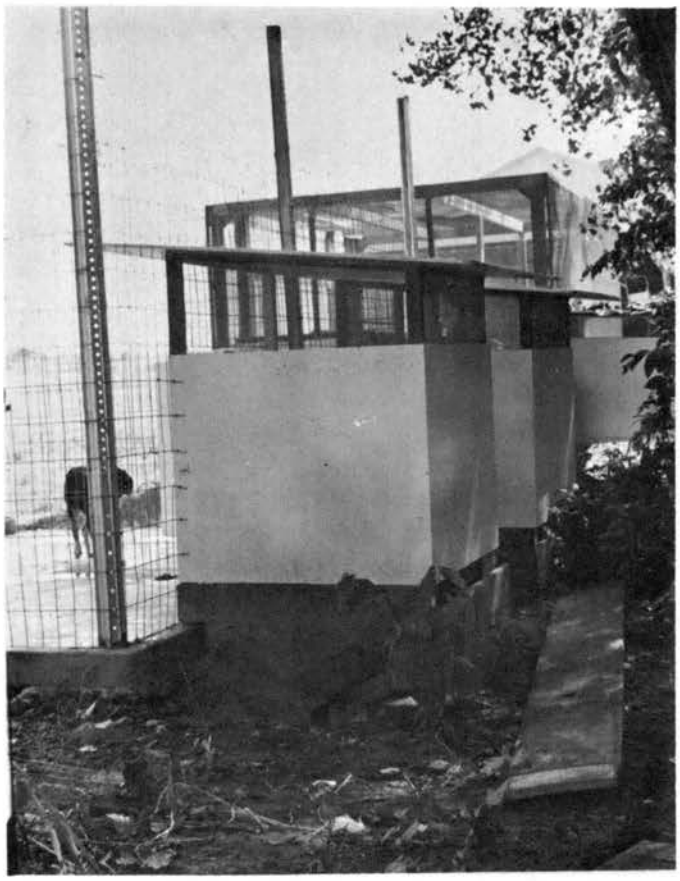


Figure 3. Dog House Showing Flea Breeding Area Underneath

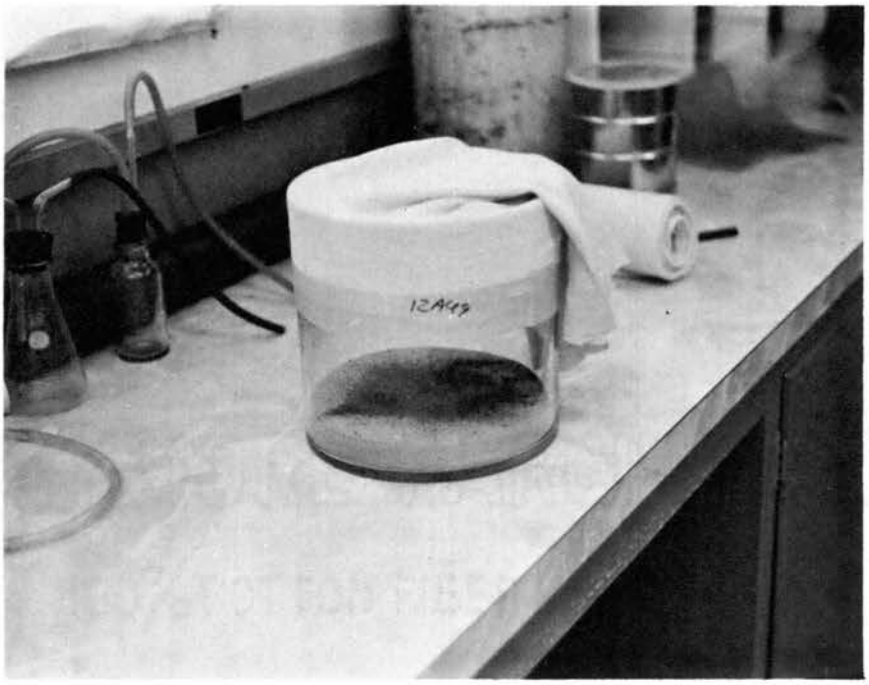


Figure 4. Flea Rearing Jar Containing a Mixture of Sand and Powdered Beef Blood

jars were sifted through a 16 mesh screen to remove the pupae. Sifting was done every other day thereafter until all the pupae had been removed. The pupae were put into a Petri dish and placed inside a tin funnel provided with a screen onto which the dish was set. The funnel was then placed into the opening of a large mouthed 3.78 ℓ jar (Figure 5). As the adult fleas emerged they fell into the jar. The jars used were 45.7 cm high which was a convenient height for only occasionally, when the funnel was removed, would a flea manage to jump from the jar. Adult fleas were removed from the jar when needed for tests by tipping the jar and allowing a few to jump out onto a white colored table top where they were collected by aspiration. Fleas could be counted rapidly and accurately by this procedure and only healthy strong fleas were obtained. The fleas to start this colony were reared from eggs collected from a cardboard box and bedding rags which had been used for a litter of pups and were obtained in Ponca City, Oklahoma. The colony was primarily a mixture of C. felis and E. gallinacea but a few Pulex simulans Baker were present. When collecting fleas from the emergence jars, if only C. felis was desired, any E. gallinacea which jumped onto the table were removed by touching them with masking tape which had been wrapped around the finger with the stickey side out. E. gallinacea were easily differentiated from C. felis because of their small size. If both species were desired two aspirators were used. Because P. simulans occurred only occasionally and then only in very few numbers, no attempt was made to differentiate them at this point.

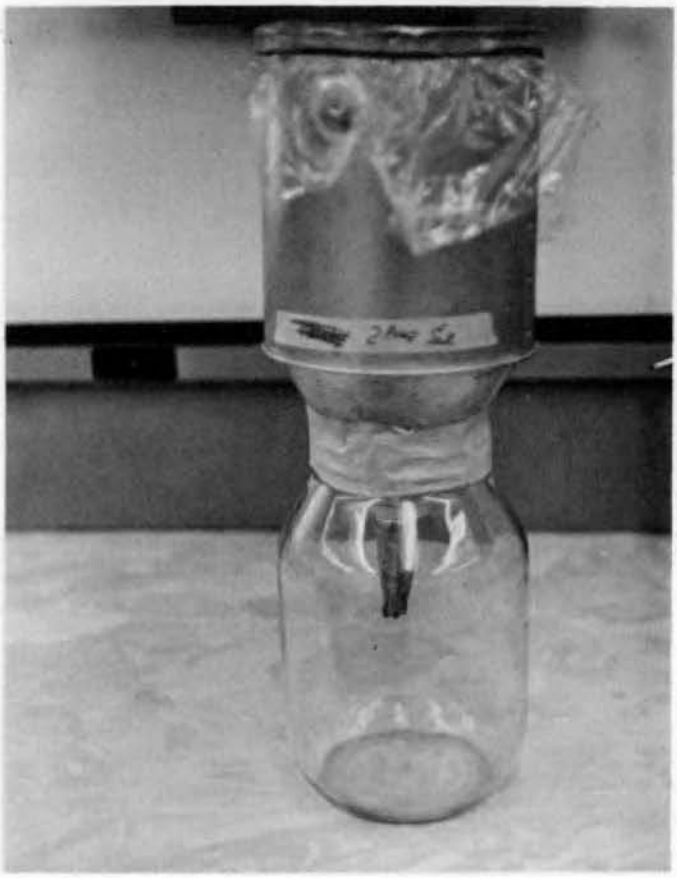


Figure 5. Flea Emergence and Adult Collection Container

Infecting Fleas

Two methods were used to feed fleas blood containing D. reconditum microfilariae. In the first method a plastic container 7 cm in diameter and 2 1/2 cm high, without a bottom and with a screw top, was securely taped to the shaved skin of an infected dog. Fleas were immobilized with CO_2 and placed on the dog's skin inside the container and were allowed to feed for varying periods of time. At the end of the feeding period CO_2 was again used to immobilize the fleas inside the container and the fleas were removed by use of an aspirator. The second method used to infect fleas was to release them on an infected dog which previously had been determined to be free of fleas. In this case the fleas were hand picked from the dog after being allowed to remain on the dog for varying lengths of time. The dog was fitted with a muzzle and all four feet were either padded with gauze taped into place or fitted with a commercial leather boot to prevent the dog from biting and scratching the fleas off its body (Figure 6). The dog was kenneled in a pen separated approximately 3 m from other dogs to reduce the chances of other fleas getting on the dog.

Several unsuccessful methods for feeding fleas blood containing microfilariae were tried. Cotton and gauze pads soaked in citrated blood containing microfilariae were placed in containers holding the fleas. This did not prove to be satisfactory. Fleas were placed in a 1 pint carton with a piece of ladies nylon hose fastened over the top to determine if the fleas would feed on a dog through the nylon mesh; however, the fleas were able to separate the threads and escape. Organza cloth was substituted for the nylon covering on the carton; this kept the fleas from escaping, but, when the cloth was placed on

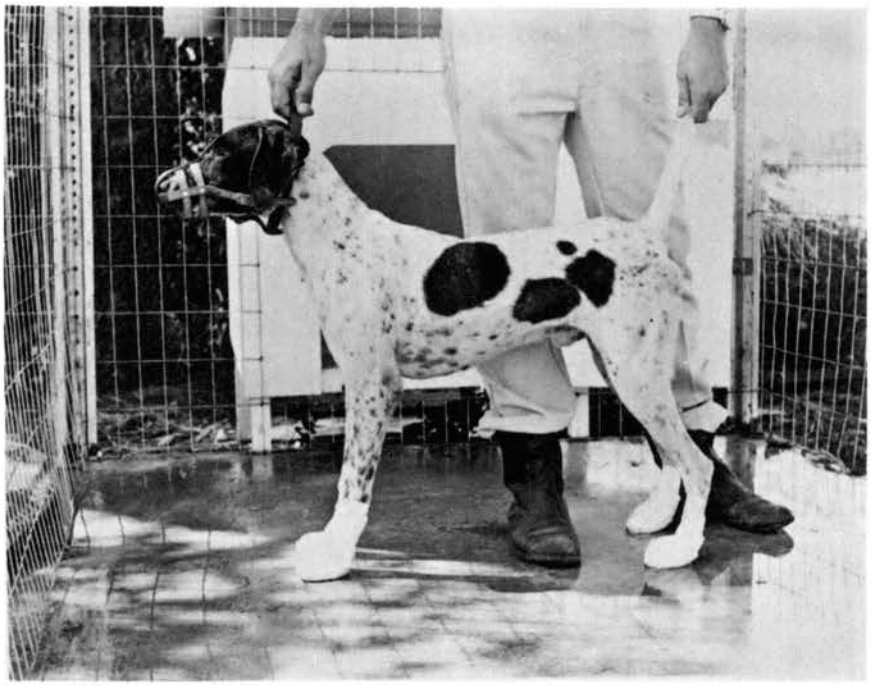


Figure 6. Dog With Muzzle and Foot Pads to Prevent the Dog From Removing Fleas From its Body

the shaved skin of an infected dog no feeding through the cloth was observed. The bottom of a 1 oz medicine cup was removed and replaced with a piece of Parafilm stretched tautly, but, no feeding through the membrane was observed.

Flea Examination

Fleas were examined for developing filariae by placing each flea into a drop of physiological saline on a clean microscope slide, and with the aid of a dissecting microscope the flea was dissected with teasing needles. When the material had been separated from the exoskeleton, the exoskeleton was removed and a coverslip was placed on the slide. The prepared temporary mount was then examined under a compound microscope at 100x magnification for the presence of developing filarial larvae. When developing larvae were found they were categorized as to stage of development and the number of larvae in each stage was recorded. When measurements and more detailed examinations were required a drop of 1:1,000 aqueous methylene blue was run under the coverslip and the larvae were killed and fixed by applying gentle heat from an alcohol flame to the underside of the slide. When semipermanent mounts were desired, selected developing larvae were removed from the dissecting slide, before the coverslip was added, and transferred to another slide containing a drop of equal parts glycerin and 1:1,000 aqueous methylene blue mixture. The larger larvae were removed by the use of a glass rod which had been heated and pulled to a fine point and the smaller ones by use of a capillary tube taking up a small amount of saline containing the larvae. The slide was gently heated over an alcohol flame to facilitate staining and then the coverslip was put into place. Mounts were made semipermanent by ringing the coverslip with synthetic resin

mounting medium (U.S. Department of the Army, 1961).

Infecting Stable Flies

Adult flies were collected near pig pens by using an insect net. The flies were placed into containers and brought into the laboratory where they were immobilized with CO_2 and the stable flies, Stomoxys calcitrans L., were separated from other flies and placed into a screen wire cage 45 cm square, one side of which was fitted with a stockinet sleeve. The cage containing the flies was kept at a temperature of about 27^{o} C and a relative humidity of 80%. The flies were offered an infective blood meal twice each day by placing the rear half of a D. reconditum infected dog through the stockinet sleeve into the cage containing the flies. The dog was held still in this position for 30 minutes each time. Flies were removed at various time intervals and examined for filariae. For examination, each fly was separated by body region, head, thorax and abdomen, and each part placed on a separate slide in a drop of physiological saline. With the aid of a dissecting microscope each body region was dissected with teasing needles, covered with a coverslip and examined under a compound microscope at 100x magnification for the presence of larvae. When larvae were found, the location, number, condition and stage of development were recorded.

Skin Penetration Test

A portion of the thigh of a dog was shaved and the skin was abraded by scraping with a surgical scalpel. Several D. reconditum infective larvae were removed from laboratory infected fleas and placed in physiological saline. A drop of saline containing the infective larvae was placed on the abraded skin and the actions of the larvae were observed

with the aid of a dissecting microscope. A coverslip was placed over the saline to retard drying until the observation was completed.

Necropsy and Recovery of Adult Worms

Attempts were made to recover adult worms from both naturally infected and laboratory infected dogs used in this study. Following euthanasia by intravenous injection of a commercial euthanasia material containing sodium pentobarbital, dogs positive for circulating microfilariae of D. reconditum were examined for the presence of adult worms in the skin, subcutaneous tissue and major muscles. The method used followed generally those described by Dunn (1932) and Lindsey (1962). Following euthanasia the hair was removed from the dog's bodies by use of an electric clipper equipped with a fine clipping head to provide a very close cut. After clipping, the dog's bodies were thoroughly washed with surgical soap and water to remove all dirt, scales and loose clipped hair which might later interfere with examination of the extract. The skin and subcutaneous tissues were removed and the larger muscles were removed and cut into 4 cm cubes. This material was then placed in 3.78 ℓ jars containing 2 liters of normal saline preheated to 40° C. The skin and subcutaneous tissues were placed in one container and the muscles were placed in other containers. The jars containing the tissues were placed in an incubator at 40° C for approximately 3 hours. The material was stirred and agitated every 15 minutes. Following the first 3 hour period, the tissues were transferred to other jars containing preheated saline and the process repeated for a period of approximately 2 hours and in one case over night. After removal of the tissues, the containers were allowed to stand for 30 minutes, and the supernatant was decanted leaving approximately 1,000 ml of extract.

The extract was poured into 500 ml beakers for ease of handling. A small amount at a time was poured into a Petri dish, held over a black paper, and examined macroscopically for adult worms. Any questionable material was examined with the aid of a dissecting microscope.

Prepatent Period

Approximately 500 laboratory reared C. felis were released on a D. reconditum infected dog and allowed to remain on the dog for a period sufficient for infective larvae to be present in a good percent of the fleas. The fleas were removed by hand picking on the 14th and 15th days after being placed on the dog. The fleas were dissected and the infective larvae were removed, pooled in 0.85% physiological saline and counted. On the 14th day 84 fleas were dissected and 112 infective larvae were collected. On the 15th day 50 fleas were dissected and 72 infective larvae were collected. Infective larvae collected and pooled in saline each day were drawn into a 6 cc syringe equipped with a 20 gauge needle and injected subcutaneously into a non-infected dog. The non-infected dog was obtained from the College of Veterinary Medicine, Oklahoma State University and was known to be free of D. reconditum and had been kenneled indoors, protected from exposure to arthropod vectors, throughout its life. From the time the dog was obtained for this study it was kenneled in an outdoor arthropod proof screened kennel (Figure 1). Periodic blood samples were taken over a four month period prior to initiation of this experiment and examined for circulating microfilariae but none were found. Following the injections of infective larvae, blood samples were taken and examined as fresh whole blood mounts for the presence of circulating microfilariae at weekly intervals throughout the study period. At necropsy, five months after injection

of infective larvae, the dog was examined for the presence of adult filarial worms.

RESULTS AND DISCUSSION

Microfilaria Survey

Rubin (1952) in a survey of the internal parasites of 100 dogs in Oklahoma County, Oklahoma, failed to find *D. immitis* although it had been diagnosed on different occasions in the Medical Clinic at Oklahoma University. *D. immitis* is known to occur in dogs indigenous to Oklahoma from cases of heartworm disease which have been diagnosed and treated at Oklahoma State University, Veterinary Clinic, Ewing (1970). As far as can be determined, a canine microfilaria survey such as the one reported here has never been conducted in Oklahoma and *D. reconditum* has never been reported to occur in the State, except for a preliminary report of the results from 100 dogs examined earlier in this study (Pennington, *et al.*, 1970).

Blood samples taken from 150 dogs were examined for the presence of microfilariae in the peripheral circulation. Microfilariae were found in 23 dogs (15.3%). Of these 14% were determined to be *D. reconditum* and 1.3% were *D. immitis*. The microfilariae of *D. reconditum* ranged from 237 μ to 282 μ long (mean 259 μ); the width was quite consistent at 4.7 μ with only minor variations. The microfilariae of *D. immitis* ranged from 284 μ to 303 μ long (mean 297 μ) and the width ranged from 5.9 μ to 7.1 μ (mean 6.6 μ). Although the means of these measurements are somewhat smaller than those reported by most other workers (Table II), they do fall within the ranges given. The slight differences may be due to variations in calibration of the eyepiece

TABLE II

MICROFILARIAL MEASUREMENTS OBTAINED BY SOME PREVIOUS WORKERS (IN MICRONS)

Investigator and Date	D. reconditum				D. immitis			
	Length		Width		Length		Width	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Newton and Wright (1956)	269 - 283	276	4.3 - 4.8	4.6	307 - 322	313	6.7 - 7.1	6.9
Wallinstein and Tibola (1960)	260 - 292	-	4.5 - 5.2	-	302 - 340	-	6.5 - 7.4	-
Lindsey (1961)	246 - 292	270.9	4.7 - 5.8	5.23	285 - 340	314	6.1 - 7.2	6.8
Lindsey (1962)	263.9 - 278.2	270.6	4.7 - 5.8	5.2	291 - 310	299	6.7 - 6.9	6.7
McKinney (1962)	233 - 260	-	4.1 - 5.4	-	304 - 343	-	6.0 - 7.4	-
Lindsey (1965)	258 - 292	270	4.7 - 5.8	5.2	286 - 340	314	6.1 - 7.2	6.8
Gubler (1966)	239 - 270	250.8	3.5 - 6.6	4.7	272 - 312	293	6.9 - 8.7	7.6
Jackson (1969)	264 - 298	-	4.7 - 5.8	-	298 - 314	-	6.7 - 6.9	-
Pennington and Phelps (1964)	230 - 285	262	3.2 - 5.8	4.7	-	-	-	-
Pennington (this study)	237 - 282	259	4.7 - 5.2	4.7	284 - 303	297	5.9 - 7.1	6.6

micrometer used to make these measurements. Sawyer, et al. (1963) point out some of the differences which can occur due to varied methods of processing microfilariae prior to measurement.

The infection rate in the study area is shown in Table III. There were no significant differences in the infection rate between male and female dogs except in the Ponca City area where the rate in female dogs was more than twice that of male dogs. This is in conflict with other workers who found male dogs infected 30-50% more often than females (Gubler, 1966; Thrasher, et al., 1963; Wallinstein and Tibola, 1960; Pennington and Phelps, 1969). The small size of the sample from Ponca City may account for this apparent difference; and, in any event, the sample is considered too small to warrent general conclusions.

Body size and hair length of dogs were recorded for 100 dogs examined in an attempt to ascertain if the rate of infection could be related to these factors. Size was recorded as small (6.8 kg or less), medium (6.8 to 12.6 kg), and large (over 12.6 kg). The hair length was noted as being short (less than 2.5 cm), medium (2.5 to 5 cm) or long (over 5 cm). Data are recorded in Table IV. Small, short-haired dogs were found to be infected more often than other dogs; however, this did not prove to be statistically significant ($\chi^2 = 3.4$, $P > 0.05$ and $\chi^2 = .72$, $P > 0.05$).

Ectoparasite Survey

Reinert (1965) in his study of host-flea relationships of Payne County, Oklahoma, found C. canis, C. felis, E. gallinacea, P. irritans, and P. simulans to occur on dogs. Hopla (1969) in his host and distribution studies of fleas found P. simulans to be much more abundant than P. irritans on coyotes and dogs in Oklahoma. Other isolated

TABLE III

DISTRIBUTION OF *D. RECONDITUM*[1] IN THE STUDY AREA

	No. Dogs Examined		No. Dogs Infected		% Infected		Total %
	Male	Female	Male	Female	Male	Female	Infected
Stillwater	29	17	4	3	13.8	17.3	15.2
Ponca City	11	13	2	5	18.2	38.5	29.2
Enid	36	34	4	2	11.1	5.9	8.5
Guthrie	4	2	1	0	25.0	0.0	16.6
Edmond	3	1	0	0	0.0	0.0	0.0
TOTAl	83	67	11	10	13.3	14.9	14.0

[1]One male and one female from Stillwater were infected with *Dirofilaria immitis* (1.3%).

TABLE IV

INCIDENCE OF INFECTION BASED ON BODY SIZE AND HAIR LENGTH

Size	% Infected	Length of Hair	% Infected
Small	23.1	Short	18.0
Medium	11.6	Medium	12.4
Large	5.6	Long	11.8

observations of the ectoparasites of dogs in Oklahoma have probably been made; but, this appears to be the first survey of a number of dogs in which the occurrence, as well as the numbers of ectoparasites found on dogs in Oklahoma is reported.

Information on the prevalence of fleas, ticks and lice infesting 93 of the 150 dogs in this study was collected. A total of 3,293 fleas representing 3 species, 171 ticks representing 3 species, and 32 lice representing 1 species, was taken from the dogs. Of all dogs examined, 86% were infested with fleas, ticks or lice. Fleas were found on 77.4% of the dogs, 40.8% were infested with ticks and 2.1% with lice. The infestation rate for C. felis was 61.4%, P. simulans 50.5% and E. gallinacea 7.5%. Both C. felis and P. simulans were found on 36.6% of the dogs examined. Only one dog was infested with E. gallinacea alone. None of the dogs examined was infested with either C. canis or P. irritans. The average number of C. felis per dog was 39, 25 for E. gallinacea and 18.2 for P. simulans.

Dermacentor variabilis (Say) infested 42% of the dogs, while only one dog each was infested by Amblyomma americanum (L.) and Rhipicephalus sanguineus (Latreille). Infestations of both fleas and ticks were found on 34.4% of the dogs. Only two dogs were infested with L. setosus and these two dogs were also infested with both fleas and ticks. Table V lists the percent of dogs infested with parasites at each of the five locations included in this survey.

Ewing and Fox (1943) state that C. felis as well as C. canis are almost cosmopolitan; however, in certain regions, one species may occur to the exclusion of the other. Hubbard (1947), Pratt and Wiseman (1962) and others list C. felis as being more abundant and generally

TABLE V

NUMBER OF DOGS EXAMINED AND PERCENT OF INFESTATIONS WITH PARASITES IN FIVE OKLAHOMA CITIES

Location	No. Dogs Examined	% Infested with Parasites	% Infested with Fleas	% Infested with *C. felis*	% Infested with *P. simulans*	% Infested with *C. felis* and *P. simulans*	% Infested with *E. gallinacea*	% Infested with Ticks	% Infested with *D. variabilis*	% Infested with *A. americanum*	% Infested with *R. sanguineus*	% Infested with Fleas and Ticks	% Infested with *L. setosus*
Edmond	4	100.0	100.0	75.0	75.0	50.0	-	75.0	75.0	-	-	75.0	-
Enid	43	79.2	76.7	70.0	32.6	30.0	2.3	32.4	30.1	-	2.3	20.9	2.3
Guthrie	6	83.3	66.7	16.7	66.7	16.7	-	50.0	50.0	-	-	33.3	-
Ponca City	15	86.7	86.7	73.0	60.0	53.3	6.7	46.7	40.0	6.7	-	40.0	6.7
Stillwater	25	92.0	76.0	48.0	68.0	44.0	20.0	60.0	60.0	-	-	48.0	-
TOTAL	93	86.0	77.4	61.4	50.5	36.6	7.5	44.4	42.0	1.1	1.1	34.4	2.1

distributed in certain regions than C. canis. Scott (1967) lists P. simulans as the common parasite of dogs in the United States. It has been the observation over a period of several years by Howell (1970) that dogs in Payne County, Oklahoma, are more commonly infested with C. felis than C. canis. In view of the observations listed above, it is not surprising that C. canis was not taken from dogs in this study.

Since Smit (1958) removed P. simulans from synonymy with P. irritans there has been confusion concerning the recorded information on the distribution and host records as to which species this information refers. This earlier confusion in classification may also concern records dealing with the vector potential of these fleas. Pointedly, this may cast doubt on the validity of classification in earlier reports of P. irritans as a vector of D. reconditum in dogs. Reinert (1965) studying the host-flea relationships in Payne County, Oklahoma, observed P. simulans to be much more abundant than P. irritans on coyotes and dogs. Hopla (1969) found the host association of P. simulans to be extremely varied from one geographic location to another, but consistent within a given area. He also found P. simulans to be much more abundant than P. irritans on coyotes and dogs in Oklahoma. Parrish, et al. (1970) discuss the diseases which may be transmitted by fleas, ticks and lice associated with dogs. It is interesting to note that 86% of the dogs in this survey conducted during late June and July were infested with ectoparasites, but, observations of some 25 dogs from the same area during early May the following year showed less than 15% were infested. Further observations of experimental dogs which were heavily infested with fleas during the summer and kenneled out doors lost their infestations with the advent of cold weather.

Development of D. reconditum in C. felis

General. Adult fertile D. reconditum females located in the dog's subcutaneous tissues liberate microfilariae which circulate in the blood stream. The microfilariae are ingested along with the blood meal when the flea feeds. Through cyclodevelopment the filarial larvae reach the infective stage and transfer back into the dog where additional development occurs to reach adulthood.

Explanation of Development Stages and Types. It is generally accepted that there are three stages of development of filarial larvae in the invertebrate host and that there are two molts, one between the first stage larva and the second stage larva and one between the second stage larva and the third stage or infective larva. Most workers agree that a cuticle is shed by the developing larva during the second molt which culminates in the third stage larva; however, there is less agreement surrounding the molt between the first and second stage larva. Pistey (1958) states that Dirofilaria tenus (Chandler) from the raccoon has two definite molts in the mosquito. Taylor (1960a) reports D. immitis from dogs has two molts in the mosquito; but, points out that after the first ecdysis the skin is not necessarily cast off immediately but may be retained until the second ecdysis occurs. Webber (1955) working with D. aethiops (Webber) from monkeys and its development in mosquitoes, found that during the first molt the external cuticle and the linings of the stoma, esophagus and rectum are shed; however, from his illustration of the second stage larva it is shown developing inside the first larval cuticle. Orihel (1969) studying D. corynodes (von Linstow) from monkeys and its development in mosquitoes found that all of the first larval cuticle with the exception of that portion

adherent to and posterior to the anal plug was shed during the first molt. Nelson (1962) in studies on D. reconditum from dogs and its development in fleas found that the prominent rounded knob containing the lancets in the first stage larva became detached with the first ecdysis and the rapidly growing second stage larva developed inside the first larval cuticle. In the present study the cuticle was observed to become detached at both the anterior and posterior ends. At this time the larva became more active than before, and growth was rapid. The exuviae were subsequently shed and separated from the larva at which time the larva was observed to have the morphologic characteristics and measurements of an infective larva.

The growth and development of the larvae of D. reconditum in the intermediate host, C. felis, is a gradual and continuous process; however, several morphologically distinct forms can be identified. Generally these are referred to as Stage I, Stage II and Stage III, the infective larva. In this study, for convenience in recording, discussing and illustrating the development, several other forms have been identified by gross morphology and size. The first stage larval development has been divided into six recognizable types. The second stage larva is represented by one type and the third stage by two types.

Stage I, Type 1 (hereafter T-1). This larva is almost identical to the circulating microfilaria in the host dog, showing very little change. The length ranges from 230 μ to 280 μ (mean 255 μ) and the width ranges from 4.0 μ to 6.0 μ (mean 4.7 μ) (Plate I, Figure 1).

Stage I, Type 2 (hereafter T-2). This type represents the first noticeable changes in the development of the larva. The larva begins to widen along the posterior 1/3 of its body just forward of the thin

Plate I

Fig. 1. Stage I, Type 1 larva

Fig. 2. Stage I, Type 2 larva

Fig. 3. Stage I, Type 3 larva

Fig. 4. Stage I, Type 4 larva

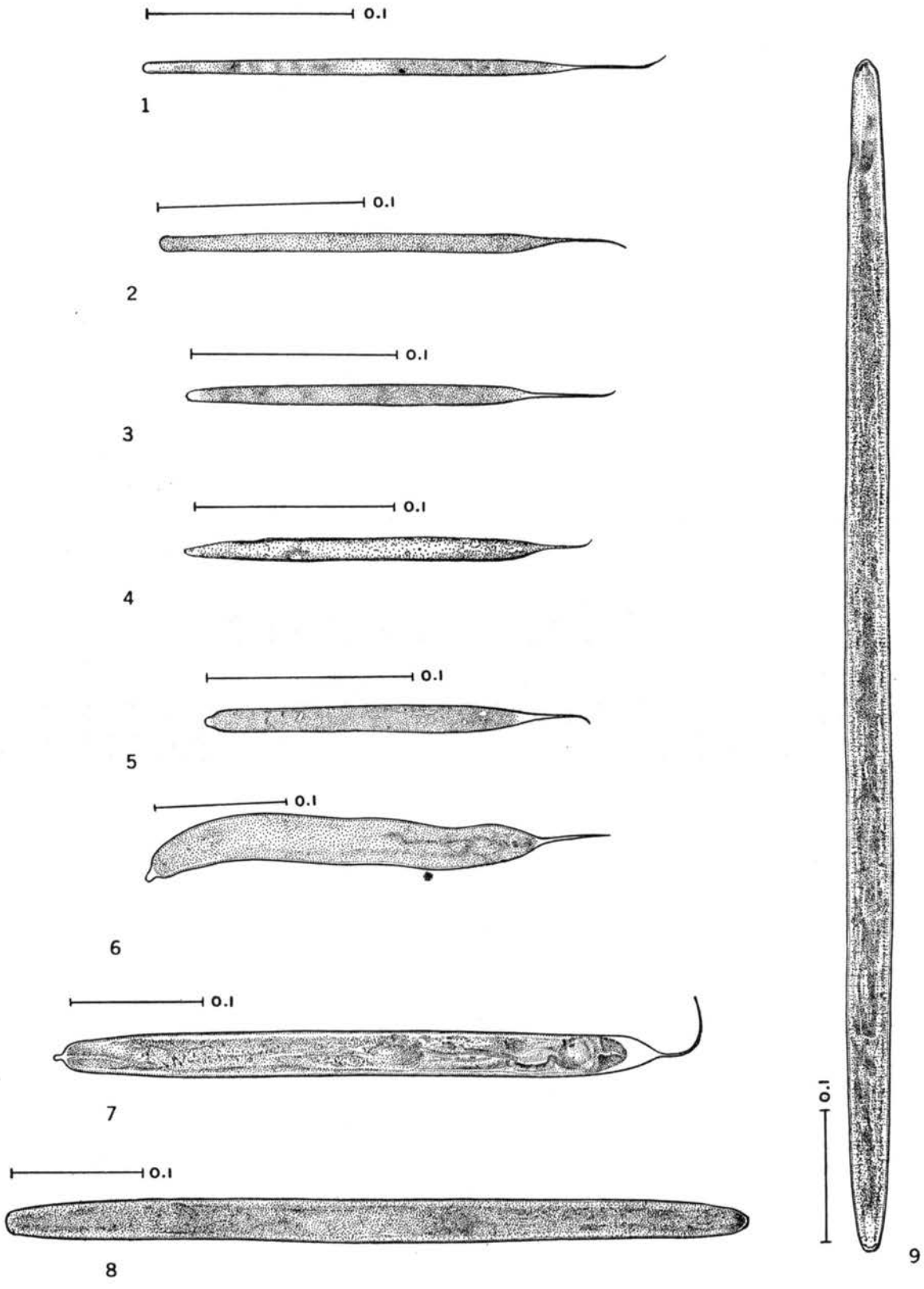
Fig. 5. Stage I, Type 5 larva

Fig. 6. Stage I, Type 6 larva

Fig. 7. Stage II larva

Fig. 8. Stage III, Type 1 infective larva

Fig. 9. Stage III, Type 1 infective larva



Figs. 1-9. D. reconditum Larval Development in C. felis

tail and becomes somewhat shorter. The length ranges from 180 μ to 230 μ (mean 212 μ) and the width, measured at the widest point, ranges from 5 μ to 7 μ (mean 6 μ) (Plate I, Figure 2).

Stage I, Type 3 (hereafter T-3). This type continues to shorten somewhat in length and the width increases considerably. The widening growth is very pronounced along the posterior 1/3 but is also quite noticeable along the rest of its length. The length ranges from 180 μ to 239 μ (mean 200 μ) and the width ranges from 6 μ to 10 μ (mean 9 μ) (Plate I, Figure 3).

Stage I, Type 4 (hereafter T-4). The larva continues to become shorter and wider and the width becomes more uniform. A cephalic knob-like structure, although detectable in the previous type, becomes more pronounced as the anterior end becomes thicker, more rounded and blunt. The length ranges from 145 μ to 200 μ (mean 170 μ) while the width ranges from 7 μ to 11 μ (mean 9.5 μ) (Plate I, Figure 4).

Stage I, Type 5 (hereafter T-5). The larva has continued to shorten and has reached its shortest length at this point. The width is much greater than that of the previous type and is now nearly uniform along the entire length of the larva. The anterior end has rounded and become very blunt with the cephalic knob protruding as a definite structure. The posterior end has also rounded and become more blunt, and the tail now appears small, short and thin compared to the width and blunt appearance of the remainder of the organism. This is the type usually referred to in the literature as the "sausage" form. The length ranges from 135 μ to 180 μ (mean 157 μ) and the width ranges from 10 μ to 20 μ (mean 15.5 μ) (Plate I, Figure 5).

Stage I, Type 6 (hereafter T-6). This type shows remarkable

growth in both length and width to almost twice the size of T-5. It retains its blunt ends and protruding cephalic knob, and the developing internal structures become more discernible. The length ranges from 200 μ to 400 μ (mean 322 μ) and the width ranges from 25 μ to 40 μ (mean 30.5 μ) (Plate I, Figure 6).

Stage II (hereafter S-II). Although there is no actual molt in the sense of casting exuviae, the larva does lay down a new cuticle and the old cuticle pulls away from the anterior and posterior ends creating a sheath like appearance at both ends. The cephalic knob has disappeared except for what appears to be the cuticle remains and the anterior portion of the larva now shows a slight indentation. The posterior end has rounded off and no longer possesses the typical tail of the previous types. The sheath retains the outline of the typical tail and cephalic knob appearance. The larva grows very rapidly in length but does not increase much in width. The length ranges from 300 μ to 700 μ (mean 502 μ) and the width ranges from 30 μ to 50 μ (mean 33 μ) (Plate I, Figure 7).

Stage III, Type 1 (hereafter III-1). The exuviae of the previous stage have been shed and the larva is now a young infective form. It has increased in length by almost 2/5 the length of the previous larva but its width has not changed. The movement is much more rapid than in the previous stage. The length ranges from 600 μ to 1100 μ (mean 715 μ) and the width ranges from 25 μ to 45 μ (mean 32 μ) (Plate I, Figure 8).

Stage III, Type 2 (hereafter III-2). This type is the mature infective larva which represents the completion of the cycle in the arthropod host. It has increased considerably in length over the previous type and has decreased somewhat in width. The anterior end of the larva

is seen to extend and contract in a probing manner (Figures 7 and 8). Posterior papillae can be seen (Figure 9) and the alimentary canal is well developed and discernible. The larva is very active and characterized by thrashing movements. The length ranges from 800 μ to 1450 μ (mean 1150 μ) and the width ranges from 20 μ to 40 μ (mean 29 μ) (Plate I, Figure 9).

It should be remembered that the growth and development of the larvae in the intermediate host is a gradual and continuous process and the above divisions are based on gross morphology with measurements as a guide. It can be expected that an overlap between each division may occur where an individual larva which falls between divisions may be placed in either a higher or lower division; but, in general the majority of larvae are easily placed in one of the divisions described.

Ingestion of Microfilariae by C. felis. Laboratory reared C. felis were placed in the plastic holder described previously which was taped to a shaved portion of a D. reconditum infected dog. The dog had a microfilariae count of 28 per .02 ml of blood. The fleas were allowed to feed for about 15 minutes and were removed and placed in a holding container. After holding for two days, 14 fleas were dissected and examined for developing larvae. One flea contained one larva which showed very little developmental change; dead larvae were found in 6 fleas and nothing was observed in the remaining 7 fleas. Since it was obvious that a feeding period of longer than 15 minutes was required to obtain a high rate of infection in the fleas, a new batch of C. felis was allowed to feed on the infected dog for one hour. These fleas were then removed and held at a temperature of about 27° C and a relative humidity of 80% for 24 hours. Sixteen female fleas were examined of which two

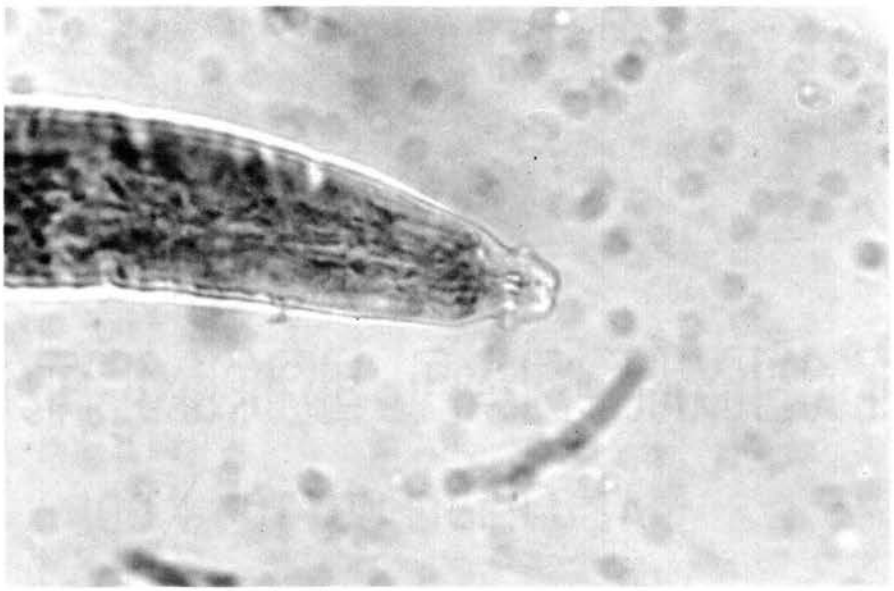


Figure 7. Anterior end of Infective Larva in Extended Position

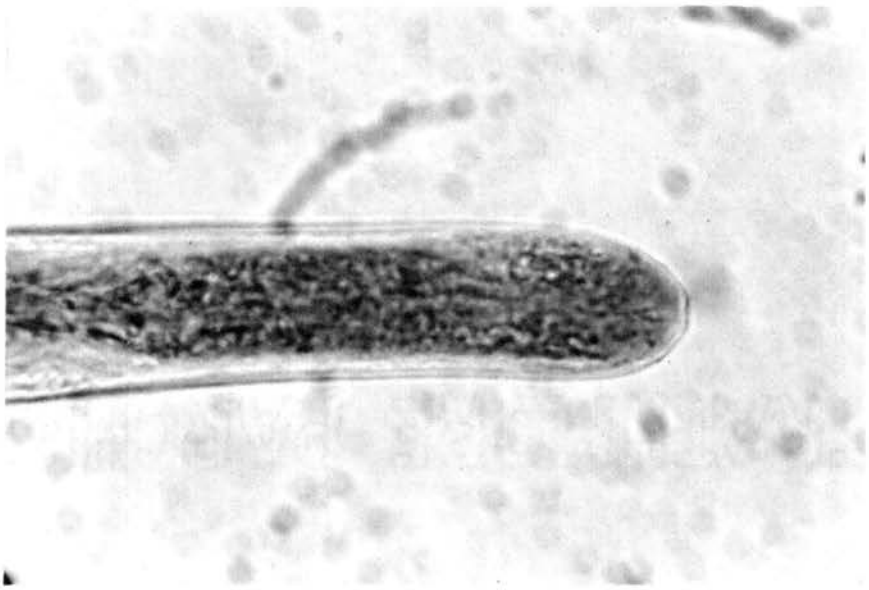


Figure 8. Anterior end of Infective Larva in Retracted Position

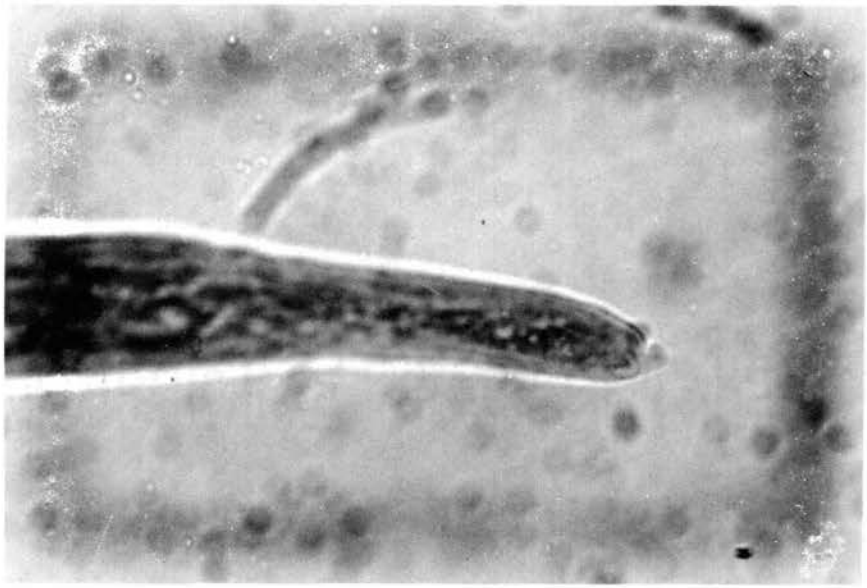


Figure 9. Caudal end of Infective Larva
Showing Papillae

fleas had one larva each, neither showing any developmental changes. The other 14 fleas were all negative for larvae. This showed that one hour feeding was still an insufficient period for the fleas to obtain a high rate of infection; therefore a two hour feeding period was tried. The fleas were held for 3 days at which time most of them had died; however, of 5 live fleas examined no larva were found.

Another batch of fleas was fed on the infected dog for 8 hours, then removed and placed in a holding container. A sample of from 10 to 14 fleas were removed from the holding container each day for 4 succeeding days and examined. On day one, 11 fleas were examined and 8 (73%) were infected with from 1 to 4 larvae (average 2.2/flea). On day two, 13 fleas were examined and 4 (31%) were infected with from 1 to 4 forms (average 1.7/flea). On day three, of 16 fleas, 6 (37%) were infected and one flea contained 14 larvae, but 3 larvae were the maximum found in any of the other 15 fleas. On day four 10 fleas were examined, 3 (30%) contained living larvae and 2 (20%) contained dead larvae. From these results it was concluded that an 8 hour feeding period will give a high rate of infection (73%); but the level of infection (2.2 larvae/flea) is low. Further, approximately 40% of the fleas may not remain infected more than about two days.

Two stray fleas which probably escaped from the feeding chamber during one of the earlier feedings were recovered from the infected dog and examined. One flea contained 85 larvae and the other contained 125 larvae in various stages of development; however, none had reached the infective stage. This finding showed that, if fleas are allowed to feed continuously over an extended period of time, very large numbers of larvae may be ingested and a large number of larvae may develop in a

single flea.

Development of Young Larvae in C. felis Transferred to a Non-Infected Dog. After inspection to insure there were no fleas on the infected dog, a number of laboratory reared C. felis were released on the infected dog. The microfilariae count at this time was 40 per .02 ml of blood. Fleas were allowed to stay on the dog for about 48 hours after which time they were removed by hand picking and aspiration. Several strips of hair the width of an electric clipper head were removed from the dog to facilitate recapture of the fleas. Five fleas were examined and all of them contained developing larvae. The number of larvae per flea ranged from 8 to 23 (average 14.6). The fleas which had been removed from the infected dog were released on a non-infected dog. The fleas were allowed to remain on the dog for 5 days after which time fleas were collected each day and examined. The results of these examinations are shown in Table VI. The infection rate in these fleas dropped from an estimated 100% at the time of transfer to 22% five days later and no infected fleas were found after the 10th day. No infective larvae were observed in any of the fleas examined. It appears that only a small percent, if any, young larvae will complete their development to the infective stage if the infected flea is transferred to a non-infected dog before the larvae have an opportunity to become well established in the flea. Additional work is necessary to determine at what stage of larval development the flea may transfer to a non-infected host and the development continue and why this occurs.

TABLE VI

PERCENT OF FLEAS RETAINING INFECTION AFTER TRANSFER TO NON-INFECTED DOGS

	At Time of Transfer	Days After Transfer								
		5	6	7	8	9	10	11	12	13
No. Fleas Examined	5	9	8	10	6	6	8	4	6	5
% Infected	100	22.0	12.5	1.0	0.0	16.6	12.5	0.0	0.0	0.0

Individual Variation in Susceptibility of C. felis to Infection. Fleas reared under identical conditions in the laboratory from the same colony and placed on an infected dog at the same time showed great individual variations in the numbers of larvae present as well as in larval development at any given time. Some individual fleas were found to contain very few larvae indicating that they either ingested relatively few larvae or possessed some rapid means of assimilating the larvae once ingested. Other individual fleas were found to contain large numbers of larvae which would indicate that they either ingested greater numbers or lacked the means to assimilate them. Fleas were also observed which contained intermediate numbers of larvae. The variation in larval development in individual fleas was independent of the variation in numbers of larvae present. The variation in larval development was characterized primarily by retardation or cessation of development of the T-2 larvae and to a lesser extent of the T-1 larvae. The degree of retardation also varied from almost complete cessation where

essentially no larvae developed beyond T-2 or T-3, to partial cessation or retardation where perhaps as many as 2/3 of the larvae present were developing normally. Secondly, dead larvae were observed in some fleas. These were mostly T-2 larvae and were usually found in fleas which also showed retardation of growth of the T-2 larvae. The larvae appear to have penetrated the gut, come to lie in the abdominal haemocoel and have just begun to show a slight thickening along the posterior 1/3 of the body when growth is stopped. Larvae continue to be ingested and the numbers of T-2 larvae build up in the haemocoel with some gradually slowing down in activity and eventually dying. The percent of dead larvae observed was usually small and signs of deterioration were noted, indicating that the larvae are probably assimilated rather rapidly after death. Death and assimilation of the larvae were not commensurate with the number of larvae ingested. Occasionally larvae developing up through T-6 were observed dead and deteriorating and these were not always restricted to fleas which showed larval retardation. There was no evidence of encapsulation of D. reconditum larvae by C. felis. Kartman (1953a) studied the factors influencing infection of mosquitoes with D. immitis and concluded that species, strain and individual variations in response to infection were probably determined in part by their genetic constitution. Kartman presented a good review of this subject and substantiated the findings of Gordon and Lumsden (1939) who showed that when mosquitoes (Aedes aegypti) fed on the web of a frog's foot, blood was taken either directly from a capillary or from an extravasation of blood derived from a previously lacerated capillary. These two methods of feeding were then correlated with the number of microfilariae taken up from the frog and it was found that feeding from extravasated blood

resulted in a lower concentration of microfilariae than when blood was taken directly from a capillary. It was also pointed out that the microfilariae concentration in different capillaries varies considerably. Kartman suggested that the extreme degree of variation in the numbers of microfilariae ingested is accounted for by the exigencies of feeding either directly from capillaries or upon intercapillary hemorrhages. Lavoipierre and Hamachi (1961) studying the feeding mechanism of the flea determined that fleas are normally solenophagous.

Effect of Developing Larvae on C. felis. It was observed that fleas which ingested and supported the development of large numbers of larvae were adversely affected by the larvae. No adverse affects were observed until about the 7th day and then only in fleas which contained large numbers of the larger and more active T-6, II and III larvae. The fleas were observed to become sluggish in their activity and leave their normal location among the hairs next to the dog's skin and crawl about on top of the hairs. The fleas obviously had stopped feeding and upon examination it was found that egg production had ceased. There was visual evidence of considerable damage to the internal organs. Fat bodies had been ruptured as the infective larvae left this site of development after molting. Other organs had been damaged by the continual thrashing of the many infective larvae. Fleas which support these large numbers to late development eventually succumb to the infection. Fleas were found in this state between the 7th and 13th days of infection. After the 13th day apparently most of the fleas which had this high susceptibility to infection had succumbed, as few were observed in this condition after this time. It appeared that fleas in this condition had a tendency to move to the surface of the dog's hair coat and

become sluggish in their activity, which made them more easily found and captured while sampling during the period in which they occurred. It is probable that this biased the results obtained during the 7th to 13th day period to some extent.

Fleas which appeared to have some means of restricting the numbers or the development of larvae did not show any adverse effects due to the infection. Their activity appeared normal and egg production continued. Stueben (1954a) working with what we now think was D. reconditum reported there was no increase in the mortality rate nor apparent effect on the fecundity of the female fleas or fertility of the male fleas harboring an infection; however in Stueben's experiment fleas were allowed to take only one infective blood meal and, on the basis of the present study, could hardly be expected to have ingested sufficient larvae to cause damage.

Transfer of Infective Larvae to the Dog. When fleas containing large numbers of infective larvae were placed in a drop of saline in preparation for dissection it was not uncommon to see infective larvae egressing from the intersclerital membranes in the area of the mouth parts. Sometimes the infective larvae could be encouraged to come out by using teasing needles to bring the mouth parts forward to the natural feeding position. When the mouth parts are brought forward the membranes are stretched thin and are more easily penetrated. During the act of feeding the mouth parts are brought forward into position for piercing the skin. The paired maxillary lacinia serve as the cutting organs and are inserted into the host followed by the epipharynx. As these mouth parts are inserted the head comes to lie in contact with the skin and the maxillae, maxillary palps and labial palps are forced

out to the sides causing additional stretching of membranes around these structures. It is probable that when the flea is thus feeding and the stretched membranes around the mouth parts are in close proximity or in contact with the feeding puncture in the skin the infective larva penetrates the thin membrane and enters the host through the puncture wound. Stueben (1954a) induced infective larvae to emerge from around the area of the mouth parts when the mouth parts were manipulated and also suggested a similar method of transfer to the dog.

Skin Penetration. Five D. reconditum infective larvae were collected from 3 infected fleas by dissection and placed in physiological saline. A drop of saline containing the infective larvae was placed on the abraded skin of a dog's thigh, covered with a coverslip and observed with the aid of a dissecting microscope for 30 minutes. Two larvae were observed to actively penetrate and enter the tissues through an opening in the abraded skin. One entered 9 minutes and the second entered 15 minutes after being placed on the dog. The remaining 3 larvae continued to move about but did not enter the tissues during the observation period.

Site of Larval Development in C. felis. Microfilariae taken in with the blood meal penetrate the midgut and enter the abdominal haemocoel soon after ingestion. In the hundreds of flea dissections made during this study, only very rarely were larvae observed in the contents of the midgut. On two occasions larvae were observed actively penetrating the wall of the midgut. Stueben (1954a) found the microfilaria left the gut as early as one hour after ingestion and very few remained in the gut after 4 hours. The primary site of development for the larvae is within the abdominal fat bodies (Figure 10); however,

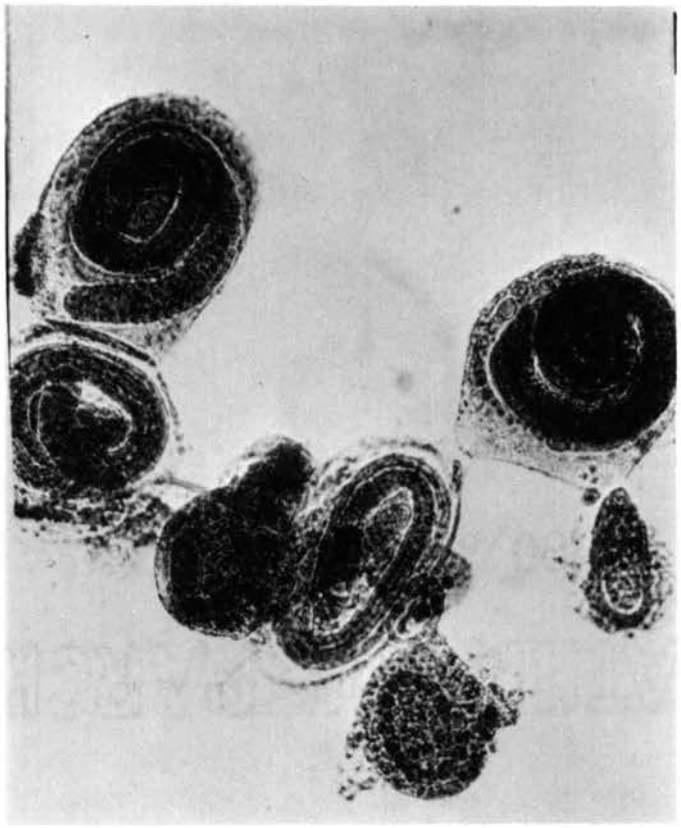


Figure 10. Larvae Developing Within Abdominal Fat Bodies

larvae also develop free in the abdominal haemocoel. Many larvae were observed in the fat bodies and free in the haemocoel. It is realized that some of the fat bodies may be ruptured during dissection, thereby liberating the larvae into the haemolymph; however, the large numbers of developing larvae observed in the haemolymph on many occasions could hardly have emerged from the small number of fat bodies ruptured during dissection. On several occasions such large numbers of developing larvae were observed in one flea that it would be a mechanical impossibility for the fat bodies to hold them all; although, on many occasions two and three developing larvae were observed within one fat body (Figure 11). Most T-1 larvae were found free in the haemocoel and only occasionally would one be found within the fat bodies. The T-2 larvae began to enter the fat bodies within the first 24 hours and this type as well as all other types through Stage II were found both in the fat bodies and in the abdominal haemocoel. For those larvae developing within the fat bodies it appeared that shortly after the Stage II larva molts the Stage III-1 larva leaves the fat body and is found free in the haemolymph. Stage III larvae were never observed within the fat bodies.

From what we know now many of the earlier workers who reported D. immitis development in fleas were probably working with D. reconditum. Breinl (1921) reported development occurred in the Malpighian tubules. Summers (1940, 1943) reported development occurred in the haemocoelic cavity and not in the Malpighian tubules as suggested by Breinl (1921); however, Brown and Sheldon (1940) reported sausage shaped larvae were recovered from the Malpighian tubules while infective larvae were found in the body cavity. Stueben (1954a, 1954b) reported development occured

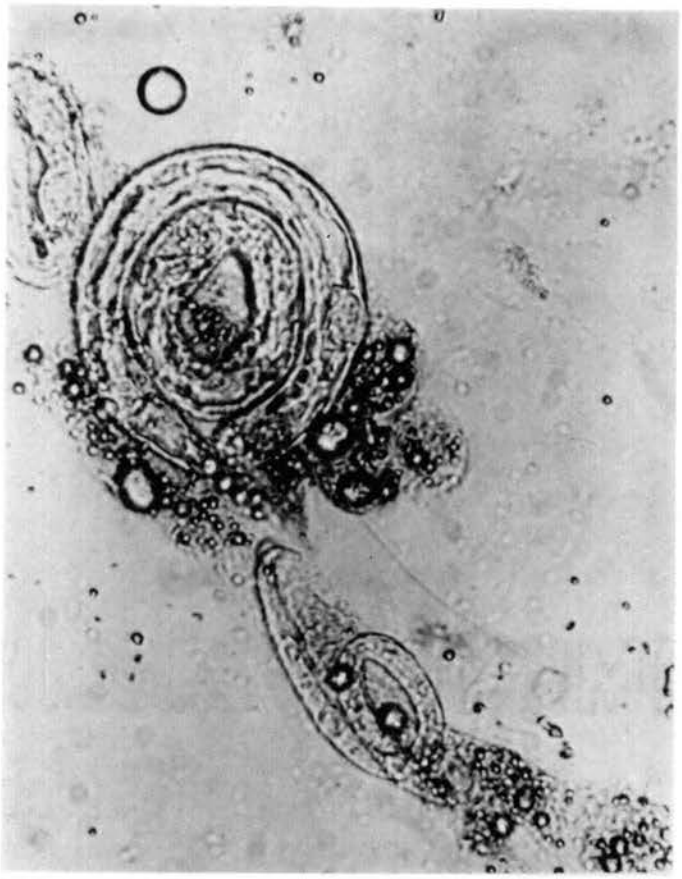


Figure 11. Multiple Larval Invasion of a Fat Body

in the haemocoel. In more recent work with D. reconditum development in fleas Newton and Wright (1956) first reported development in the haemocoel but later (1957) reported development in the fat bodies. Nelson (1962) listed the fat bodies as the site of development.

Daily Progress of Infection in C. felis. Approximately 400 laboratory-reared C. felis were placed on a D. reconditum infected dog. The dog had previously been examined and found to be free of any fleas and the dog's blood contained 17 microfilariae per .02 ml of blood. A sample of the fleas was taken daily and examined for larvae. The results for each day are presented in Table VII.

Day 1 - Nine fleas were examined at the end of the first 24 hours and the number of larvae found ranged from 6 to 38 per flea (average 15.4). The maximum development was to T-2 and 58.5% of the larvae were found to be of this type. Many T-2 larvae had already entered the fat bodies.

Day 2 - The number of larvae found was almost twice that of Day 1, ranging from 13 to 54 (average 28.6) larvae per flea. The maximum development was to T-3 which occurred in 58.3% of the fleas examined. A few dead larvae were observed in 3 of the 12 fleas. The average number of dead larvae found in fleas containing dead larvae was 2.6. In some of the dead larvae the tissues were breaking down and were partially dissolved.

Day 3 - The number of larvae per flea was again twice that of the previous day. The numbers ranged from 23 to 135 (average 63.4) larvae per flea. The majority of the fleas showed maximum development of larvae to T-4; however, two fleas already contained larvae which had developed to T-5. Approximately 3/4 of the larvae, however, had not

TABLE VII

THE NUMBERS AND DEVELOPMENT OF D. RECONDITUM LARVAE IN C. FELIS AFTER FEEDING ON AN INFECTED DOG FROM DAY 1 TO DAY 16

Flea No.	Day 1									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	2	4								6
2	1	5								6
3	4	4								8
4	5	3								8
5	7	3								10
6	5	11								16
7	7	15								22
8	9	16								25
9	19	19								38
10										
11										
12										
Total	59	80								139
Avg/ Flea	6.6	8.8								15.4

TABLE VII (Continued)

Flea No.	Day 2									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	4	9								13
2	1	9	6							16
3	7	11	1							19
4	10	11	1							22
5	4	14	4							22
6	10	14								24
7	9	16								25
8	13	19								32
9	13	22	1							36
10	8	24	6							38
11	10	26	6							42
12	21	33								54
Total	110	208	25							343
Avg/ Flea	9.2	17.3	2.1							28.6

TABLE VII (Continued)

Flea No.	Day 3									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	2	18	3							23
2	4	20								24
3	3	3	8	10	2					26
4	5	32	2	1						40
5	10	46	2							58
6	8	45	3	2						58
7	5	24	19	11						59
8	6	55	10	6						77
9	10	40	24	15	2					91
10	22	64	19	2						107
11	29	77	24	5						135
12										
Total	104	424	114	52	4					698
Avg/ Flea	9.4	38.5	10.4	4.7	0.4					63.4

TABLE VII (Continued)

Flea No.	Day 4									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	3	17	0	2						22
2	7	8	18	9	7	2				51
3	5	41	5	3	2					56
4	7	79	3	1						90
5	4	83	3	2						92
6	7	90	4	2	1					104
7	10	14	26	19	28	14				111
8	6	88	10	7	3					114
9	13	61	52	59	14					199
10										
11										
12										
Total	62	481	121	104	55	16				839
Avg/ Flea	6.9	53.4	13.4	11.6	6.1	1.8				93.2

TABLE VII (Continued)

Flea No.	Day 5									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	0	6	1	1	0	0	1			9
2	4	13	0	0	1					18
3	0	51	0	1	2	3	2			59
4	10	48	1	1						60
5	7	66	9	5	6	3	1			97
6	10	27	31	23	14	15	6			126
7	12	95	7	6	4	8	2			134
8	18	36	35	25	18	22	3			157
9	30	59	55	34	30	27	9			244
10	25	67	70	42	33	29	37			303
11										
12										
Total	116	468	209	138	108	107	61			1207
Avg/ Flea	11.6	46.8	20.9	13.8	10.8	10.7	6.1			120.7

TABLE VII (Continued)

Flea No.	Day 6									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	0	13	0	0	0	0	1			14
2	2	25	1							28
3	4	33	1	1	1	1				41
4	3	48	1	0	1	1	1			55
5	12	24	31	17	30	27	22	2		165
6	4	32	29	12	23	34	34	6		174
7	8	39	32	30	25	28	16	14		192
8	4	33	68	52	56	46	32	2		293
9										
10										
11										
12										
Total	37	247	163	112	136	137	106	24		962
Avg/ Flea	4.6	30.9	20.4	14.0	17.0	17.1	13.3	3.0		120.3

TABLE VII (Continued)

Flea No.	Day 7									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	1	29	0	0	0	1	2			33
2	5	18	14	10	10	14	1			72
3	1	42	7	7	13	17	4			91
4	6	23	24	8	10	34	19	3	1	128
5	3	77	12	10	5	15	7	1		130
6	8	39	38	24	29	33	8			179
7	5	23	23	23	31	58	18	7	1	189
8	4	28	32	16	18	66	39	17	7	227
9	8	30	30	23	52	48	27	14	1	233
10	20	58	35	22	30	52	17	1		235
11	27	127	61	72	71	108	43	7		516
12	11	134	90	74	85	154	76	19		643
Total	99	628	366	289	354	600	261	69	10	2676
Avg/ Flea	8.3	52.3	30.5	24.1	29.5	50.0	21.8	5.8	0.8	223.0

TABLE VII (Continued)

Flea No.	Day 8									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	5	46	0	0	0	1	1			53
2	4	44	3	0	0	0	2	1	1	55
3	8	41	3	2	2	2	4	4		66
4	4	69	6	4	1	3	1			88
5	8	72	9	2	2	1				94
6	11	63	37	17	23	22	5			178
7	6	23	22	34	45	60	31	39	16	276
8	11	57	43	47	32	72	41	12	11	326
9										
10										
11										
12										
Total	57	415	123	106	105	161	85	56	28	1136
Avg/ Flea	7.1	51.9	15.4	13.3	13.1	20.1	10.6	7.0	3.5	142.0

TABLE VII (Continued)

Flea No.	Day 9									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	3	35	2	1	4	8	1			54
2	7	52	31	12	19	23	15	3	5	167
3	10	52	40	24	33	28	6	6	13	212
4	12	69	38	26	23	57	22	11	5	263
5	8	40	44	20	27	56	36	24	18	273
6	1	29	28	12	30	43	31	33	85	292
7	8	52	44	27	34	75	37	30	31	338
8	2	53	78	31	42	75	29	24	72	406
9	3	39	43	35	52	98	61	68	58	457
10										
11										
12										
Total	54	421	348	188	264	463	238	199	287	2462
Avg/ Flea	6.0	46.8	38.7	20.9	29.3	51.4	26.4	22.1	31.9	273.6

TABLE VII (Continued)

Flea No.	Day 10									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	8	21	3	2	2	2	3	1		42
2	7	53	3	1	0	1	2	4	2	73
3	8	29	39	8	13	14	4	2	2	119
4	3	24	25	13	14	30	9	10	14	152
5	0	27	36	16	24	36	17	28	72	256
6	2	11	30	15	33	39	35	57	75	297
7	1	37	44	33	31	35	29	33	57	300
8	2	20	30	26	37	80	46	65	186	492
9										
10										
11										
12										
Total	41	222	210	114	154	237	145	200	408	1731
Avg/ Flea	5.1	27.7	26.2	14.2	19.2	29.6	18.1	25.0	51.0	216.4

TABLE VII (Continued)

Flea No.	Day 11									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	4	57	2	2	1	0	0	1		67
2	9	49	15	4	1	7	9	7		101
3	4	19	32	19	9	48	12	8	16	167
4	2	47	45	14	10	33	9	10	9	179
5	3	29	36	11	23	51	17	18	28	216
6	3	11	30	21	27	31	26	38	51	238
7	0	9	9	10	31	58	29	27	67	240
8	0	7	8	7	23	39	28	44	104	260
9	7	60	31	23	13	44	49	29	28	284
10										
11										
12										
Total	32	288	208	111	138	311	179	182	303	1752
Avg/ Flea	3.6	32.0	23.1	12.3	15.3	34.6	19.9	20.2	33.7	194.7

TABLE VII (Continued)

Flea No.	Day 12									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	0	6	6	0	0	1				13
2	5	15	2	1	1	1	1			26
3	4	22	7	1	1	2				37
4	3	67	13	5	5	6	2	3	3	107
5	11	66	17	8	11	15	2			130
6	1	15	11	14	24	58	27	34	41	225
7										
8										
9										
10										
11										
12										
Total	24	191	56	29	42	83	32	37	44	538
Avg/ Flea	4.0	31.8	9.3	4.8	7.0	13.8	5.3	6.2	7.3	89.7

TABLE VII (Continued)

Flea No.	Day 13									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	2	18	1	0	1	1				23
2	2	31	1	0	1	1	0	1		37
3	19	38	7	0	5	5	1			75
4	2	29	10	7	12	14	2	13	5	94
5	4	92	17	8	8	14	12	11	13	179
6	8	21	90	26	19	25	5			194
7	2	67	43	23	26	62	54	67	63	407
8										
9										
10										
11										
12										
Total	39	296	169	64	72	122	74	92	81	1009
Avg/ Flea	5.6	42.3	24.1	9.1	10.3	17.4	10.6	13.1	11.6	144.1

TABLE VII (Continued)

Flea No.	Day 14									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	3	15	0	0	0	0	0	6	3	27
2	4	20	3	3	1	2	1			34
3	3	18	9	3	2	1	2	1		39
4	8	30	3	3	2	3	3	3		55
5	2	38	2	3	2	4	3	3		57
6	5	34	3	5	4	5	2	1		59
7	3	40	11	4	2	7	6	9	3	85
8	10	81	6	4	8	14	4	3	1	131
9										
10										
11										
12										
Total	38	276	37	25	21	36	21	26	7	487
Avg/ Flea	4.7	34.5	4.6	3.1	2.6	4.5	2.6	3.2	0.9	60.9

TABLE VII (Continued)

Flea No.	Day 16									
	1st Stage						2nd Stage	3rd Stage		Total
	T-1	T-2	T-3	T-4	T-5	T-6	II	III-1	III-2	
1	0	7	4	4	1	6	3	8	3	36
2	1	7	0	1	1	3	12	16		41
3	5	28	6	0	0	4	1	5	3	52
4	3	24	3	0	4	4	4	10	1	53
5										
6										
7										
8										
9										
10										
11										
12										
Total	9	66	13	5	6	17	20	39	7	182
Avg/ Flea	2.2	16.5	3.2	1.2	1.5	4.2	5.0	9.8	1.8	45.5

developed past T-2. Dead larvae were observed in 2 of the 11 fleas examined.

Day 4 - Larval development in the fleas after 4 days on the infected host showed that the number of larvae per flea had increased by about 30 which is essentially the same as the increase between Day 2 and Day 3. The numbers ranged from 22 to 199 (average 93.2) larvae per flea. Development had progressed to T-5 in 2/3 of the fleas and 2 of the 9 fleas examined supported T-6 larvae. An average of 5 dead larvae per flea was observed in 5 of the fleas. Individual variations in infection among the fleas, although present in Day 3, became prominent at this time. In fleas number 1, 3, 4, 5, 6 and 8 75% to 90% of the larvae had not developed beyond T-2 indicating some method for limiting or retarding growth is present. In fleas 1 and 8 there is a large variation in the numbers of larvae found (22 and 114, respectively); but by comparing the percent of T-2 larvae found (80% to 77%, respectively) the degree of retardation of development seemed about equal. Fleas 2, 7, and 9 do not show any larval retardation and development appears to be unrestricted; however, there are also variations in the numbers of larvae found in these fleas (51 to 199).

Day 5 - Larval development for Day 5 showed that the number of larvae per flea had continued to increase by about 30 as it did for Days 2, 3 and 4. The numbers ranged from 9 to 303 (average 120.7) larvae per flea. Development had reached Stage II in 80% of the fleas. Sixty percent of the fleas showed retardation in development of larvae for from 65% to 85% of the larvae found in these fleas were still T-2. The individual variations in numbers of larvae found per flea were greater than for Day 4. One flea had as few as 9 larvae while another had as

many as 303. Here, as in Day 4, the number of larvae found cannot be correlated with the degree of retardation. Flea number 1 had a total of only 9 larvae of which 66.6% were T-2 while flea number 7 had a total of 134 larvae of which 72% were T-2. Fleas 6, 8, 9 and 10 showed a rather typical unrestricted type of development although there was considerable variation in the numbers of larvae found in these fleas (126 to 303).

Day 6 - Fleas examined on Day 6 did not show any increase in the number of larvae per flea. The numbers ranged from 14 to 293 and the average remained the same as for Day 5 with 120 larvae per flea. Development had reached Stage III-1 in half of the fleas examined and in the other half retardation of larval growth was observed with only an occasional larva found to be developed further than T-2. Individual variations in both numbers and degree of development were seen. Dead larvae were observed in 4 fleas and in one of these there was no evidence of retardation of larval growth, and the dead larvae were T-5 and T-6. It should be noted that on Day 6 the first Stage III-1 infective larvae were observed.

Day 7 - The number of larvae per flea almost doubled that for Day 5 and Day 6 with 223 larvae per flea (range 33 to 643). Development had reached Stage III-1 in 60% of the fleas and 30% contained mature infective larvae (Stage III-2). In this sample only 3 fleas (numbers 1, 3 and 5) were collected which showed retardation of larval development and in two of these (numbers 3 and 5) 50% of the larvae had developed beyond T-2. Fleas number 11 and 12 had 516 and 643 developing larvae, respectively, and these were the two largest numbers of larvae found in fleas during this study.

Day 8 - In five of the 8 fleas examined retardation of larval development was observed and the number of larvae averaged less than 75 per flea. In the other 3 fleas retardation of larval development was not observed and the average number per flea was 260. Due to the mounting individual variation among fleas, together with the small sample size, comparison of totaled data between samples is no longer valid. However, by comparing the more susceptible fleas it can be seen that 10% of the larvae were in the infective stage (III-1 and III-2) on Day 8 as compared to 3.2% for Day 7. Among the more susceptible fleas, the average number of infective larvae per flea on Day 8 was 39 compared to 8.7 for Day 7.

Day 9 - In 1 of the 9 fleas examined retardation of larval development was observed. In the remaining 8 fleas good development occurred and most harbored relatively large numbers of infective larvae. As mentioned earlier, fleas which have large numbers of infective larvae become sluggish and crawl out on top of the hair coat where they are more easily seen and captured during hand picking of the sample.

Day 10 - In 6 of the 8 fleas examined good larval development was observed, although there was considerable variation in the number of larvae per flea (119 to 492). In the more susceptible fleas, the number of infective larvae per flea reached its greatest density at this time. One flea had a total of 492 larvae of which 151 were in the infective stage. When the abdomen of this flea was opened it appeared to be filled completely with a massive ball of wriggling, thrashing worms.

Day 11 - In one of 9 fleas examined retardation of larval development was observed, while in a second flea approximately 50% of its larvae had developed beyond T-2. In the remaining 7 fleas good development

of larvae was observed. The number of larvae per flea among the more susceptible fleas was still heavy and the percent of infective larvae remained high.

Day 12 - There was a reduction in the percent of fleas in which good development of larvae occurred. In fact only 2 of the 6 fleas contained infective larvae, and in one of these less than 50% of the larvae had developed beyond T-2. The reduction in percent of fleas found in which good development occurred was probably due to death of many fleas which had supported growth and development of large numbers of larvae to the larger stages. This probably accounts also for the decline in average number of larvae per flea, as well as average number of infective larvae per flea for the sample.

Day 13 - Larval development in fleas examined on Day 13 was about the same as was seen on Day 12. Three of the 7 fleas contained Stage III-2 larvae, but in one of these less than 50% of the larvae had developed beyond T-2.

Day 14 - In all of the fleas examined on Day 14 larval development was retarded in varying degrees. A few larvae had developed to Stage III in 7 of the 8 fleas; however, less than 50% of the total larvae from all fleas had developed beyond T-2.

Day 16 - No samples of fleas were taken on Day 15; however, there were no noticeable changes in the samples taken on Day 16 from those taken on Day 14. Sampling was stopped at this time because no further changes appeared to be occurring.

The fleas used in this study were genetically heterogeneous and had been reared through several generations on non-infected dogs. The infected dog used in these studies was obviously susceptible to flea

infestation because at the time of acquisition several fleas were found on him and the dog later proved susceptible to the laboratory infestation. The D. reconditum infecting the test dog appeared to be compatible with the C. felis used in this study as evidenced by the large numbers which developed in some fleas. As suggested by Kartman (1953a) for mosquitoes, the variation in flea susceptibility to infection by D. reconditum is probably determined by their genetic make up. This test was not designed to determine the ratio of the various reactions to infection, but, the results clearly show that: (1) There is a certain percent of the fleas, probably relatively small, which does not possess the means to restrict, retard or otherwise exert any influence upon the numbers of larvae ingested nor the complete development of most ingested larvae. Egg production in these fleas stops, and they probably all eventually die from the infection. These fleas probably play an important part in the initial transmission of the infection because of the large numbers of infective larvae being produced prior to cessation of feeding and because of their restless wandering which makes them easily transferred to a new host. (2) There is a certain percent of the fleas, probably relatively large, which possess some means of restricting, retarding or otherwise exerting an influence upon the number of larvae ingested and on the development of larvae ingested. In this case the larval infection never becomes severe enough to adversely affect the normal activities of the flea. In most cases, however, a small percentage of the larvae manage to complete development to the infective stage. These fleas live what possibly would be a near normal life span and are capable of prolonged transmission of infection. They continue what appears to be normal egg production, thereby, maintaining the flea population.

Prepatent Period

A total of 184 D. reconditum infective larvae were collected from 134 C. felis by dissection after the fleas had been allowed to feed on an infected dog for periods of 14 and 15 days approximately 8 days after infective larvae were noted. The infective larvae were injected subcutaneously into a non-infected dog on two consecutive days. A total of 12 pre-exposure blood samples had been taken and examined for the presence of circulating microfilariae over a period of 5 months and none was found. Following injection of infective larvae, blood samples were taken at weekly intervals and examined as whole blood slide preparations for the presence of circulating microfilariae. Circulating microfilariae were first observed in the blood 81 days after the infective larvae had been injected. The measurements and appearance of the microfilariae were consistent with those of D. reconditum. The length ranged from 248 μ to 282 μ (mean 265 μ) and the width was quite consistent at 4.7 μ. To the author's knowledge this is the first time any information on the prepatent period of D. reconditum has been reported. Following the appearance of circulating microfilaria, blood samples were taken at weekly intervals for 8 weeks. Five aliquots of 0.02 ml were examined and the average number of microfilariae per slide was as follows: 0.2, 3.3, 2.2, 2.8, 8.0, 3.4 and 6.6. To further substantiate that the microfilariae observed were those of D. reconditum, the dog was euthanitized and examined for presence of adult worms. Four female worms were recovered from the subcutaneous tissues. Their morphology was consistent with that given by Newton and Wright (1957) and Nelson (1962) for D. reconditum. The heart, lungs and associated great vessels were examined for the presence of adult D. immitis and none were found.

Microfilariae from the dog upon which the fleas were fed to obtain their infection were determined to be D. reconditum, and adult male and female worms recovered from this dog at necropsy were also determined to be D. reconditum.

The prepatent period for D. reconditum was found to be much shorter than that reported for D. immitis. The prepatent period for D. immitis has been reported by various workers to range from a little less than 6 months up to 9 months (Bancroft, 1904; Webber and Hawking, 1955; Newton, 1957; Orihel, 1959; Kume, et al., 1962; Warne, et al., 1967; and others).

E. gallinacea as a Vector of D. reconditum

Laboratory-reared E. gallinacea were placed on a D. reconditum infected dog and samples of fleas were then removed and examined at various time intervals. Although the tests with E. gallinacea were not as extensive as those conducted with C. felis during this study, the development of D. reconditum in E. gallinacea roughly paralleled that found in C. felis, except that the large numbers found in C. felis did not occur in the smaller E. gallinacea. The greatest number recorded from one flea was 16. Stueben (1954a) reported development in E. gallinacea. Nelson (1962) reported development of D. reconditum larvae from E. larina taken from infected hyenas and jackals in Kenya. Because of the sedentary habits of E. gallinacea it probably is a source of reinfection for its host, but probably is less important in transmission from dog to dog.

P. simulans as a Vector of D. reconditum

In the heterospecific flea colony maintained for these studies an occasional P. simulans was found during the tests with C. felis. The development of D. reconditum larvae was the same as that observed in the tests with C. felis. When P. simulans was collected during the daily sampling the progress of development was found to be identical to that of C. felis. P. simulans has not previously been reported to be a vector of D. reconditum; however, it is likely that some of the reports of development in P. irritans (Summers, 1940 and 1943; Stueben, 1954a) were P. simulans according to the classification of Smit (1958).

D. variablis as a vector of D. reconditum

D. variablis adults in varying degrees of repletion were collected from a D. reconditum infected dog and examined for developing larvae. No larvae were observed in any of the ticks examined. Nelson (1962) examined ticks collected from D. reconditum infected animals with negative results. Pennington and Phelps (1969) examined R. sanguineus collected from D. reconditum infected dogs, but no developing forms were found. Highby (1938) reported finding the larvae of Dirofilaria scapiceps (Leidy) in the gut of ticks he collected from an infected snow shoe rabbit. It appears from this study and from the literature that ticks in general, and D. variablis and R. sanguineus in particular, do not serve as vectors of D. reconditum.

S. calcitrans as a Vector of D. reconditum

Phillips (1939) examined S. calcitrans for larvae after allowing them to feed on a dog infected with D. immitis and found larvae in the stomach but failed to find development beyond a slight thickening of

the larvae. In the present study 21 stable flies were allowed to feed for 30 minutes on a dog infected with D. reconditum. Eight flies were examined between 10 minutes and 3 1/2 hours after feeding and in all cases living larvae were found in the blood meal contents of the fly's midgut. The number ranged from 3 to 26 (average 12.6) living larvae per fly. On two occasions, dead larvae were found in the abdominal haemocoel. The remaining 13 flies were examined 24 hours after feeding and no larvae, either living or dead, were found. It appears that although microfilariae are readily taken in with the blood meal by the fly and may penetrate the gut into the abdominal haemocoel, they do not survive and are assimilated by the fly within 24 hours.

C. felis as a Vector of D. immitis

Laboratory-reared C. felis were placed on a dog infected with D. immitis and were removed and examined for larvae at various time intervals. One live, but undeveloped larva was observed in 1 of 5 fleas examined after being allowed to feed 1 day on the infected dog. After examination of many fleas over a 6 day feeding period with negative results it was concluded that C. felis will not serve as a vector of D. immitis. Many workers have examined fleas from dogs infected with D. immitis and have not found development (Grassi, 1888; Bancroft, 1901; Kosuge, 1924; Phillips, 1939; Taniguchi, et al., 1944; Rosen, 1954; Newton and Wright, 1956; and Orihel, 1959). It is now well accepted that those workers who reported development of D. immitis in fleas were all working with some other filarid, probably D. reconditum (Breinl, 1921; Brown, 1939; Brown and Sheldon, 1940; Summers, 1940 and 1943; Bradley, 1952; and Stueben, 1954a and 1954b).

SUMMARY AND CONCLUSIONS

A survey of the incidence of canine filariasis and ectoparasites was made and the capability of C. felis, P. simulans, E. gallinacea, S. calcitrans and D. variabilis to support development of D. reconditum was examined. The cyclodevelopment of D. reconditum in C. felis was studied and the time required for development in the definitive host was observed.

The survey of canine filariasis showed 15.3% of the dogs infected of which 14% were D. reconditum and 1.3% were D. immitis. These results show that the prevalence of dirofilariasis is quite low in the general dog population in North-Central Oklahoma, but, the incidence of D. reconditum is relatively high.

The survey of ectoparasites on dogs from animal shelters showed 61.4% were infested by C. felis with an average of 39 fleas per dog and 50.5% were infested by P. simulans with an average of 18 fleas per dog. E. gallinacea infested only 7.5% but these dogs had an average of 25 fleas per dog. D. variabilis infested 42% of the dogs, while only about 1% were infested by A. americanum or R. sanguineus. L. setosus was found to infest about 2% of the dogs and the numbers found were usually low. Poorly cared for dogs in Oklahoma have a relatively high incidence of ectoparasites in that 86% are infested with ectoparasites. Fleas infest over 75% of the dogs and over 40% are infested with ticks.

Development of D. reconditum larvae to the infective stage was found to occur in C. felis, P. simulans and E. gallinacea.

S. calcitrans and D. variabilis do not support the development of D. reconditum. Studies on the development of D. reconditum in C. felis showed that it required about 6 days for ingested microfilariae to develope to infective stage larvae. The daily progress of development was followed and the larvae were found to develop within the fat bodies, as well as free in the abdominal haemocoel. Individual variation, both in the number of larvae ingested and the number of larvae which would develop was observed in C. felis. Fleas which ingested large numbers of larvae and in which large numbers reached the infective stage, usually succumbed to the infection; whereas, those which either did not ingest large numbers or otherwise restricted the numbers of larvae developing to the infective stage, showed no adverse effects from the infection.

The time required for the larvae to complete their development, mate and begin producing microfilariae after being introduced into the definitive host was found to be 81 days or less.

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