STUDIES ON EXPERIMENTAL AND NATURAL INFECTIONS OF STRONGYLOIDES STERCORALIS (BAVAY, 1876) GRASSI, 1879, IN DOGS

By

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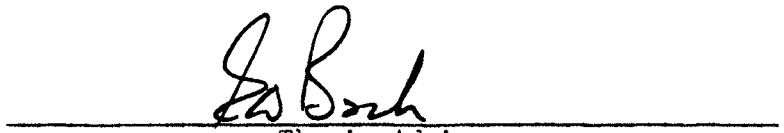

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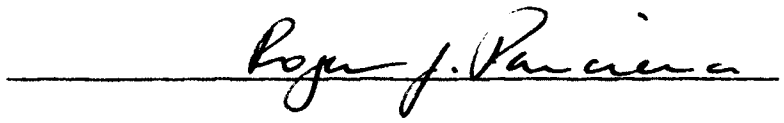

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CHAPTER I

INTRODUCTION

It is necessary to become acquainted with the concepts of biological research in order to determine the relationships involved in helminth parasitism. Acquisition of this knowledge aids in the understanding of biological associations, assists in the identification of the deficiencies associated with available literature and defines the methods by which problems can be solved.

There are many species in the genus Strongyloides that have been recorded as occurring in man and animals from various parts of the world. Species of the genus are considered to be host-specific parasites, however, some species appear to have the ability to establish in more than one host. Strongyloides stercoralis (Bavay, 1876) Grassi, 1879, has been reported to infect man, the cat, the chimpanzee and the dog (Faust, 1964, Sandground, 1925).

It is considered by some workers that the species in the dog and man are identical; others state that the human species is host-specific while still other workers have reported there are two species in the dog, and that only one of these infects man. It is apparent that biology associated with those species infecting man and dog is not completely understood nor is the taxonomy associated with these species stabilized.

Strongyloides is unusual among the parasitic nematodes of the

domestic animals because there is an alternation of generations in the usual life cycle. This phenomenon causes the formation of two distinct types of development outside the host body. The first stage larvae may either metamorphose directly to infective larvae or indirectly into free-living adults, male and female, and in turn the hatched eggs give rise to first stage larvae which develop to infective larvae.

The objectives of this study were:

1. To determine some aspects of the biology of the species of Strongyloides obtained from naturally infected dogs.
2. To determine the prepatent and patent periods of experimental strongyloides infection of the dog and cat.
3. To determine whether environmental factors may influence the development and infectivity of the filariform larvae of Strongyloides in the dog.
4. To determine what laboratory animals are acceptable experimental hosts for dog Strongyloides sp.

CHAPTER II

REVIEW OF LITERATURE

The confusion associated with the naming of certain Nematodes gives an insight to the problems involved in taxonomy. Numerous nomenclatures have been defined and many biologists have tried to develop a taxonomic scheme for Strongyloides spp. The difficulties associated with the taxonomy of this genus are related to the morphological variations and biological characteristics that have been reported for the various species.

The species of Strongyloides was first observed by Normand (1876) in the feces of French colonial troops in Cochin China. The free-living generation worms were designated by Bavay (1876) as Anguillula stercoralis, and the parasitic generation, as Anguillula intestinalis. Grassi (1879), Perroncito (1881), and Leukart (1883) demonstrated that these two "species" constituted separate parts of the same life cycle, which was heterogonic, and represented parasitic as well as free-living generations.

According to Whitlock (1960), Grassi (1879) reported finding a parasite which in his opinion was similar to Strongylus but which was sufficiently different to induce him to erect a new genus, Strongyloides. He included Anguillula intestinalis Bavay (1876), in this genus.

In 1902, the human Strongyloides was designated by Stiles and

Hassal as Strongyloides stercoralis. They corrected the name Strongyloides intestinalis on the basis of priority and homonyomy. Further, they stated Ehrenberg (1838) gave the name Anguillula intestinalis to a parasite which von Gleichen (1776) found in the earthworm.

Several systems of schemes for the classification of the genus Strongyloides are available in the current literature. The classification scheme outlined by Yamaguti (1961) for the genus Strongyloides is accepted for purposes of this thesis.

Class:	Nematoda	
Order:	Rhabdiasidea	Railliet, 1916
Family:	Strongyloididae	Chitwood et McIntosh, 1934
Genus:	Strongyloides	Grassi, 1879

Dogs have been found naturally infected with a species of nematodes identical, morphologically and biologically, to Strongyloides stercoralis. According to Chandler (1925), Fülleborn (1914) found Strongyloides in a Chinese dog. Morphologically this species closely resembled Strongyloides stercoralis, and was considered by Fülleborn as identical with the human species. Fülleborn succeeded in experimentally producing Strongyloides infections in dogs by means of larvae derived from a human case. These infections did not persist long and did not become chronic as in the case of the naturally infected Chinese dog. He observed development only to be indirect while Brumpt (1921) reported that both direct and indirect development occurred in dogs infected with larvae from human infection. On the basis of this type of development, Brumpt (1921) prefers to regard this parasite as a distinct species, Strongyloides canis. According to Sandground (1925),

Grassi and Braun (1899) observed that dogs were difficult to infect when exposed, per os, to infective larvae derived from a human case.

Sandground (1925) disagreed with Brumpt's conclusion and pointed out that a change in the development of the parasite, when infections of Strongyloides are transferred from one host to another (not necessarily a new host species), does not indicate the non-specificity of the parasite for the new host. He stated that exposure to Strongyloides resulting in infections in man depends mostly on soil contamination with infective stages and on the social habits of the individual. Sandground considered the validity of the species of Strongyloides in man and in dogs, and preferred to regard the parasite of dogs to be identical with Strongyloides stercoralis. He based his conclusions on observations after he infected himself with 20 indirect infective larvae derived from a dog. The infection was established eight days after his exposure to the infective larvae.

Galliard (1940) also stated that Strongyloides spp. of the dog and of man were the same species, and that the rarity of natural infection in the dog is due to the fact that the animal is less exposed to larval contamination of the environment than man.

The specific identity of Strongyloides occurring in dogs has not been determined and there seems to be no valid reason to suggest that the parasite of dog is a different species from that of man. In all probability it is a variety of the human species.

Cats may be infected with Strongyloides stercoralis. The first report of a species Strongyloides in cats appears to be that by Chandler (1925).

Sandground (1925) infected three cats with Strongyloides

stercoralis from the dog. These infections were relatively light and were of short duration. He concluded from these experiments that Strongyloides stercoralis is not a normal parasite of cats.

Rogers (1939) considered strongyloides from the cat as a distinct species, Strongyloides cati, and based this conclusion on biological and morphological differences observed for this species.

The biology of Strongyloides is very complex. Although the life cycle is direct, development of this nematode includes a alternation of generation with sexual reproduction in the free-living generation and parthenogenesis in the parasitic generation. A parasitic male does not occur in domestic animals or man although Faust (1933) and Kries (1932) claimed that parasitic males were observed in the lungs of the dog at necropsy. Lucker (1942) believed that the occasional presence of such males probably indicated the development of the free-living male, under unusual circumstances, occurs in vivo.

According to Thayer (1901), Leukart (1883) suspected the possibility of two strains of the parasite, one having only a parasitic generation, and the other having both a parasitic and a free-living generation. The parasitic generation was regarded by Leukart as hermaphroditic, and by Rovelli (1888) as parthenogenetic. According to Premvati (1958), Leichtenstern (1899 & 1905) agreed with Leukart's suggestion of two strains, and believed that an indirect life cycle was the predominant cycle in tropical climatic areas while a direct one is predominant in temperate regions.

Darling (1911) reported that the parasitic female, embedded in the intestinal wall, produced two types of progeny which were expelled into the crypts. Graham (1938) recorded that neither the age of the

parasite nor the age of the host had any significant role in determining the mode of development by which the progeny of a single homogenically derived Strongyloides ratti proceeded. Chang and Graham (1957) described three types of eggs from parthenogenetic females of Strongyloides papillosus. Some of these eggs contained the normal number of chromosomes, six, others showed four, while still others had two. Little (1962) claimed that there are two kinds of eggs produced by the parasitic females of Strongyloides fülleborni. One develops to the free-living adult male, while the other may develop to either the free-living adult female or to the infective larvae, depending on the conditions of the culture.

A number of workers have attempted to determine the influence of varying environmental conditions on the direction of the life cycle direct or indirect.

Nishigori (1928) stated that if first stage larvae passed with the feces meet favorable conditions, such as nutriment, humidity, temperature and oxygen, they develop into sexually mature free-living adults. Beach (1936) reported he could influence the mode of the developmental cycle by changing the culture media of Strongyloides simiae in monkey. The poorer the osmotic and nutritional properties of the medium, the greater was the tendency for the rhabditiform larvae to develop directly into infective filariform larvae rather than into sexual adults. Whereas a favorable medium produced totally free-living sexual forms.

Graham (1939) recorded a seasonal variation in the frequency of the heterogonic cycle of Strongyloides ratti: the yield of heterogonic development in homogonic forms was greater in spring and summer, while in winter there was a considerable drop in the percentage of the in-

direct phase. The factors involved apparently were temperature and humidity acting on the rat host and affecting the development of the eggs of the parasitic females. Later Graham (1940) studied a homogonic line derived from a heterogonic line and found that its characteristics closely resembled the latter. Graham's conclusion indicates that the type of life is determined by genetic factors in the eggs of the parasitic females, but external factors may play some role.

Darling (1911) pointed out with Strongyloides, cold temperature limited the egg production in the free-living stages and decreases the probability for eggs to develop into larvae of the indirect phase. The lower temperature prevented the growth of protozoa, bacteria and yeasts usually seen in successful cultures kept at room temperature, and thus limited the growth of larvae by inhibiting the food supply.

Galliard (1950) observed that temporary exposure of Strongyloides infective larvae to cold temperatures, between each passage in dogs, reduced their virulence, diminished their infectivity and increased the percentage of direct development. He also found that the application of X-rays to the duodenal region caused a reduction in the level of parasitism and increased the probability of the direct development of larvae.

Premvati reported (1958) that under optimum conditions Strongyloides fülleborni has an indirect life cycle but under adverse conditions of pH and consistency of media, nutriment and temperature, the chance toward indirect life cycle is reduced and the tendency to direct life cycle is increased.

Stewart (1963) found that the homogonic development of Strongyloides alone occurred in nutrient agar with B. coli, while both

homogonic and heterogonic development took place in cultures of feces mixed with water, granular animal charcoal or sphagnum moss.

Some investigators have determined that the type of development a species may undergo can be influenced by the host and that attempts to infect an unfavorable host with a definite strain may change the mode of the life cycle.

Sandground (1926) determined the mode of the life cycle change when abnormal hosts were infected. He claimed that direct development in certain species originally arose following the introduction of the more primitive heterogonic parasites into new species of host.

Brumpt (1921), according to Premvati (1958), reported the progeny of Strongyloides papillosus were changed from a predominately direct development to a predominately indirect development when rabbits were infected with the sheep strain of this species.

The effect of varying temperatures on the development of Strongyloides was reported by Leukart (1883) who stated that cold was lethal to Strongyloides larvae, but did not give any details on the lethal temperature range. Sandground (1926) believed the optimum temperature for development of the free-living stages to be 26 to 28 C.

Kries (1932) reported that Strongyloides simiae eggs were destroyed by eight days exposure to 1 to 2 C. and rhabditiform larvae in less than two days. Cordi and Otto (1934) determined the optimum temperature for development of Strongyloides fülleborni was 23 to 30 C. in fecal charcoal cultures. Molinari (1956) reported that Strongyloides fülleborni did not survive 30 seconds at -180 C. and considered the death was due to ice crystal formation. Stewart (1963) found that development and survival of Strongyloides ransomi larvae

was best at 15 C., and he also observed that development did not occur at temperatures below 9 C. or above 35 C. in nutrient agar.

CHAPTER III

MATERIALS AND METHODS

For purposes of this thesis, the nematode parasite was identified as Strongyloides stercoralis. Experimental infections in dogs were established with infective larvae recovered initially from feces of two naturally infected dogs obtained from different parts of Oklahoma. The miniature French Poodle female, raised in Sapulpa, Oklahoma, and was eight weeks old when the infection was diagnosed in September, 1966. The Chihuahua male, raised in Ardmore, Oklahoma, and was about seven months old when received in July, 1967. Larvae recovered from experimentally infected dogs were used for subsequent infections.

The dogs that were experimentally infected were kept in stainless steel dog cages. Each dog was fed once every day in the morning with a prescribed amount of a commercial feed (F/D and EZ/D) especially formulated for the maintenance of continuously caged dogs. Clean water was supplied at all times. The cage floor was covered with papers every morning and each was cleaned daily. The possibilities for re-infection were reduced by removal of the fecal material soon after the dogs defecated. Stools were collected several times each day. The same procedure was applied for cats. The laboratory animals: mice, rats and guinea pigs were kept in small stainless steel cages. Sufficient amount of a commercial pelleted feed was always available. Clean water was supplied at all times.

Smear preparations made with saline and feces from infected dogs were used initially to demonstrate the presence of first stage rhabditiform larvae. This method may be employed for routine fecal examination to determine Strongyloides infection but low numbers of larvae in some stool samples are not likely to be observed by this technique. Further, it is difficult to determine the morphological characteristics of the rhabditiform larvae in smears due to the large amount of debris. Moreover, changes in the concentration of the saline solution in these preparations may cause changes to occur in larval structures.

The Baermann apparatus was used to isolate the first rhabditiform larvae from the fresh stool and third stage infective larvae from the various culture substrates. The procedure involved the placing of recently passed feces on a wire screen suspended in a funnel. The funnel was closed at the bottom by means of a clamped rubber tube, and filled with distilled water warmed to a temperature of about 80 F. The fecal mass was separated and spread on the screen by means of a wooden applicator stick to facilitate migration of the first rhabditiform larvae from the stool.

The larvae gradually accumulated in the stem of the funnel due to gravitation. The first withdrawal of water from a Baermann funnel was made after 2 to 4 hours. A second withdrawal was taken 4 to 12 hours to collect more larvae from the Baermann funnel. The water sample was distributed into stender dishes, diluted with a small amount of distilled water and observed for larval stages.

The first stage larvae collected from Baermann water samples were suspended in distilled water, distributed into several petri dishes

and incubated at a temperature ranging from 25 to 28 C. The petri dishes were examined for about 6 hours to follow the progress of the developmental stages. It was noted that some distilled water batches obtained from the College supply had a detrimental effect on the larvae. The reasons for these effects were not determined.

To observe microscopically the larval and adult parasites, a small drop of water bearing the free-living adults and larvae was transferred to a slide by means of a fine dropping pipette. The slide was then heated to inactivate the larvae before being covered by a coverslip. This method of killing the larvae and free-living adults was used because the parasites are relaxed, enzymes are inactivated and the specimens can be measured easily.

Several culture techniques were used to obtain the optimum growth of the developmental stages of Strongyloides. These included the water culture technique and the sphagnum moss or granular charcoal culture techniques. The former involved taking a water sample from the Baermann apparatus, placing it in petri dishes and maintaining these at culture temperatures. The latter technique was accomplished by mixing the fecal sample with a sufficient quantity of distilled water to make a thin paste-like fluid which was thoroughly mixed with either sphagnum moss or granular animal charcoal, adding enough additional water to make the mixture moist, but not wet. Wet cultures enhanced fungal growth which appeared to have a detrimental effect on the larval development.

Sphagnum moss has several advantages for maintaining the growth of the larvae. They include: retention of moisture for a long period, absorption of fecal odor and control of bacterial growth. Granular

animal charcoal provides a suitable substrate for the growth of strongyloides larvae at optimum temperatures. Although filariform larvae were more easily removed from charcoal by the Baermann technique, these cultures had a tendency to lose moisture and to leave an undesirable odor. All sphagnum moss and charcoal cultures were maintained in polystyrene plastic utility dishes.

The cultures were kept in the controlled temperature box at 25 to 28 C. for about five to six days. Under these conditions infective larvae will develop readily. The cultures with an excess of moisture caused an increase in fungal growth. After incubation, the cultured material was placed in warm distilled water in a Baermann apparatus. The first withdrawal of water was made after 2 to 4 hours. It was observed that in most instances the majority of the larvae was collected from the Baermann funnel in the first water sample. The larvae were collected, concentrated, and counted with the use of a steroscopic microscope.

The infective larvae tend to settle to the bottom and when exposed to the light and heat they start moving upward and become excited. Large numbers of the larvae tend to migrate toward the side of the watch glass. In most instances a variable amount of debris was found mixed with the larvae, depending on the kind of cultural substrate used. It is very difficult to remove larvae from the debris by use of a pipette.

An alternate method of collecting and concentrating the larvae was used. This included the collection and suspension of larvae in distilled water for several times to remove debris. The counted larval suspension was mixed with 10,000 I.U. crystalline penicillin plus 10

mg. of dihydro-streptomycin sulphate to control bacterial contamination.

All dogs and abnormal hosts were infected with a known number of infective larvae of Strongyloides. The dose ranged in size from 80 to 4200 larvae. The animals were injected subcutaneously in the shoulder region where the skin is loose and where better diffusion of the suspension could be achieved. After injection, the syringe was washed with additional distilled water and the washings similarly transferred to the animal. Afterwards the syringe was washed again and the washing itself examined microscopically to determine the number of larvae remaining.

No difficulties were encountered in controlling the animals during the dosing procedure. Care should be taken to avoid oozing of the suspension at the injection site by holding the skin firmly between two fingers for about 10 seconds before releasing the animal.

Necropsies were performed on dogs and abnormal hosts to determine the presence of the parasitic female in the small intestine. The dogs and cats were euthanetized by subjecting them to a continuous 110 volt electric charge. Immediate death resulted and necropsy was performed without delay. Rats, guinea pigs, and mice were euthanetized with the use of chloroform.

The mucosa of stomach and the small intestine were thoroughly washed with normal saline and scraped by gloved fingers. Intestinal and stomach washings were sedimented in normal saline were fixed in hot formal-saline and transferred to specimen jars for later parasitological examination. Fecal material was collected from both the small and large intestine of each animal necropsied and was cultured with

either sphagnum moss or charcoal. The remaining small intestine was cut into several short sections of about 10 to 15 centimeters long and subjected to artificial digestion. As advocated by Schwartz (1939) the pepsin-hydrochloric acid-digestion technique was used for the recovery of the parasitic females from the intestinal mucosa. The formula of the pepsin-hydrochloric acid-mixture is as follows: 600 ml. of distilled water, 6 grams of pepsin powder, and 10 ml. concentrated hydrochloric acid. The intestinal segments were placed with the solution in a graduated glass container and left for 12 hours in an incubator at 37 C. The entire mucosa usually was digested by this technique. An aliquot of the digested material was fixed with 10 per cent formal saline.

Because of the small size of the parasitic female its recovery from the small intestine may be overlooked. Therefore careful examination of the washing and digestant samples of the small intestine was necessary. Recovery of the parasitic female was achieved by placing a small quantity of the sediment from fixed sample in a petri dish and diluting it with a few drops of distilled water before observing with a stereoscopic microscope. The parasitic females and larval stages were siphoned off with a pipette and stored in jars containing 10 per cent formal saline.

Anatomical measurements were made on various developmental stages of larvae and adults of the strongyloides by means of a compound microscope at 100 and 480 magnifigations. All measurements were made with the aid of a calibrated ocular micrometer. The same steps were followed in isolation and examination of free-living larvae, adults or infected larvae from different cultural media.

CHAPTER IV

RESULTS AND DISCUSSION

Studies on the Development of the Preparasitic Stages of Strongyloides stercoralis

The study of modifications in Strongyloides species life cycle and the effect of micro-environmental factors on the free-living stages has been discussed by several workers (Nishigori, 1928; Beach, 1936; Graham, 1939). These investigators experimented with the free-living stages in an attempt to explain the effects of variations in the external environment on them such as nutriment, temperature, relative humidity and oxygen.

According to Premvati (1958) the question of explaining the mixed types that may be observed within the same culture media probably depends upon whether early development and hatching of the eggs of the parthenogenetic female occur inside or outside of the host.

One of the objectives of this study was to determine some of the conditions that influence the formation and development of the various life cycle stages of Strongyloides stercoralis in the dog. No attempt was made to determine all of the factors that may be involved nor to verify the observations reported by previous investigators. Data summarized were obtained from a limited number of experiments to obtain life cycle stages from cultures, with different substrates, maintained at 25 to 28 C.

It was observed that either the homogonic (direct) or heterogonic (indirect) life cycles, or both, may occur in charcoal, sphagnum moss or water cultures of dog feces containing first stage rhabditiform larvae.

Data obtained from four cases of experimental canine strongyloidiasis indicate that the homogonic (direct) mode of development predominated early in the patent period and the heterogonic (indirect) mode of development predominated later in the course of infection. The change of homogonic to heterogonic development was gradual. Only homogonic filariform larvae were obtained from water cultures of feces from a naturally infected dog while both homogonic and heterogonic development occurred in charcoal and sphagnum moss cultures of feces from this animal. In the latter instance, the direct mode of development was predominant. No attempt was made to determine those factors that may influence the mode of development of larvae in feces from experimentally or naturally infected animals.

Development of the preparasitic stages by homogonic or heterogonic development was observed to occur in water cultures maintained at 25 to 28 C. These data are summarized in Table I and illustrated in Figure 1.

Homogonic filariform larvae were observed to occur in water cultures at 31 hours. Body length measurement of these larvae obtained from water cultures of feces from a naturally infected dog were in the range of 0.47 to 0.60 mm., average, 0.55 mm. Body length measurements of larvae isolated from cultures of feces from an experimentally infected dog were in the range of 0.43 to 0.59 mm., average, 0.50 mm.

During the course of these experiments, no attempt was made to

TABLE I

AVERAGE LENGTH AND WIDTH MEASUREMENTS OF S. STERCORALIS FREE-LIVING MALE AND FEMALE ADULTS OBTAINED FROM WATER CULTURES DURING THE FIRST 100 HOURS

Free-living Adult Female			Free-living Adult Male		
Average Body Length*	Width in micron	Age in hour	Average Body Length*	Width in micron	Age in hour
0.70 (0.69-0.71)	23 (22-24)	43	0.68 (0.67-0.69)	22 (22-22)	43
0.80 (0.79-0.81)	24 (23-25)	50	0.76 (0.75-0.77)	23 (22-24)	50
0.96 (0.96-0.97)	33 (25-35)	72	0.83 (0.82-0.85)	26 (24-33)	72
0.98 (0.97-0.99)	35 (32-38)	90	0.86 (0.85-0.87)	33 (25-35)	90
1.10 (1.05-1.11)	36 (35-38)	100	0.93 (0.90-0.98)	34 (32-35)	100

*All measurements are in millimeters. Figures in parenthesis indicate range of measurement.

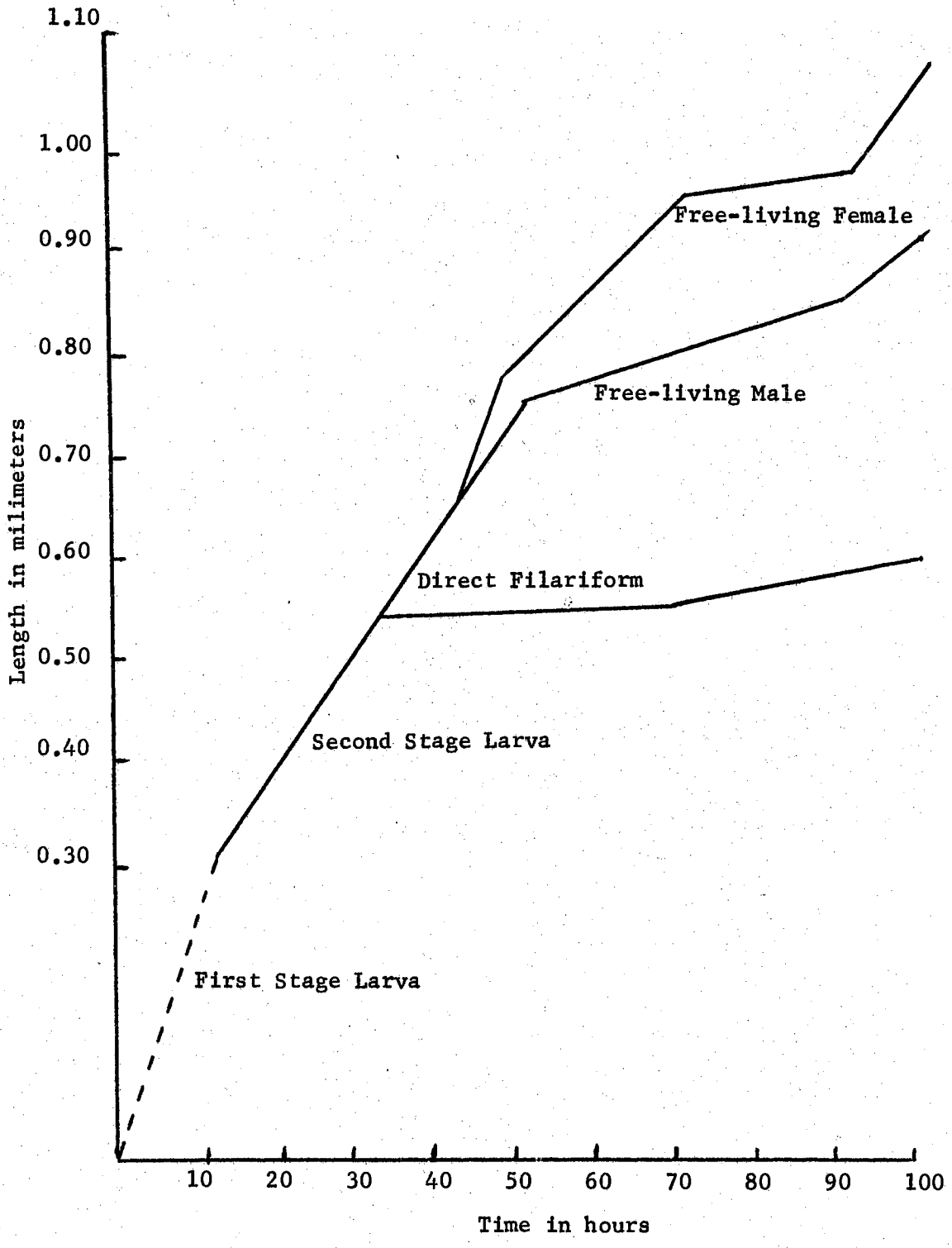

Figure 1 - Growth curve of the developmental stages of Strongyloides stercoralis in the dog obtained from water culture during the first 100 hours.

differentiate the first and second stage larvae as to type of development prior to the first 31 hours. Sexual differentiation of the free-living adults occurred 43 hours after the feces were passed. The male could be identified by size (0.68 mm.), the presence of spicules, a twisted posterior end, and sluggish movement. Females could be identified by their larger size (0.70 mm.) and morphology, by the vulvar opening located about midway of the body length, and by the characteristic movement which is comparatively faster than that of the male. The female moves forward with a serpentine-like movement on the bottom of the culture dish and, at times, moves upward in the fluid medium.

Shortly after sexual differentiation, or at 44 hours, gravid females were observed with 0 to 3 eggs in the uterus; while at 55 hours, as many as 10 eggs were observed in some females. From examination of data illustrated in Figure 2, it is apparent that the number of eggs formed in female uteri varies with the number of hours elapsed after sexual differentiation. After 68 hours, 13 to 15 eggs were observed in some female uteri, however, first stage larvae from eggs of free-living females were observed in 66 hours and second stage larvae appeared in cultures after 62 to 72 hours. Heterogonic filariform larvae were observed to occur in water cultures in 90 to 93 hours after the feces were passed.

Figure 2 illustrates graphically the growth of the various developmental stages during the first 100 hours of culture. It is clear from this curve that either the direct or indirect life cycle is established about 31 hours after defecation. Direct infective filariform larvae, after their initial formation, did not increase in size. The growth of the free-living female occurred at a rate faster than

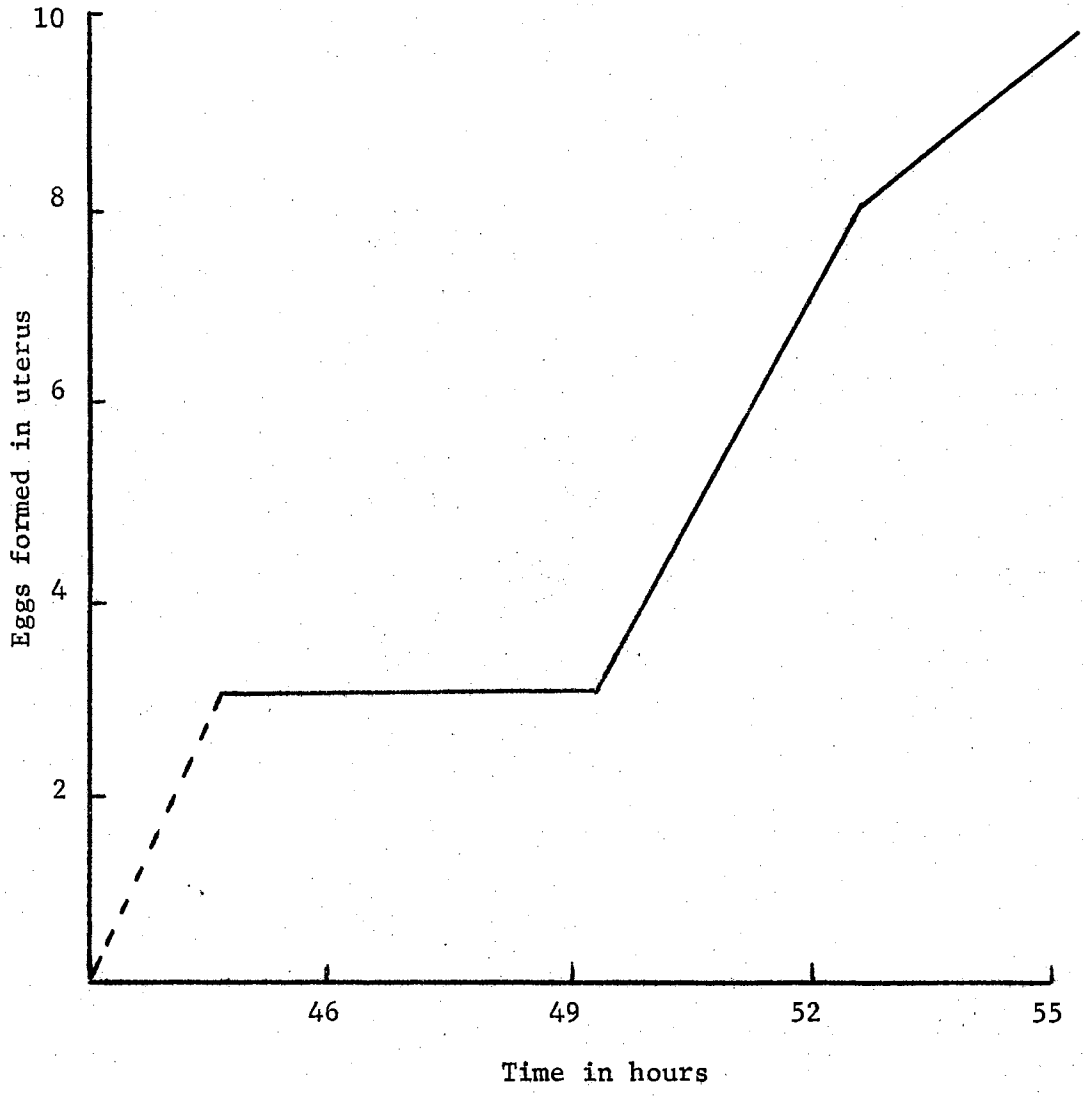

Figure 2 - The eggs number formations curve in free-living female uterus of Strongyloides stercoralis in water culture.

that of the free-living male and attained an average length of 1.01 mm. in 100 hour cultures. Growth continued after sexual differentiation and during the egg laying period. Beach (1936) reported as many as 80 filariform larvae developed from a free-living female with an average of 60. Besch (1967) reported an average of 24 larvae per free-living female in water cultures held at 25 C. The free-living male continues to grow after sexual differentiation attaining an average length of 0.93 mm. in 100 hour cultures. Kries (1932) observed that the adult free-living male was necessary for propagation. This observation was verified during this study.

Only one generation of larvae was observed for each free-living generation. Besch (1967) observed the free-living female to persist in water cultures for 65 to 156 hours with an average persistance of 72 to 84 hours. Longevity of free-living males was for a shorter period, i.e., less than 72 hours.

According to Lucker (1942), the homogonic mode of development can be completed in 48 hours after defecation if the fecal cultures are kept at 25 C. In the heterogonic cycle, rhabditiform larvae differentiate into males and females in about 36 hours and attain sexual maturity in 48 hours. Heterogonic filariform larvae were observed to occur in about 96 hours after feces were deposited. He concluded both cycles could occur in the same culture, however, the heterogonic mode of development predominated in all cultures.

Observations on the Morphology of the Filariform Larvae of Strongyloides

The filariform larva can be identified by characteristics of the strongyliform body; it is slender and the esophagus is nearly one-half

the length of the body. Posteriorly, the tail terminates with a tripartite process. A small genital primordium is located near the middle of the intestine and ventral to it.

Measurements of various body structures of filariform larvae obtained from water cultures of stools from experimental infections in two dogs of different breeds, and a cat were compared to measurements made on filariform larvae obtained from water cultures of stools from a naturally infected dog. These data are summarized in Table II.

Interpretation of these data indicate that the heterogonic filariform larvae obtained from the experimental cat and poodle infections are longer than the homogonic filariform larvae obtained from experimental beagle and natural Chihuahua infections. These data concur with the observation of Lee (1930) in that heterogonic filariform larvae are larger than the homogonic forms. Measurements have not been reported previously concerning the average width of filariform larvae from dog infections nor is information available on the average length of the esophagus, the distance from the genital primordium to the anterior end and the distance from the anal pore to the posterior end. Statistical analyses were not done to determine the significance of differences noted in the measurements of the various body structures.

Average measurements of various anatomical parts of filariform larvae obtained from different culture media of experimental infections in dogs and cat were made. These measurements were compared with the average measurements of larvae from a human host reported by Little (1966), as well as those obtained from a natural canine infection recorded in Oklahoma. These data are summarized in Table III.

Results based on these data indicate that the average length

TABLE II

AVERAGE MEASUREMENTS OF BODY STRUCTURES OF FILARIFORM LARVAE OF S. STERCORALIS OBTAINED FROM WATER CULTURES, EXPERIMENTAL AND NATURAL INFECTIONS

Source and Type of Larvae	Number Measured	Average* Length	Average** Width	Average* Length of Esophagus	G.P.[a]*	A.P.[b]**
Experimental infection American Short-haired cat Heterogonic Larvae	25	0.56 (0.54-0.60)	17 (14-20)	0.25 (0.23-0.28)	0.34 (0.33-0.35)	72 (62-81)
Experimental infection - Poodle - Heterogonic Larvae	25	0.57 (0.52-0.60)	15 (14-16)	0.26 (0.24-0.28)	0.36 (0.32-0.38)	78 (59-88)
Experimental infection - Beagle - Homogonic Larvae	25	0.50 (0.43-0.59)	15 (14-16)	0.22 (0.18-0.25)	0.31 (0.27-0.38)	68 (57-74)
Natural infection - Chihuahua Homogonic Larvae	25	0.55 (0.47-0.60)	15 (14-16)	0.24 (0.22-0.27)	0.35 (0.34-0.38)	66 (57-70)

G.P.[a]* = distance from genital primordium to the anterior end
A.P.[b]** = distance from anal pore to the posterior end
Figures in parentheses indicate the range of measurements

*All measurements are in millimeters.
**All measurements are in microns.

TABLE III

AVERAGE MEASUREMENTS OF FILARIFORM S. STERCORALIS LARVAE FROM DIFFERENT CULTURE MEDIA AND FROM A NATURAL HUMAN INFECTION

Source of Larvae	Culture	Number Measured	Average Length Millimeters	Average Width Microns	Average Length of Esophagus Millimeters	Average Genital Pore to Ant. End Millimeters	Average Anus to Tail Microns
Experimental infection - poodle	Water	25	0.57	15	0.25	0.36	78
	Charcoal	25	0.60	17	0.26	0.37	74
	Sphagnum Moss	25	0.60	17	0.26	0.38	76
Experimental infection - beagles	Water	25	0.50	15	0.22	0.31	68
	Charcoal	25	0.60	17	0.27	0.37	80
	Sphagnum Moss	25	0.55	17	0.24	0.34	69
Experimental infection - cat	Water	25	0.56	17	0.25	0.34	72
Natural infection - Chihuahua	Water	25	0.55	15	0.24	0.35	66
Human infection (Little, 1966)	--	31	0.56	16	0.24	--	69

measurements of filariform larvae from the poodle were longer in sphagnum moss cultures than those obtained from charcoal or water cultures while those from the beagles obtained from charcoal cultures were longer than the larvae obtained from sphagnum moss or water cultures.

It is obvious from Table III that the average measurements of filariform larvae obtained from experimental or natural infections in dogs and from an experimental infection in cat were similar to the average measurements reported from a natural human infection.

Body length measurements and two indices, body length versus body width and body length versus length of the esophagus, of filariform larvae from experimental and natural infection in dogs and experimental infection in a cat were compared with those data reported in the literature by several workers. These data are presented in Table IV.

TABLE IV

VARIOUS BODY MEASUREMENTS OF FILARIFORM LARVAE OF S. STERCORALIS FROM DOG AND CAT INFECTIONS COMPARED TO DATA REPORTED IN THE LITERATURE

Source of Larvae	Length In Millimeters	αx	B^{xx}	Number Measured
Experimental infection, poodle	0.52-0.64	37.1-37.3	2.2-2.3	75
Experimental infection, beagles	0.46-0.63	33.1-36.4	2.22-2.37	75
Natural infection, Chihuahua	0.47-0.61	35.1-35.4	2.31-2.37	25
Experimental infection, cat	0.54-0.60	34.9-35.04	2.24-2.34	25
Cat, Rogers, 1939	0.49-0.67	35.0-46.2	2.1-2.5	--
Cat, Chandler, 1925	0.52-0.61	37.5-38.4	2.3	--
Dog, Kries, 1932	0.54-0.60	14.2-19.5	3.2-5.1	--

$$\alpha x = \frac{\text{Body Length}}{\text{Body Width}} \qquad B^{xx} = \frac{\text{Body Length}}{\text{Length of Esophagus}}$$

Prepatent Period and Patent Period of Experimental Infections

The prepatent period is that period of time between the invasion of the host by an infective larva and the recovery from that host of some new stage of the parasite. The length of the prepatent period of a particular parasite may be influenced by many factors including: age and sex of the host, size of the larval dose, biology of the parasite and compatibility of the host to the parasite. Failure to demonstrate the prepatent period in an experimental animal might indicate the incompatibility of the parasite and the host.

The patent period of infection refers to the length of time the infection can be demonstrated in the host by use of accepted laboratory methods. The length of the patent period may be influenced by the biology of the parasite, by age and sex of the host, by diet of the host, by development of host resistance and by other non-determinable factors. The postpatent period of infection is that period in which the parasite persists in the host habitat but during which its presence can not be detected. This latter period is associated with the development of senescence in the female parasite.

The prepatent and patent periods of experimental infections of S. stercoralis in dogs and cats was determined by the demonstration of first stage rhabditiform larvae in recently passed stools by means of the Baermann apparatus and by use of water cultures of feces. Data were obtained from infections, experimentally produced, in seven dogs (six beagles and one poodle) and in two American short-haired cats. These data were summarized and are presented in Table V.

TABLE V

PREPATENT AND PATENT PERIODS OF EXPERIMENTAL
S. STERCORALIS INFECTION IN DOGS AND CATS

Animal	Larval Dose	Prepatent Period (Days)	Patent Period (Days)
Dog B1 (6 mo. female)	200	21	56
Dog B2 (6 mo. female)	180	15	39
Dog B3 (8 mo. female)	550	12	60
Dog B4 (10 mo. female)	1100	5	31
Dog B5 (1 yr. female)	500	9	17
Dog B6 (8 mo. male)	1100	9	At least 91 days
Dog poodle (10 mo. female)	350	18	49
American Short-haired cat 1 (4 mo. female)	1500	16	Not determined
American Short-haired cat 2 (4 mo. female)	700	16	7

The length of the prepatent period was observed to vary in dog infections and appeared to be associated with the size of the larval dose. For all dog infections, the length of the prepatent period was in the range of 5 to 21 days, average of 13 days. For three infections in which the larval dose was less than 350 larvae, the prepatent period was in the range of 15 to 21 days, average 18 days; the four infections with larval dose in excess of 500 larvae, the prepatent period was in the range of 5 to 12 days, average 8 days. No attempt was made to

determine the influence of age and sex of the host on the length of the prepatent period of infection in Strongyloides infections in dogs.

Sandground (1928) infected 20 dogs with doses of 100 to 430,000 filariform larvae and observed the length of the prepatent period to be in the range of 5 to 9 days, average 7 days. There was no correlation between size of the infective dose and the length of the prepatent period. Augustine (1939) reported the length of the prepatent period for experimental Strongyloides infections in dogs to be 7 to 11 days. He gave no indication of the number of dogs infected or the size of the infective dose. Galliard (1950) stated the prepatent period of S. stercoralis experimental infections in three dogs to be 9 to 11 days but concluded that in light infections it may be much longer.

The length of the patent period of infection observed for experimental infections in dogs was in the range of 17 to at least 91 days, average 49 days. Determination of the patent period was dependent on the demonstration of first stage larvae in feces by use of the Baermann apparatus or by use of one of the culture techniques. During the initial period of infection, reproduction in the parasitic female was apparently continuous and larvae could be isolated with ease from stools. This phase often ended abruptly. Then, larvae were observed only occasionally, in lower numbers, and after irregular intervals. In one instance, (poodle) the non-reproductive period was observed to be longer than four weeks. Adult parasitic females with eggs in uterus were obtained from two dogs, B2 (33 females) and poodle (139 females), at necropsy. Attempts to isolate larvae from these animals were unsuccessful for at least two weeks prior to necropsy. However, cultures made from the contents of the small intestine and large

intestine of both animals produced large numbers of filariform larvae. Apparently, as the infection progresses, most first stage larvae are unable to survive and disintegrate as they traverse the length of the digestive tract. This phenomenon has been observed by Faust, et al. (1934). He concluded that usual examination procedures were unsatisfactory for the demonstration of rhabditiform larvae in the stools of infected individuals. Current methods used in the diagnosis of strongyloidosis of dogs are dependent on the demonstration of rhabditiform larvae in recently passed stools or on the occasionally observed characteristic egg. It is apparent that determination of incidence of infection would be difficult in those cases in which the irregular shedding of larvae has occurred. Jones and Abodie (1955) reported results of demonstrating Strongyloides rhabditiform larvae in duodenal fluid aspirated from infected patients. They concluded this technique was most efficient for the diagnosis of strongyloidosis in man. More work needs to be done to establish effective diagnostic techniques to demonstrate presence of parasitic females during all stages of infection in dogs.

The duration of the S. stercoralis, human origin, infections in dogs was discussed in detail by Sandground (1928). Using the daily output of larvae in the stool as the criterion of infection, he reported that the patent period, in experimental infections, was at least 2 months and persisted for more than 11 months with an average of 18 weeks. Galliard (1940) stated that young dogs are easily infected with S. stercoralis, human origin, and that, depending on dose and virulence of larvae, the animal may die in less than 2 weeks to several months. He indicated the low incidence of infection of the dog with this species is due to the lack of exposure to human fecal contamina-

tion. Also various strains of this species have been identified and each strain has a different infectivity. He reported a direct relationship between the capability to infect the host and the fecundity of the parasitic female. Apparently strains with low infectivity for man also have low infectivity for dogs.

Sex may have an influence on the length of the patent period of infection in dogs. This is indicated in the data summarized in Table V. Only one male was infected experimentally and, at the time of writing, it had a patent period in excess of three months. More work needs to be done to determine the influence of age as well as sex on the susceptibility of the dog to S. stercoralis.

The length of prepatent period of infection was the same in both cat infections, 16 days, although one animal received more than twice the larval dose of the other.

The patent period was not determined for the infection in the cat that was given the larger dose. The length of the patent period for infection in the other cat was 7 days. Augustine (1939) failed to produce a demonstratable infection in two cats experimentally infected with larvae obtained from a naturally infected dog. Sandground (1928) experimentally infected eight cats with filariform larvae obtained from dogs infected with S. stercoralis. Larval dose was in the range of 500 to 40,000 larvae, the length of the prepatent period was in the range of 9 to 16 days, average 13 days. Age of host and size of larval dose did not appear to influence length of prepatent period. Duration of infection in cats was 2 to 7 weeks, average 3 weeks.

Observations on Parasitic Females of Strongyloides Species Obtained from Experimental and Natural Infections

Measurements were made of selected anatomical structures of the adult parasitic females of Strongyloides spp. obtained from experimental and natural infections in the dog and from an experimental infection in the cat. These were compared to similar measurements reported by various workers for parasitic Strongyloides females from dog and cat infections. These data are summarized in Table VI.

It is obvious from these data that variations occur in the length and width of parasitic Strongyloides females collected from experimental and natural infections in the dog. Average size, length and width, for worms collected from the intestinal mucosa of the naturally infected dog was larger than those collected from experimental infections. Reasons for these differences were not defined because of apparent variations in collection and preservation techniques. Average length and width measures for parasitic females collected from cat experimentally infected with filariform larvae of dog origin were slightly smaller than those reported for the dog infections. However, the range for length and width measurements between host species was not determined.

Although much information is available in the literature concerning the biology of Strongyloides species in the dog, only a few workers have reported measurements of the various stages of development of these species. In several instances these measurements are reported as a ratio but without the specific values. Consequently, comparison of measurements made during this study to those reported in the literature is of limited value and will not be discussed.

TABLE VI

AVERAGE MEASUREMENTS OF SELECTED ANATOMICAL STRUCTURES OF ADULT PARASITIC FEMALES OF STRONGYLOIDES SPECIES

Source of the Parasite Female	Number Measured	Average* Length	Average** Width	Average* Length of Esophagus	Genital* Primordium to Tail	Anus** to Tail
Experimental infection, poodle	20	1.65 (1.53-1.74)	33 (30-38)	0.51 (0.47-0.53)	0.47 (0.43-0.50)	56 (0.52-0.62)
Experimental infection, Beagle 2	20	1.83 (1.62-2.03)	40 (36-41)	0.54 (0.52-0.64)	0.58 (0.50-.065)	59 (56-62)
Natural infection dog, Oklahoma (Besch)	20	2.14 (1.96-2.30)	42 (40-45)	0.60 (0.53-0.68)	0.66 (0.60-0.72	60 (52-66)
Experimental infection, cat	20	1.58 (1.46-1.72)	36 (31-38)	0.50 (0.46-0.54)	0.51 (0.45-0.56)	58 (56-60)
Lucker, 1942, dog***	--	2.0	30	0.55	0.58	69
Chandler, 1925, cat	--	2.6-2.9	--	--	--	--
Kries, 1932, dog	--	1.8	--	--	--	--

*All measurements are in millimeters.
**All measurements are in microns.
*** These figures are approximations.

Experimental Strongyloides Infections in Abnormal Hosts

The study of animal parasites has shown that host species differ among themselves in their susceptibility to parasites. Some species offer definite resistance to parasites which readily infect other species. These might be highly infective parasite to one host species, yet harmless to another, even closely related, species. All host species are completely resistant or immune to some form of infection or parasitism. Smith (1963) believed that underlying all host-parasite conflict is the universal function of digestion and assimilation in both host and parasite.

One of the objectives of this study was an attempt to determine whether certain laboratory animals could serve as acceptable experimental hosts for the species of Strongyloides known to infect dogs. Infective filariform larvae obtained from Baermann water samples of dog fecal cultures were injected sub-cutaneously into 12 mice, 4 rats and 5 guinea pigs. Dose levels were in the range of 80 to 400 filariform larvae for mice, 80 to 185 larvae for rats and 200 to 500 for guinea pigs. All cultures were made with feces containing larvae collected from experimentally infected dogs. Results obtained from these studies indicate that these experimental animals apparently are refractory to infection with filariform larvae of Strongyloides stercoralis. No immature or adult parasitic females were observed in washings taken from the stomach or small intestines at necropsy, from sediment in saline washes of the lungs or liver left at incubator temperatures for 12 hours nor from samples obtained from artificial digestion of the stomach and small intestine of each animal. No attempt was made to determine the extent of migration nor the extent of

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development of the parasite that occurred, in the various species of laboratory animals. Based on the results obtained from this limited study it is concluded that the white laboratory mouse, the laboratory rat and the guinea pig can not be used as experimental hosts for the Strongyloides species of the dog.

Augustine and Davey (1939) failed to establish the infection in 4 guinea pigs after exposing them to 2000 to 5000 infective larvae obtained from cultures of feces on an infected dog.

Sheldon and Otto (1938) reported that larvae or adults were recovered from 5 to 18 guinea pigs given infective larvae of Strongyloides ratti infected percutaneously or sub-cutaneously. Adults were found in the lungs but not the intestinal tract of two animals and rhabditiform larvae were observed in the lungs of one of these laboratory animals.

CHAPTER V

SUMMARY AND CONCLUSIONS

The taxonomic scheme for the genus Strongyloides Grassi, 1879, is reviewed and discussed. The biology associated with Strongyloides species infecting man and dog is not completely understood nor is the taxonomy associated with these species stabilized.

Strongyloides spp. are unusual among the parasitic nematodes of the domestic animals because of an alternation of generation in the usual life cycle with sexual reproduction in the free-living generation and parthenogenesis in the parasitic generation.

A series of experiments was conducted to determine the influence of the environmental conditions on the mode of the life cycle of Strongyloides species.

Results of data indicate that either homogonic (direct) or heterogonic (indirect) life cycle, or both, may occur in charcoal, sphagnum moss or water cultures of dog feces containing first stage rhabditiform larvae. The homogonic mode of development predominated early in the patent period and heterogonic mode of development predominated later in the course of infection. The change from homogonic to heterogonic was gradual. Only homogonic filariform larvae were obtained from water cultures of feces from a naturally infected dog while both homogonic and heterogonic development occurred in charcoal and sphagnum moss cultures of feces from this animal. In the latter instance, the

direct mode of development was predominant.

In water cultures, homogonic filariform larvae were observed to occur in 31 hours and heterogonic filariform larvae in 90 to 93 hours after the feces were passed. Sexual differentiation of the free-living adults occurred 43 hours after the feces were passed. Shortly after the sexual differentiation, gravid females were observed in with 0 to 3 eggs in uterus; while at 56 hours 10 eggs were observed in some females and at 68 hours, 13 to 15 eggs. First stage larvae from eggs of free-living females were observed in 66 hours and second stage larvae 62 to 72 hours. Only one generation of larvae was observed for each free-living generation. The heterogonic filariform larvae obtained from the experimental cat and poodle infections are longer than the homogonic filariform larvae obtained from experimental beagle and natural Chihuahua infections. Average measurements of various anatomical parts of filariform larvae obtained from different culture media of experimental infections in dogs and cat were made. Results based on these measurements indicate that the average length measurements of filariform larvae vary depending on the host and on the culture medium used.

The length of the prepatent period was observed to vary in dog infections and appeared to be associated with the size of the larval dose. For all dog infections, the length of the prepatent period was in the range of 5 to 21 days, average of 13 days. The length of the patent period of infection observed for experimental infection in dogs was in the range of 17 to at least 91 days, average 49 days. The length of prepatent period of infection in two cats were 16 days. The patent period for the infection in one cat was 7 days.

Adult parasitic females, with eggs in uterus, were obtained from two dogs at necropsy. Attempts to isolate larvae from these animals were unsuccessful for at least two weeks prior to necropsy.

Measurements were made of selected anatomical structures of the adult parasitic females of Strongyloides spp. were obtained from experimental and natural infections in dog and from experimental infection in the cat. Average length and width measurements for worms collected from the intestinal mucosa of the naturally infected dog was larger than those collected from experimental infections while the same measurements collected from cats experimentally infected with filariform larvae of dog origin were slightly smaller than those reported from the dog infection.

Attempts to determine whether certain laboratory animals, mice, rats and guinea pigs, could serve as acceptable experimental hosts for the species of Strongyloides known to infect dogs indicate that these experimental animals apparently are refractory to infection with filariform larvae of dog strongyloides.

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