A STUDY OF THE NEMATODE *CAPILLARIA BOEHMI* (SUPPERER, 1953): A PARASITE IN THE NASAL PASSAGES OF THE DOG

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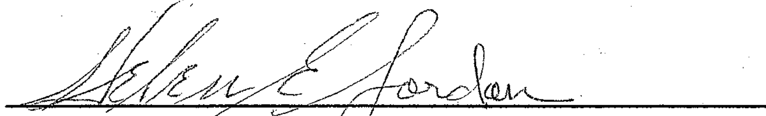

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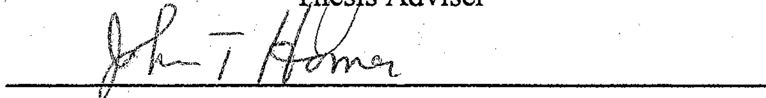

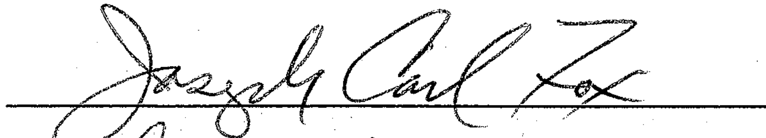

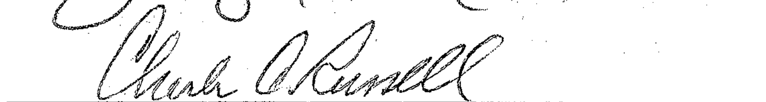

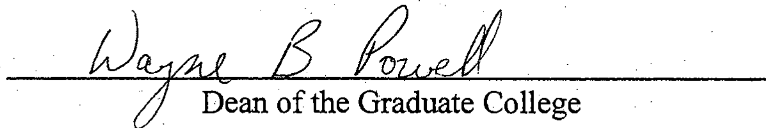

Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Capillaria boehmi (Supperer, 1953) is a minute, thread-like nematode which inhabits the nasal passages and sinuses of canines, felines and related mammals. In descriptions and reports it has often been confused with a closely related nematode, *Capillaria aerophila* (Creplin, 1839) which inhabits the trachea and lungs of similar hosts. Some reports (Levine, 1980; Williams, 1980; Barsanti and Prestwood et al., 1983; Evinger et al., 1985; King et al., 1990) state that the nasal capillarid, *C. boehmi*, is the same species as *C. aerophila.*

Although *C. aerophila*, commonly known as the fox lungworm due to its location in the lungs and trachea of its host, has for some time been regarded as a health problem in foxes and occasionally in dogs, *C. boehmi* was not reported as a serious health threat or considered to be of great economic importance. Neither species has routinely been treated successfully. Little was known or understood of the life cycle of either species, with numerous conflicting reports. A portion of this confusion is due to the fact that *C. boehmi* eggs are difficult to distinguish from those of *C. aerophila.* Additionally, the eggs of both bear a strong resemblance to those of *Trichuris vulpis*, the common whipworm, creating another possibility for incorrect diagnosis.

The lack of clinical signs and observable pathological changes resulting from the presence of *C. boehmi* in the nasal areas also contributed to the limited interest in it as a

parasite of dogs.

Occasionally, a diagnosis of capillariasis was made following a coprological examination which revealed the presence of *Capillaria* spp. eggs, or following the discovery of the worms at necropsy. In all likelihood, the nematode's presence went undetected due to its diminutive size and the small number of eggs produced.

During the late 1980's and early 1990's, a number of greyhounds which had been retired by a racing-greyhound kennel, were donated to Oklahoma State University College of Veterinary Medicine to be used in the veterinary student training program. Fecal examinations, performed as part of the induction procedure, revealed that many of the dogs were passing *Capillaria* spp. eggs. This information, which was not of primary concern in the use of these dogs, was, nevertheless, routinely recorded.

Later, after the dogs were euthanatized, an examination was made to determine the location of the worms. Specimens of *C. boehmi* were found in the nasal passages and sinus cavities of the dogs. No worms were found in the tracheal or bronchial areas.

The question arose as to why so many of these dogs, which had been raised in a confined situation, were infected. Was it possible that many of the dogs selected for retirement did not race as well due to the infection with the worms, or from pathological changes associated with the worms? If that was the case, then the infection in the dog with the nasal capillarid might, indeed, be worthy of additional study from an economic point of view as well as from one of scientific interest.

The economic impact of greyhound racing is great. According to an Associated Press feature article in 1993, greyhound racing that year drew 30 million people to 56

tracks in 18 states, betting $3.4 billion. Of that amount, $230 million went to state funds (Shulins, 1993). This is not to say that the infection in pets, utility or show dogs is of any lesser importance than in the greyhound, but possibly that the infection manifests itself more fully in a dog whose importance, and very existence, is dependent on its ability to run and utilize each breath of air to its fullest.

The potential that this nematode has to adversely affect the racing greyhound, the lack of information available on the worm, and the conflicting reports concerning it made it attractive and interesting for a research project. The hope is that this project may serve to augment that which is known about *C. boehmi* and to organize the previous reports, many of which are in conflict with each other, in a reasonable manner.

CHAPTER II

LITERATURE REVIEW

Confusion both in clinical diagnosis and in species distinction between *C. aerophila* and *C. boehmi* dictated that a literature search should include both species. As recently as 1980 it was routinely reported in textbooks such as Current Veterinary Therapy (Williams, 1980) that only one capillarid, *C. aerophila,* was found in lungs, trachea and nasal passages of dogs, with no indication that the lung and nasal parasites might be different species.

A literature review revealed that the primary locations in which *C. aerophila* was normally found were the trachea and lungs of dogs and other carnivores, with only occasional reports of recovery from the nasal passages and sinuses. Most of the reports were based on fecal findings. Few contained data describing locations within the host. Many of the reports dealt with finding capillarid eggs in foxes raised for fur production.

A few studies reported a similar nematode, *C. boehmi*, as the species found in the nasal passages and sinuses. Several investigators recorded the finding of both species in both locations, thereby adding to the confusion. *Capillaria boehmi* was not described until 1953 following studies by Supperer. Reports prior to that time did not consider the possibility that the species of capillarid in the nasal passages was different from the one in the lungs.

Taxonomy

Taxonomic organization of capillarid nematodes presents one of the more difficult challenges for helminth taxonomists, mainly because of limited knowledge of the species morphology resulting from incomplete descriptions. Numerous attempts have been made to reorganize the taxonomy, but the current classification remains confused (Yamaguti,1961; Anderson, et al., 1974; Anderson, et al., 1982; Maggenti, 1991; Baylis, 1937; Freitas and Lent, 1936).

One reason so many have attempted additional divisions of the genus is the great number of species presently included within this genus. Yamaguti (1961) listed 37 capillarid species in fish, 13 in amphibians, 13 in reptiles, 104 in birds and 88 in mammals.

The generic name *Eucoleus* rather than *Capillaria* has been chosen by some recent authors (Campbell and Little, 1991; Schoning et al., 1993). *Capillaria aerophila* or *E. aerophila* is generally used to describe the nematode found in the lungs and trachea. Most current authors have refered to the nasal capillarid as either *C. boehmi* or *E. boehmi,* but some have refered to it as *C. aerophila* or *E. aerophila*. Possibly these authors were unaware of the descriptions published by Supperer in 1953.

Capillaria Zeder, 1800

Synonyms of *Capillaria* include *Trichosoma* Rudolphi, 1819; *Trichosomum* Creplin, 1829; *Liniscus* Dujardin, 1845; *Calodium* Dujardin, 1845: *Eucoleus* Dujardin,

1845; *Thominx* Dujardin 1845; *Hepaticola* Hall, 1916; *Capillostrongyloides* Freitas et Lent, 1935; *Skrjabinocapillaria* Skarbilovitsch, 1946; *Aonchotheca* Lopez-Neyra, 1947; *Gessyella* Freitas, 1959; *Pterothominx* Freitas, 1959; *Pseudocapillaria* Freitas, 1959; *Ritaklossia* Freitas, 1959; *Pearsonema* Freitas and Mendonca, 1960; *Orthothominx* Freitas and Silva, 1960; *Paracapillaria* Mendonca, 1963; *Schulmanela* Ivashkin, 1964; *Armocapillaria* Gagarin and Nazarova, 1966; *Paratrichosoma* Ashford and Muller, 1978 (Anderson, et al., 1982; Yorke and Maplestone, 1962; Butterworth, 1976). The genus *Capillaria* contains a large number of species, many of which are inadequately described (Read, 1949a). Accordingly, controversies arise as to the criteria used in these classifications. There have been numerous taxonomic rearrangements of the capillarids in attempts to present a valid and logical taxonomic structure (Dujardin, 1845; Travassos, 1915; Yorke and Maplestone, 1926; Freitas and Lent, 1935; Lopez-Neyra, 1947; Skryabin et al., 1957; Freitas, 1959; Freitas and Mendonca, 1960; Freitas and Silva, 1960; Mendonca, 1963; Gargarin and Chulkova, 1971; and Moravec, 1982).

<u>Partial Listing of Capillarid Species</u>

Capillarids have been found in a multitude of hosts and locations, from the intestine of domestic cats to the highly pathogenic capillarid in the skin of *Xenopus laevis,* the clawed toad of northern South America (Georgi, 1976).

A partial listing of *Capillaria* species and the hosts in which they occur is presented in Table I, page 7, and is limited to capillarid parasites of cats, dogs, and related carnivores in North America (Freitas, 1936; Georgi and Georgi, 1990).

TABLE I

PARTIAL LISTING OF *CAPILLARIA* SPECIES

Parasite	Described by	Hosts and location
C. hepatica	Bancroft, 1893	Liver of rats, muskrats, woodchucks, other rodents and a wide range of occasional hosts including carnivores
C. putorii	Rudolphi, 1819	Small intestine of bear, hedgehogs, racoon, swine, various mustelids and domestic cats
C. plica	Rudolphi, 1819	Urinary bladder of dogs, cats, foxes, wolves
C. feliscati	Diesing, 1851	Urinary bladder of dogs, cats, foxes, wolves
C. aerophila	Creplin, 1939	Lungs and trachea of foxes, cats, dogs and various mammals
C. boehmi	Supperer, 1953	Nasal passages and sinuses of foxes, cats, dogs and various mammals

Morphology

Capillaria spp. Adults

The genus *Capillaria* is described as having a hair-like, elongate, slender body, about the same width its entire length. The mouth is simple, without lips. It has a stichosome, which is long, gradually increasing in diameter posteriorly and is made up of a serial duplication of esophageal glands individually known as stichocytes, exterior of the esophagus (Holmes and Kelly, 1973; Caveness, 1964). In the male, the anus is terminal or subterminal and there are usually small membranous caudal alae or a bursa-like structure. The spicule of the male is single and often difficult to see, and has a smooth or spinose sheath. The female's vulva is near the end of the esophagus (Beck and Beverley-Burton, 1968; Yamaguti, 1961).

Capillaria boehmi (Supperer, 1953) Adults

In 1953, Rudolph Supperer published the description, based on three males and five females, of *C. böhmi*, a new species, in Z.f.Parasitenkunde. He had recovered the worms from the frontal sinuses of a silver fox. The species name *böhmi* was chosen out of respect for Professor L.K. Böhm who had done previous studies regarding this parasite.

Capillaria boehmi Male

Male length is 15 - 25 mm and width is 75 - 113 μ. Ratio of esophagus length to body length in the male is 1:1.5. The spicule is slender and has a chitinous covering. The

spicular sheath is completely covered with fine spines. The spicular sheath is largest at the terminal end of the worm and tapers at the anterior. Krull (1969) mentions that the spicule might easily be overlooked as it can be retracted to an extensive degree.

Capillaria boehmi Female

Female length is 30 - 41.25 mm and width is 120 - 210 μ. The number of esophageal cells is 29 - 34. Esophagus length is 6 - 7 mm. Ratio of esophagus length to body length is 1:5 - 1:5.89. The vulva, which does not extend over the surface of the body, is located directly behind the intersection of the esophagus and the intestine. The anal opening is terminal (Supperer, 1953; Read, 1949b).

Morphological Comparison of *C. boehmi* and *C. aerophila* Adults

Differentiation of *C. aerophila* and *C. boehmi* is difficult due to numerous similarities and overlaps in sizes of structures. The greatest difference between the two species is found in the ratio of esophagus length to body length for the females. The ratio for *C. aerophila* is 1:3-1:4.4 and for *C. boehmi* is 1:5-1:5.89. The ratio for esophagus length to body length for males is of little use due to overlaps. Morphological comparisons of *C. aerophila* and *C. boehmi* were made by Supperer (1953) in his description of *C. boehmi* as a new species. Information he presented at that time is included in the following Table II, page 10.

TABLE II

COMPARISON OF MORPHOLOGICAL CHARACTERISTICS OF *C. AEROPHILA* AND *C. BOEHMI* ADULTS (Supperer, 1953)

	C. aerophila	*C. boehmi*
	Female	
Body length	20 - 40 mm	30 - 41.25 mm
Body width	100 - 180 μ	120 - 210 μ
Esophagus cells	40-50	29 - 34
Esophagus length	7.4 mm	6 - 7 mm
Ratio esophagus length to body length	1:3 - 1:4.4	1:5 - 1:5.89
	Male	
Body length	15 - 25 mm	15 - 25 mm
Body width	100 μ	75 - 113 μ
Ratio esophagus length to body length	1:1.8 -1:2	1:1.5
Maximum width of spicular sheath	18 μ	21.11 μ
Length of spicular sheath	900 μ	500 μ

Capillaria boehmi Eggs

The eggs *C. boehmi* are amber to clear in color and are barrel-shaped with a blister-like plug at each end. They are 60.64 - 72.33 μ long and 30.1 - 33 μ wide, with the majority 63.85 - 65.2 μ. The surface is pitted. The pits, which are round- to-oval and different in size, are irregularly located. The pitting gives the shell a finely-striated or granular appearance when viewed microscopically (Supperer, 1953).

Morphological Comparison of *C. boehmi* and *C. aerophila* Eggs

Capillaria boehmi and *C. aerophila* eggs bear marked similarities to each other. *Capillaria boehmi* eggs average length is 64 μ; *C. aerophila* eggs average length is 65 μ (Christenson, 1938). Supperer (1953) reported that the pitting of the surface of *C. boehmi* was slightly deeper than that of *C. aerophila*, the result being that *C. aerophila* eggs have a netted appearance while *C. boehmi* has definite pitting. The blister-like plugs, located at the ends of *C. boehmi* eggs, are slightly more protruding than those of *C. aerophila*. Campbell and Little (1991) reported that a difference exists as *C. aerophila* eggs contain an undifferentiated embryo which completely fills the interior, while *C. boehmi* has a multicellular developing embryo which does not fill the interior. This criterion for differentiation is questionable as it appears only to be a result of development. The following Table III, page 12, summarizes morphological characteristics of *C. aerophila* and *C. boehmi* eggs.

TABLE III

COMPARISON OF MORPHOLOGICAL CHARACTERISTICS
OF *C. AEROPHILA* AND *C. BOEHMI* EGGS (Supperer, 1953)

	C. aerophila	*C. boehmi*
Length	68 - 73 μ	60.6 - 72 μ
average	65 μ	64 μ
Width	35 - 40 μ	31 - 33 μ
average	38 μ	32 μ
Shape	Elongated oval	Broadly oval
Surface appearance	Netted	Pitted
Polar plugs	Protruding	Pronounced protruding

Specificity of Host and Location

Members of the genus *Capillaria* are rather diverse, with a wide range of hosts. At least 255 species were reported by Levine (1978). Host specificity varies among the capillarids although most are quite specific in regard to their location within the host (Beck and Beverley-Burton, 1968). Most tend to stay within a group of animals, such as mammals, birds, reptiles, or amphibians. Little host specificity is demonstrated by some species, such as *C. hepatica*, which may reside in the liver of practically any mammal throughout the world (Levine, 1978). Other capillarids, while still specific for location within the host, have a much narrower host range. For example, *C. caudinflata,* a parasite which inhabits the small intestine, is restricted in host range to the domestic fowl. Another capillarid, *Capillaria collaris,* is only found in the cecum of domestic fowl. These species are restricted to the locations mentioned, although both may occur in several different hosts.

Representative *Capillaria* spp. species in mammals have been reported as inhabitants of the alimentary tract, respiratory system, genito-urinary tract and subcutaneous tissues urinary bladder and liver (Read, 1949a).

It appears that although a capillarid nematode may show specificity for a certain location within its host, there remains the possibility that it may be found in an abnormal or aberrant location due to chance or unusual circumstance. Greve and Kung (1983) reported that *C. putorii,* which is normally a parasite in the small intestine of cats, was found embedded in the gastric mucus of 18.3% of 60 cats examined. In all cats with *C. putori* in the stomach, Greve and Kung found evidence of intestinal reflux, leading them

to believe that all instances were due to mechanical movement of the worms to an unusual location. Some observers might have taken a situation like this and reported that the nematode had taken up residence on a regular basis in a different location within the host. Others might have attempted to reclassify it taxonomically based on its location and identified it as a different species or subspecies. A situation such as this helps point out the importance of finding morphological differences in the nematode before separating it taxonomically due to location.

The majority of reported findings of *C. aerophila* are from the trachea and bronchi of the fox. As is true with many of the very small nematodes in which the females are considerably larger than the males, findings of females are much more common than the findings of males (Wakelin, 1968). Since it is often only the females which are recovered, additional difficulties arise in identification when only male characteristics are used to differentiate species.

Christenson (1938) published an in-depth study of life history and epidemiology of the fox lungworm, which he identified as *C. aerophila*. He did not address the possibility of two different species residing in different areas of the respiratory tract. At one point he discusses numbers of worms recovered from one fox as more or less typical of the nematode's distribution. A total of 1,361 adult worms were recovered; 19 occurring in the nasal sinuses; 12 from the laryngeal region; 514 from the trachea and 716 from the bronchi, bronchioles and smaller air passages. He found that the more mature forms were in the larger ducts where he reported fertilization took place.

In addition to reports of *C. aerophila* from the fox, there has been documentation

of it in numerous other wild animals and in the domestic dog and cat from a wide variety of geographical areas and climates (Christenson, 1938; Endres, 1976; Greenlee and Noone, 1983). Additional hosts include the bobcat (Stone and Pence, 1973), marten (Butterworth, 1976), coyote (Morrison and Gier, 1979), black bear (Greenlee and Noone, 1983) and raccoon (Hamir et al., 1997).

Beck and Beverley-Burton (1968) reported that Semenova (1952) had described a case of a woman patient in Russia infected with *C. aerophila*. Treatment consisted of intratracheal injections of iodine and potassium iodide. According to the report, this was the fourth case of human infection described in the literature.

Table IV, page 16, presents a summary of host animals from which *C. aerophila* and /or *C. boehmi* have been recovered.

TABLE IV

HOST ANIMALS REPORTED TO HARBOR
C. AEROPHILA OR *C. BOEHMI*

Reported as *C. aerophila*		
Host	Reported by*	Year
Grey Fox	Creplin	1839
Human	Semenova	1952
Silver Fox	Wakelin	1968
Bobcat	Stone and Pence	1973
Cat	Endres	1976
Marten	Butterworth	1976
Coyote	Morrison and Gier	1979
Red Fox	Dibble et al.	1983
Black Bear	Pence et al.	1983
Dog	Greenlee and Noone	1983
Raccoon	Hamir et al.	1997
Reported as *C. boehmi*		
Host	Reported by*	Year
Silver Fox	Supperer	1953
Dog	Campbell and Little	1991

*This column is not intended to indicate that the report is the first or only publication of the findings of the parasite in this particular host

Surveys and Prevalence Reports

Although both *C. aerophila* and *C. boehmi* occur in many areas other than the North American continent, the surveys and prevalence reports listed herein were restricted to those concerning North America. Many of the surveys based identification of the parasite upon findings of eggs within feces. Few determinations were based on recovery of worms from lungs and trachea. Seldom was there any reason to believe the nasal passages were examined or that *C. boehmi* was even considered as a possible parasite.

Black Bears

Pence et al. (1983) surveyed black bears from the southeastern United States. Thirty-seven of 98 (38%) bears were positive for *C. aerophila.* The worms were recovered from lungs and trachea of the animals. The number of worms recovered from each animal varied from 4 to 367 for *C. aerophila* and from 1 to 3 for those identified only as *Capillaria* spp.

Bobcats

Stone and Pence (1978) reported a parasite survey that was made of bobcats in west Texas. Sixty-six animals were collected between December, 1973, and January, 1977. The bobcats were from an area that was principally used as rangeland. Most animals included in the study were carcasses obtained from fur trappers. *Capillaria aerophila* nematodes were recovered from three of the animals.

Watson et al. (1981) reported that 143 bobcats from West Virginia were examined for parasites. A total of 25 (17%) were positive for *C. aerophila*. Necropsies were performed on the animals, so that the actual location of the parasite in the lungs was determined. They noted that the parasite occurred more frequently in older animals than in young and frequency increased in areas where the host population increased in density. It was not mentioned if the nasal passages were examined for the presence of parasites.

Cats

Christenson (1938) mentioned an earlier paper by Chandler who, in 1922, recovered *C. aerophila* from seven of twenty-seven cats examined in Michigan.

A fecal examination survey of 217 cats from laboratory colonies, a humane society shelter and homes in east central Illinois showed 4% to be positive for capillarid eggs. No speciation was attempted in this survey. At the time the survey was done in 1975, little consideration was given to the possibility of a respiratory tract capillarid. As both *C. feliscati* and *C. plica* may be present in the bladder of the cats and were well known at the time, it appeared that the author assumed that the eggs found were a contaminant from the urine (Guterbock and Levine, 1977). It was not reported in the article how the fecal samples were obtained. If the samples were collected from litter boxes, it is certainly possible that they were contaminated with urine. Eggs may also appear in the feces after passing through the digestive tract following ingestion during grooming.

Coyotes

Surveys of coyotes in 1977 and 1978 showed infection rates of up to 50% with *C. aerophila*. No distinction was made between the nasal capillarid or the lung capillarid and no report was made as to the location within the host from which the nematodes were recovered (Morrison and Gier, 1979). The article did not state whether or not the nasal passages were checked for the presence of nematodes. Coyote carcasses were obtained from Kansas, western Oklahoma, northern Texas, Colorado, New Mexico, Arizona and southern California. *C. aerophila* was present in 7 (4%) of 181 coyotes. The increased prevalence was based on comparison with a survey made one year previous which had used the presence of thick, stringy mucus in the respiratory passages as an indication of the presence of the worms.

In 1983 it was reported that a 1977-78 survey of 291 Montana coyotes was made to determine the parasites with which they were infected. Only one was found to harbor *Capillaria* spp., and this determination was made by finding capillarid eggs in the intestine (Seesee et al., 1983).

Dogs

In 1974 and 1975 a survey was done of 500 stray dogs in Columbus, Ohio, to determine prevalence of various intestinal parasites. Findings showed that 1% of the dogs examined had *Capillaria* spp. eggs in their feces. No attempt was made to distinguish the species of capillarid (Streitel and Dubey, 1976).

A 1977 to 1980 parasite prevalence survey of 4058 dogs at the Louisiana State

University Teaching Hospital stated that eggs identified as *C. aerophila* were found in only six of the dogs examined. Egg recovery was by means of fecal flotation. At the time the article was written, few made the distinction between *C. aerophila* and *C. boehmi*. No capillarid eggs were recovered from dogs less than six months of age (Hoskins et al., 1982).

A 1980 survey of 110 fecal samples from animal shelter dogs in Chicago showed a finding of five dogs positive for capillarid eggs in their feces. These were identified as *C. aerophila*. Two previous surveys of the area completed in 1970 of 846 dogs and in 1979 of 806 dogs had revealed no capillarid eggs (Jaskoski et al., 1982).

A survey of endoparasites in dogs in Oklahoma showed findings of *C. boehmi* in dogs occurred consistently during the years 1983 to 1990. During that time, an average of 1,250 dogs per year were given fecal examinations at the Oklahoma State University veterinary medical teaching hospital. Actual numbers of dogs infected with the parasite were not reported. Graphic representation of the findings showed a prevalence rate of a high in 1986 of 5%. Percent of positive animals for the remaining seven years was positive but less than 5% (Jordan et al., 1993).

A report by Schoning et al. (1993) showed that four of 230 greyhounds surveyed were positive for *C. boehmi* in the state of Kansas. Identification was based on differentiation of the eggs of *C. boehmi* from those of *C. aerophila* as well as recovery of adult worms from the nasal passages. This is the first published account of a survey conducted to show prevalence of *C. boehmi*.

Foxes

A survey of red foxes in west central Wisconsin revealed 9% of 78 animals were positive for *C. aerophila* recovered from the bronchi. The findings were recorded from necropsy results (Dibble et al., 1983).

Butterworth and Beverley-Burton (1981) conducted a survey *Capillaria* species in wild carnivores in Ontario, Canada, over a period of 18 months. *Capillaria aerophila* was found in 44% of the 48 red foxes examined. One to eight worms were recovered from within the mucosa of the trachea and bronchi of each animal. Most of the worms (66%) were found in the trachea.

Martens

Capillaria aerophila was recovered from 15% of martens examined in a survey of carnivores in Ontario, Canada. One worm was recovered per animal (Butterworth, 1976).

Opossums

Capillaria aerophila has also been reported in the opossum as a naturally ocurring infection in northeastern Georgia (Prestwood et al., 1977). Three of four opossums trapped in the wild had capillarid nematodes within their lungs. Well-defined foreign body granulomas were associated with the worms. Viable *Capillaria* spp. seldom were found, but the granulomas containing the characteristic eggs and cuticular debris were commonly see. The granulomatous reaction was intense and caused extensive damage to adjacent tissue. This type of reaction is often indicative of a host that is not suitable for

the parasite.

Raccoons

Hamir et al. (1997) reported finding *Capillaria* spp. in the trachea of a raccoon. Although raccoons are reported to have capillarids in the mucosa of the alimentary and urinary tracts, there had been no previous reports of these nematodes in the respiratory tract of this host. They reported that more than 600 raccoons had been examined over a ten year period and that this was the first in which the capillarids had been found in the trachea. This report may represent a previously known parasite of raccoons in an aberrant location, may represent a previously described pulmonary *Capillaria* spp. in aberrant host, or perhaps a new species in its natural site and host species. In the report it was chosen to treat the finding as the known species *C. aerophila*, reported from a new host, as site selection is quite specific for these nematodes but host selection is less specific.

Life Cycle

Little definitive information is available on the life cycle of *C. boehmi*, and only slightly more on *C. aerophila*. Much that has been reported has been based on assumptions and reports by others, many of which appear to be assumptions in the original reports.

Direct or Indirect?

Most authors accept Christenson's (1938) study that demonstrated that the life

cycle of *C. aerophila* is probably direct. He reported that infection results from the swallowing of embryonated eggs, as is the case with the closely related common whipworm, *Trichuris vulpis*. Infective larvae are released, penetrate the gut wall and are carried to the lungs by the circulation. The worms pass to the alveoli, bronchi, bronchioles. The prepatent period is about 42 days (Beck and Beverley-Burton, 1968).

Christenson (1938) made numerous attempts to transmit the lungworm infection to guinea pigs, rabbits, cats, dogs and foxes, with only limited success. Results with the guinea pigs, rabbits, and dogs were negative. Of the 22 cats fed larvated eggs, seven developed a cough by seven to ten days post infection. One animal passed capillarid eggs on the forty-third day. All animals appeared negative at necropsy. He attempted to infect foxes, feeding approximated 50,000 embryonated eggs to each. He observed that many eggs passed through the digestive tract unchanged. The failure to transmit the infection led to attempts to achieve it by mixing egg-contaminated soil with feed. This was unsuccessful until scrapings from the nestbox were used. Ten days after feeding, typical clinical manifestations of lungworm disease were present. A necropsy conducted 21 days post infection showed partially developed worms in the lower air passages. From these results, he determined that the life cycle was direct.

The adults live in the major air passages, where fertilization takes place. Typical eggs are produced, coughed up, swallowed, and passed out in the feces. Given favorable conditions, the eggs develop to a larvated stage in 35 to 50 days. The eggs are then infective. According to most reports, the infective eggs are ingested with contaminated food or water. It takes seven to ten days for the larvae to reach the lungs. The route from

the digestive tract to the lungs is unknown, but it is assumed that the larvae are carried there by the blood stream. By forty days the worms have reached maturity. Christenson did not separate *C. aerophila* and *C. boehmi* in this early life cycle description, but reported both as *C. aerophila*, making a distinction only by the location in which the nematodes are found (Christenson, 1938).

There are several closely related species, which are reported to have indirect life cycles, some requiring passage through an invertebrate as does *C. plica* or another mammalian host, as does *C. hepatica* and *Trichinella spiralis*. Enigk (1950) produced a massive report on *C. plica* as it occurred in the urinary bladder of the dog, fox and wolf. He fed larvated eggs to young red foxes but was unable to transmit the infection. He found many of the eggs passed through the intestines unchanged. From this he determined that the life cycle was not direct. He then placed earthworms in soil containing capillarid eggs and allowed the mixture to incubate three weeks. The worms were then fed to the fox which, after 58 days, passed capillarid eggs in its urine. Further studies concerning the earthworm showed that the capillarid eggs hatched in the earthworm but did not continue development. As Enigk felt that ingestion by the earthworm was necessary for hatching, he regarded the earthworm as a necessary intermediate host. He reported finding up to eighty larvae in a single earthworm. Enigk proposed that his studies left no doubt that the earthworm was a necessary intermediate host.

A report on *C. plica* by Senior et al. (1980) posed the proposition that perhaps the life cycle was direct after all. The study included prevalence of the nematode in dogs

maintained in two purebred dog breeding kennels. Of the mature dogs examined, 62.5% were positive in one kennel, and 74.8% were positive in the other. The authors suggested the high prevalence of infection in these kennels presented the probability of direct transmission. Although transmission trials in this study also supported the indirect life cycle due to failure of the eggs to develop in helminth-naive pups whereas ingestion of earthworms collected from the yards of one kennel caused patent infection 61 to 68 days later, it was nevertheless suggested that the poor palatability of earthworms, demonstrated by refusal of dogs which had been fasted, suggested that they are not ingested directly. He does, however, suggest that the dogs might ingest soil contaminated with infective larvae from dead earthworms when grooming themselves. Inspection of the kennels did not present suggestion of an alternate intermediate host.

Capillaria hepatica is unusual in its life cycle as reported by Singleton and McCallum (1990). They report it is the only known helminth of mammals with a direct life cycle that depends on the death of the definitive host for transmission. The female worm deposits eggs into sinuous tracts in the liver, where they remain until liberated by cannibalism, necrophagy or possibly predation. Predators, however, are not at risk from eating animals infected with *C. hepatica* as only the embryonated eggs are infective. The eggs do no become embryonated until they have been allowed to remain in the external environment for an extended period of time.

A closely related parasite within the same family which has an unusual life cycle is *Trichinella spiralis.* This nematode also is dependent upon predation for the completion of its life cycle. Cysts of *T. spiralis* containing infective larvae are found

primarily in striated muscles of infected animals. When a cyst is eaten, the larvae are liberated in a few hours by digestive processes. They enter the mucosa of the small intestine and become sexually mature two to six days after ingestion. There are about twice as many females as males. The worms reach maturity, mate, and the males die soon after. The females burrow through the mucosa into the lymph spaces where they deposit 1,000 to 10,000 larvae. The life span of the female is about six weeks. The larvae pass into the circulatory system and are distributed throughout the body, finally coming to rest in the striated muscles. The diaphragmatic, masseter, lingual and intercostal muscles are the most heavily parasitized. A capsule is formed around them by the muscle fiber. The cysts begin to calcify in six to nine months, but the larvae in them may remain alive for as long as eleven years. No further development takes place until the muscle has been eaten by another animal. The cysts are then liberated during digestion in the stomach and the cycle is repeated (Levine, 1968).

Cyclical Egg Production

The production of eggs by *C. boehmi* was reported by Schoning et al. (1983) as being cyclical, with a peak every seven weeks. This conclusion was made from results using one dog kept for 24 weeks. It was, however, not determined if the egg production was indeed cyclical or if the variations in egg findings might have been related to host factors controlling the concentration of eggs in the feces. The author noted that if a fecal examination took place during the time that the occurrence of the eggs in the feces was at a low, that the infection might be missed or discounted.

Clinical Signs and Pathological Findings

Greenlee and Noone (1984) reported the case of a ten year old dog that had a chronic cough of two years duration which had ranged from mild to severe with the dog "gasping" for breath. Cytologic analysis of bronchial washings revealed *Capillaria* spp. eggs mixed with purulent eosinophilic inflammation with abundant neutrophils. At that time, *C. aerophila* was still considered by many to reside in both the nasal passages and the bronchial areas. All fecal examinations were negative for capillarid eggs. The dog described was one which reportedly was in contact with wildlife. The case was described as "*Capillaria aerophila*, a parasite of the nasal cavity, trachea, bronchi and bronchioles", but it was never determined if the nematode was established in the nasal areas, as necropsies were not performed. The author, who first treated the dog with corticosteroids for chronic coughing, felt that the immediate improvement indicated that a hypersensitivity or inflammatory reaction to the worms, rather than clinical signs due to an airway obstruction.

Barsanti and Prestwood (1983), in their generalized description of parasites in the nasal passages, stated that clinical signs are seldom seen. However, in some severely infected animals, sneezing and nasal discharge may be present. Their comments dealt mainly with the nasal mite *Pneumonyssus caninum* and the pentastome nasal worm *Linguatula serrata*. The authors described clinical signs of *C. aerophila* infections, in the trachea, bronchi and occasionally nasal passages and frontal sinuses. This infection was responsible for a harsh, dry cough such as that often associated with infectious tracheobronchitis or kennel cough. Occasionally a white, foamy mucus was produced by

coughing. They further stated that *C. aerophila* was the most commonly encountered lungworm infecting dogs and cats in North America. It was mentioned that *C. aerophila* is dependent upon the earthworm as an intermediate host for transmission. The article contained photos of *Capillaria* spp. eggs which have details matching the description of *C. boehmi* eggs by Campbell and Little (1991) rather than *C. aerophila.*

Evinger et al. (1985) reported signs of sneezing and a mild serous discharge in a dalmatian dog treated for nasal capillariasis. The sneezing worsened, and was accompanied by bilateral mucous discharge which gradually became purulent. Paroxysmal sneezing increased in frequency, duration, and intensity until the owner reported episodes occurring approximately ten to twelve times per day. The dog would strike his nose on the floor during these episodes and would expel abundant purulent nasal discharge. The nasal discharge as well as the feces contained many capillarid eggs. These eggs were identified by the authors as *C. aerophila*. Photomicrographs of the eggs were included in the article and matched in detail the eggs described by Campbell and Little (1991) as *C. boehmi* eggs.

A case was reported by King et al. (1990) of a hound with nasal capillariasis. He described a dog in good overall physical condition but with intermittent right-side nasal discharge of two months' duration. The discharge usually was reddish-brown and tenacious. There was no history of coughing or sneezing. It was suspected that secondary bacterial infection was responsible for the excessive nasal discharge. It was certainly possible that the irritation caused by the worms established a good media for abundant bacterial growth. Again, as with several previously mentioned articles of recent

times describing nasal capillariasis, the nematode was identified as *C. aerophila*, giving no consideration to the published descriptions of *C. boehmi*. Photomicrographs were included with this article, and they matched in detail the photograph presented by Campbell and Little (1991) in their published description of the egg of *C. boehmi*.

Although sneezing, coughing and a possible nasal discharge might often not be considered of serious importance, when it interferes with the breathing efficiency of a dog such as a racing greyhound, it takes on a greater significance. As greyhounds are a commercial animal, and represent a big business, with approximately 48,000 whelped each year (Schoning and Cowan, 1993), it may be that these rather insignificant pathological changes have a much greater overall effect on the greyhound breed than on other breed of dogs.

As an side note, a similar nematode, *C. plica*, had long been considered of minor clinical significance but, following surveys of kennels, was found to be the probable cause of hematuria, dysuria and pollakiuria, with or without secondary infection, when occurring as a heavy infection (Senior et al., 1980).

Diagnosis

Methods of Diagnosis

The diagnosis of nasal capillariasis may be difficult, and is based on the findings of the nematode's egg from nasal washings, nasal swabs, nasal lavage or fecal examinations or from necropsy findings of the adults worm (Krull, 1969; Barsanti and Prestwood, 1983; Schoning, et al., 1993; Wolf and Turnwald, 1996).

Various techniques have been used to determine number of parasite eggs per gram of feces. Sloss and Kemp (1987) report that these methods are of value in the study of parasite life cycles or in determining the effects of experimental therapy for the removal of parasites. However, quantitative fecal techniques are of little value in clinical diagnosis.

Differentiation of Eggs

Identification of *C. boehmi* eggs in a canine fecal sample depends greatly on the observer's ability to differentiate it from other eggs in the sample, especially those which have bi-polar plugs and are of a similar shape and size.

Trichuris vulpis, the whipworm of dogs, may sometimes be confused with *Capillaria* spp. by those examining a fecal preparation. According to personal communication with Campbell (1991), most technicians still identify *Capillaria* spp. eggs as *Trichuris* sp. He stated that many of the surveys listing the variety of the eggs recovered do not include *Capillaria* spp. Since the eggs were mistakenly identified as "whipworm".

Although rarely, cats also are infected with *C. aerophila* as reported by Hass (1978). Eggs also must be differentiated from *Trichuris campanula*, a feline whipworm. Additionally, the eggs of the whipworm in cats are smaller than those found in dogs, thereby diminishing one of the criteria by which the two genera are distinguished.

Another nematode parasite of cats which produces similar eggs is *C. putori*, which resides in the intestinal tract. Greve and Kung (1983) reported that eggs of *C. aerophila*

and *C. putori* have differences that make differentiation possible. Eggs of *C. aerophila* tend to be larger than those of *C. putori,* eggs of *C. aerophila* are broadly oval whereas eggs of *C. putori* are parallel-sided, and the network of ridges on the shell surface is delicate in *C. aerophila* but coarse and obvious in *C. putori.*

Newcomb et al. (1984) discussed another possible area of confusion in his discussion of bi-polar-plugged eggs. In examining a fecal sample from a cat they found eggs later identified as *C. hepatica* and *Trichuris muris*, both probably just passing through the cat's system as a result of prey consumption. Following euthanasia, necropsy revealed no parasites present, but certainly the egg findings would cause the examiner to consider either an infection with one of the parasites known to produce this type of egg or possibly to confuse these eggs with similar eggs of parasites of the same family.

The *Capillaria* spp. eggs also must be distinguished from *Anatrichosoma* spp., a trichurid nematode described by Georgi (1985), normally residing in the nasal passages of African monkeys, which has an egg similar to that of *Capillaria* spp. Although considered unusual, this egg has been reported in a canine host. Hendrix et al. (1987) reported finding the embryonated, double-plugged egg in the fecal sample of a five-month-old boxer dog. The dog also had the subcutaneous nodules described as symptomatic of this genus and containing eggs identical in description. The eggs measured 62.5 microns by 50 microns.

The description of the *Anatrichosoma* sp. eggs by Pence and Little (1972) would suggest that they could be differentiated due to the fact that they are embryonated when passed. However, it is not uncommon for *Capillaria* spp. eggs also to be embryonated

when passed.

Treatment

Numerous researchers have tried a variety of therapeutic regimes for the treatment of *C. boehmi* and *C. aerophila*. Several anthelmintics used alone or in a combination with antibiotics, antihistamines and corticosteroids have been found in most reports to give temporary relief and disappearance of clinical signs. Total clearance of eggs has been observed in several reports, but removal of worms has not been documented.

Dogs

Several different approaches have been used in an attempt to resolve infections with *Capillaria* spp. and to relieve the dog of the symptoms. Since coughing, sneezing, wheezing and occasionally nasal discharge are many times the only symptoms present, especially in the dog, initial treatment is often centered around use of antibiotics and antihistamines. Add to this the fact there are often few eggs in the feces and, even when found, those eggs are often mis-identified as *Trichuris* sp., the result is that the animal may be treated for a bacterial or viral respiratory infection, for an allergic reaction, or for whipworms and not *Capillaria* spp. *per se.*

Pepper (1978) reported treating two dogs which had chronic coughs and which were positive for *Capillaria* spp. eggs with the anthelmintic levamisole, 5 mg/lb for ten days. Both dogs showed improvement, but the cough returned four months later. Treatment was repeated and again there was improvement. Again, coughing returned in

four months.

Another scenario was reported by Greenlee and Noone (1984) where a dog was first treated with antibiotics, then with antihistamines, then with prednisolone before *Capillaria* spp. eggs were found. When finally eggs were observed in bronchial washings, the dog was treated with oral levamisole (10 mg/kg sid for five days, stopping for nine days and repeating). No *Capillaria* spp. eggs were found one month following treatment. Although this report on the use of levamisole was published as treatment for *Capillaria* spp., it did not prove removal of the parasite from the host, only the disappearance of the eggs from the bronchial washings.

Evinger et al. (1985) reported treatment of a dog with nasal discharge and respiratory distress that began with administration of the antihistamine chlorpheniramine for symptoms. The treatment relieved the symptoms, but three weeks after treatment was discontinued, the symptoms resumed. The addition of prednisone and ampicillin did not alleviate the symptoms. At the time of symptomatic treatment, the animal had not yet been diagnosed with capillariasis. Cytologic and histologic examinations of specimens from the nasal passages revealed numerous eggs identified as *C. aerophila*. Similar eggs were found in the feces using fecal flotation. The dog was treated with ivermectin 0.2 mg/kg of body weight orally. Within seven days the nasal discharge was gone. Five fecal examinations for eggs during the next four months were negative. After reviewing the article, it appeared that the parasite which Evinger et al. reported was *C. boehmi*, rather than *C. aerophila* due to the location of recovery from the host.

King et al. (1990) reported on a five-year-old intact female blue-tick hound which

was evaluated for chronic nasal discharge. Nasal infection caused by *C. aerophila* was diagnosed by identification of adult nematodes and eggs in the nasal flush sediment and by nasal biopsy samples and eggs in fecal flotations. The dog was presented with a severe nasal discharge. The dog was treated with fenbendazole 50 mg/kg orally each day for 10 days. Five days following treatment, the discharge was resolved. Four months later, the dog was back with a nasal discharge and capillarid eggs in its feces. The dog was then treated with ivermectin at a dose of 0.2 mg/kg body weight orally. The nasal discharge resolved within two weeks, but fecal examinations remained positive for the next three months despite three additional treatments with ivermectin plus antibiotics for secondary infection. It was stated that the reason for the persistence of the infection was lack of sanitation leading to reinfection. There is the possibility that the improvements seen in clinical signs were merely related to the control of the bacterial infection rather than the removal of the worms.

Cats

Treatment of the cat for respiratory tract capillariasis is still in the experimental phase. Georgi (1975) suggested that treatment for *C. aerophila* in the cat might include the use of methyridine, thiabendazole, levamisole or dichlorvos. At that time, reports of the use or success of these drugs were not available.

Treatment of a kitten with respiratory problems was described by Endres (1976). The kitten was first thought to have pneumonia and was treated with antibiotics. It responded well, but the moist cough returned soon after antibiotic treatment was

discontinued. When capillarid eggs were discovered in the feces, the kitten was given levamisole, a cattle anthelmintic, diluted for accurate dosage and injected subcutaneously into the flank, then repeated two weeks later. No problems were observed and the coughing did not return. All subsequent fecal examinations were negative for the presence of capillarid eggs. However, removal of the worms was not proven as there was no necropsy.

Another levamisole treatment was described by Smith (1979) for use on naturally parasitized domestic short-haired cats for internal helminths. Levamisole for cattle was given to the cats at the rate of 8 mg/kg of body weight for one treatment. Fecal flotations were checked for the presence of eggs five days post-treatment and seven days post-treatment. The levamisole showed an efficacy rate of greater than 95% for *Ancylostoma* spp., *Toxocara* sp. and *Toxascaris* sp. but had no apparent effect on the *C. aerophila* being passed by the two cats. No capillarids were recovered at the necropsy. One possibility not mentioned in the article is that since the capillarid eggs must traverse a rather tortuous path from their origin in the respiratory tract to exit the body in the feces that the time of one week between treatment and euthanasia might not have been long enough to rid the cat's system of eggs already in transit.

Corwin et al. (1984) were unsuccessful in treating five naturally infected cats with fenbental/praziquantel paste. Diagnosis of *C. aerophila* was based on fecal examinations. These animals were among seventeen random-source cats and nineteen random-source dogs which were treated with a formulation of 3.4% fenbental and 0.34% praziquantel at a rate of 0.29 gr/kg of body weight, once daily for three days to determine

the effect on internal parasites. Necropsy revealed no apparent effect on *C. aerophila*. Efficacy was 100% for the removal of *Ancylostoma caninum, Toxocara canis* and *Taenia* spp. and 99.9% in clearance of *Trichuris vulpis* in dogs, and 100% in removal of *Ancylostoma tubaeformis*, *Toxocara cati* and *Taenia* spp. In dogs, *Dirofilaria* sp. appeared unaffected, and in cats, there was no apparent effect on *Capillaria* spp. or on *Paragonimus* sp.

Foxes

Brannian (1985) reported treatment of young foxes which were passing eggs identified as *C. aerophila*. Although *C. aerophila* and *C. boehmi* seldom cause significant clinical signs in dogs, in young foxes the signs are often serious. The disease in these particular animals progressed from coughing spasms to a generally debilitated condition. First treatment was with amoxicillin with no improvement noted. At this point *Capillaria* spp. eggs were found during a fecal examination. Next treatment was with oral levamisole, 13 mg/kg, for 7 days with no apparent improvement. One week following the levamisole treatment, the foxes were treated orally with fenbendazole, 30 mg/kg for two consecutive days every two weeks for four treatments. During treatment the foxes improved remarkedly. Results of fecal examinations were not reported. Eighteen months later the foxes again showed symptoms and fecal examinations revealed a single *Capillaria* spp. egg. Direct examination of sputum revealed numerous eggs. Fenbendazole treatment was repeated and respiratory signs were again alleviated. It is uncertain if the parasite was removed with the treatment or just reduced in numbers

which allowed clinical signs to subside. The author assumed a direct life cycle and that reinfection was a possibility.

Blagburn et al. (1986) did efficacy testing on gray foxes using mebendazole (22 mg/kg), fenbendazole (50 mg/kg), febantel (10 mg/kg) and ivermectin (0.4 mg/kg) against *Ancylostoma* spp., *Capillaria aerophila* and lungworm larvae. Treatment was given once, with fecals taken prior to treatment and one and three weeks following treatment. Specific identification of mature parasites was not possible because the animals were not necropsied. His reported findings concerning *Capillaria* spp. eggs showed that of four positive animals treated with mebendazole, three remained positive; one was treated with fenbendazole and remained positive; two were treated with fenbantel and one remained positive. None of the foxes chosen for the ivermectin treatment portion of the study were positive for capillarid eggs so there was no report on its efficacy.

Species Distinction Using DNA Technology

During the last two decades, great technological advances have occurred concerning the use of DNA to compare and distinguish various species (Liu et al., 1996). The impact of these advances in the fields of taxonomy and biological investigation have been immense. In the early studies using DNA technology, only limited research was carried out on helminths (Rollinson et al., 1986). Even so, as early as 1972 it was suggested by Simmons et al. that techniques using DNA might be a useful method in parasite taxonomy. This suggestion was made after the researchers noted that DNA

differences strictly adhered to the morphological differences noted between the species being compared (Simmons et al., 1972).

The use of DNA technology in taxonomy has had a wide variety of results, some predictable and some surprising. For instance, in the areas of grouping various animals together in terms of their evolutionary origin, one of the more surprising groupings was the placement of nematodes with arthropods. Although these two groups would appear very diverse, according to Aguinaldo et al. (1997) phylogenetic analysis of DNA sequences indicate a close relationship between arthropods and nematodes. This was not surprising, in retrospect, as both are molting animals, which separates them from others.

DNA Isolation and Purification. Due to the cuticle found on nematodes, it is often difficult to extract pure DNA from them. The cuticle will often co-precipitate with the DNA during isolation and inhibit subsequent enzymatic reactions.

Gasser et al. (1993) described a method for isolation and purification of DNA he has found to work well with parasitic helminths. The worms were suspended in 250 - 500 μl 20 mM Tris-HCl, pH 8.0, 100 mM EDTA, 1% sodium dodecyl sulfate (SDS) containing 500 μg/ml Proteinase K (Boehringer), homogenized with a polytron at slow speed for one minute and incubated at 37°C for ten minutes. He states that the polytron homogenizer is more effective for DNA extraction from nematodes with their tough outer cuticle than simple digestion with Proteinase K. He also feels that the damage done to the DNA by the homogenizer is so slight as to be negligible. After homogenizing, the mixture was centrifuged for three minutes (10,000 g). Then the supernatant was transferred to a fresh tube and extracted using phenol/chloroform/isoamyl alcohol

(25/24/1). The aqueous phase was precipitated with 2X the volume of absolute ethanol, immediately centrifuged for 2 minutes, and the DNA suspended in 100 μl of distilled H_2O. Gasser et al. describe the desired DNA precipitate as having the appearance of cotton wool. This is in contrast to the DNA mixed with the co-precipitate of a 'white flocculate substance', probably polysaccharides which often inhibit PCR or may cause amplification of non-specific products. This procedure yielded DNA in adequate amounts and sufficiently clean for effective PCR.

DNA Amplification Using PCR. The polymerase chain reaction (PCR) can provide a rapid and effective method to generate template with only minute or limited quantities of material or even a single nematode egg. Using PCR, and beginning with a single molecule of the genetic material DNA, over one billion similar molecules may be generated in a single afternoon using relatively simple laboratory equipment and reagents. The DNA sample can be pure or a minute part of an extremely complex mixture of biological materials (Mullis, 1990).

DNA Sequencing. Ribosomal DNA (rDNA) sequences have been used extensively for speciation (Liu et al., 1996).

CHAPTER III

RESEARCH OBJECTIVES AND PROTOCOL

The overall objective of this study is to add to the current knowledge regarding *C. boehmi*, a parasite of the nasal passages and sinuses of the dog.

Specific Objectives

1. To elucidate morphological characteristics of *C. boehmi* adults and eggs, see Figure I, page 41.

2. To expand the knowledge of the biology of *C. boehmi*, see Figure II, page 42.

3. To describe pathological effects of *C. boehmi* in the dog which are associated with the presence of the parasite, see Figure III, page 43.

4. To describe epidemiological factors associated with nasal capillariasis in the greyhound, see Figure IV, page 44.

5. To determine anthelmintic effect of ivermectin on *C. boehmi* in greyhounds, see Figure V, page 45.

Figure I: Protocol for Elucidation of Morphological Characteristics of *C. boehmi* Adults and Eggs

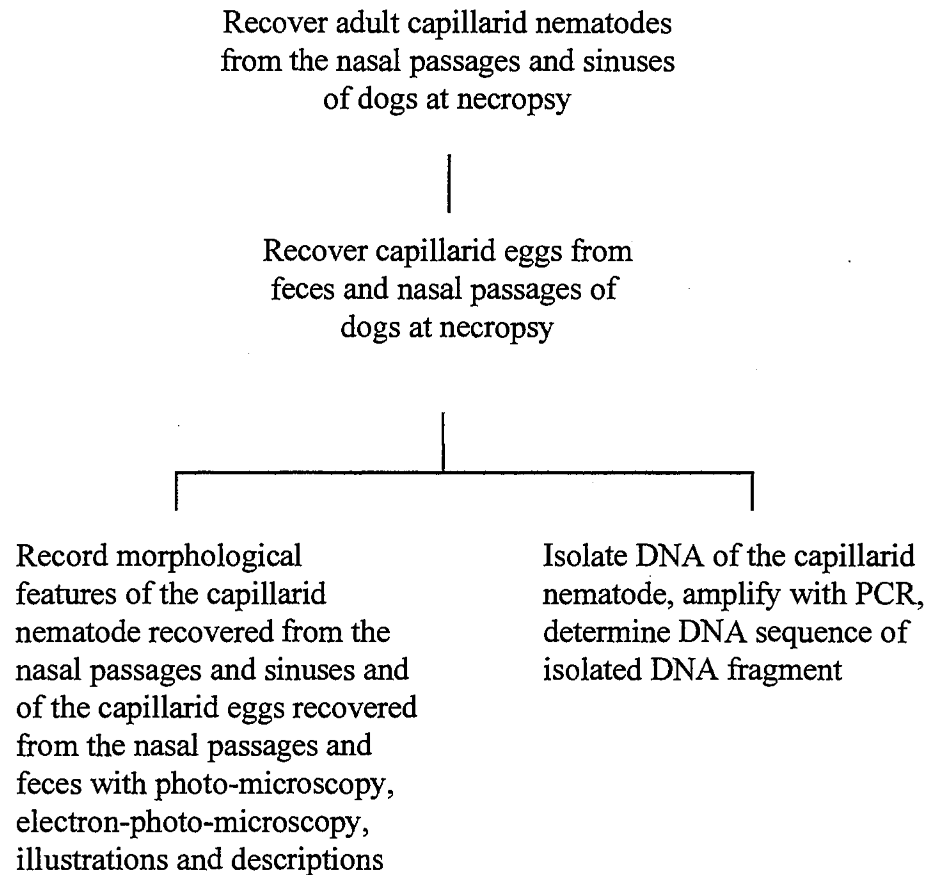
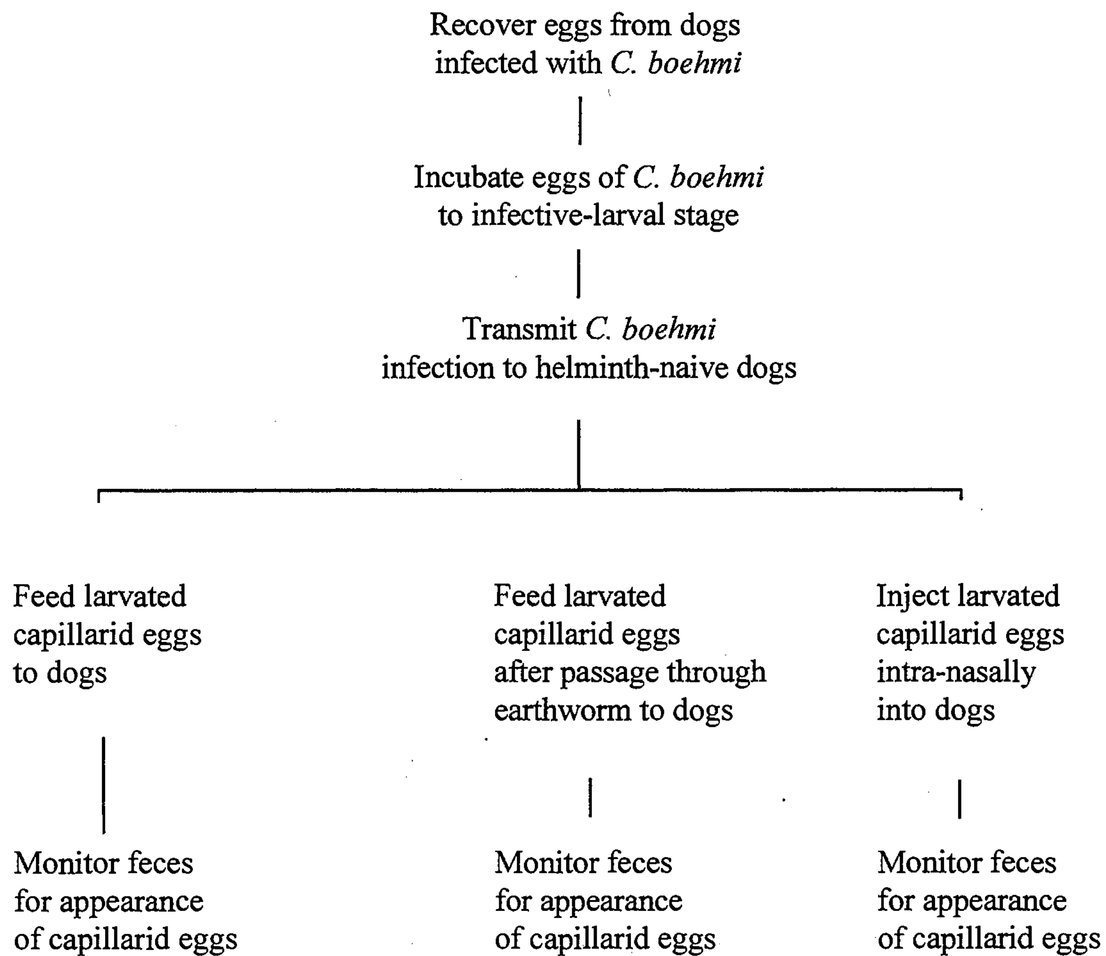


Figure II: Protocol for Biological Studies of *C. boehmi*



Dogs will be euthanatized and necropsied at termination of study to determine location of adult nematodes.

Figure III: Protocol for Determining Pathological Effects Associated with *C. boehmi* Infection in the Dog

Euthanatize and necropsy dogs
shown positive for *Capillaria* spp. infection
by coprological examination

Examine nasal passages, sinuses,
trachea and lungs for presence
of adults capillarid parasites

Observe and record any pathological changes
or signs of immunological response associated
with presence of the worms

Photograph and describe findings

Figure IV: Protocol for Determining Epidemiological Factors Associated with *C. boehmi* in the Greyhound

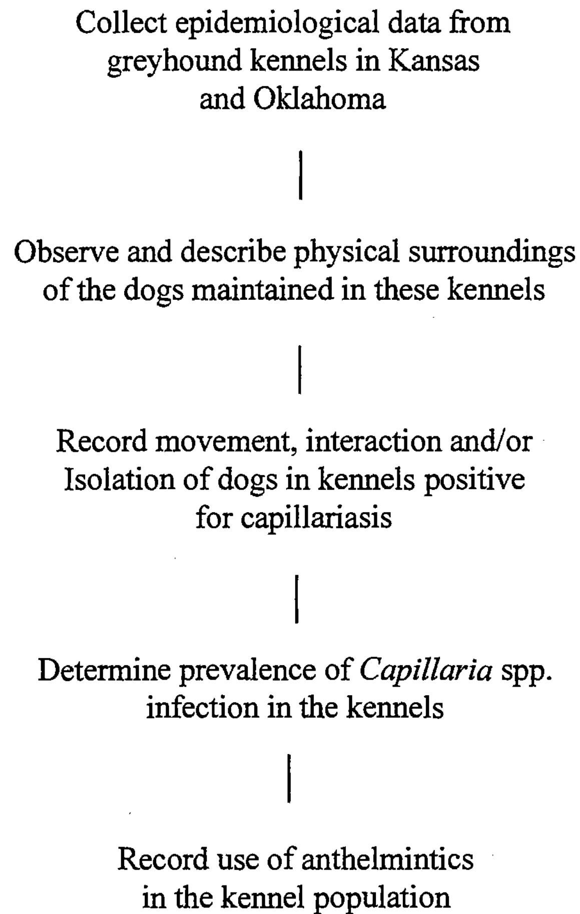
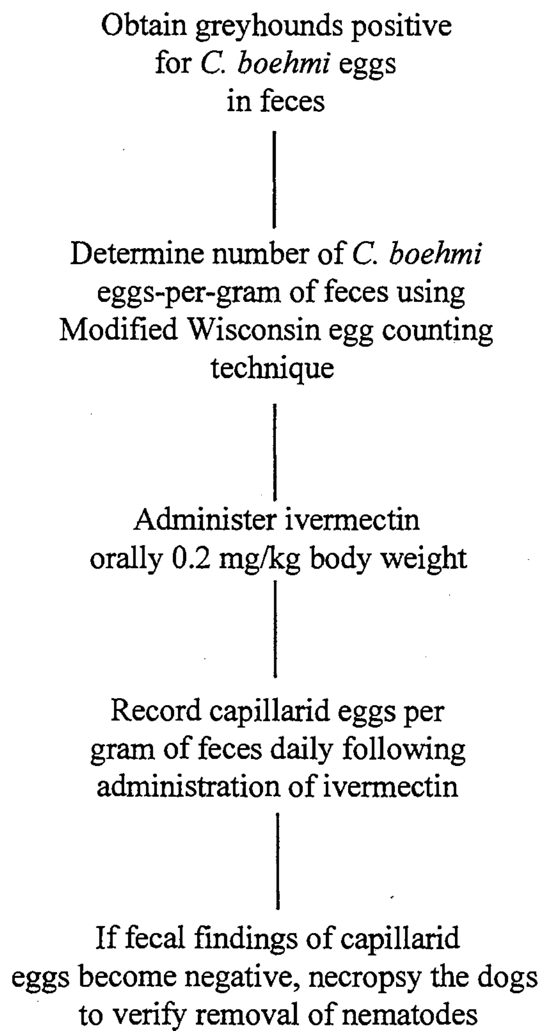


Figure V: Protocol for Determining Anthelmintic Effect of Ivermectin on *C. boehmi* in the Dog



CHAPTER IV

MORPHOLOGICAL STUDIES OF *C. BOEHMI*

Capillaria boehmi adults and eggs were photographed and measured shortly after removal from the nasal passages and sinuses of an infected dog. Measurements of adult *C. boehmi* males and females and *C. boehmi* eggs are presented in Table V, page 48.

Capillaria boehmi Adults

Measurements of adult worms were within the size range recorded by Supperer (1953) in his original description of *C. boehmi.* Males measured 15 - 25 mm in length by 100 - 180 μ in width. Females were 30 to 40 mm in length by 120 - 210 μ in width. To increase visibility when photographing internal structures, the worms were placed in lactophenol.

When first removed from the host and placed in saline, the worms remained in a graceful, gently curved pattern as shown in Figure VI, page 49. The finely-tapering anterior end has few distinguishing characteristics available for diagnosis prior to the esophageal area in which stichocytes can be observed. Differentiating male from female with this portion of the worm is not feasible as the anterior ends of male and female were indistinguishable. The anterior of a female *C. boehmi* is shown in Figure VII, page 50. The stichosome esophagus was presented in series of three photograph of increasing magnification to show the individual stichocytes making up the stichosome in Figures VIII - X, pages 51 - 53.

A photo of a non-gravid female showing the vagina and vulva at the junction of the esophagus and intestine is shown in Figure XI, page 54. A gravid female's vulva with eggs passing to the exterior is shown in Figure XII, page 55. Figure XIII page 56, shows a mid-length section of body cavity of a gravid female completely filled with eggs within the uterus. Many of the worms were found to be coated with a mixture of mucus and eggs on the cuticle as shown in Figure XIV, page 57. The mixture adhered tightly to the worms and would remain even after the worms were handled and placed in 70% ETOH for storage. Figure XV, page 58, shows the posterior end of the male with the two fleshy lobes of the copulatory bursa-like structure and their connecting membranous flap. The posterior end of the female clearly showing the terminal opening of the digestive tract is depicted in Figure XVI, page 59. An electron-photomicrograph of the posterior end of a female is shown in Figure XVII, page 60. This photograph also shows a portion of the body revealing a surface covered by fine, spiny structures. For a parasite residing in the nasal passages on the surface of the mucosal lining, spines on the cuticle would be expected to aid the worm in maintaining its position.

TABLE V

DIMENSIONS OF *C. BOEHMI* ADULTS AND EGGS

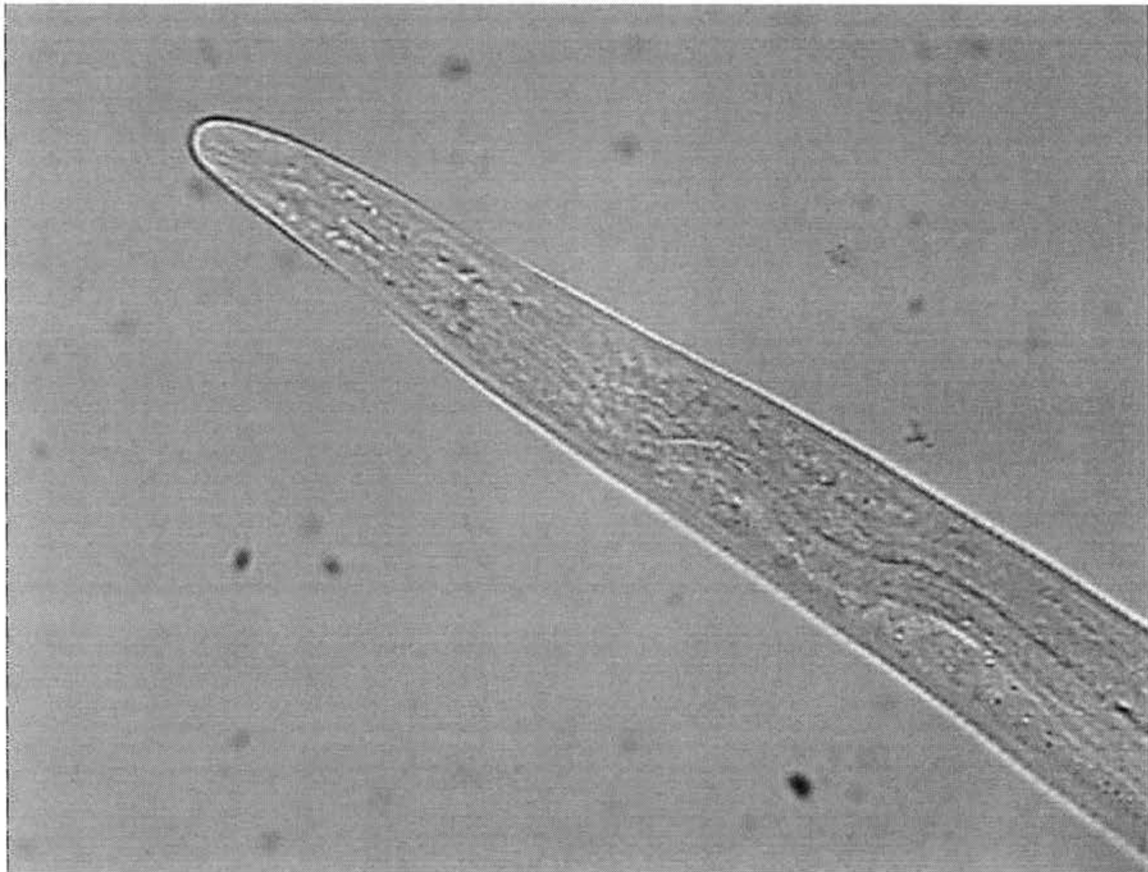
	Adults	
	Male	Female
Body length	15 - 25 mm	30 - 42 mm
Body width	100 - 180 μ	120 - 210 μ
Number stichocytes		29 - 34

	Eggs
Length	53 - 64 μ
Width	30 - 35 μ
Ratio Width : Length	1 : 1.8

_______________ = 875 μ

Figure VI: Adult *C. boehmi* Female Removed from Nasal Passage of Greyhound

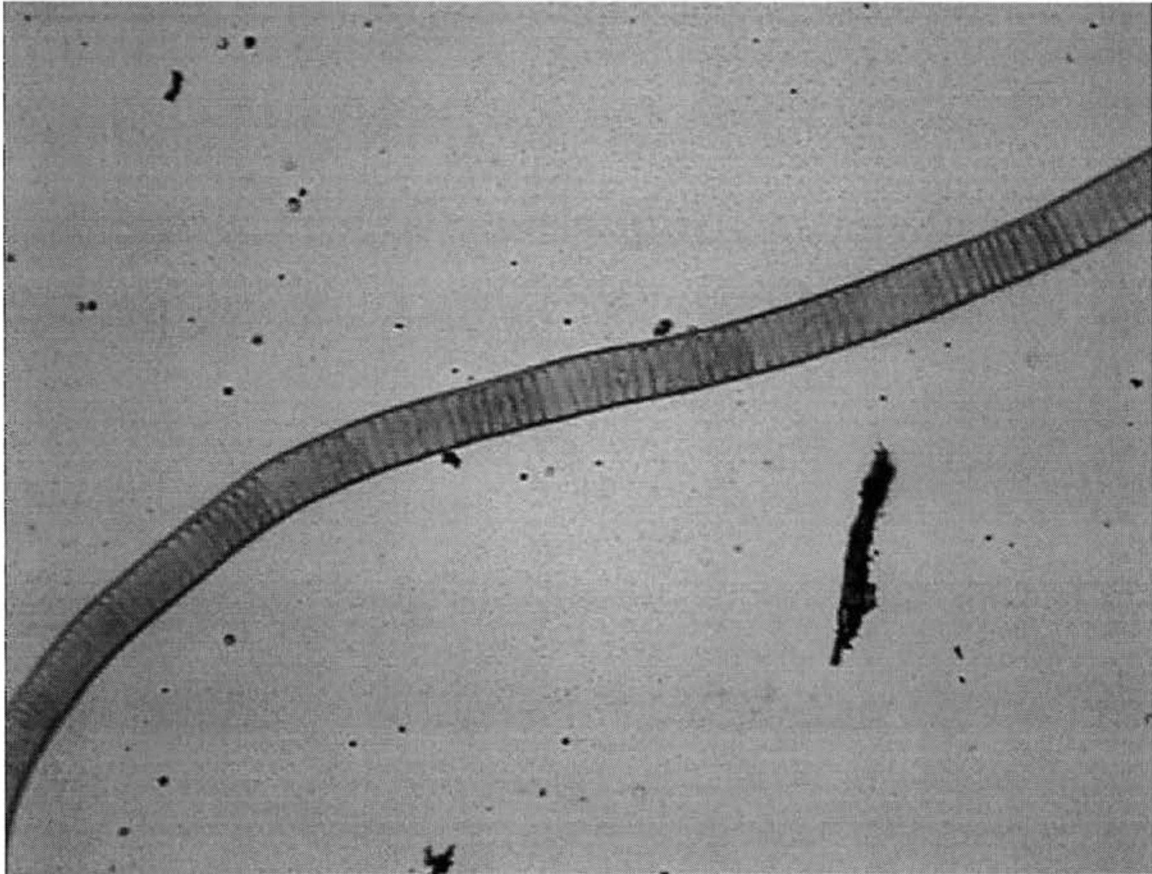
Freshly-removed nematodes were placed in 0.85% sterile saline where they remained alive for up to two months. The twisted and coiled pattern seen here was typical of the appearance retained by the worms.



______________ = 120 μ

Figure VII: Anterior end of Adult Female *C. boehmi*

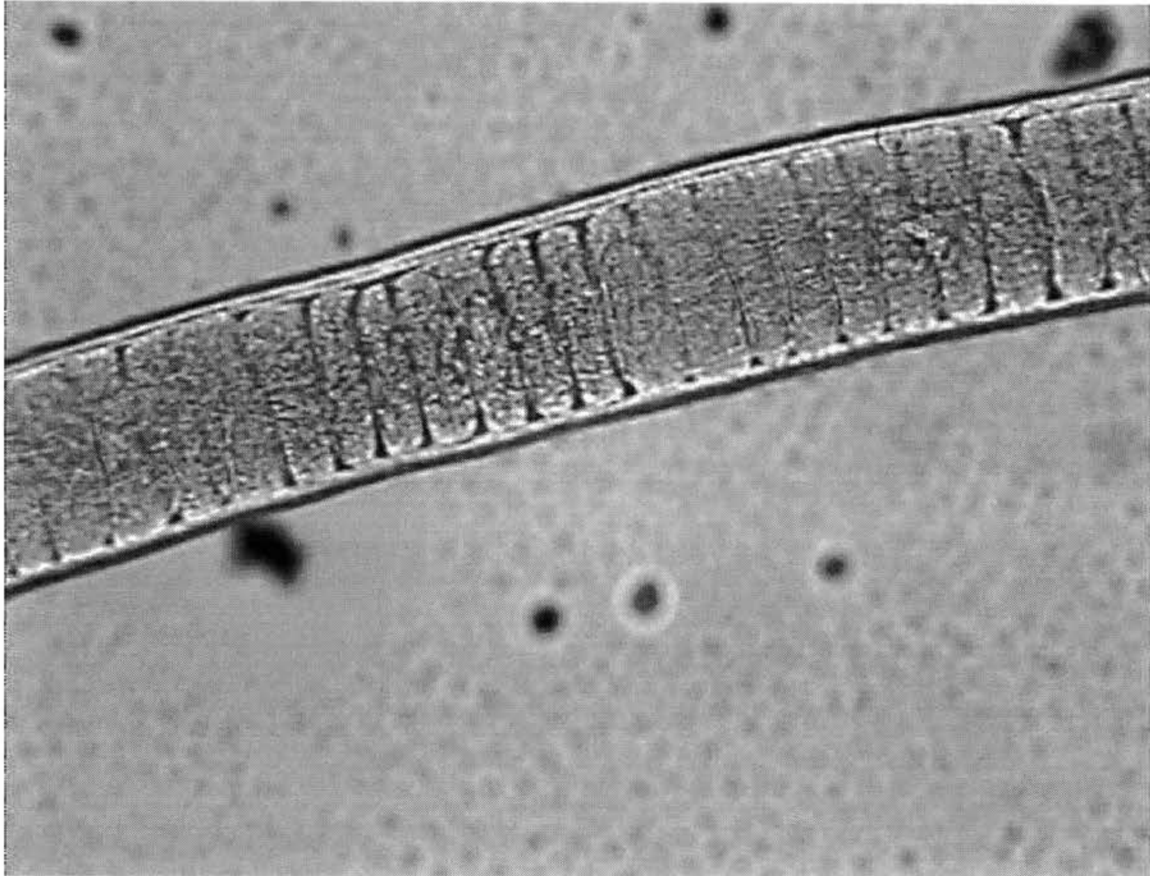
Anterior ends of male and female *C. boehmi* worms appeared so similar as to be indistinguishable except for size.



______________ = 547 μ

Figure VIII: Stichosome Esophagus of *C. boehmi*

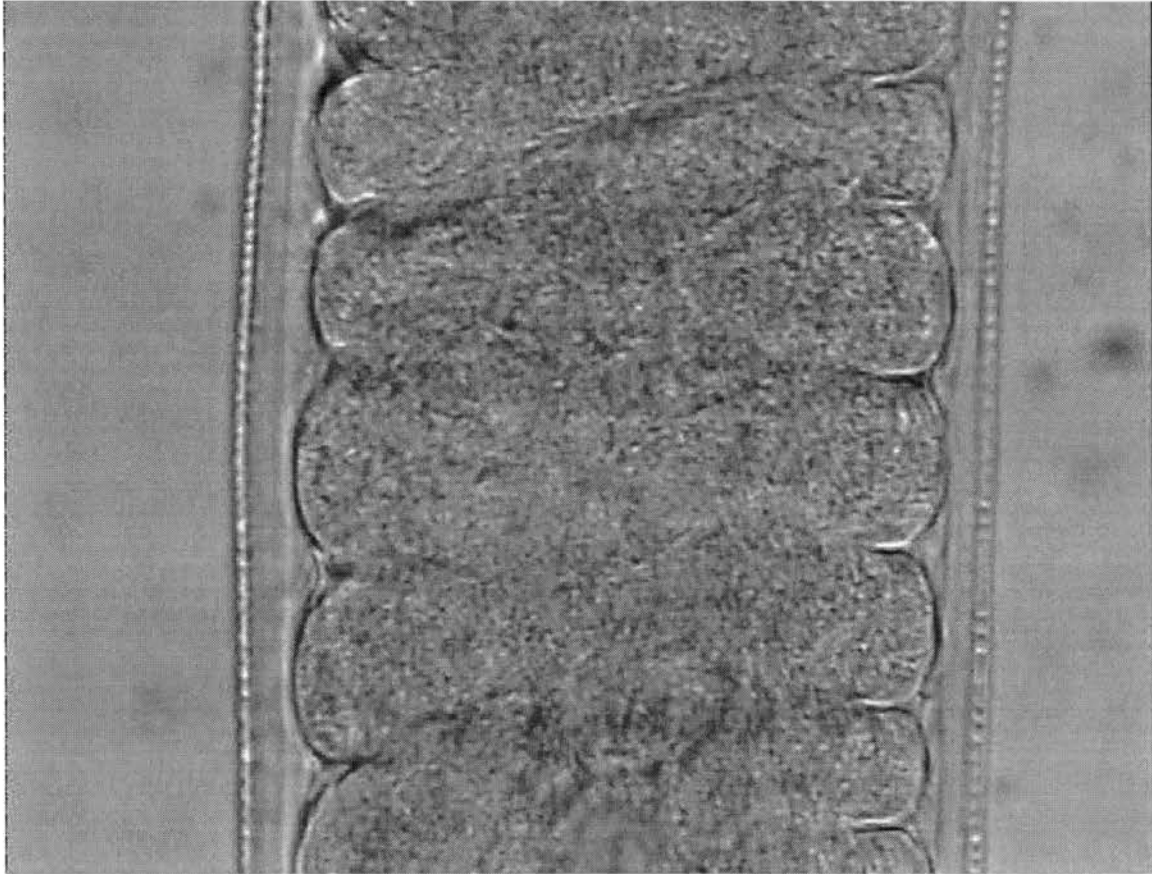
The stichosome esophagus is a distinguishing characteristic of nematodes in the family Trichuridae. It is made up of glandular cells stacked singly outside the contour of the esophagus proper in *C. boehmi*.



______________ = 146 μ

Figure IX: Stichosome Esophagus with Stichocytes

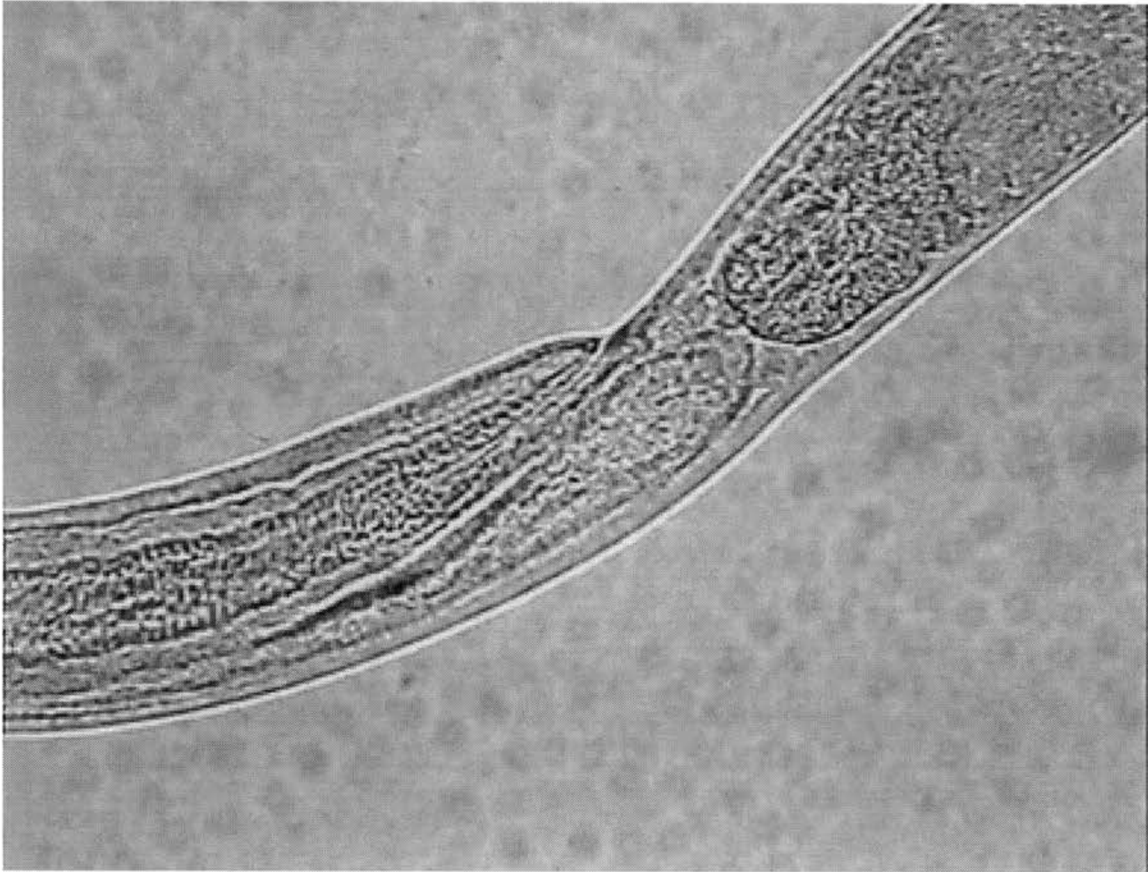
Large cells called stichocytes surround the esophageal tube running from the anterior end of the worm to the junction with the intestine.



_____________ = 44 μ

Figure X: Stichocytes Which Make Up Stichosome Esophagus

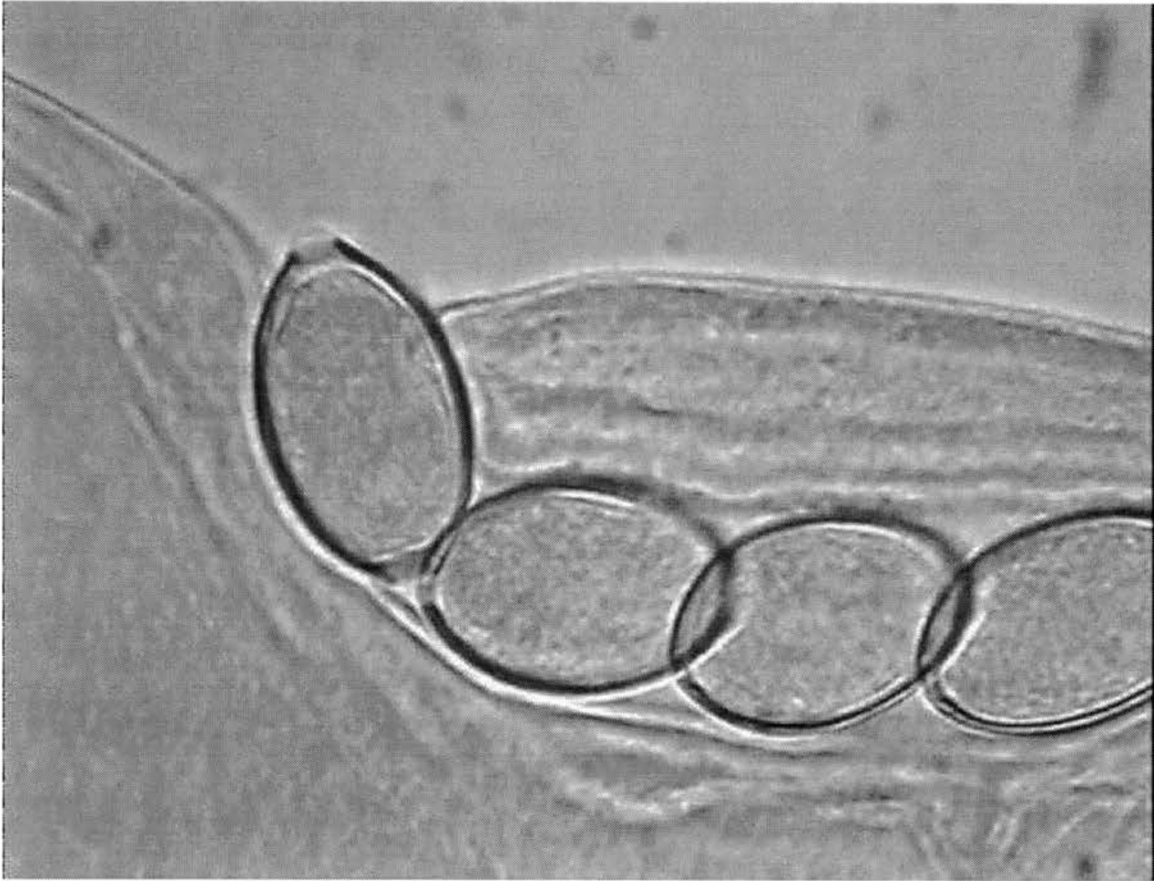
Number of stichocytes which make up the stichosome esophagus in the female worm are used as one of the distinguishing characteristic between *C. boehmi* and *C. aerophila*



______________ = 146 μ

Figure XI.: Vulva of Non-gravid Female *C. boehmi*

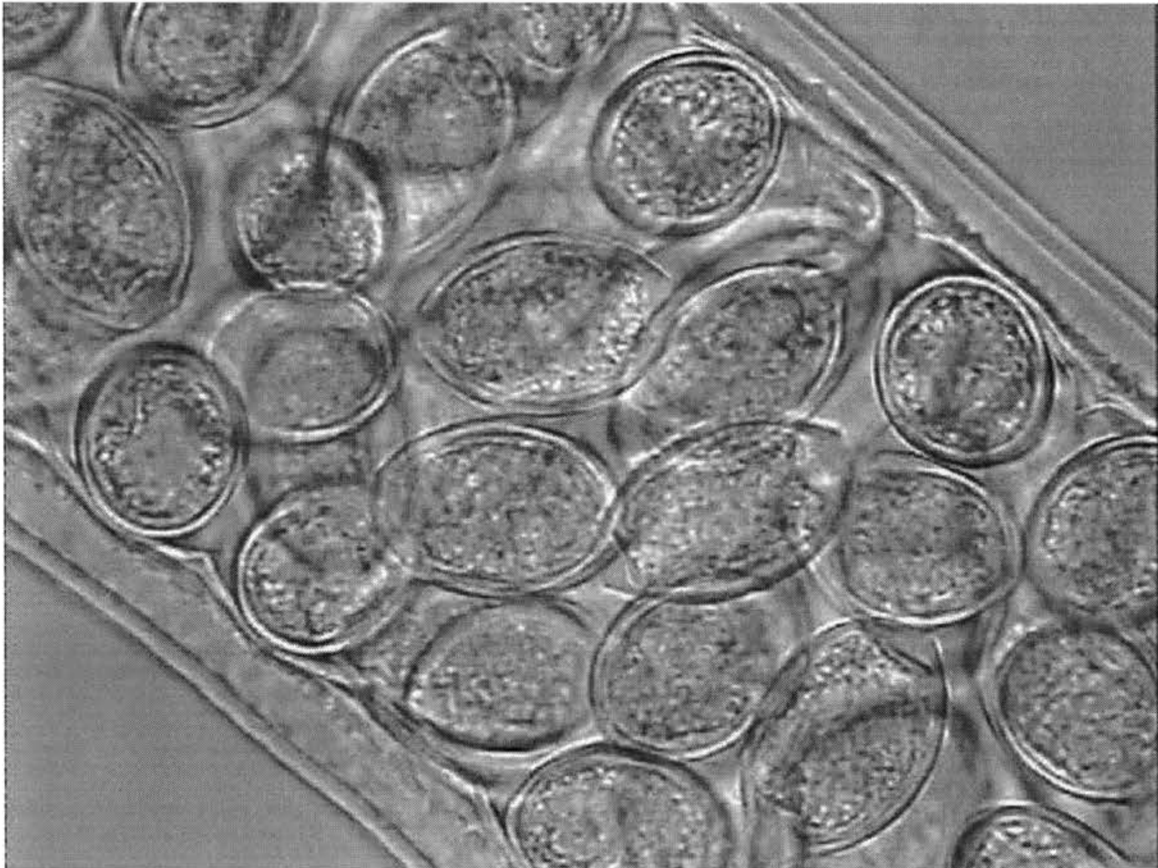
The vulva of *C. boehmi* is located near the junction of the esophagus and the intestine.



______________ = 32 μ

Figure XVIII: *Capillaria boehmi* Eggs Passing to Exterior Through Vulva of Female

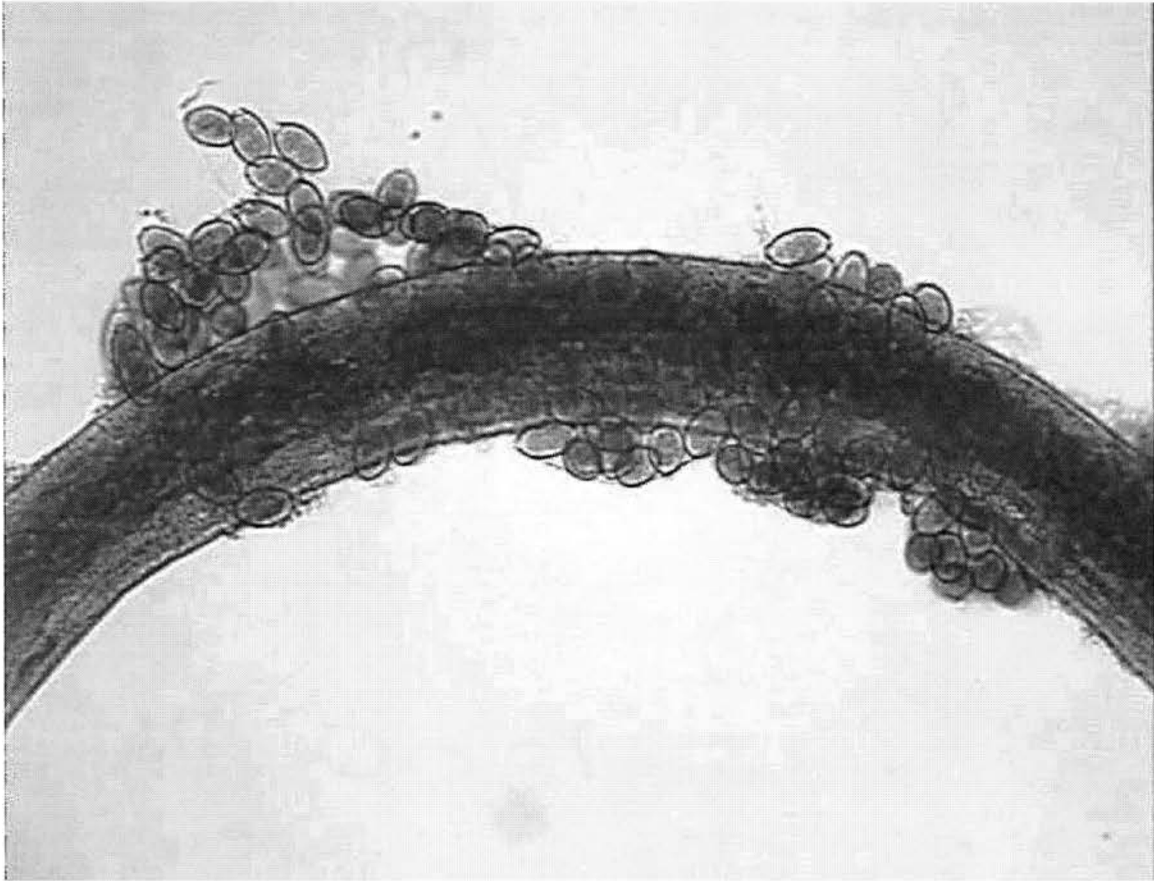
In the mature female *C. boehmi*, the is an almost continuous, though gradual, exit of eggs from the vagina through the vulva.



______________ = 37 μ

Figure XIII: Eggs in Uterus of Female *C. boehmi*

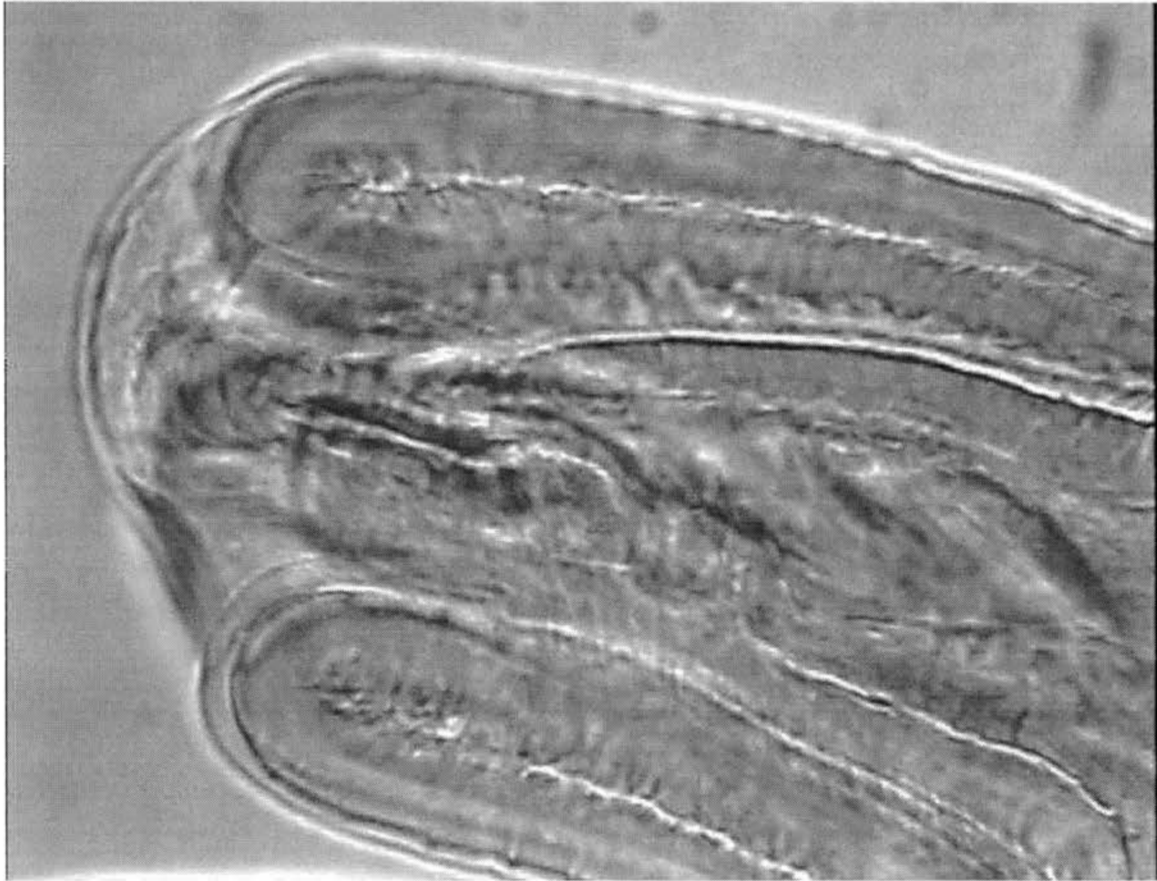
The body of the mature, gravid female *C. boehmi* is so packed with eggs in the uterus that there appears to be little room even for the passage of the intestine.



________________ = 230 μ

Figure XIV: Eggs Adhering to Cuticular Covering of Adult *C. boehmi*

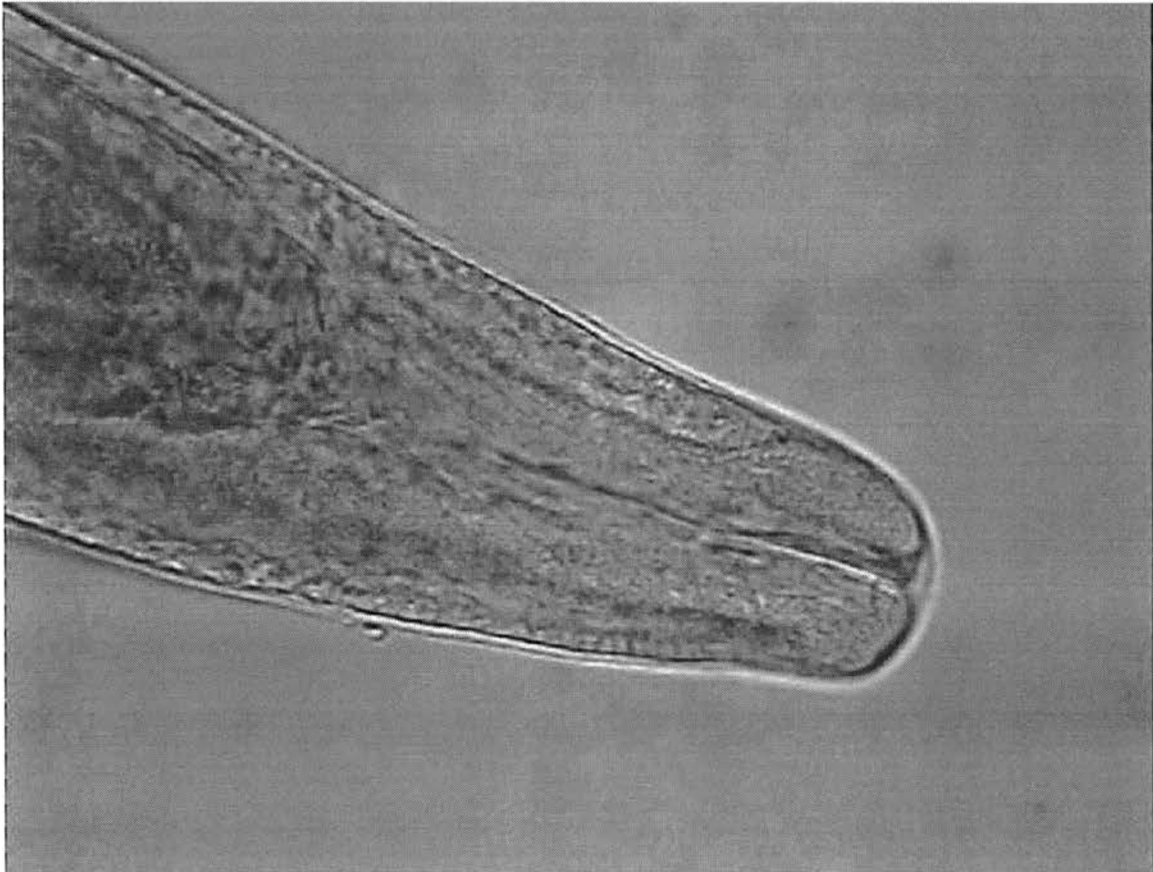
Masses of eggs mixed in mucus were often found adhering to the cuticular surfaces of both male and female nematodes.



——————— = 25 μ

Figure XV: Caudal End of Male *C. boehmi*

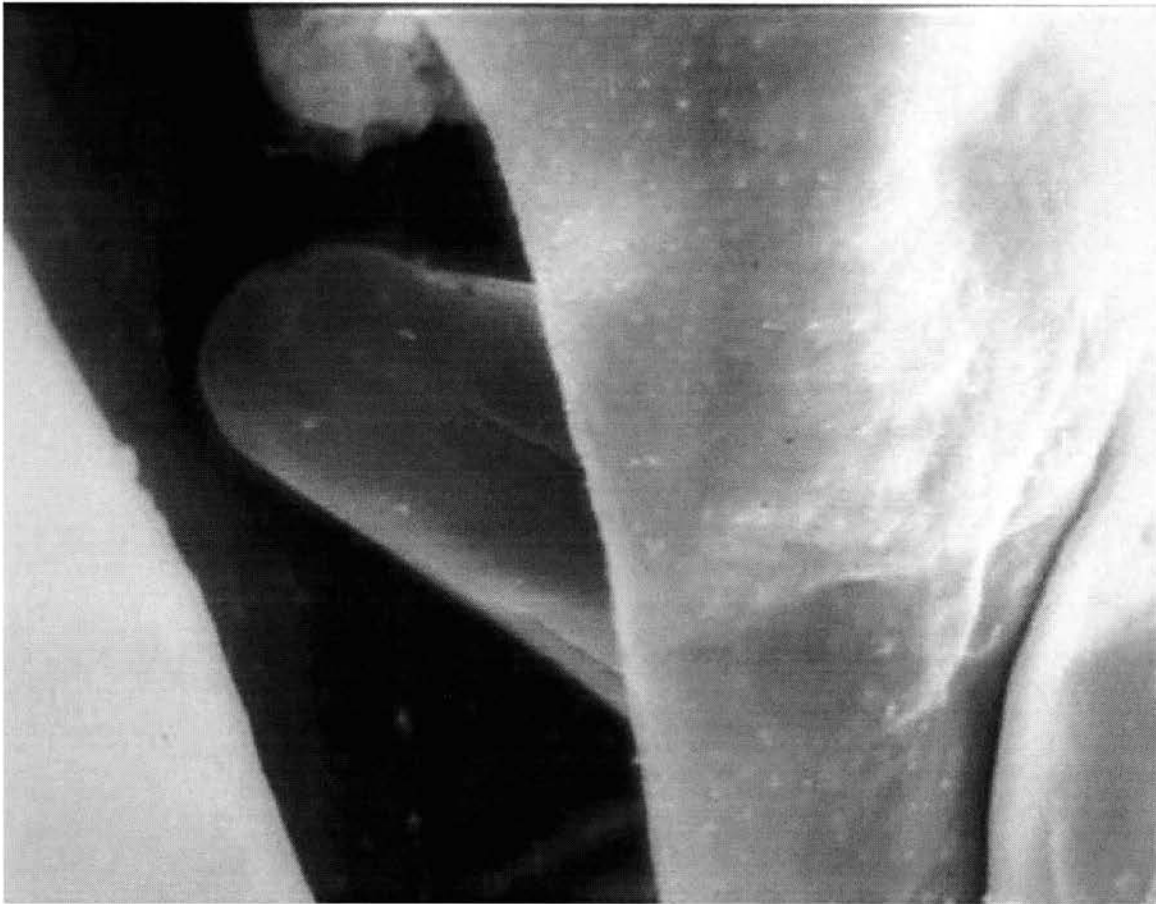
Two fleshy lobes and a membranous flap make up the bursa-like structures located on the caudal end of the male *C. boehmi*.



——————— = 50 μ

Figure XVI: Caudal End of Female *C. boehmi*

The intestine of *C. boehmi* empties through a terminal anus.



——————— = 50 μ

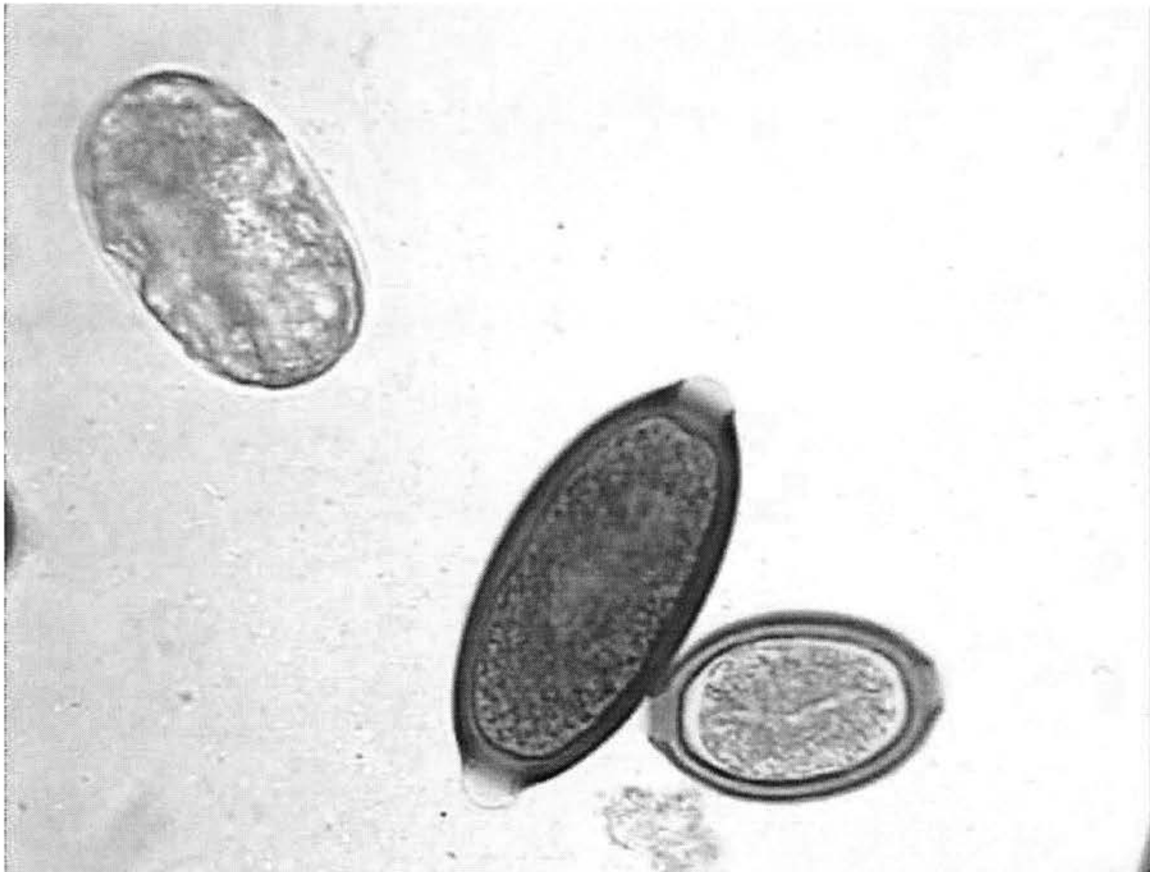
Figure XVII: Electron Photomicrograph of Adult Female *C. boehmi*

Electron photomicrograph of the posterior end and a portion of the body surface of an adult female *C. boehmi* shortly after removal from sinus passages of infected greyhound.

Capillaria boehmi Eggs

Eggs were collected at the time of the dissection of the nasal passages and sinus areas so that developmental stages could be compared with the eggs collected from the feces. The eggs were in various stages of development as shown in Table IX, page 90. These eggs, as well as eggs from the feces were measured. Measurements of *C. boehmi* eggs were 30 - 35 μ in width by 53 to 64 μ in length. These measurements were shorter in length and greater in width than those reported by Supperer (1953). The result was that the eggs found in this study had a greater width to length ratio which resulted in a more rounded appearance than the original description.

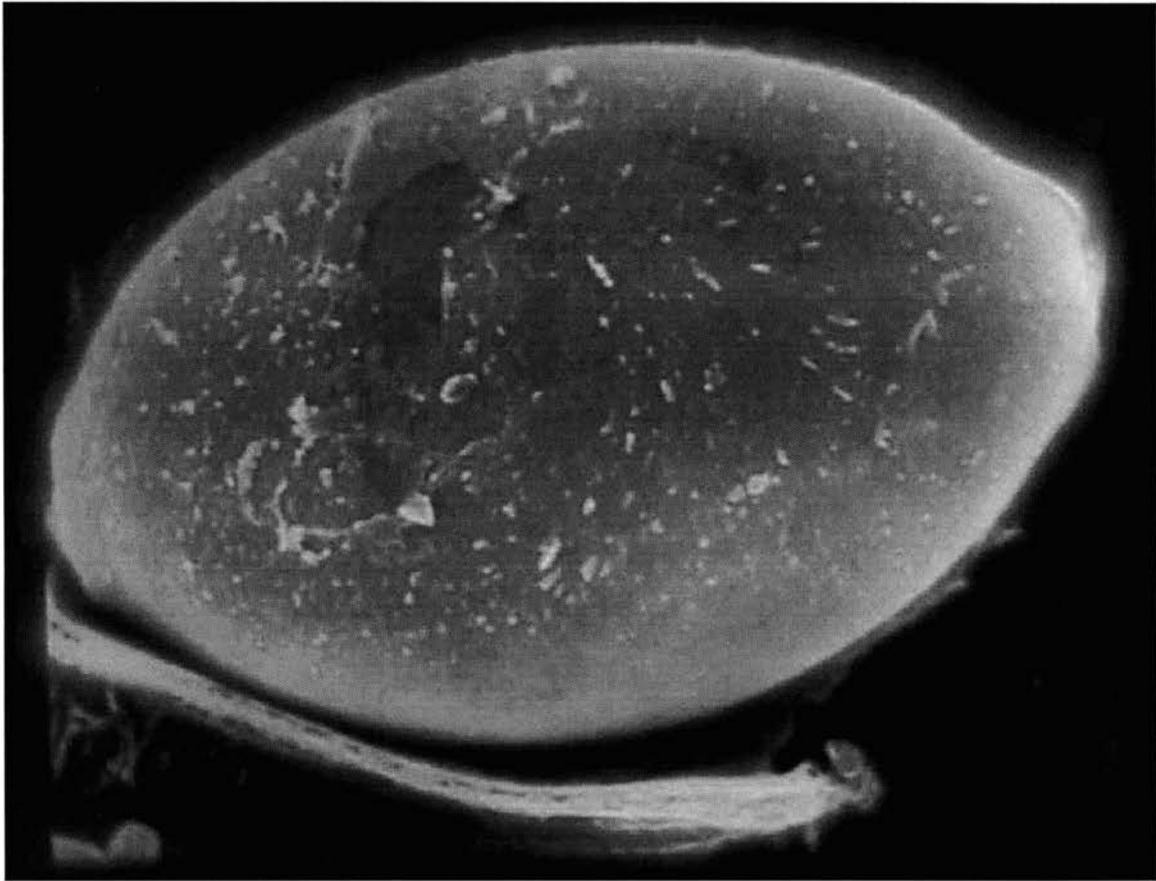
Eggs of the three nematode species with which the dog was infected were collected from the feces and are shown for comparison in Figure XVIII, page 62, An electron photomicrograph of one of the eggs removed from the sinus passages at the time of necropsy is shown in Figure XIX, page 63. Variations in appearance of the eggs occurred in the opposition of the bi-polar plugs, in the length-to-width ratio, in the orientation of the egg, and one egg with three plugs rather than two as shown in Figures XX - XXIII, pages 64 - 67.



______________ = 34 μ

Figure XVIII: Eggs of Three Species of Nematodes Recovered from Greyhound Feces

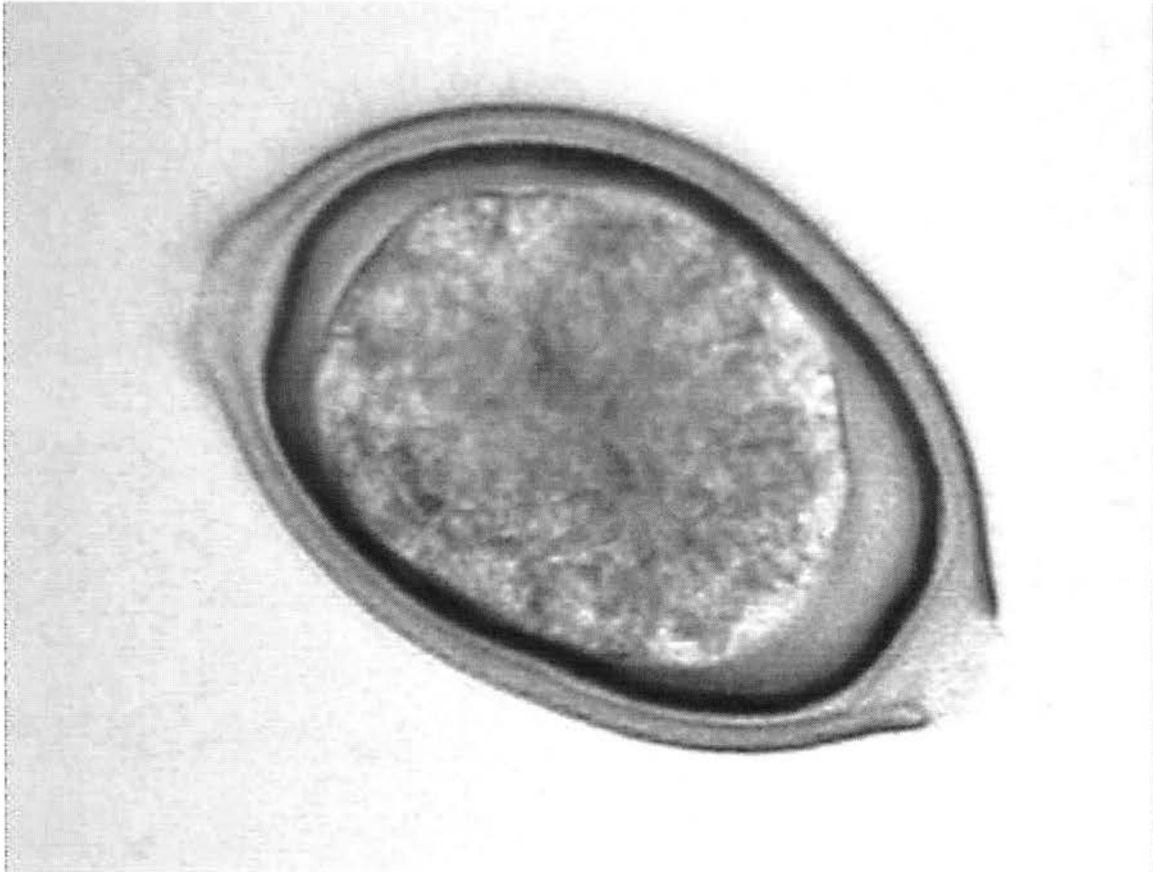
The three nematode eggs, *Trichuris* sp. (center), *Ancylostoma* sp. (upper left) and *C. boehmi*. (Lower right), were recovered from one greyhound fecal sample using $NaNO_3$ as a flotation medium.



______________ = 10 μ

Figure XIX: Electron Photomicrograph of *C. boehmi* Egg

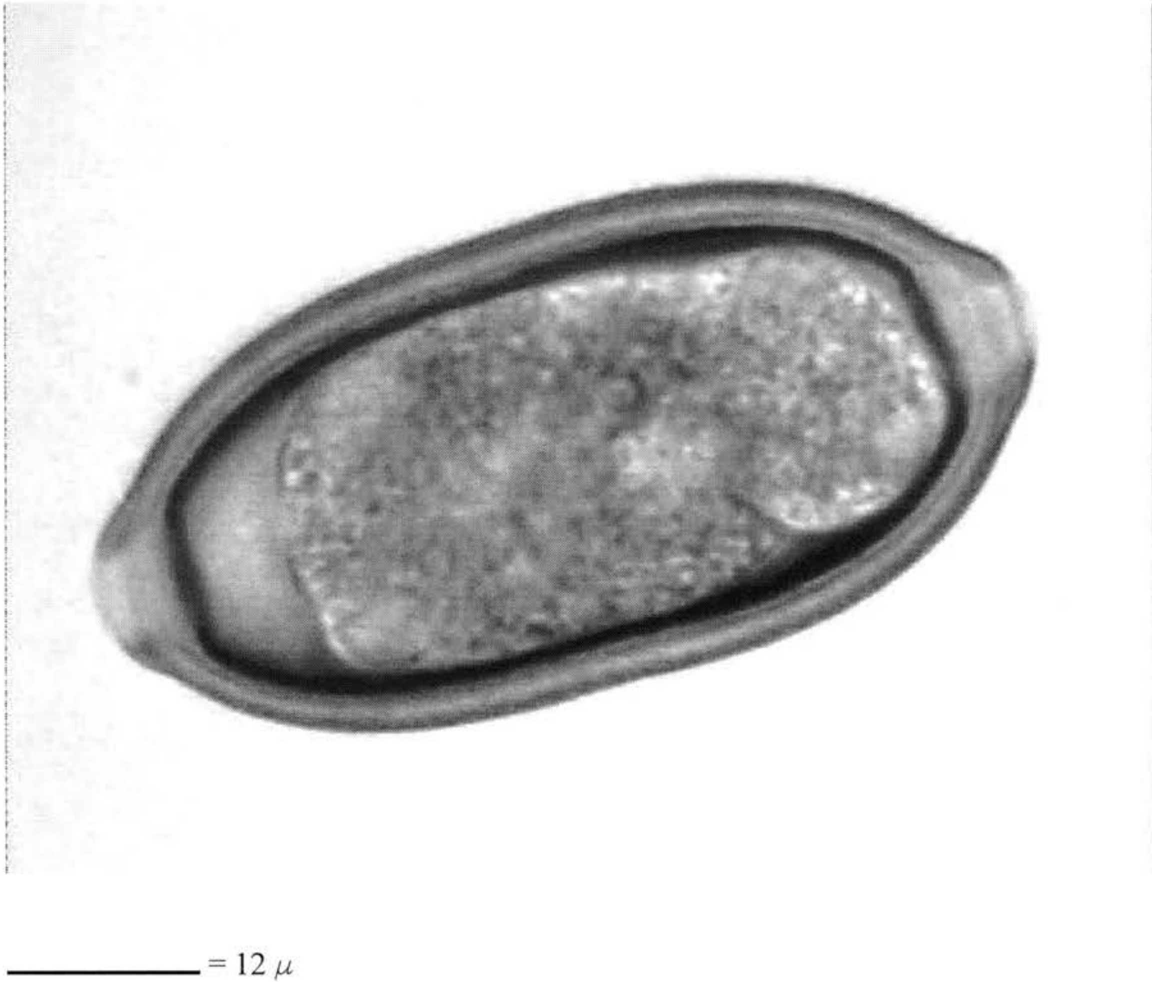
Electron photomicrograph of *C. boehmi* egg recovered from nasal washings from an infected greyhound. Note delicate pitting on surface where the film of mucus is peeled away.



______________ = 12 μ

Figure XX: Variation of *C. boehmi* Egg Showing Asymmetrical Arrangement of Bi-polar Plugs

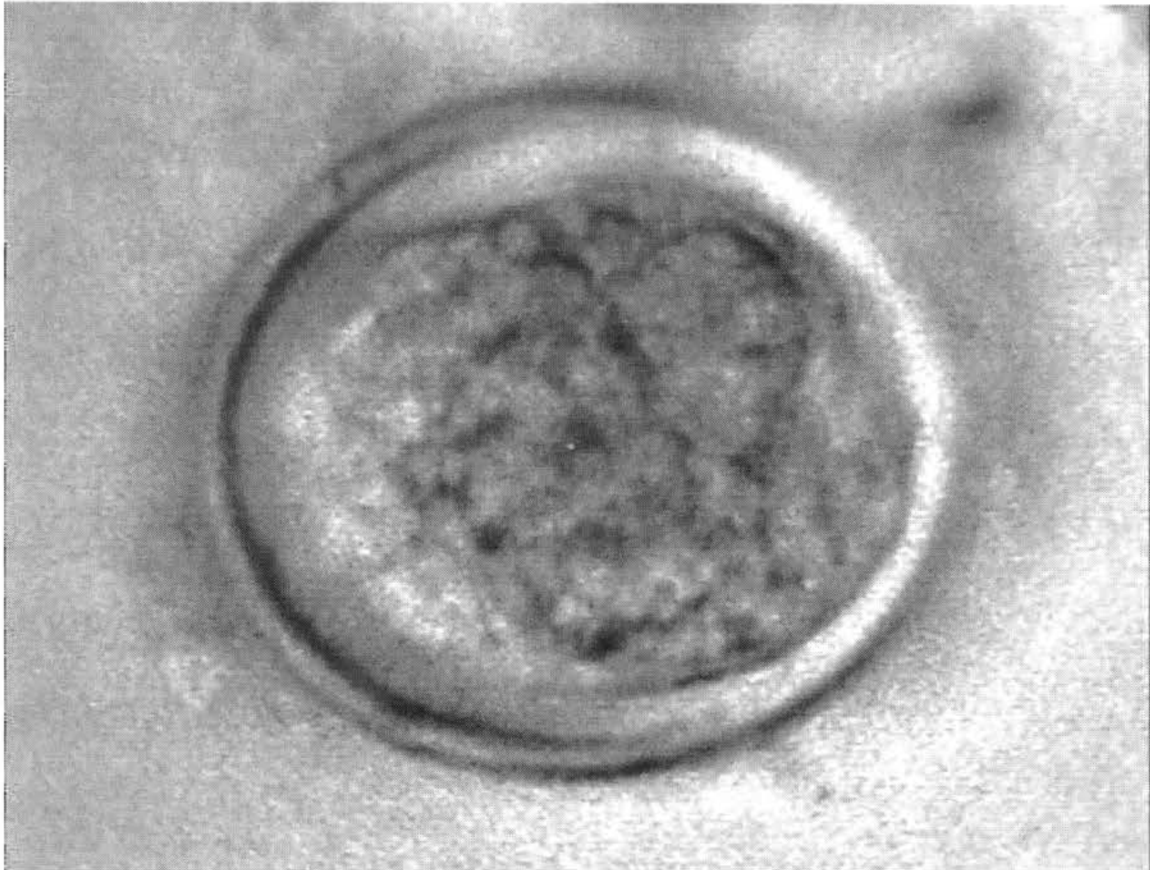
The bi-polar plugs on this egg were quite asymmetrical, thereby producing an appearance of an egg which had one very flat side and one extremely rounded side.



——————— = 12 μ

Figure XXI: Variation of *C. boehmi* with Greater than Normal Length-to-width Ratio

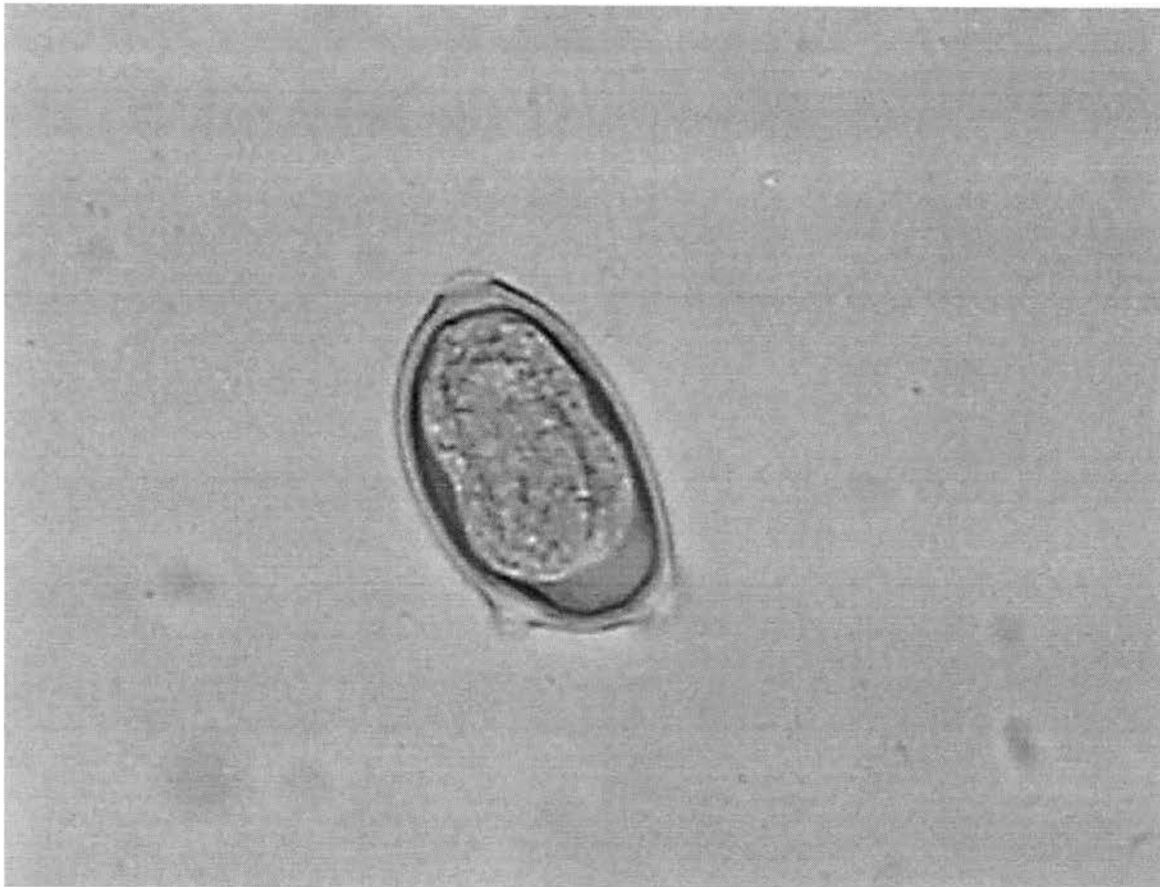
In addition to the greater than normal length to width ratio, the shell shows irregularity in curve and greater than typical thickness.



———————— = 9 μ

Figure XXII: *Capillaria boehmi* Egg Viewed from Polar Plug End

This was a characteristically shaped egg which was trapped in an on-end position under the coverslip when the fecal flotation was made, creating the atypical appearance.



_____________ = 27 μ

Figure XXIII: *Capillaria boehmi* Egg with Three Plugs

This *C. boehmi* egg had three plugs instead of the usual two.

Examination of *C. boehmi* DNA

Morphology has been and will, in all likelihood, continue to be the backbone of nematode taxonomy. It is fundamental to the higher level classification of nematodes and in many cases affords a fast and accurate diagnosis to species. However, the identification of some specific and sub-specific groups may require the application of other taxonomic techniques. Other methods available to date include host range testing, protein electrophoresis, immunological techniques and most recently, DNA sequence analysis. Since, with DNA sequencing, the genotype of the nematode is examined directly, problems associated with phenotypic variations are avoided (Curran, 1991).

Prior to the use of DNA sequencing, taxonomic organization on the species level has been based on criteria as diverse as life cycles and locations within the host. Almost invariably, each time a decision was made regarding taxonomy, it was followed closely by a list of exceptions to the rule, or challenged as lacking validity.

DNA sequencing and comparison would appear to be the 'end all' answer for taxonomic separation. However, the electron microscope was hailed as such, as has been each new technique prior to that. Although still in its youth as a procedure, DNA sequencing has become widely accepted and has contributed vast amounts of data which may be used for taxonomic pursuits. The use of sequencing holds great promise for providing a definitive resolution to the puzzle. With the advent of the polymerase chain reaction (PCR), the usability of DNA sequencing increased many-fold.

Materials and Methods

Extraction and Isolation of Nematode DNA

DNA isolation and purification methods were designed following the description by Gasser et al. (1993) for sequencing of DNA from single worms and eggs of parasitic helminths. *Capillaria boehmi* nematodes, preserved in 70% ETOH and fresh specimens in 0.85% saline, were chosen.

Nematodes were suspended in 250 μl of premixed 20 mM Tris-HCl (pH 8.0), 100 mM EDTA, 1% sodium dodecyl sulfate (SDS) to which was added 500 ug/ml Proteinase K. The mixture was homogenized with a Microson (T.M) ultrasonic cell disrupter for one minute, and incubated for ten minutes at 37°C in a water bath. The suspension was centrifuged at 10,000 g for three minutes. Supernatant was then transferred to a fresh tube and extracted once with phenol/chloroform/isoamyl alcohol (25/24/1). The aqueous phase was then precipitated with 2X volume absolute ethanol and immediately centrifuged for two minutes at 10,000 g. The DNA was suspended in 110 μl H_2O. The resulting product was frozen, to remain until the primers to be used could be obtained. The procedure was repeated for the other two nematodes.

Amplification of Nematode DNA

The oligonucleotide primers used for the worms for the polymerase chain reaction (PCR) were chosen based on the publication by Liu et al.(1996). The primers were designed based on invariant regions of published nematode 5S rRNA sequences.

The primers were 5Ss.F (5'-GCGAATTCTTGGATCGGAGACGGCCTG-3') and 5Ss.R (5'-GCTCTAGACGAGATGTCGTGCTTTCAACG-3').

Procedure for amplification of the DNA was done following guidelines set forth in descriptions published by Gasser et al. (1993) with only slight modifications. Amplification of the sequences designated by the previously described primers was done using PCR. PCR mix (final concentration 10 mM Tris - HCl (pH 8.8) / 50 mM KCl / 2.0 mM $MgCl_2$ / 0.1% Triton X-100 / 200 μM each of dATP, dCTP, dGTP, dTTP, 1 μM of each primer and 1 unit Taq DNA polymerase, (Promega).

Worm DNA in H_2O was added to 45 μl PCR mix to bring the total to 100 μl. Four drops from an 18 gauge needle of mineral oil was added to the top of mixture to retard evaporation. The PCR tube was preheated to 94°C for 2 minutes then subjected to PCR (DNA Thermocycler, Perkin Elmer Cetus) 94°C for one minute (denaturation); 55°C for one minute (annealing); 72°C for one minute (extension) for 37 cycles. On the final cycle the denaturation time was increased to two minutes and the extension time was increased to five minutes.

Each of the three 15 μl aliquots of the PCR amplified fragment products was mixed with 5 μl of loading buffer (50% glycerol, to keep the sample heavy enough to stay in the well / 10 mM EDTA, to stop further DNA reactions / 0.025% Bromphenol blue, to dye the sample and make it visible as it proceeds across the gel) then placed in a well on the 1.5% agarose gel in TAE buffer (40 mM Tris-Acetate and 2 mM EDTA). Following migration of the DNA, the gel was stained in 0.5 mg/ml Ethidium bromide. The image was captured on Bio-Rad Gel Doc system using Multi-Analyst software

program.

Results

Extraction of the *C. boehmi* DNA according to the methods described yielded a DNA product. The amplification of this product and the following migration and staining of it in the agarose gel allowed the DNA fragment to be visualized with the Bio-Rad Gel Doc system. The product was approximately 150 base pairs and is shown in Figure XXIV, page 72. This product, however, was not stable and degraded before the resultant band of DNA could be purified and submitted for sequencing. Repeated efforts to reproduce the band from DNA isolated from the worm failed.

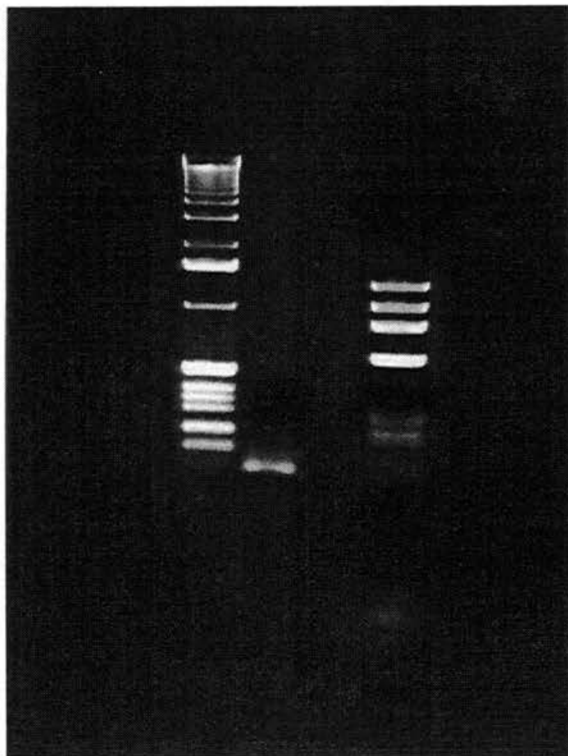


Figure XXIV: Migration of *C. boehmi* DNA on Agarose Gel

The center represents the band of *C. boehmi* DNA on the gel representing approximately 150 base pairs.

Discussion

Morphological characteristics of *C. boehmi* were photographed soon after removal of the nematodes from the dogs' nasal passages and sinuses. The lactophenol in which the nematodes were submersed on the microscope slide facilitated visibility of internal structures. One of the disadvantages of photography in comparison to pen-and-ink drawings of the worms is that only structures at one level are shown due to the limited focal range of the camera.

Conclusion

The photomicrographs contained herein give excellent representation of *C. boehmi* morphological structures not available until now, in particular those of the caudal ends of both male and female worms. The electron photomicrographs show specifics of the *C. boehmi* adult nematode cuticle and of the pitting in the egg shell at a levels not previously seen. This collection of pictures should prove invaluable for species distinction and differentiation in later studies.

No studies of DNA investigation of *C. boehmi* had been presented until now. Further work with DNA isolation, amplification and sequencing is needed. It was, however, demonstrated that the use of a previously described primer for Trichuroidea nematodes would yield a DNA product from *C. boehmi*.

CHAPTER V

BIOLOGICAL STUDIES OF *C. BOEHMI*

Long Term Observation of *C. boehmi* Infected Dogs

Study 1: Four *C. boehmi* Positive Greyhounds

Materials and Methods

Greyhounds. Four greyhounds, two males and two females, were selected from dogs donated to be used for research or teaching. The dogs had been obtained from a Kansas racing/breeding greyhound kennel known to have dogs positive for *C. boehmi*. These dogs had been removed from the racing and breeding programs for failure to perform at required levels. The four greyhounds, obtained in March, 1991, were kept in a home-based kennel facility for observation for six years. They were all positive for capillarid eggs as ascertained by fecal examinations. Recovery of worms six years later at necropsies verified presence of *C. boehmi* in the nasal passages and sinuses.

The number of dogs selected was limited to four due to the amount of kennel space available, costs associated with upkeep of the large dogs and labor and time required to maintain the them.

Criteria used in the selection of the four dogs included:

1. The dogs were infected with *Capillaria* spp. at the time of their induction into the program.

2. The dogs' overall health status and condition were good, and they were free of

limiting injuries.

3. The dogs were young enough that aging would not limit their use in the program.

4. The dogs' dispositions were such that they could be handled with ease.

Photographs of the greyhounds after they had been maintained for six years following their acquisition from the breeding/racing kennel, are included in the Results section of this chapter, Figures XXV - XXVIII, pages 82 - 85. A description of these dogs is included in Table VI, page 76.

TABLE VI

GREYHOUNDS WHICH WERE POSITIVE FOR
C. BOEHMI AND MAINTAINED FOR SIX YEARS

Dog Number Weight	Sex	Date Whelped	Color
Greyhound 1 70 lbs.	Male	June, 1990	Brindle
Greyhound 2 73 lbs.	Male	July, 1989	Brindle
Greyhound 3 67 lbs.	Female	July, 1990	Brindle
Greyhound 4 64 lbs.	Female	Aug., 1990	Fawn

Kennel Facilities. The kennel in which the dogs were kept for the six years they were maintained for study had three indoor/outdoor adjacent kennel runs. During the first five years the two females were together in the center run, with one male in each of two side runs. During the last year of maintenance, following the removal of one male from the program, each dog had a separate kennel run. The runs were separated by six foot high chain-link fencing. The floor surface was concrete with a continuous slope to the gate-end of each run for drainage. Runs were roofed with corrugated metal. They were attached to a small building which had separate wooden housing compartments inside for each run.

Care and Feeding. Bedding in winter was prairie hay. Cedar shavings were used for bedding in summer. Plastic five-gallon buckets were in each run for water. Feed was Hill's Science Diet Canine Growth formula fed dry.

Dogs were vaccinated yearly against distemper, hepatitis, leptospirosis, parainfluenza, parvo and rabies.

Fecal material was picked up daily, runs were washed down 2 - 3 times weekly and rinsed weekly with Roccal disinfectant diluted 1:200.

Fecal Examinations. Coprological examinations were conducted twice monthly on the feces of the four dogs for the duration of this project. The method used to determine if there were capillarid eggs present in the feces was flotation with saturated $NaNO_3$. A saturated solution of $NaNO_3$, was prepared by adding 850 grams $NaNO_3$ salt to one liter of water which resulted in a specific gravity of 1.4. Two grams of feces were

mixed with 15 mls flotation solution. The solution was then filtered through a coarse strainer to removed large particles and undigested material, and poured into a shell vial until a convex meniscus was formed. A glass coverslip was placed on top of the container to provide a surface to which the eggs adhered. After 7 - 8 minutes, the coverslip was removed and placed on a glass slide for microscopic examination at 100X magnification.

To determine eggs per grams of feces, the Modified Wisconsin egg counting technique was used.

Modified Wisconsin Procedure for Egg Counts. The Modified Wisconsin Procedure of egg counts, based on the procedure described by Benbrook and Sloss (1948), is one method used where it is desirable to count the number of eggs per gram of feces. The sugar solution used in this procedure is prepared by adding 454 gms sugar to 355 mls tap water with 6.7 mls liquified phenol crystals added as a preservative. The procedure is as follows:

1) Place two grams fecal sample into a small beaker.

2) Add 60 mls of tap water to sample.

3) Mix well and allow to stand five minutes to facilitate mixing.

4) Pour the mixture through an ordinary tea strainer into a second beaker, stirring the material in the strainer while pouring, until most of liquid has passed through the strainer. 5) Add another 60 mls water to original beaker and pour through sieve into second beaker.

6) Stir solution in second beaker and immediately pour into two conical centrifuge tubes,

filling them to within 1/4 inch of the tops.

7) Centrifuge tubes at 1500 rpm for five minutes.

8) Decant and discard supernatant.

9) Fill centrifuge tubes to within 1/4 inch of top with sugar solution. .

10) Stir solution with applicator stick to resuspend sediment.

11) Add sugar solution with pipette to bring solution to level with top of each centrifuge tube.

12) Place #2 coverslip on top of each tube.

13) Centrifuge both tubes at 1500 rpm for ten minutes.

14) Remove coverslips, being careful to retain drop of fluid adhering to bottom side, and place on glass microscope slide.

15) Count eggs under each coverslip using 100X power.

16) Total eggs counted times four equals number of eggs per two grams of feces.

Necropsy. Following euthanasia, each dog's entire nasal, tracheal and bronchial areas were checked for the presence of *C. boehmi*. The trachea and lungs were opened and inspected with the use of a dissecting microscope. Tissue from these areas was then carefully flushed into a collection dish and the liquid examined for the presence of worms which might have been dislodged.

For examination of the nasal passages, the entire head was split down the midline. The two halves were examined with use of a dissecting microscope. Nasal and sinus tissues were removed and teased apart to reveal all surfaces. Finally, remaining surfaces were flushed with water into a collection dish to salvage as many worms as

possible.

The worms which were removed from the nasal passages were collected in 0.85% saline then counted. Due to the delicate nature of the worms and the difficulty in finding and removing them from their location, some were broken. Therefore, when counts were made, consideration was given to the fact that there would be two halves to make up a whole worm for the count. The worms were preserved in 70% ETOH. Using a dissecting microscope, gravid female worms could be identified by the presence of eggs in the uterus. Males and non-gravid females were difficult to differentiate without higher magnification. Whole worms were removed from the alcohol, placed on a glass slide in a drop of lactophenol, and covered with a glass coverslip in order to view morphological structures and determine sex.

Results

Greyhound Physical Condition. The dogs stayed in good-to-excellent physical condition and appearance throughout the entire project. The one symptom observed which was likely related to the capillarid infection in the greyhounds was that they would often wheeze, especially when excited. The wheeze had a monophonetic tone and was associated with the indrawn breath, much as might be expected with a nasal or soft palate obstruction. No records of wheezing occurrences were kept but the frequency of the episodes were frequent enough to be considered possibly associated with the capillarid infection.

The following photographs of the four greyhounds, Figures XXV - XXVIII,

pages 82 - 85, are included to show the excellent apparent physical condition the dogs retained, even after having harbored the capillarid nasal parasite for at least six years. All photos were made in the summer of 1997.

Figure XXV: Greyhound #1, Adult Brindle Male Positive for *C. boehmi* and Maintained for Six Years

This brindle male greyhound was given the kennel name 'Scar', due to a very visible three-inch scar on his left shoulder received in an altercation with another dog during his race track days. Scar would wheeze when excited or exercising more than the other three greyhounds, but then he was more exuberant than the other three and as a result was often jumping with excitement at the least provocation.

Figure XXVI: Greyhound #2, Adult Brindle Male Positive for *C. boehmi* and Maintained for Six Years

This brindle male greyhound was given the kennel name No-Scar, to differentiate him from the other brindle male which was very similar in appearance but which had a large scar on his shoulder.

Figure XXVII: Greyhound #3, Adult Brindle Female Greyhound Positive for *C. boehmi* and Maintained for Six Years

Although housed in the same run with the fawn female which had the highest egg count of the four dogs, the egg count on this dog had little fluctuation and remained low throughout her stay.

Figure XXVIII: Greyhound #4, Fawn-colored Female Positive for *C. boehmi* and Maintained for Six Years

This female maintained the highest *C. boehmi* fecal egg count of the four greyhounds throughout her entire six year stay.

Coprological Findings. During the six years that the greyhounds were maintained, they remained positive for *C. boehmi* eggs. The fecal egg counts for the capillarid eggs varied slightly, but not with consistent fluctuations, nor were the feces at any time negative for eggs. Rather than having a cyclic variation of egg concentration within the feces, it appeared that the variations were due to dilution resulting from the amount of food consumed that day and the activity in the alimentary canal. Feces which were well-formed, even to the extent of being slightly dry, would contain far more eggs per gram of feces than those collected from a soft fecal sample without form. Typical results of fecal flotations for the four greyhounds are shown in Table VII, page 87, with complete findings listed in Appendix 1, page 157.

TABLE VII

TYPICAL FINDINGS OF *C. BOEHMI* EGGS IN FECAL FLOTATIONS OF FOUR GREYHOUNDS

Greyhound	*Capillaria boehmi* eggs/100X field
#1 (male)	2 - 4
#2 (male)	1 - 3
#3 (female)	0 - 1
#4 (female)	6 - 8

* Eggs were collected on a coverslip by flotation using a saturated $NaNO_3$ solution and viewed microscopically using 100X magnification.

<u>Correlation of *C. boehmi* Adult Numbers to Egg Numbers</u>. In the two dogs examined, the was no correlation found between numbers of worms present and numbers of egg found in the feces. At the time of euthanasia, a fecal sample was collected from each dog and the capillarid eggs-per-gram of feces were counted using the Modified Wisconsin egg counting technique, as shown in Table VIII, page 89. This allowed comparison between numbers of eggs recovered and eggs per gram of feces to determine if a correlation existed. Greyhound #2 had 138 adult worms and 574 eggs per gram of feces and Greyhound #4 had 68 adult worms and 1704 eggs per gram of feces.

<u>Developmental Stages of Eggs Recovered from the Nasal Passages</u>. Developmental stages of the eggs recovered from the nasal passages were recorded and compared with developmental stages of eggs recovered from feces at the time of euthanasia. Eggs recovered from the feces were farther along in development than those from the nasal passages. Developmental stages of eggs from the two locations are listed in Table IX, page 90.

TABLE VIII

ADULT *C. BOEHMI* RECOVERED FROM NASAL PASSAGES AND SINUSES OF GREYHOUNDS AND EGGS PER GRAM OF FECES AT TIME OF NECROPSY

Date	Dog	Worms Recovered	Eggs per Gram of Feces*
Aug., 1997	#2	138	574
Nov., 1997	#4	68	1704

*At time of euthanasia as counted using Modified Wisconsin egg counting technique

TABLE IX

DEVELOPMENTAL STAGES OF *C. BOEHMI* EGGS REMOVED FROM NASAL PASSAGES AND FROM FRESHLY PASSED FECES

Developmental Stage of Egg	Collected from Nasal Passages	Collected from Fresh Feces
Undifferentiated zygote completely filling interior area of egg	18%	6%
Multicellular embryo with space between the embryo and the egg shell	80%	90%
Vermiform larvae, not yet fully developed	1%	2%
Fully developed motile larvae	1%	2%

Discussion

The dogs remained positive for capillarid eggs throughout the six years they were maintained for this study. The kennel conditions in which they were kept were designed to approach as nearly as possible what one would expect in a commonly encountered kennel situation with good sanitation. It would appear that the infection was limited only by the life span of the dog. The life span of the individual worms is not known. If a life span of one year were assumed, then it would be necessary to add at least five newly-established worms each month to have a population of sixty after one year's time. Certainly a reinfection rate of five worms per month would not be many for a direct life cycle. But if the life cycle were indirect and required an intermediate host, in a well-monitored controlled kennel situation that number begins to sound excessive. Perhaps maximum numbers of worms were controlled by partial immunity, or by a crowding effect within the nasal passages and sinuses.

As stated by Christenson (1938), the epidemiological factors governing survival of infective stages of parasites of the respiratory system are cold, heat, ultra-violet radiation and desiccation. With these factors in mind, it is possible that, even with routine cleaning and careful management, the maintained dogs still could be continuously reinfected due to:

1. Oklahoma has only short periods of intense heat with about two weeks of temperatures exceeding 38°C which usually occur during the months of July and August, and intense cold with temperatures below -18°C, usually occurring during the months of January and February, which would be lethal to the survival of the infective

stages.

2. The outdoor kennel runs were covered by a roof to provide protection from the heat and ultra violet radiation of direct sunlight and from precipitation, therefore providing protection for the parasites and well as the dogs.

3. Even though the runs were cleaned regularly, there was always the chance eggs could have remained in a small crack or crevice until infective.

4. Also, a small percentage of the eggs may have been retained in the nasal passages where they may have larvated, hatched and reinfected the dog.

Although all of the preceding scenarios are possible for reinfection, under the conditions in which the dogs were maintained, the only two which appeared credible were:

1. Autoinfection from parasites never leaving the host.

2. Even very low reinfection rate could maintain a continuous infection if the worms are long-lived.

This led to the conclusion that a *C. boehmi* infection could remain with a dog for a period exceeding six years, due to reinfection, longevity of the worms or a combination.

Few of the worms collected could be identified as male. It was relatively easy to identify the sex of the gravid females by the presence of eggs. Worms other than gravid females were examined with the compound microscope to determine sex by finding the vagina and vulva at the base of the esophagus, even if the posterior of the worm was missing. One possible reason for finding more females than males might be that the

females were larger than the males and therefore be easier to find. Given the smaller size of the males and the fact that the nematode with which we were dealing was very delicate in nature, it is possible that more males were broken or damaged than females. The possibility exists that the males are shorter-lived than the females, and perhaps die after copulation, as with *Trichinella spiralis*, a closely related nematode included in the same superfamily as *C. boehmi* (Georgi and Georgi, 1990). Another possibility is that there are fewer males existing in an infection than females, as is the case with *T. spiralis* (Levine, 1968). That scenario would help explain the numerous nongravid females discovered.

Developmental stages of the eggs recovered from the nasal passages as listed previously in the Results section were noted. The eggs of *C. boehmi* were described in great detail by Campbell and Little (1991). Granted, these descriptions were for eggs in freshly passed feces, not those recovered directly from the nasal passages. However, their published description might lead the reader to infer that all *C. boehmi* eggs could be expected to be found in the condition of containing a multicellular embryo. This certainly was not the case for eggs removed directly from the nasal mucosa. This difference became important when it was considered that one of the main distinguishing characteristics as listed by Campbell and Little (1991) between the eggs of *C. boehmi* eggs and those of *C. aerophila* was the developmental stage. The photos in that article showed and the description stated a difference between the two species as *C. boehmi* having a multicellular embryo with a space remaining between the embryo and the shell, and *C. aerophila* having only a one or two cell embryo completely filling the interior of

the shell. This was the description of eggs found in fresh feces. It would appear from the present study that the slow passage of the eggs through the nasal areas and sinuses contributes to the varying stages of development. Therefore, developmental stage of the egg and a gap between embryo and shell, which is a direct result of the developmental stage, should not be used as the primary criterion to distinguish these two species.

The various developmental stages of the recovered eggs led to the consideration that the life cycle might include utilization of autoinfection. Possibly the eggs could develop to an infective stage while still in the host, either trapped in the nasal tissues or as they progressed through the alimentary tract. Given the location of these worms and eggs in an area inherently rich with a fresh and ample supply of oxygen, continuously moist and in a mucous coating rich in nutrients, it would be an easy step to consider that the eggs might be able to hatch *in situ* and the larvae remain and develop at or near the location of hatching. This reasoning might help explain the buildup of large numbers within a host.

Study 2: *C. boehmi*-Positive Blue-tick Hound

Materials and Methods

Blue-tick Hound. In the summer of 1980, a blue-tick hound was introduced into the kennels of Oklahoma State University, College of Veterinary Medicine, Department of Veterinary Parasitology, Microbiology and Public Health, Laboratory Animal Resources, to remain as a permanent resident. The dog was an adult, intact, female blue-tick hound weighing 75 pounds. She was housed in the Laboratory Animal

Resources kennel for a period of eight years and maintained until her death in January of 1988.

At the time of admission to the kennel, the hound's feces was positive for *Capillaria* spp. eggs (Mullins, 1997). The dog retained the infection throughout her stay, despite numerous anthelmintic treatments. At necropsy, the only capillarids recovered were from the nasal passages and sinuses.

Kennel Facilities. The kennels were indoor and had concrete floors with chain-link fencing. The animals and facilities were kept clean, washed down and disinfected on a daily basis. The dog was cared for by laboratory animal.

Fecals and Anthelmintics. Weekly fecal examinations were performed during the dog's residence. These were done using a saturated $NaNO_3$ flotation solution and a two-gram sample of fecal material. Fecal results were reported as number of eggs per 100X field (Jordan, 1992).

Anthelmintic treatment was administered by laboratory personnel (Jordan, 1977). Antiparasitic preparations which were used were used Spotton (Fenthion), DNP (Disophenol), Nemex (Pyrantel Pamoate), Strongid T (Pyrantel), Task (Dichlorvos), and Nemex 2 (Pyrantel Pamoate with Oxantel Pamoate). A complete listing of anthelmintics and dosages where available and dates administered are included in Appendix 4, page 166.

Necropsy. The dog was euthanatized in 1988. A complete necropsy was performed including examination for parasites. *Capillaria boehmi* adults were found in

the nasal passages and sinuses only. No parasites were found in the lungs and trachea.

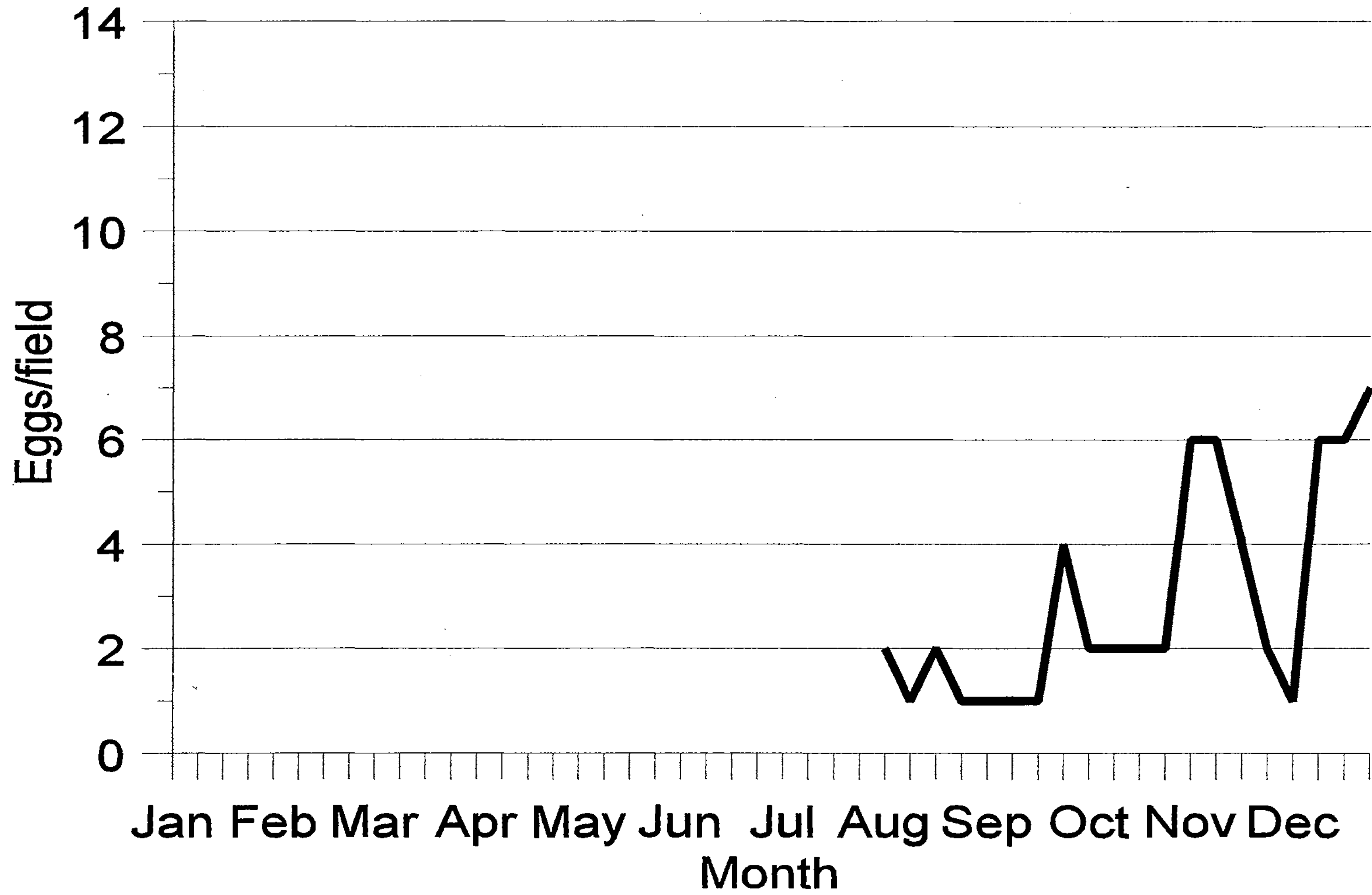
The dog was an adult, intact, female blue-tick hound weighing 75 pounds. She was housed in the Laboratory Animal Resources kennel for a period of eight years. The dog was an adult, intact, female blue-tick hound weighing 75 pounds. She was housed in the Laboratory Animal Resources kennel for a period of eight years.

Results

In the early days following the dog's entrance into the program, some records were incomplete. During the years 1980-84, findings of *C. boehmi* eggs were reported as positive or negative, with no indication as to concentration of eggs in the fecal sample. These reports have been presented, as they were originally recorded, in Appendix 4, page 166, along with a record of anthelmintics administered. Although a variety of anthelmintics were administered to the dog for control of *Ancylostoma* spp., the canine hookworm, the capillarid infection, as evidenced by the presence of eggs in the feces, was never eliminated.

The following Figures XXIX - XXXII, pages 97 - 100, graphically represent results of the fecal examinations of the blue-tick hound by year for the years 1984 through 1987.

Fig. XXIX: 1984 Capillarid Egg Findings from a Blue-tick Hound



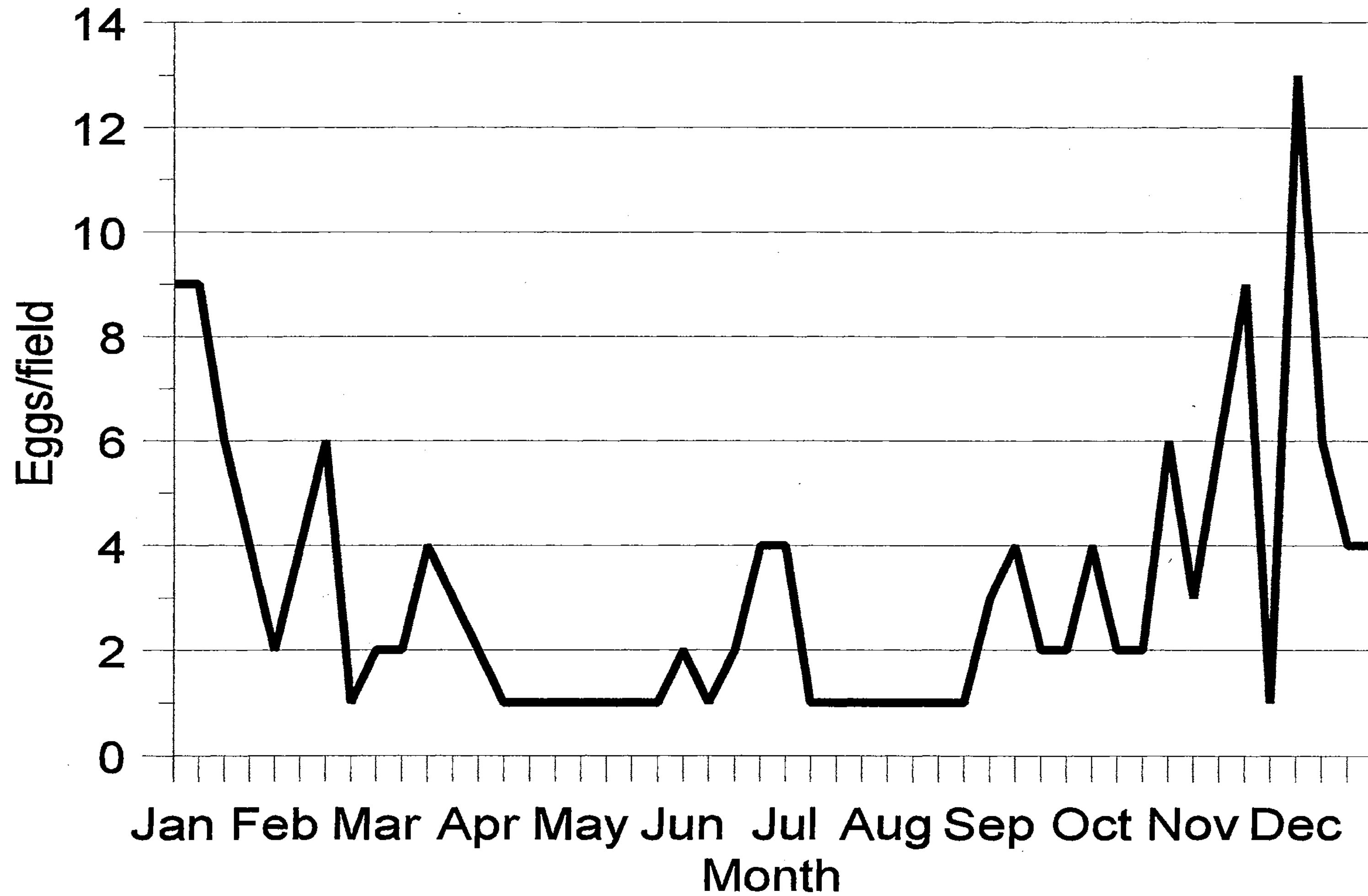
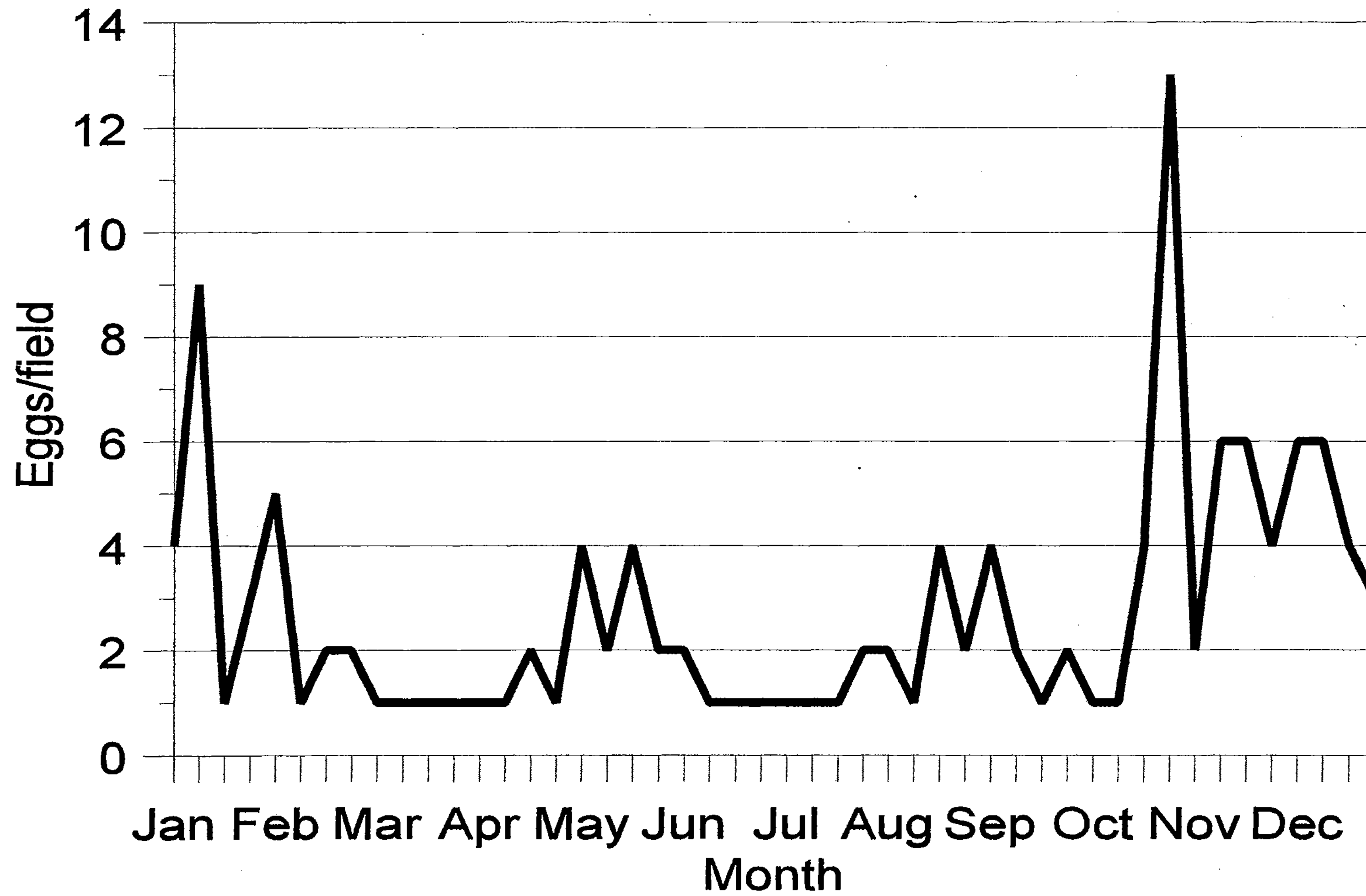


Fig. XXX: 1985 Capillarid Egg Findings from a Blue-tick Hound
Eggs/field
0
2
4
6
8
10
12
14
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month

Fig. XXXI: 1986 Capillarid Egg Findings from a Blue-tick Hound



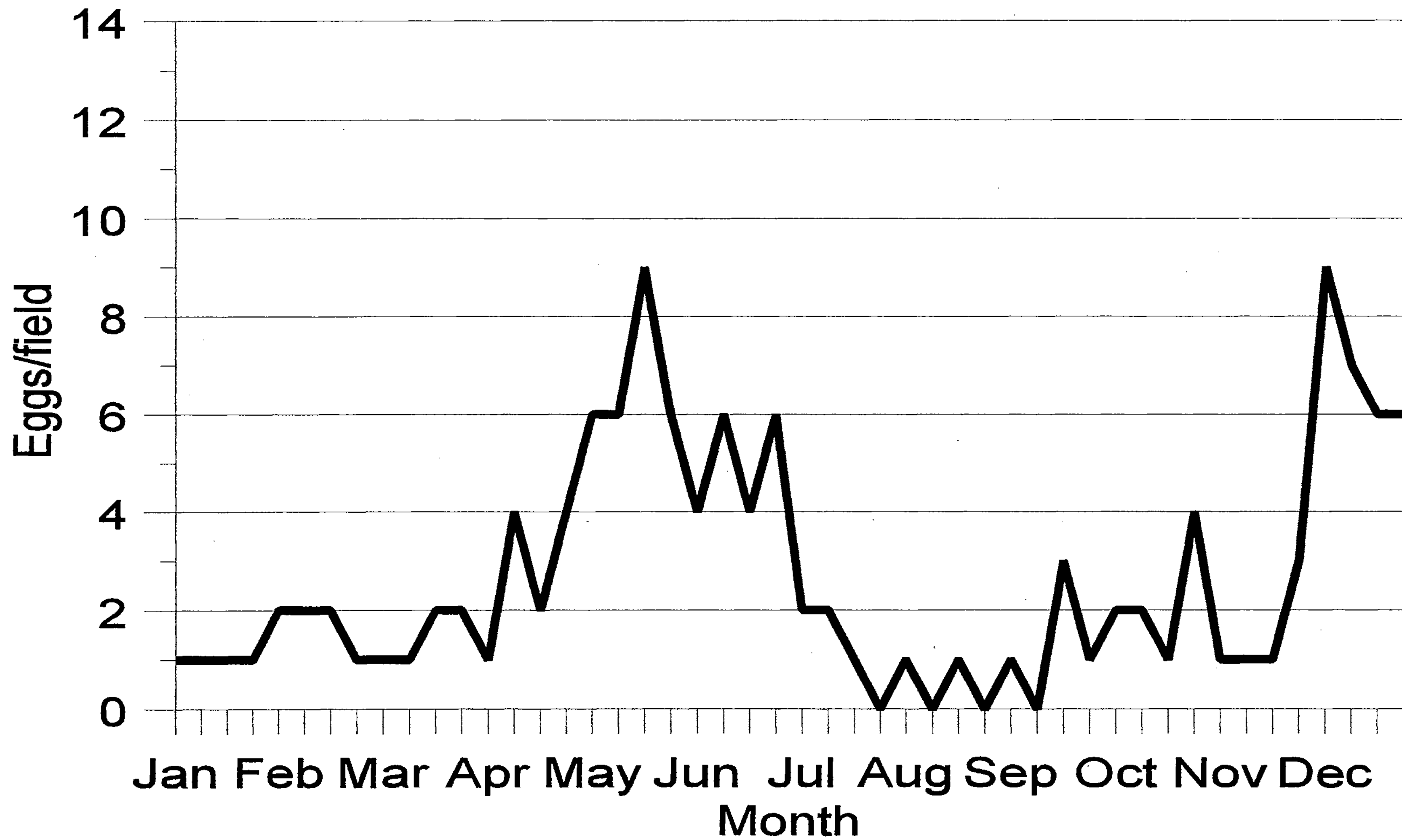


Fig. XXXII: 1987 Capillarid Egg Findings from a Blue-tick Hound

Discussion

It is noteworthy that the blue-tick hound was never free of the *C. boehmi* infection for the eight years of her stay, although maintained in a clean environment and given anthelmintics on a regular basis. The egg count varied from negative to as many as fourteen eggs per 100X field of magnification as shown on the graphs on the preceding four pages. During the time the dog was maintained, there were only four fecal examinations negative for capillarid eggs. Maximum time between positive readings was twenty-one days. This indicates that egg output at that time may have been quite low and therefore not detected during the fecal examination, but does not indicate that the infection was absent from the dog.

The study of egg-shedding patterns shown in the Figures XXIX - XXXII, pages 97 - 100, spanned a period of three-and-a-half years with weekly monitoring. The fluctuations peaked every five to seven months with distinct valleys separating the peaks. Schoning et al. (1993) reported cyclical egg shedding of *C. boehmi* occurring at five week intervals. For that study the egg numbers were monitored weekly over a period of twenty-four weeks. The findings in the current study differed from that report in regard to spacing of fluctuations. The fluctuations would certainly indicate cyclical egg production of *C. boehmi*. It would, however, be presumptuous to state that these observed fluctuations were unequivocal proof of cyclical egg production.

The changes in concentrations of eggs in the feces may have been related to a variety of reasons which include the amount of food consumed as a dilution factor as well as the speed at which it progressed through the alimentary canal. Fecal collection times

were not recorded and may have varied, giving a more concentrated sample at one time of day than at another. The dog was allowed into an outside run for exercise in good weather, which may have affected the speed with which ingested material progressed through the alimentary canal. Under the best of controlled circumstances, often fecal egg findings will vary somewhat even from the same dog on the same day and within the same fecal sample regardless of egg production by the nematode in question.

Another possible explanation for the fluctuations existed in that the dog involved in this study was an intact female which experienced estrous cycles. These were not recorded as part of her residence history, but probably occurred approximately every six months. The peaks and valleys of egg recovery also occurred approximately every six months.

Observations of additional animals would need to be conducted under strict physical and environmental controls before definite conclusions could be drawn showing absolute fluctuations. These additional controls would need to include amount and type of food consumed, amount of exercise and thorough mixing of the complete fecal production over a 24 hour period before a sample is taken. This could be expected to reduce but not eliminate fluctuations in egg recovery not related to fluctuations in egg production by the worm. Even so, determination of eggs per gram of feces or any of the similar egg counting techniques are questionable in their value to interpret accurately number of nematodes present or even numbers of eggs produced.

Conclusion: Study 1 and 2

The fact that the blue-tick hound and all four greyhounds remained positive for C. boehmi eggs in their feces during the years they were kept for observation and study led to the conclusion that the infection would remain with a dog throughout its life, once it became infected..

Although infected with *C. boehmi* for most of their adult life, the five dogs remained in apparent excellent physical condition, virtually free of clinical signs. From this it can be concluded that observable physical signs can not be relied upon to give an indication of the presence of the infection in a dog.

Cyclical egg-recovery patterns were seen in coprological examinations of the blue-tick hound, but not in the greyhounds. The only conclusion that can be reached from these observations is that there may be cyclical *C. boehmi* egg recovery patterns in some animals but not in others. Although not necessarily cyclical, there was a variation in numbers of eggs found. This could easily result in failing to diagnose an infection of *C. boehmi* in a dog if the sample was taken at a time of minimum egg counts. If a *C. boehmi* infection is suspected, several fecal examinations should be made over a period of days to minimize the chances of missing the eggs.

Transmission of *C. boehmi* Infection

Published reports on the *C. boehmi* life cycle, including method of transmit between hosts, is poorly understood, mostly unknown and even speculative, often based on extrapolation from other species.

As with other parasites, control and prevention of this nematode in a host population is best accomplished by finding a weak link in the life cycle chain and attacking at that point. Although anthelmintic treatment may, for many parasites, give temporary relief, true control cannot be expected to be achieved without a full understanding of the complete life cycle and instigation of epidemiological and environmental controls associated with that life cycle.

An overview of the literature available concerning this parasite suggested that the life cycle was, in all probability, direct. The approach chosen was to determine if a direct life cycle could be demonstrated under experimental condition. If accomplished, the result would substantiate the probability that such could occur in nature.

Most articles published concerning transmittal of the nematode in question either stated or implied that a larvated egg was ingested, hatched in the small intestine, then the larva somehow migrated to the airway passages.

Numerous attempts at transmitting the infection through oral administration of the larvated eggs were made. When no positive results occurred with this method, attempts at intra-nasal infections were made.

Materials and Methods

Collection of Eggs

Eggs to be larvated were collected and treated in two ways: 1. The eggs were left in the fecal material, thereby providing a natural surrounding for the larvation process, and 2. The eggs were removed from the feces in an effort to reduce the amount of

bacterial growth and to increase the concentration of eggs.

Eggs Remaining in Feces. Freshly passed, formed fecal samples were collected from the greyhounds. A two gram portion of each sample was checked for egg concentration by flotation using a saturated sodium nitrate solution. The feces containing the highest concentration of eggs was chosen for use.

Eggs Separated from Feces. Fresh, formed, fecal samples were collected from the greyhounds. These were placed in a gallon of tap water overnight to soften. The mixture was stirred, then rinsed through a #30-mesh screen into a five gallon bucket using a mild pressure with a hose sprayer to reduce large particles. The three-to-four gallons of fecal/water mixture was then allowed to settle. After twelve hours, the supernate was decanted and the bucket refilled with fresh water. This procedure was repeated every 12 hours, five to six times until the liquid became quite clear. The final sediment was removed and centrifuged 1500 rpms for five minutes. The supernate was discarded and the sediment was resuspended in a Wisconsin sugar formulation for egg counts (Benbrook and Sloss, 1948) flotation solution and centrifuged, 1500 rpms for five minutes. Eggs were collected on coverslips and washed with distilled water into pertri dishes.

Larvation of Eggs

Various methods were used in an attempt to provide an environment for the eggs to larvate without being overwhelmed by bacterial and fungal growth. Feces from the

greyhounds contained not only *Capillaria* spp. eggs, but also *Ancylostoma* spp. and a few *Trichuris* sp. eggs, see Figure XVIII, page 62. However, it soon became apparent the two nematode contaminants were not a problem. If the feces or solution of water and eggs was allowed to at remain at or near room temperature two to three days, the *Ancylostoma* spp. eggs would hatch and the larvae would soon die and disintegrate, leaving only the *Capillaria* spp. eggs and a few *Trichuris* sp. Larvation of the *Trichuris* sp. progressed at a much slower rate that of the capillarids. Eggs or eggs-and-feces mixtures were placed in six-inch Pyrex glass petri dishes with loose fitting glass lids. Incubation of the eggs was attempted at room temperature of approximately 22 °C, and/or in an incubator at 35 °C, and /or in a refrigerator at 1 °C.

Distilled water was added as needed to compensate for evaporation. The mixtures were stirred three to four times a week to allow for even distribution of moisture and to help control the fungal growth by aeration and breaking up of the fungi. An incubation period of approximately 40 days was chosen after reviewing Christenson's (1938) life history and epidemiological studies on *C. aerophila*. Christenson noted that under normal summer conditions the eggs were embryonated in 35 to 50 days. Following are descriptions of techniques used to achieve larvation of the eggs. A summary of these procedures is shown in the Results section in Table XI, page 113.

Feces and earthworms. Feces containing eggs was mixed with an equal amount of top soil to which twelve earthworms were added. The mixture was allowed to remain at room temperature of 22 °C for 40 days. The earthworms were then cut into sections and, along with a portion of the soil in which they had been living, were added to the feed of

the dogs. The purpose of feeding both soil and earthworms to the dogs was to include not only any capillarid eggs or larvae that might be retained in the earthworm, but also any which might have passed through the earthworms' digestive tract.

Feces, Animal Charcoal. The procedure as outlined in the Manual of Veterinary Parasitological Laboratory Techniques (Ministry of Agriculture, Fisheries and Food, 1971) using animal-source charcoal, was used as a guide in the preparation of a fecal culture to induce larvation of the capillarid eggs. The feces were finely broken up using a spatula. Fresh fecal material was mixed with an equal amount of granulated animal-source charcoal and water was added until the mixture was moist. The mixture was divided between two petri dishes, one of which was incubated at 22°C and the other at 35°C.

Feces, Plant Charcoal. A variation on the above technique was set up as a culture, using an easily accessible form of plant charcoal. Plant charcoal of the type used in fish-aquarium filters (Hartz brand) was mixed with an equal amount of fresh fecal material and placed in petri dishes. Water was added until the mixture was moist. The mixture was maintained in two petri dishes, one at 22°C and the other at 35°C.

Feces, Potassium Dichromate. Enigk (1950), in his life cycle and epidemiological studies of *C. plica*, the capillarid nematode of the urinary bladder, reported that he was successful in larvating eggs in 2% potassium dichromate. Due to the similarity in species, it was decided to try that method. Feces containing eggs were placed in a glass petri dish to which 10 mls of 3.5% solution of potassium dichromate was added in an

attempt to retard bacterial and fungal growth. The mixture was allowed to stand at 22°C.

Eggs, Antibiotics. Eggs in distilled water were placed in three petri dishes. A mixture of Fungizone (20 mls, Gibco Labs)/Penicillin G (3170 units, Gibco Labs)/Streptomycin Sulfate (1434 units, Gibco Labs)/distilled water (1 liter) was added to the eggs to a depth of 2 mm in each petri dish. Dishes containing these mixtures were maintained one each at 1°C, 22°C and 35°C.

Eggs, Bleach. Eggs in distilled water were placed in two petri dishes. Household bleach (Sodium hypochlorite, 5.25% by weight), diluted 1:400 with distilled water was added to the samples to a depth of 2 mm. These were incubated one each at 22°C and 35°C.

Eggs, Roccal. Eggs were placed in two petri dishes with Roccal disinfectant diluted 1:200 with distilled water added to a depth of 2 mm. These were incubated one each at 22°C and 35°C.

Exposure of Susceptible Dogs

Fecal samples from all dogs used as recipients of the larvated capillarid eggs were checked three times over an ten day period and found to be negative for parasitic eggs prior to the use of the dogs. The repeated fecal examinations were performed to lessen the chances of missing a low-grade parasitic infection. The dogs came from a purebred dog breeding kennel facility which was maintained with excellent management and cleanliness. The dogs were worm-free when obtained and were produced by worm free

parents. Table X, page 110, includes a description of the recipient dogs.

Exposure by Ingestion. The six dogs designated with "F" numbers were given larvated *Capillaria* spp. eggs in their feed. Following the administration of the larvated eggs, total fecal volume was checked daily for two days for the presence of eggs. Fecal samples were collected each day during the following week and examined for eggs.

These recipient dogs were maintained for an additional period of two years. During this time, fecal examinations were done on all dogs on a monthly basis. All six of the dogs were then euthanatized. Nasal passages, sinuses, trachea and bronchi were examined.

Exposure by Intra-nasal Inoculation. Eggs used for intra-nasal inoculation were larvated according to the procedure described previously with eggs only and 1:200 dilute Roccal. The two dogs designated with "N" numbers were used as recipients. Both dogs were anesthetized using Rompum/Ketamine mixture to insure that they would remain stationary for thirty minutes after inoculation. The dogs were placed in a dorsal-side-up recumbency position, with its nasal passages angled upward at a thirty degree angle. One-half ml of eggs-in-water mixture containing approximately one thousand eggs per ml, was administered to each side of each dog's nasal passages.

TABLE X

DOGS USED AS RECIPIENTS FOR
LARVATED EGGS OF *C. BOEHMI*

Exposure Method	Dog Number	Breed	Sex	Age*
Ingestion	F 1	Poodle	Male	Six months
Ingestion	F 2	Poodle	Male	Six months
Ingestion	F 3	Beagle	Male	Three months
Ingestion	F 4	Westie	Male	Six months
Ingestion	F 5	Shih tzu	Male	Six months
Ingestion	F 6	Maltese	Female	Three years
Inhalation	N 1	Bichon	Male	Six months
Inhalation	N 2	Maltese	Male	Six months

*Age at time of induction into program

Results

Larvation of Eggs

Room temperature yielded far greater survival rates of the eggs, fewer survived in the incubator, and little or no development occurred in the refrigerator.

If the egg solution was left with no attempt to control bacteria and fungus, the eggs were soon surrounded, covered and clumped together. Production of proteolytic enzymes by the rapidly growing bacteria was soon followed by disintegration of the eggs. The most success in retarding bacterial growth was achieved by the addition of Roccal disinfectant diluted 1:200. Even so, bacteria and fungus remained a constant problem.

Of the eggs that were collected by sugar flotation to be subjected to larvation techniques, 1 - 2% contained fully-developed, motile larvae. By day 24 of incubation in Roccal, 95% of the eggs which were still intact, having withstood the onslaught of fungi and bacteria, had motile larvae, see Figures XXXIII - XXXVIII, page 113 - 118.

Every other day, a sample of the solution containing the eggs was placed on a slide with a coverslip to determine the stage of development of the eggs. On day 24, when most of the eggs were larvated, it was noticed that one larva emerged from the egg on the slide being observed, see Figure XXXIX, page 119. This was probably a result of the pressure of the coverslip which was applied to the solution.

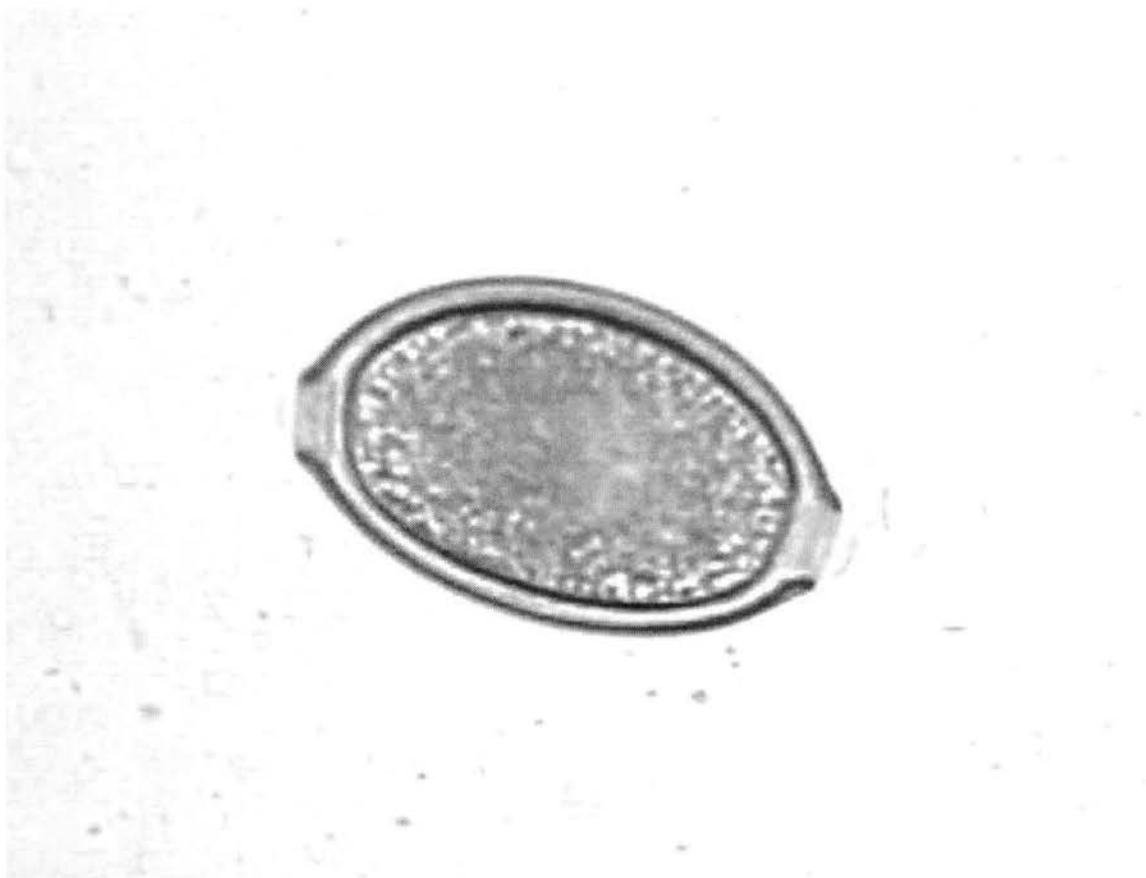
Results of the various techniques used to larvate the capillarid eggs are shown in Table XI, page 112.

TABLE XI

TECHNIQUES USED TO LARVATE
C. BOEHMI EGGS

Technique	Larvae Development	Bacterial Growth	Fungal Growth
Feces and Earthworms			
22°C	++	+	-
Feces, Animal Charcoal			
22°C	++	++	+++
35°C	-	+	+
Feces, Plant Charcoal			
22°C	++	++	+++
35°C	-	+	+
Feces, Potassium Dichromate			
22°C	-	-	-
Eggs, Antibiotics			
1°C	-	-	-
22°C	++	++	++
35°C	-	-	-
Eggs, Bleach			
22°C	++	++	++
35°C	-	-	-
Eggs, Roccal-D			
22°C	+++	-	-
35°C	-	-	-

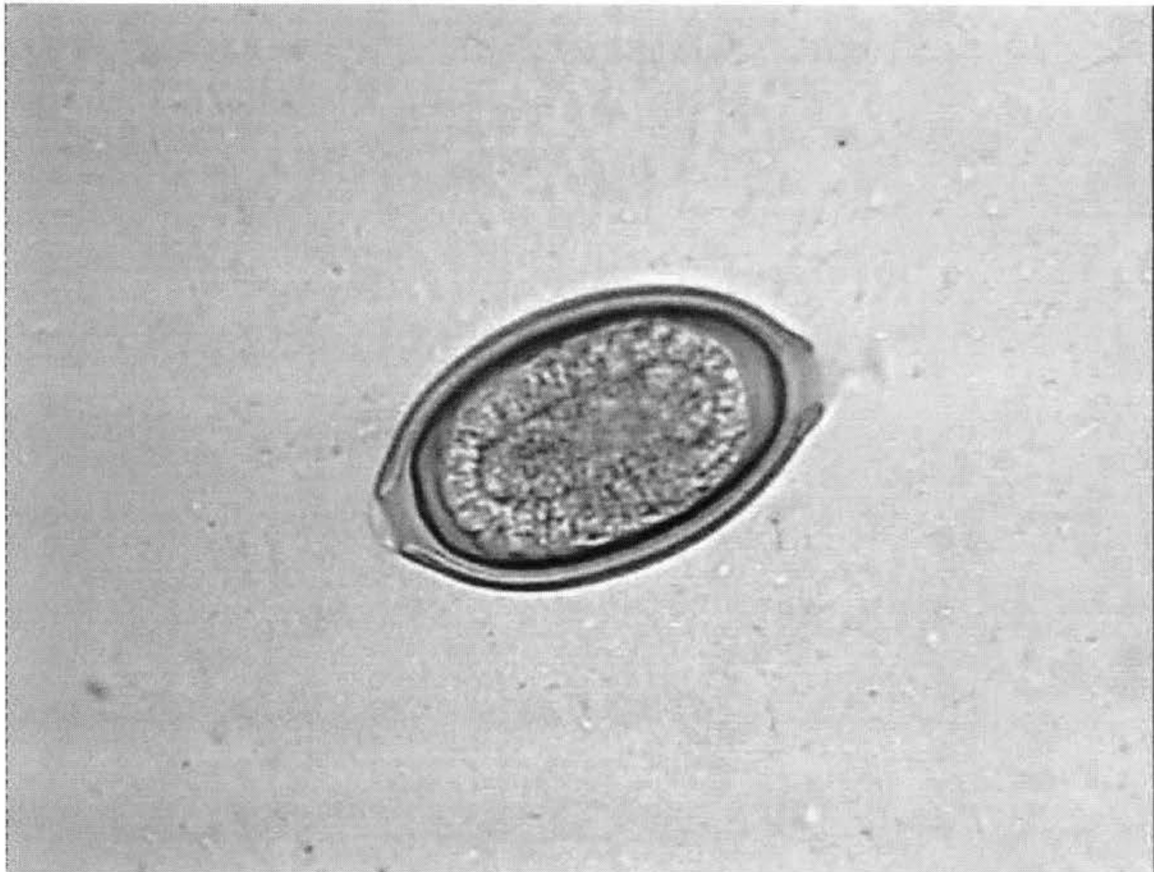
- None
+ Few
++ Moderate
+++ Many



______________ = 18 μ

Figure XXXIII: *Capillaria boehmi* Egg with Undifferentiated Embryo

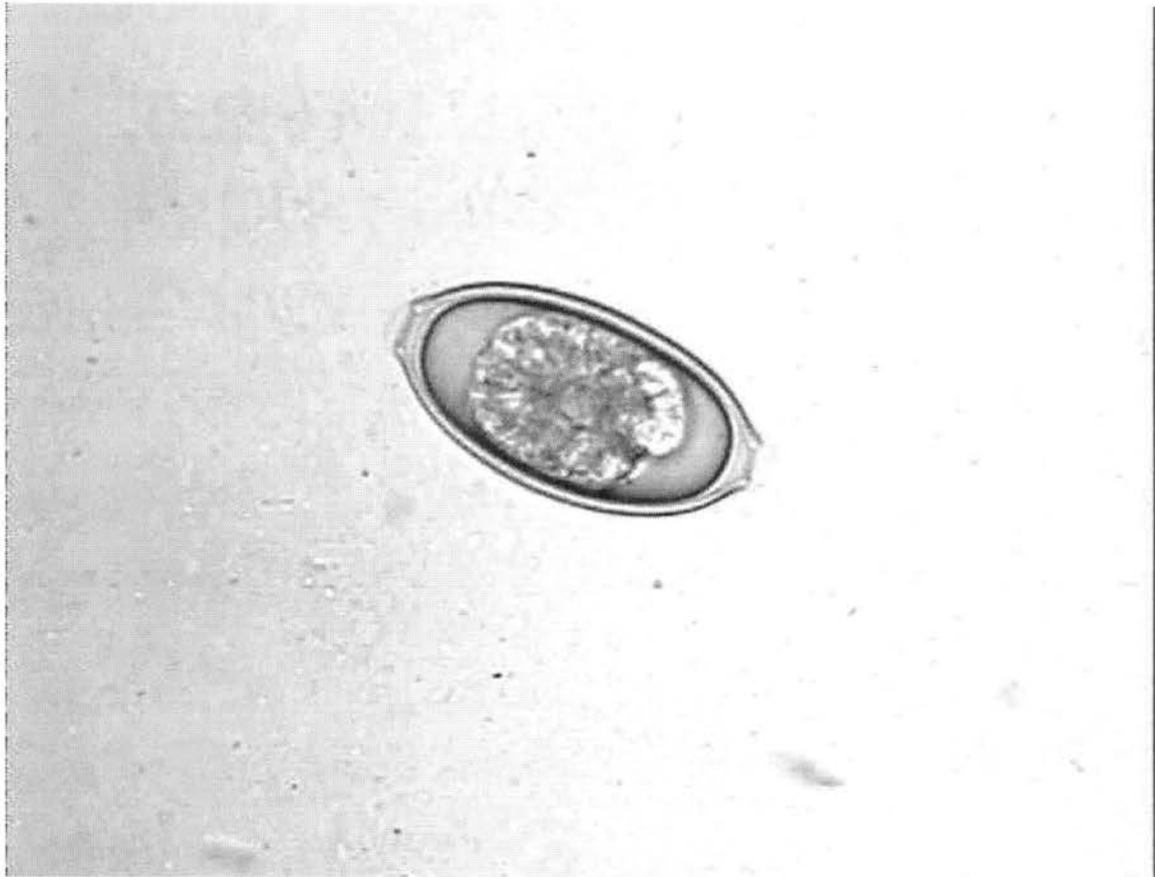
This egg was recovered directly from the nasal passages of a greyhound at necropsy. Note that the undifferentiated embryo completely fills the interior space of the egg.



——————— = 22 μ

Figure XXXIV: *Capillaria boehmi* Egg with Blastula Stage Multicellular Embryo

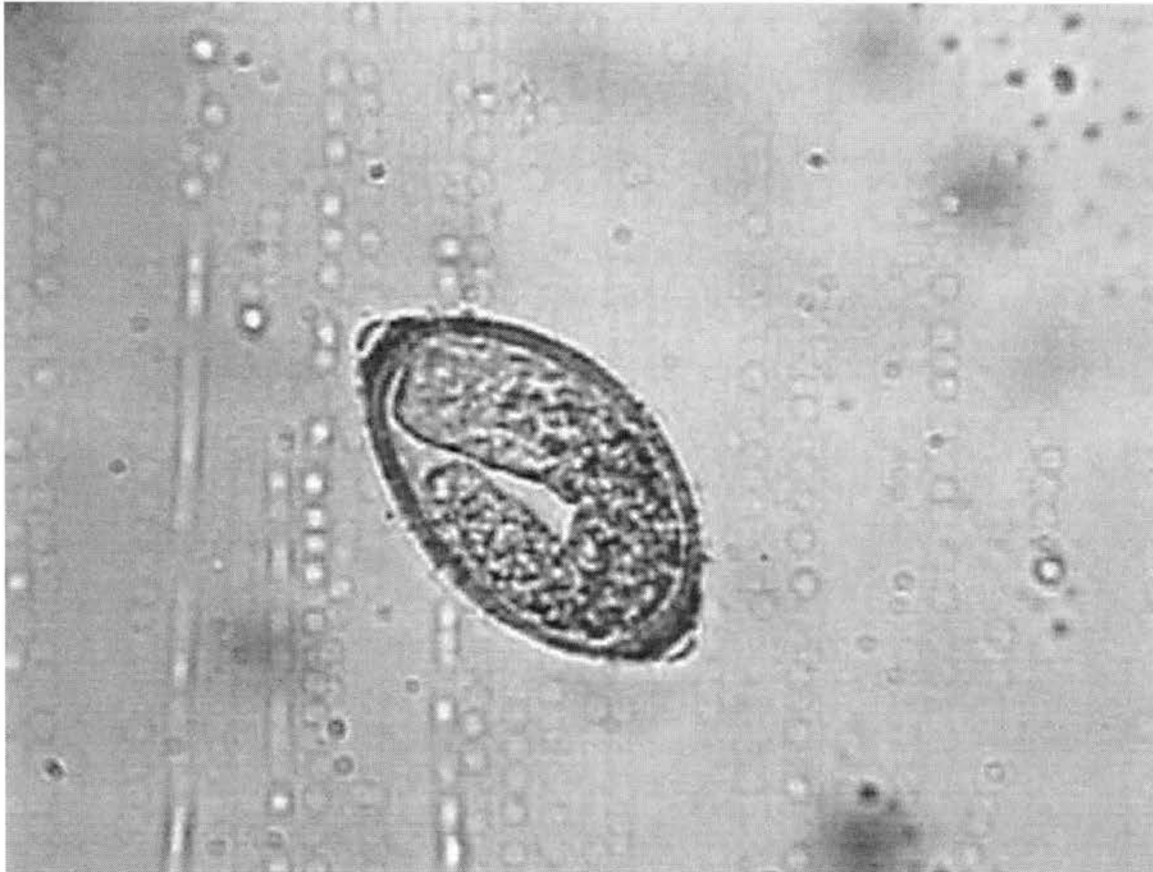
This egg containing a blastula stage multicellular embryo was removed from washings from the nasal passages and sinuses of a greyhound at necropsy. This is the developmental stage of the *C. boehmi* egg so often described as typical of the species when recovered during the process of performing a fecal examination.



______________ = 30 μ

Figure XXXV: *Capillaria boehmi* Egg with Early Gastrula Stage Developing Larva

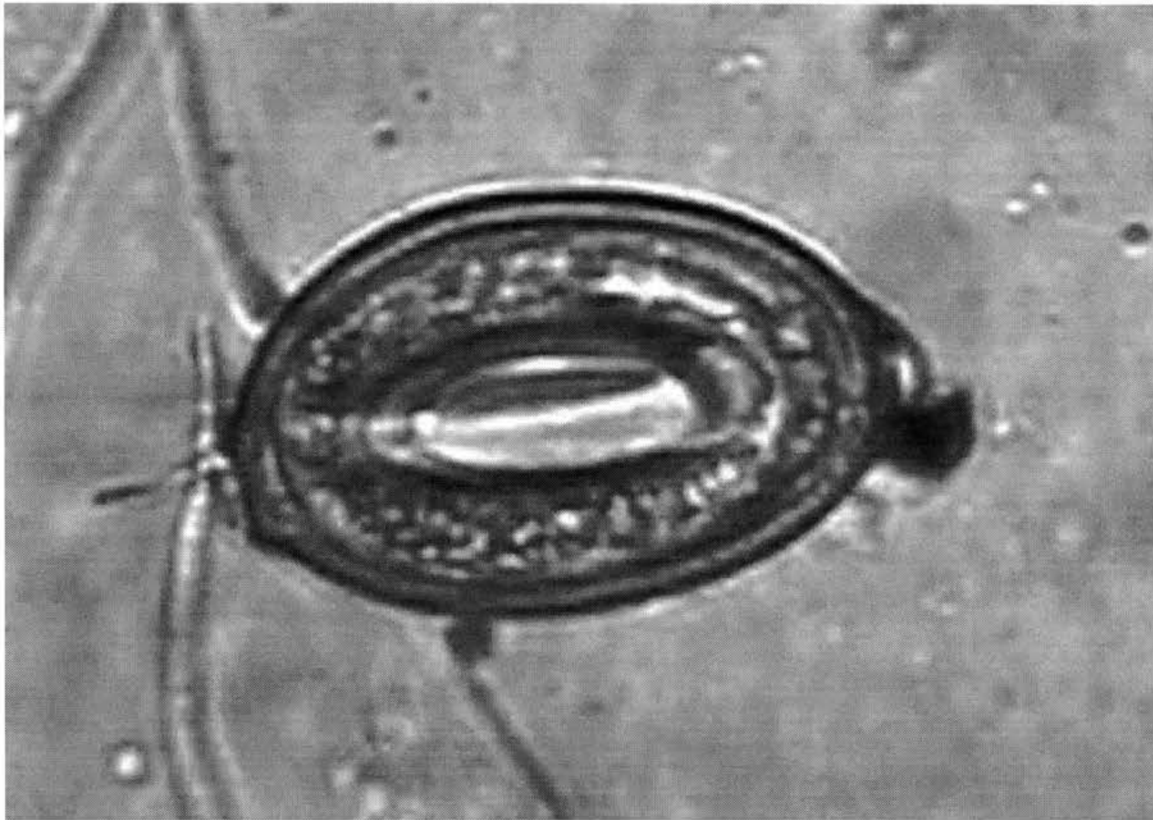
This early developmental stage gives the first indication of the shape of the developing nematode.



_____________ = 23 μ

Figure XXXVI: *Capillaria boehmi* Egg with Mid-to-late Gastrula Stage Developing Vermiform Larva

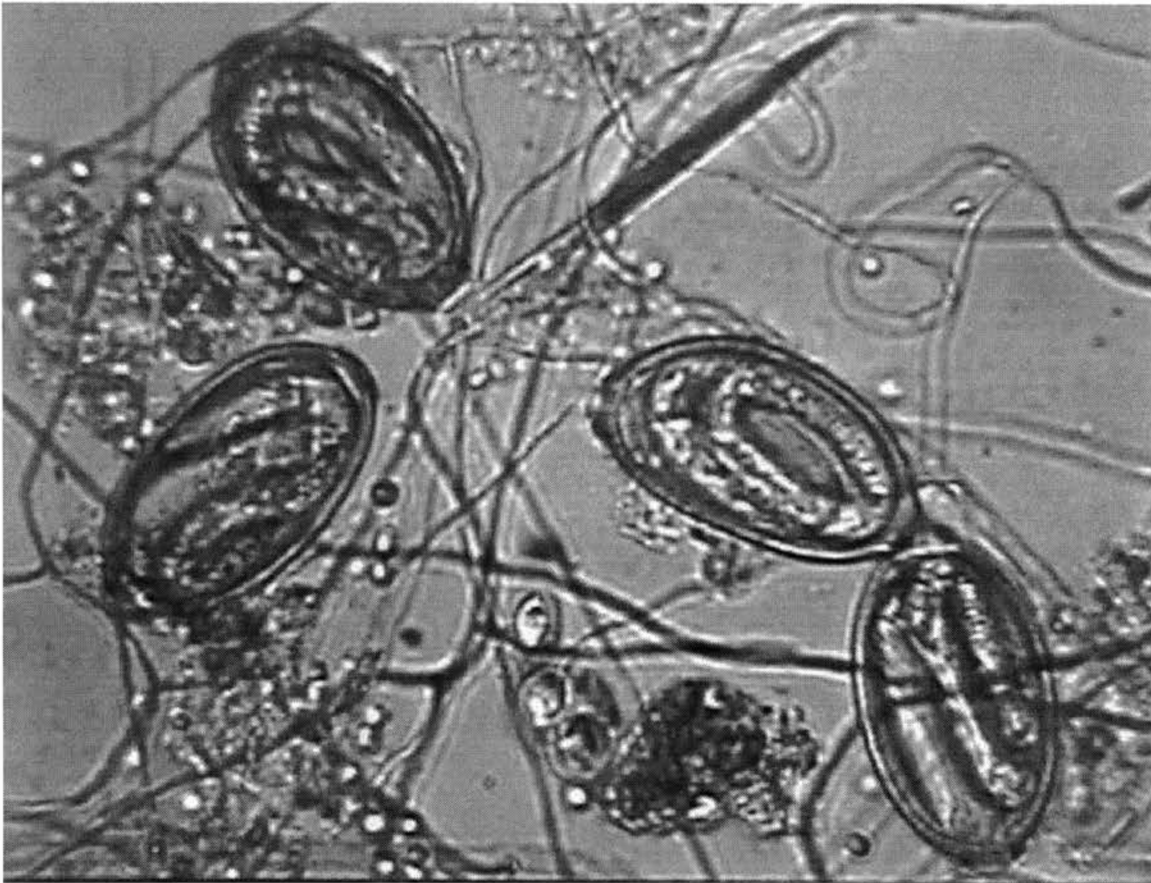
This egg was recovered from washings from a greyhound's nasal passages and sinuses at necropsy. This phase of the capillarid egg is of short duration, existing only as a developmental stage prior to becoming a fully developed, motile larva. As more eggs are becoming larvated in feces than when still retained in the nasal passages, this stage, like the next which contains the fully developed larva, is more often seen in fecal samples.



________________ = 15 μ

Figure XXXVII: *Capillaria boehmi* Egg with Fully Developed Motile Larva

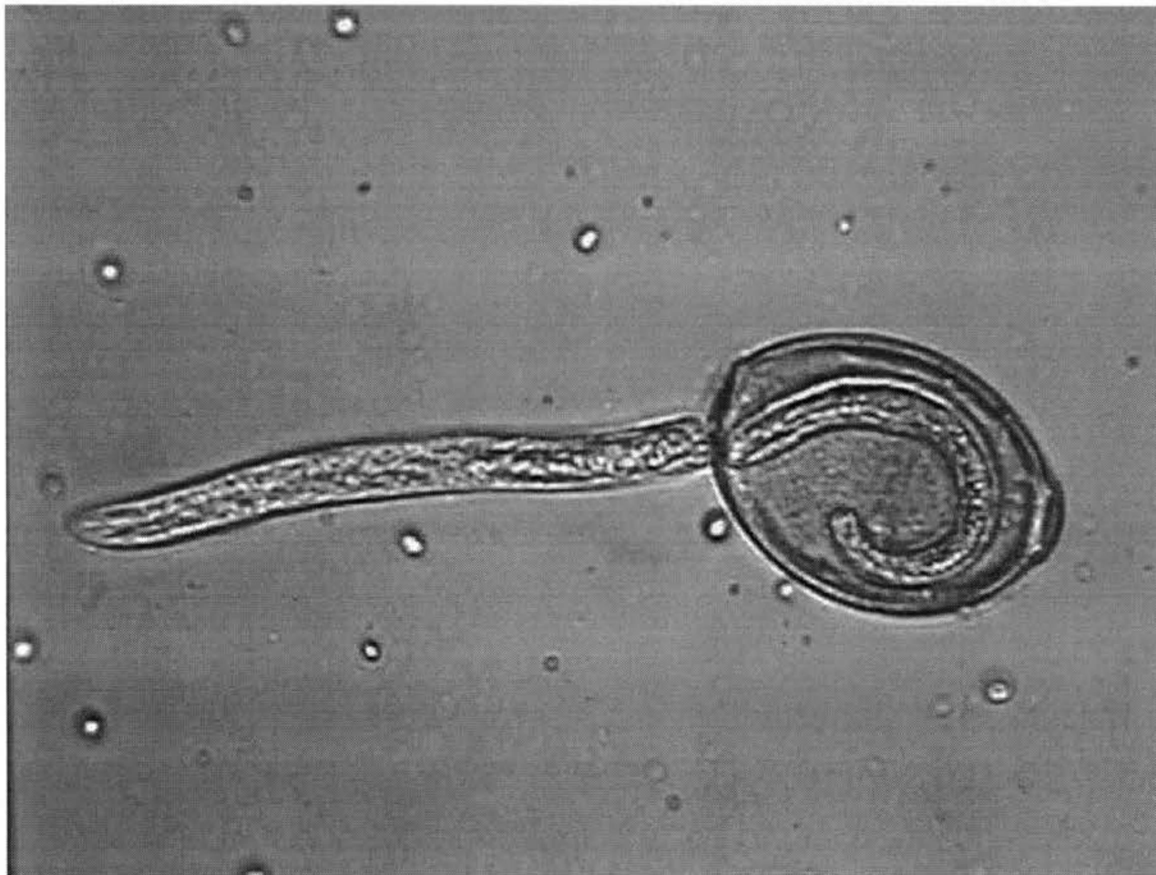
This egg was recovered from washing of the nasal passages and sinuses of a greyhound at necropsy. The fully developed larva contained within the egg was motile at the time of recovery.



——————— = 31 μ

Figure XXXVIII: *Capillaria boehmi* Eggs with Motile Larvae After 24 Days Incubation

These eggs were recovered from freshly passed feces from a greyhound. They were allowed to develop for 24 days in 1:200 Roccal at room temperature, 22°C. At that time, 95% of the eggs examined contained motile larvae. A profusion of fungi is intermingled with the eggs.



______________ = 25 μ

Figure XXXIX: Motile Larva Emerging from *C. boehmi* Egg

As eggs were being checked for developmental stage during incubation, this larva was noticed as it emerged from within the egg.

Exposure by Ingestion

Total fecal output of the six dogs which had been given larvated *Capillaria boehmi* eggs with their feed was checked daily for two days and a fecal sample was examined each day thereafter for one week. No eggs were observed..

Although the recipient dogs were maintained for a period of two years, they at no time exhibited any clinical signs of infection nor were any of the routine fecal examinations positive for eggs. All six of these dogs were euthanatized two years after the attempted infection. Nasal passages, sinuses, trachea and bronchi were examined. No *C. boehmi* worms were found in any of the animals.

Exposure by Intra-nasal Inoculation

Total fecal volume of the two dogs which had larvated capillarid eggs inoculated into their nasal passages was checked for two days and a fecal sample was examined each day thereafter for one week. No eggs were observed. The dogs were maintained for an additional three months and at no time exhibited any signs of infection.

Discussion

Larvation of Eggs

Although numerous techniques were used to produce a suitable environment in which the *C. boehmi* eggs could larvate, it became apparent that the simplest technique was the best. The eggs which developed most successfully to the stage of containing a

motile larvae were those maintained at room temperature, 22°C, with diluted Roccal disinfectant added to retard bacterial growth. Very likely, most general purpose disinfectants would be acceptable if they had bacterial and fungal retarding capabilities.

Transmission of Infection

Christenson (1938) demonstrated a direct life cycle for *C. aerophila* with the parasite transmitted by the fecal-to-oral route. However, he also reported several failed attempts to reproduce the infection in foxes by feeding them embryonated eggs, both eggs alone and eggs mixed naturally with soil.

Assuming the life cycle for *C. aerophila* is direct, it would then be an easy step to place *C. boehmi* in the same pattern, except that the developing adults continue to migrate from the lungs and trachea to the nasal passages and sinuses where they become residents for their reproductive lives.

The current study produced findings similar to his reports of lack of success in transmitting the *C. boehmi* infection with ingestion of embryonated eggs. Additionally, no success in the transmittal of the infection was achieved by feeding earthworms which had been maintained in egg-contaminated soil or by intra-nasal inoculation of embryonated eggs.

Conclusion

Larvation of *C. boehmi* can be achieved at room temperature using a mild disinfectant to control the growth of bacteria and fungus. This observation leads to the

conclusion that kennels should not expect to control the spread of *C. boehmi* in the dog population by using a mild disinfectant to clean runs and living areas.

Although generally accepted that the life cycle of *C. boehmi* is direct and passed to the next host through the fecal-to-oral route, transmission of the infection was not accomplished in this study. Further life-cycle and transmission studies should be encouraged until it is possible to reproduce transmission findings.

CHAPTER VI

PATHOLOGICAL STUDIES OF *C. BOEHMI*

Necropsy Findings

Location of *C. boehmi* within the Dog

Fourteen dogs were necropsied to determine the location of *C. boehmi* within the host. These dogs included a blue-tick hound which was positive for *C. boehmi* for at least eight years, two greyhounds, positive for *C. boehmi* at least six years, and eleven greyhounds held as research and teaching animals for less than two months.

Following euthanasia, the dogs' heads were opened from front to back along the midline. When the nasal passages and sinuses were opened so that the adult *C. boehmi* could be removed, it was noted that no worms were found in the inch-and-a-half of nasal passage nearest the external opening. The worms were uniformly distributed throughout the remaining sinus and nasal areas. The worms lay in the mucus, closely associated with the epithelial lining of the nasal passages and sinuses but not attached to it, such that their presence was not obvious to the casual observer during a necropsy. The lack of coloring of the worms produced an excellent camouflage effect, making them almost impossible to detect without magnification. The nature of the tissues in these areas is such that it contains many twists, turns and convolutions to increase surface area. In order to systematically search, it was necessary to take small areas of tissue and tease the surfaces apart to locate the worms. All sinuses had to be opened and internal surfaces examined.

When the worms were located, they were gently removed with a probe and placed in 0.85% saline. Trachea and bronchial areas of the lungs were searched, but no worms were discovered.

Pathological Findings

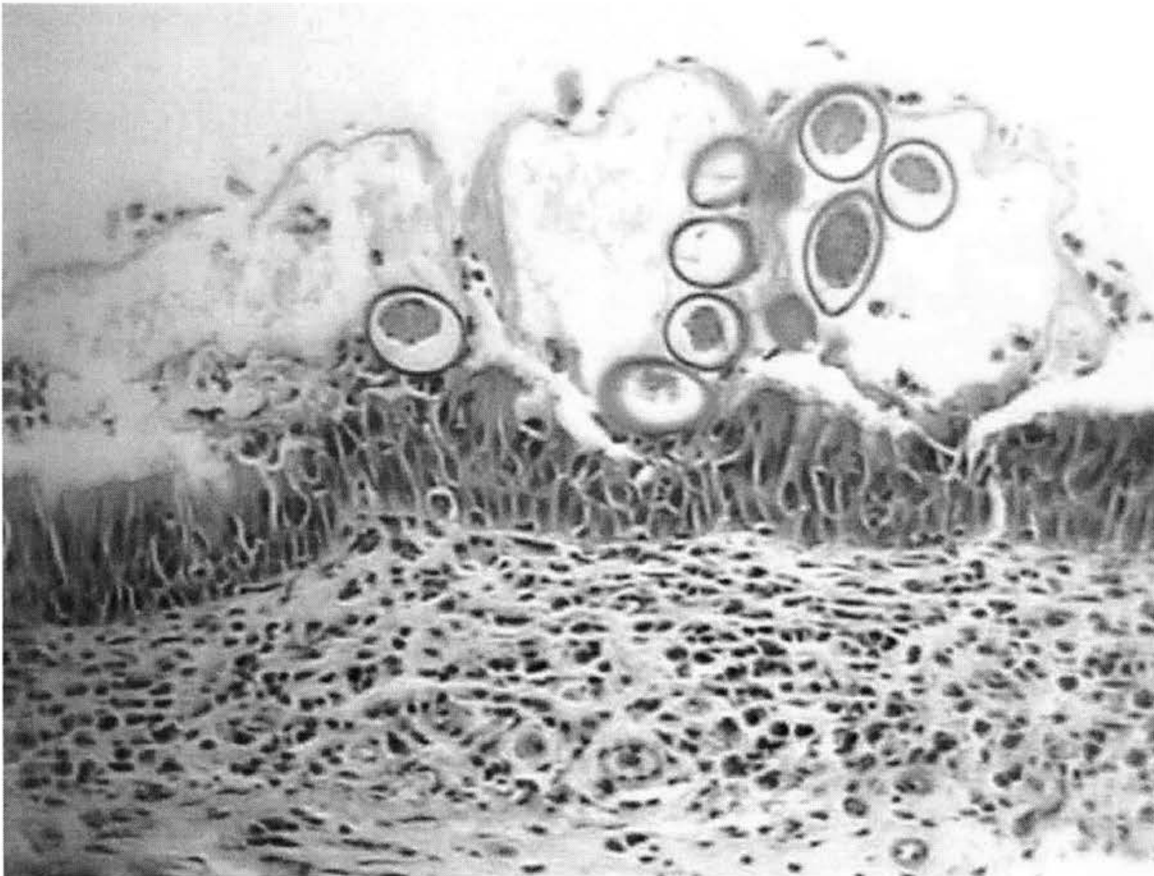
A complete necropsy, including microscopic examination of nasal tissues, was completed on Greyhound #4, the fawn-colored female.

Gross observations revealed no significant lesions in any of the internal organ systems. The nasal passages grossly appeared essentially normal with the possible exception that they were moist with an excess amount of mucus. Microscopic examination of the lungs showed the pleural surfaces, alveolar walls and airways essentially normal. There were numerous small clusters of phagocytic cells containing grayish, somewhat crystalline debris, found in terminal bronchiolar locations.

Examination was made of several specimens of nasal mucosa, many of which appeared essentially normal. Often a modest population lymphoplasmacytic cells were seen in the lamina propria, but few had transmigrated the epithelium. Some areas of the lamina propria seemed loosely arranged. Several areas of epithelium contained a preponderance of goblet cells and other segments had very few. Many segments of epithelium were nicely ciliated. Some specimens had a deeply pseudostratified columnar epithelium rather than a columnar epithelium which was rather well ciliated. Some areas were more prominently and intensely inflamed with modest thickening of the epithelium and containing scattered transmigrating cells and dense proprial infiltrates with

lymphoplasmacytic cells. In these areas there were nearby parasitic forms enmeshed in surface mucus. The forms were chiefly parasite eggs with a medium-thick shell and bi-polar plugs. Additionally, there were multiple cross-sections of one or more parasitic nematodes. The mucous layer which contained the parasite eggs also contained scattered granulocytic cells. A few of these cells were visible in the lamina propria.

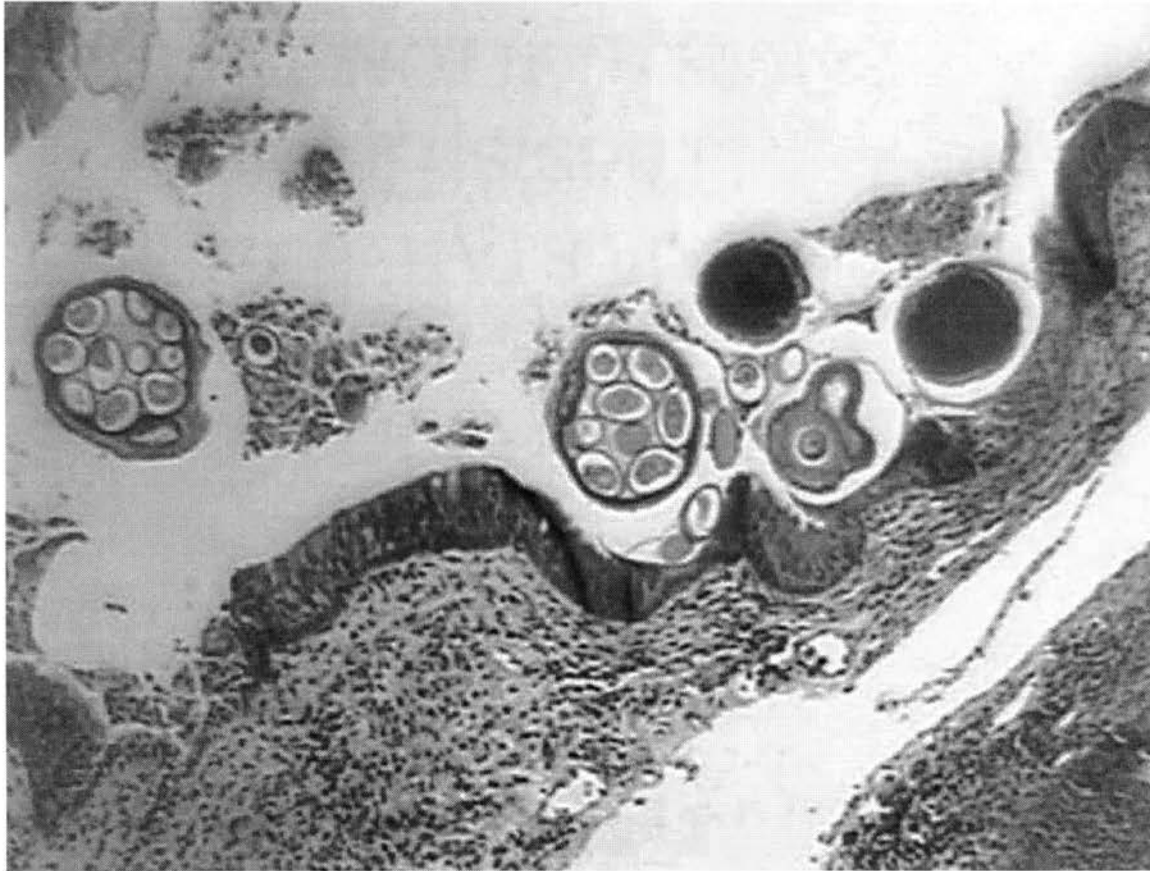
Photomicrographs of nasal tissue sections made at time of necropsy follow in Figures XXXX - XXXXII, pages 126 - 128.



______________ = 110 μ

Figure XXXX: Greyhound Nasal Tissue with Cross-sections of *C. boehmi*

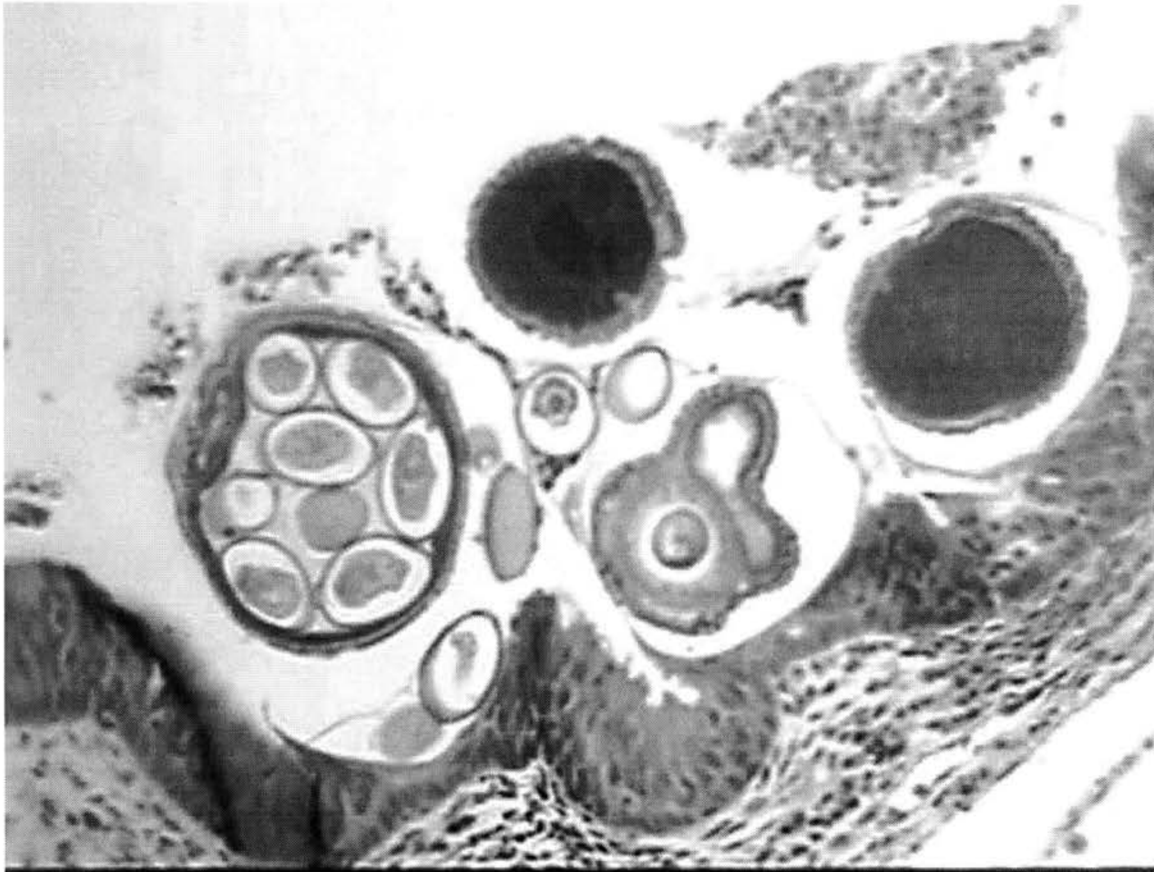
Capillaria boehmi is shown here lying on the surface of the nasal epithelium. Adjacent tissues contain increased numbers of inflammatory cells including plasma cells, neutrophils and eosinophils.



______________ = 200 μ

Figure XXXXI: Greyhound Nasal Tissue with Deformed Epithelial Layer and Cross-sections of *C. boehmi*

Surface tissues in contact with *C. boehmi* had a distinctly deformed appearance conforming to the body impression of the worm. Ciliated columnar cells immediately under the body of the worm appeared attenuated and shortened. Increased amounts of mucus were present in the passages, surrounding and covering the worms.



——————— = 110 μ

Figure XXXXII: Greyhound Nasal Tissue with Cross-sections of *C. boehmi* Showing the Nematode Closely Associated with Nasal Tissues

Capillaria boehmi was never found located within or under the tissue surface. Instead, it was lying in close proximity with the surface and covered with a layer of host mucus. Many of the eggs deposited by the worms remained, for a time, trapped in the mucus.

Conclusion

All of the fourteen dogs, positive for capillarid eggs in their feces and necropsied, had *C. boehmi* present in the nasal passages and sinuses only. This finding verified these locations as the sole domain of this nematode.

Microscopic examination of tissue sections prepared following necropsy revealed damage to the delicate tissues lining the nasal passages. The depressions in the epithelial tissue as it conformed to the body shape of the worm resulted in misshapen and deformed cells. Areas of inflammation were present in tissue adjacent to each worm. Increased amounts of mucus were present throughout the nasal passages. These findings left no doubt that the nematodes had a pathological effect on the dog.

Perhaps it will become apparent, as more is learned about *C. boehmi*, that there are more severe clinical signs associated with infections with nasal capillarids then previously thought, especially when associated with secondary bacterial infections.

CHAPTER VII

EPIDEMIOLOGICAL STUDIES OF *C. BOEHMI*

Prevalence Surveys

In 1991, greyhounds from a breeding and racing kennel were donated to the Oklahoma State University College of Veterinary Medicine to be used in the teaching program. Routine fecal examinations of these dogs showed that several harbored *Capillaria* spp.

Necropsies of the dogs revealed that the capillarid nematodes in the dogs were confined to the nasal passages and sinuses. None were found to reside in the bronchi or trachea. The worms were identified as *Capillaria boehmi.* None of the dogs examined showed any obvious clinical signs.

As several of the dogs removed from the kennel had capillarid eggs in their feces, it posed the question if the dogs remaining in the kennel were infected at the same prevalence rate as those dogs removed. It was speculated that the possibility existed that the removal of the dogs from the racing program might have been influenced by the presence of the parasite.

Materials and Methods

Surveys of the greyhound kennel in Kansas and another, similarly operated greyhound kennel in Oklahoma were conducted to determine the prevalence of the capillarid infection in the resident dogs.

Kennels Surveyed

Kennel 1. The kennel from which the donated greyhounds were obtained was located in rural southern Kansas. It had been in operation in excess of twenty years and contained approximately 120 adult greyhounds used for racing and breeding. Adults of both sexes were frequently transported across the country to compete in races and for breeding.

Dogs being conditioned for the race track were housed in individual metal cages. Twice a day the dogs were let out to exercise in a large community pen, ten to twelve animals at a time. Prior to their release from their individual cages, a muzzle was placed on each animal and held in place with a strap behind the ears. The muzzles fit closely enough to prevent biting, but were loose enough to allow the animals to pant with open mouths, bark, and drink water. Muzzles were chosen at random for use on the dogs, and one muzzle might be used on several different dogs during the course of a day during the different exercise periods. Exercise periods were thirty minutes to an hour each.

The outdoor pens in which the dogs exercised were twenty feet wide by fifty feet long, bounded by five foot high wire mesh fencing. Several five gallon plastic water buckets were wired to the fence. The surface of the pens was soil, and, although well-drained, would be muddy in wet weather. Feces were picked up daily, but often would be mixed with the soil or mud due to the activity of the dogs in the runs.

The dogs were fed after returning to their cages following the morning exercise. Feed consisted of boiled and raw chicken and chicken parts and commercial kibble.

Anywhere from zero to ten litters of puppies might be present at any one time.

Nursing females and their puppies had large individual indoor/outdoor runs with concrete floors inside and soil outside. A large whelping box with bedding was located inside.

After weaning, young dogs were kept two, three or four to a run in large indoor/outdoor pens similar to those used for the nursing females.

Health care included vaccination for distemper, hepatitis, leptospirosis, parainfluenza, parvo and rabies. Ivomec anthelmintic for large animals was the treatment chosen for control of parasitic nematodes and was administered orally or by injection once every one to two months. Fecal examinations were seldom performed, and then only in conjunction with veterinary care required for illness or injury. The kennel owner was unaware of the capillariasis in his dogs.

Kennel 2. The location of this kennel was in rural northern Oklahoma and it housed approximately 100 dogs. It was in many ways quite similar to Kennel 1. Differences included that it was located in an area with sandy soil and it had been in operation less than five years. Fewer of the dogs were kept in cages, with the majority kept in long outdoor runs about 10 feet by 100 feet, two to three dogs per run.

Fecal Collection and Examination

Specimens were obtained by turning out one dog at a time and collecting the feces immediately after defecation, being careful to exclude contamination from the ground. Samples were returned to the laboratory and checked for presence of eggs of parasitic helminths.

Two grams of feces were added to 20 mls saturated $NaNO_3$ levitation solution.

The specific gravity of the solution was 1.400, obtained by mixing 910 grams $NaNO_3$ crystals with 1000 mls distilled water. The solution was mixed thoroughly, then filtered through cheesecloth into a shell vial, bringing the fluid level to the rim with a positive meniscus. A coverslip was placed on top of the vial and allowed to remain seven minutes. The coverslip was then removed, with care taken not to lose the drop of fluid suspended from the coverslip, and placed on a glass microscope slide (Jordan, 1992). The entire slide was then examined using 100X magnification. Numbers of eggs per 100X field were recorded. This is not to be confused with eggs per gram, but does give a basis for comparison between animals.

Results

Kennel 1

The survey of seventy-five adult dogs from the Kansas kennel revealed that thirty-six (48%) of the dogs were positive for *Capillaria* spp. eggs in their feces. Only dogs more than six months of age were included in the survey. *Ancylostoma* spp. eggs were found in fifteen of the dogs, *Trichuris* sp. in six and *Toxocara* sp. in one.

A total of eighteen dogs of the seventy-five surveyed (24%) had parasitic nematodes eggs other than *Capillaria* spp. eggs in their feces. Of those eighteen, eight were negative for capillarid eggs. A complete list of coprological findings of *Capillaria* spp., *Ancylostoma* spp., *Trichuris* sp., and *Toxocara* sp. eggs per 100X field is included in Appendix 2, page 160.

Kennel 2

The survey of thirty-seven adult dogs from the Oklahoma kennel did not show that any of the dogs were infected with *Capillaria* spp. *Ancylostoma* spp. eggs were found in the feces of one dog, *Trichuris* sp. in two dogs and *Toxocara* sp. in four dogs. Thus seven of the thirty-seven dogs examined (19%) were positive for parasitic nematode eggs, although none were *Capillaria* spp. A complete list of coprological findings is included in Appendix 3, page 163.

Discussion

The prevalence of *C. boehmi* in the Kennel 1, the Kansas kennel, was high. Occurrence of *C. boehmi* in this kennel had not previously been known. As the general perception is that *C. boehmi* is harmless in dogs and of no consequence, it is possible that even if it had been recognized, it would not have caused concern.

There may be several reasons *C. boehmi* was present in so many of the dogs in Kennel 1, including:

1) The kennel had been established for a long period of time. That would increase the likelihood of an infection having been introduced into the kennel population. Once in the population, if untreated or difficult to treat, if the life cycle is direct or if the intermediate host is abundant, an infection could well become established and eventually spread to all susceptible animals.

2) The fact that the dogs had soil runs which were very difficult to clean and lent themselves to the perpetuation of parasite infections. Also these runs were often muddy

in inclement weather, thereby providing a moist environment conducive to the long term survival of the nematode eggs.

3) The dogs were allowed to mingle at exercise time, which allowed direct contact between animals and possibly permitted exchange of parasites between hosts if the life cycle is direct.

4) Muzzles were picked at random for use on the dogs during excise periods. If the life cycle for *C. boehmi* is direct and the egg did not require prolonged exposure to the external environment to become infective, this would be an excellent method of transferring the infection.

Kennel 2, located in Oklahoma, did not yield any fecals positive for capillarid eggs. The possibility exists that the source dogs from which Kennel 2 was established did not harbor *C. boehmi*. The fact that the kennel was relatively new presented the probability that *C. boehmi* simply had not yet been introduced into the population. The kennel did, however, have present the other three parasitic nematodes found in Kennel 1. If these other three parasites, all of which have direct life cycles have maintained a presence in the kennel population, in all likelihood, once introduced, *C. boehmi* could be expected to do as well, or perhaps better due to the difficulty of successful treatment.

The percentage of dogs infected with parasitic nematodes other than *Capillaria* spp. did not vary significantly between the two kennels. Of the eighteen dogs in Kennel 1 which were positive for nematode eggs other than *Capillaria* spp., eight (44%) were negative for capillarid eggs. This would indicate that the capillarid infection was not related to infections with other parasitic nematodes. The management and cleanliness of

operation did not vary greatly between the two kennels. However, the difference in finding of capillarid eggs in the dogs' feces varied tremendously with one kennel at 48% and the other at zero. Given the similarities in the two kennels, their management and the fact that they were geographically situated within one hundred miles of each other, it would be expected that findings of parasite species would be in similar proportions. It was not expected to find the dramatic difference in capillarid infection.

Conclusion

Kennels managed in the manner in which a typical greyhound kennel is managed would be prime targets for the spread of *C. boehmi* infection once it is introduced. These kennels, by design, allow the intermingling of dogs from different areas of the kennel with each other. Additionally, the dogs are transported across the country for the purposes of racing, training and breeding, thereby presenting the opportunity to carry the infection to a new kennel.

It appears, at this time, that the *C. boehmi* infection in the domestic dog is more prevalent in the greyhound than in other breeds of dogs. Further prevalence studies are needed to determine if this is the case. When the occurrence of *C. boehmi* in greyhounds was noted, the question arose of why this parasite was being found in this particular breed of dog. The possibility exists that the infection is normally one of wildlife, in particular the coyote and fox. In the Oklahoma and Kansas areas it is a common practice to hunt coyotes with greyhounds. Perhaps the infection was transmitted from the coyotes to the hunting greyhounds. It would not be difficult to envision the occasional introduction of a

greyhound from a racing program to a hunting program, or vice versa.

In the last few years a determined effort been made to find homes for greyhounds removed from the racing program. This creates an focus of concern that infected greyhounds will be brought into contact with other dogs and possibly transmit the infection to them. For this reason, if for no other, additional studies of this parasite should be pursued to determine the life cycle and methods of transmission of the infection.

CHAPTER VIII

TREATMENT STUDIES OF *C. BOEHMI*

Anthelmintic Treatment

Two case reports were found documenting treatment described as successful for nasal capillariasis in dogs using the anthelmintic ivermectin (Evinger, et al., 1985; King, et al., 1990). The dosage reportedly used by both authors was 0.2 mg/kg of body weight given orally. Neither author had the opportunity for a necropsy examination of the dogs to determine if the adult worms were removed by the treatment.

Materials and Methods

With the information from the two articles, and the fact that the greyhound breeding and racing kennels which were surveyed used Ivomec brand of ivermectin as the anthelmintic of choice, it was determined to administer Ivomec to two greyhounds, a male and a female.

Ivomec, produced by Merck and Co., Inc., AgVet Division, is specifically labeled for use as an injection in cattle and swine only. The anthelmintic was given to the dogs orally, 0.2 mg/kg of body weight.

The two dogs were seven-and-a-half years old and had been infected with *C. boehmi* continuously for at least six years. Both were also infected with *Ancylostoma* spp.

Fecal egg counts were determined using the Modified Wisconsin egg counting technique. Fecal examination results were recorded every day for both dogs, beginning one day prior to administration of the anthelmintic. If the dogs' feces became negative for capillarid eggs, euthanasia and necropsies were planned to follow with examination of the dogs' lungs, trachea, nasal passages and sinuses.

Results

The dogs had a dramatic reduction in *C. boehmi* egg numbers during the first week post treatment. By one week post treatment, egg counts were near zero. However, during the second week, *C. boehmi* egg numbers increased until they were back to original values by the end of the two week period.

By the third day post treatment, both dogs were negative for *Ancylostoma* spp. eggs in their feces and remained negative throughout the two-week period post treatment that they were monitored.

At the time of treatment, the male had 180 *C. boehmi* and 184 *Ancylostoma* spp. eggs per gram of feces. By the second day post treatment, the *Ancylostoma* spp. egg count was zero and the *C. boehmi* egg count was decreasing. One week post treatment the *C. boehmi egg* count was zero By day eight post treatment, egg numbers had started to climb and had returned to normal two weeks post treatment.

The female had 60 *C. boehmi* and 1188 *Ancylostoma* spp. eggs per gram of feces prior to treatment. The first day post treatment, the *Ancylostoma* spp. egg count had been reduced to only two eggs, and the *Capillaria* spp. egg count was 96. The third day the

Ancylostoma spp. egg count was zero and remained so for the remainder of the eight day observation period.. The *C. boehmi* egg count decreased to 44 eggs per gram by the third day, and by the eighth day had dropped to 4 eggs per gram. By day ten egg numbers were increasing and continued to show an increase back to original numbers. Egg count numbers for the female are graphically represented in Figure XXXXIII, page 142. A complete list of eggs per gram of feces following administration of the anthelmintic to the female is shown in Appendix 5, page 172.

Discussion

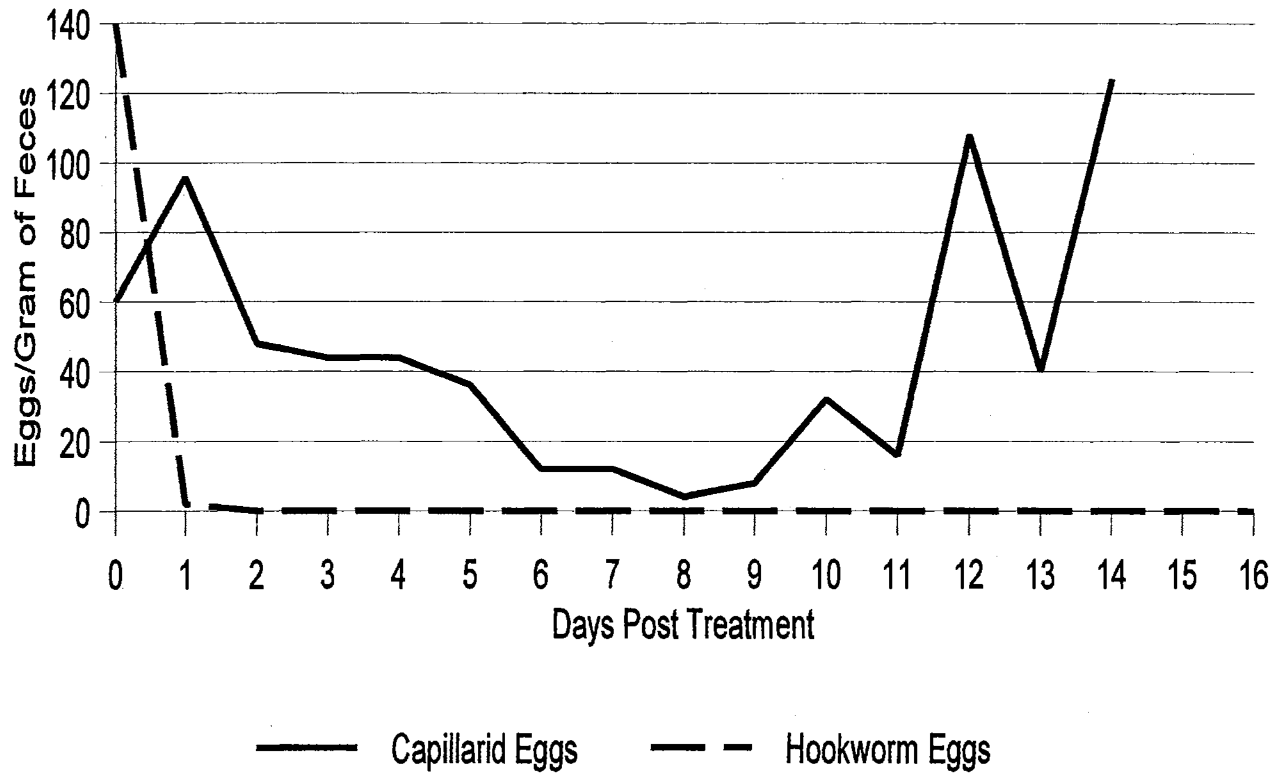
The *Ancylostoma* spp. infection in both dogs served as an excellent control to demonstrate that the anthelmintic was effective. The treatment dramatically reduced the number of *C. boehmi* eggs recovered from the dogs, but clearly did not remove the adult worms. If only one fecal examination was made one week following treatment when egg counts were near zero, it would be easy to conclude that the treatment had successfully eliminated both the *Ancylostoma* spp. and the *C. boehmi* infections.

Conclusion

Ivomec brand ivermectin, given orally at a rate of 0.2 mg/kg of body weight, did not remove the infection of *C. boehmi*, from the dogs. There was obviously some effect on the nematodes from the anthelmintic, as egg counts were reduced almost to zero one week post-treatment. It would be recommended that further studies with ivermectin and with other anthelmintics be done which involve repeated treatments, increased dosages,

and follow-up necropsies to determine anthelmintic effect on adult nematodes.

Fig. XXXXV: Daily Egg Counts Following Anthelmintic Treatment



CHAPTER IX

SUMMARY AND CONCLUSION

Morphological Studies

The photomicrographs contained herein show specific morphological characteristics of *C. boehmi* previously unavailable which should facilitate future studies of the nematode. Illustrated are the anterior of the worm, stichosome esophagus, vulva at the junction of the esophagus and intestine, uterus filled with eggs and caudal ends of both male and female. Electron photomicrograph shows caudal end of female as well as section of cuticle covered with fine spines.

Illustrations include typical eggs as recovered from both nasal passages and feces as well as variations and atypical eggs. Electron photomicrograph shows egg surface with pitting pattern distinctive for *C. boehmi*. A series of photomicrographs shows progressive steps of embryonic development within the egg.

The studies which produced this report supported Supperer's (1953) decision to name *C. boehmi* as a new species separate and distinct from the very similar nematode, *C. aerophila*, based on differences in morphological characteristics of both adults and eggs, combined with differences in location of the parasite within the host.

In the adult worm, the body width of the female and the number of esophageal cells are sufficient to warrant separation of *C. boehmi* from *C. aerophila*. Body width for *C. boehmi* female was found to be 120 - 210 μ compared to 100 - 180 μ for *C. aerophila*. Number of esophageal cells in the female *C. boehmi* were found to be 29 - 34 compared

to 45 - 50 for *C. aerophila.*

The *C. boehmi* egg is shorter than the egg of *C. aerophila*, and the difference width-to-length is less, resulting in the *C. boehmi* egg having a more rounded appearance. *Capillaria boehmi* eggs measured 53 - 64 μ in length by 30 - 35 μ in width, yielding a width-to-length ratio of 1 : 1.8 compared to 1 : 2 ratio for the more elongated *C. aerophila* egg.

DNA investigations demonstrated that the use of a previously described primers for Trichuroidea nematodes would yield a DNA product from *C. boehmi.*

Biological Studies

This study is the first report of long term observations of dogs infected *with C. boehmi*. The infection remained in four dogs kept six years and one dog kept eight years. This indicates that, unlike some parasites, the *C. boehmi* infection could be expected to remain in a dog throughout its life.

Egg recovery of *C. boehmi* showed cyclical patterns with peaks about every six months. This study was by far the longest period of time that patterns in egg production have been reported for the capillarid. As fewer eggs of *C. boehmi* are recovered from the feces than for many other parasites, especially those dwelling in the intestine, a fluctuating egg-recovery pattern could easily produce negative findings which would lead to failure to diagnose the infection. Therefore, if an infection with *C. boehmi* is suspected, it should be recommended to make separate assessments of fecal findings over a period of several weeks to increase the chances of observing capillarid eggs, if present.

Despite the fact that repeated attempts to transmit the infection of *C. boehmi* directly from one dog to the next failed, it is still presumed that the life cycle is direct. The similar nematode, *C. aerophila*, has been shown to utilize a direct life cycle, although many of the attempts at transferring that infection were unsuccessful.

Pathological Studies

Necropsies of fourteen dogs revealed that the parasite location within the host was restricted to the nasal passages and sinus areas.

Clinical signs are seldom observed in dogs infected with *C. boehmi*. Dogs infected with the nematode and observed for more than six years maintained an excellent outward physical appearance. There have been sporadic reports of sneezing and nasal discharge in individual animals. Normally the infection exists in such a way that the first, and often only, sign of infection is the finding of capillarid eggs in the feces. Infection with *C. boehmi* may predispose the host to secondary bacterial infections which may, in a some cases, show clinical signs as associated with respiratory tract infections.

That the parasite in the nasal passages of dogs can cause pathological changes was shown beyond doubt by the microscopic examination of nasal tissues following necropsy. The ciliated epithelial cells were altered and deformed in the vicinity of the worms. When numerous worms were present, large areas of these tissues were affected. Inflammatory cells were present and large amounts of mucus produced in response to the presence of the worm. The result was a restriction of the air passageway as well as setting the stage for the invasion of bacteria.

Epidemiological Studies

Prevalence reports for *C. boehmi* are scarce as most authors have presented reports identifying the capillarid eggs recovered in their studies as *C. aerophila*. Prevalence reports in this study were combined with necropsies to determine the actual location of the parasite within the host as well as morphological characteristics both of adult worms recovered and of eggs. As awareness of *C. boehmi* increases, it is hoped that future surveys will distinguish between the two species.

Treatment Studies

Treatment for *C. boehmi* has not been proved to be effective. Although reports have been published that indicate various anthelmintics have been used successfully, there were an equal number of contradictory reports concerning the use of the same drugs indicating lack of success. None of the reports indicated that necropsies were performed after anthelmintic treatment to demonstrate actual removal of the worm from the host. Reports of effective treatment with anthelmintics were based on negative fecal findings, which were probably temporary.

Two dogs naturally infected with *C. boehmi* were treated with ivermectin. Numbers of eggs in the feces fell to near zero one week following treatment, but were back to original counts by two weeks post treatment. This would indicate that it would be possible to obtain a negative fecal egg count it a treated dog was examined only once, one week post treatment.

Conclusion

Species distinction between *C. boehmi* and the very similar nematode *C. aerophila* were reemphasized due to morphological differences of the adults and eggs as well as a difference in location that the nematode inhabits within the host.

That *C. boehmi* has a pathological effect upon the dog was shown in necropsy examinations showing inflammatory cell infiltration of tissues adjacent to the parasite as well as an increased production of mucus.

The importance of *C. boehmi* as it affects greyhounds and the potential impact it has on their ability to race competitively overshadows other effects occasionally seen in dogs. The high percentage of animals infected in a kennel in which the parasite is present indicates that it can spread throughout the population with little restraint.

To date no anthelmintic can be depended upon to successfully rid the host of the parasite. Therefore, it is imperative that owners of greyhound kennels be made aware of this parasite and take preventive measures to assure that it is not introduced into their animals. If introduced, controls should be implemented to prevent the spreading to other susceptible dogs.

Recommendations for Further Study

Additional investigation will provide sequencing of the DNA of *C. boehmi* which will prove helpful both in accurately identifying the parasite within the host as well as being useful for species comparisons.

Further life cycle and transmission studies are needed for this nematode.

Although it is generally accepted that transmission is direct from host to host, this needs to be verified with reproducible trials.

There is a need for future anthelmintic efficacy testing which includes necropsies of the treated animals. Studies should be conducted which include a variety of anthelmintics, variations in dosage and necropsies to support findings.

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APPENDICES

Appendix 1

Coprological Findings of *C. boehmi* Eggs in Four Greyhounds During a Six-Year Period

Date	Greyhound Number 1 eggs/field *	Greyhound Number 2 eggs/field *	Greyhound Number 3 eggs/field *	Greyhound Number 4 eggs/field *
1991				
Mar	3 - 5	2 - 3	0 - 1	7 - 9
Apr	3 - 5	1 - 3	0 - 1	8 - 10
May	1 - 3	1 - 3	0 - 1	6 - 8
Jun	2 - 3	2 - 3	2 - 3	4 - 6
Jul	3 - 5	2 - 3	0 - 1	8 - 10
Aug	3 - 5	3 - 4	1 - 3	8 - 10
Sep	1 - 3	0 - 1	1 - 2	8 - 10
Oct	2 - 3	0 - 1	1 - 3	4 - 6
Nov	3 - 5	2 - 3	2 - 3	8 - 10
Dec	1 - 3	2 - 3	1 - 3	7 - 9
1992				
Jan	3 - 4	3 - 4	0 - 1	8 - 10
Feb	1 - 3	1 - 3	1 - 3	7 - 9
Mar	1 - 3	1 - 3	1 - 3	7 - 9
Apr	3 - 4	0 - 1	2 - 4	8 - 10
May	0 - 1	1 - 3	1 - 3	4 - 6
Jun	3 - 5	2 - 3	3 - 4	7 - 9
Jul	3 - 4	2 - 3	1 - 3	5 - 6
Aug	1 - 3	0 - 2	2 - 3	4 - 6
Sep	3 - 4	1 - 3	1 - 3	3 - 5
Oct	3 - 5	1 - 3	3 - 4	10 - 12
Nov	2 - 4	0 - 1	1 - 3	8 - 10
Dec	3 - 4	0 - 1	1 - 3	7 - 9
1993				
Jan	1 - 3	1 - 3	0 - 1	8 - 10
Feb	4 - 5	2 - 4	1 - 2	7 - 9
Mar	1 - 3	1 - 3	2 - 3	4 - 6

* Eggs recovered from two grams of feces and counted in 100X magnification field

continued

Appendix 1 (continued)

Date	Greyhound Number 1 eggs/field *	Greyhound Number 2 eggs/field *	Greyhound Number 3 eggs/field *	Greyhound Number 4 eggs/field *
Apr	1 - 3	1 - 3	0 - 1	6 - 8
May	3 - 4	2 - 4	1 - 3	7 - 9
Jun	2 - 4	0 - 1	0 - 2	6 - 8
Jul	1 - 3	1 - 2	1 - 3	4 - 6
Aug	1 - 3	1 - 3	2 - 3	6 - 8
Sep	1 - 3	3 - 4	1 - 2	8 - 10
Oct	2 - 4	2 - 3	0 - 2	4 - 6
Nov	2 - 3	1 - 3	1 - 3	6 - 8
Dec	1 - 3	1 - 3	1 - 3	4 - 6
1994				
Jan	2 - 3	1 - 3	0 - 1	10 - 12
Feb	3 - 5	2 - 4	1 - 3	4 - 6
Mar	1 - 3	2 - 4	1 - 2	8 - 10
Apr	4 - 6	2 - 4	1 - 2	9 - 10
May	2 - 3	1 - 2	0 - 1	4 - 6
Jun	3 - 5	1 - 3	1 - 2	9 - 10
Jul	1 - 2	1 - 3	1 - 2	4 - 5
Aug	1 - 2	2 - 3	2 - 3	7 - 8
Sep	1 - 3	2 - 3	1 - 3	8 - 9
Oct	2 - 3	2 - 4	0 - 1	8 - 10
Nov	3 - 5	2 - 4	0 - 1	8 - 9
Dec	1 - 3	1 - 2	0 - 1	7 - 8
1995				
Jan	1 - 3	1 - 3	0 - 1	7 - 9
Feb	1 - 3	1 - 3	1 - 2	4 - 6
Mar	2 - 3	2 - 4	1 - 3	8 - 9
Apr	1 - 2	2 - 4	1 - 2	9 - 10
May	3 - 5	2 - 3	1 - 3	2 - 3
Jun	1 - 3	1 - 3	0 - 1	6 - 7
Jul	3 - 5	0 - 1	2 - 3	8 - 10
Aug	2 - 4	2 - 3	1 - 3	4 - 5

* Eggs recovered from two grams of feces and counted in 100X magnification field

continued

Appendix 1 (continued)

Date	Greyhound Number 1 eggs/field *	Greyhound Number 2 eggs/field *	Greyhound Number 3 eggs/field *	Greyhound Number 4 eggs/field *
Sep	1 - 3	2 - 4	1 - 3	5 - 6
Oct	1 - 3	1 - 3	2 - 3	4 - 6
Nov	2 - 4	1 - 3	0 - 1	8 - 9
Dec	3 - 4	0 - 1	1 - 2	7 - 8
1996				
Jan	3 - 5	0 - 1	0 - 1	8 - 9
Feb	1 - 3	2 - 4	3 - 4	4 - 6
Mar	2 - 3	0 - 1	0 - 1	3 - 5
Apr	1 - 3	0 - 1	1 - 2	4 - 5
May	3 - 5	0 - 1	1 - 3	6 - 8
Jun	3 - 5	2 - 4	1 - 2	6 - 7
Jul	3 - 4	3 - 5	0 - 1	7 - 8
Aug	1 - 3	1 - 3	1 - 3	5 - 6
Sep	1 - 2	0 - 1	1 - 2	3 - 5
Oct	2 - 4	1 - 2	1 - 2	4 - 6
Nov	3 - 5	2 - 4	0 - 1	4 - 5
Dec	1 - 3	1 - 3	1 - 3	7 - 8
1997				
Jan	1 - 2	0 - 1	1 - 2	7 - 8
Feb	3 - 5	2 - 3	2 - 3	8 - 9
Mar	1 - 3	0 - 1	0 - 1	3 - 4
Apr	2 - 3	2 - 3	1 - 3	6 - 8
May	2 - 3	1 - 2	1 - 2	7 - 9
Jun	3 - 4	2 - 3	2 - 3	3 - 5
Jul	2 - 3	1 - 3	1 - 3	5 - 7
Aug	1 - 3	2 - 3	0 - 1	5 - 6
Sep	3 - 4	-----	2 - 3	7 - 9
Oct	2 - 3	-----	0 - 1	6 - 8
Nov	4 - 5	-----	3 - 4	10-12
Dec	2 - 3	-----	2 - 3	-----

* Eggs recovered from two grams of feces and counted in 100X magnification field

continued

Appendix 1 (continued)

Date	Greyhound Number 1 eggs/field *	Greyhound Number 2 eggs/field *	Greyhound Number 3 eggs/field *	Greyhound Number 4 eggs/field *
1998				
Jan	3 - 4	-----	0 - 2	-----
Feb.	4 - 5	-----	0 - 2	-----
Mar.	5 - 6	-----	2 - 3	-----

* Eggs recovered from two grams of feces and counted in 100X magnification field

Appendix 2

Coprological Findings of *Capillaria* sp., *Ancylostoma* sp., *Trichuris* sp. and *Toxocara* sp. Eggs from Adult Dogs in a Kansas Greyhound Kennel (Kennel 1)

Dog # / Sex	Date	*Capillaria* eggs/field *	*Ancylostoma* eggs/field *	*Trichuris* eggs/field *	*Toxocara* eggs/field *
1 / Fe	03/29/91	6			
2 / Fe	03/29/91	10		1	
3 / Ma	03/29/91	0 - 1			
4 / Ma	03/29/91	4 - 5			
5 / Ma	03/29/91	1 - 2			
6 / Ma	03/29/91	2 - 3			
7 / Fe	03/29/91	neg			
8 / Ma	03/29/91	4 - 5			
9 / Ma	03/29/91	2 - 3			
10 / Ma	03/29/91	10 - 12			
11 / Ma	03/29/91	neg			
12 / Ma	03/29/91	neg			
13 / Ma	03/29/91	neg			
14 / Fe	03/29/91	neg			
15 / Ma	03/29/91	neg			
16 / Ma	03/29/91	0 - 1			
17 / Fe	03/29/91	neg	6	1	
18 / Ma	03/29/91	8 - 10			
19 / Ma	03/29/91	3 - 4			
20 / Ma	03/29/91	neg			
21 / Ma	03/29/91	1 - 2			
22 / Ma	03/29/91	5 - 6		1	
23 / Fe	03/29/91	6 - 8			
24 / Ma	03/29/91	neg			
25 /	03/29/91	neg	4		
26 /	03/29/91	2 - 3	3		
27 /	03/29/91	2 - 3	1		
28 /	03/29/91	neg			
29 /	03/29/91	neg	4		

* Eggs recovered from two grams of feces and counted in 100X magnification field

continued

Appendix 2 (continued)

Dog # / Sex	Date	*Capillaria* eggs/field *	*Ancylostoma* eggs/field *	*Trichuris* eggs/field *	*Toxocara* eggs/field *
30 /	03/29/91	0 - 1	1	1	
31 /	03/29/91	2 - 3		9	
32 /	03/29/91	5 - 6	4		
33 /	03/29/91	4 - 5			4
34 /	03/29/91	6 - 8			
35 /	03/29/91	0 - 1			
36 /	03/29/91	neg	1		
37 /	03/29/91	neg			
38 / Ma	04/20/91	neg			
39 / Ma	04/20/91	neg			
40 / Ma	04/20/91	neg			
41 / Fe	04/20/91	2 - 3			
42 / Fe	04/20/91	0 - 1			
43 / Fe	04/20/91	neg			
44 / Ma	04/20/91	neg			
45 / Fe	05/24/91	neg			
46 / M/F	05/24/91	neg			
47 / Fe	05/24/91	neg			
48 / Ma	06/07/91	10			
49 / Fe	06/07/91	neg			
50 / Fe	06/07/91	neg			
51 / Fe	06/07/91	0 - 1	1	1	
52 / Ma	06/07/91	neg			
53 / Fe	07/11/91	12			
54 / Ma	07/11/91	1			
55 / Ma	07/11/91	3			
56 / Ma	07/11/91	neg			
57 / Ma	07/11/91	neg			
58 / Fe	07/11/91	neg			
59 / Fe	07/11/91	neg			
60 / Ma	08/20/91	2 - 3			
61 / Ma	08/20/91	1	3 - 4		

* Eggs recovered from two grams of feces and counted in 100X magnification field

continued

Appendix 2 (continued)

Dog # / Sex	Date	*Capillaria* eggs/field *	*Ancylostoma* eggs/field *	*Trichuris* eggs/field *	*Toxocara* eggs/field *
62 / Fe	08/20/91	neg	2		
63 / Ma	08/20/91	0 - 1			
64 / Fe	08/20/91	neg			
65 / Ma	08/20/91	neg			
66 / Ma	08/20/91	2			
67 / Ma	08/20/91	neg			
68 / Ma	08/20/91	neg			
69 /	09/30/92	neg			
70 /	09/30/92	neg	3		
71 /	09/30/92	2			
72 /	09/30/92	neg	2		
73 /	09/30/92	neg			
74 /	09/30/92	neg	1		
75 /	09/30/92	3 - 4	3 - 4		

* Eggs recovered from two grams of feces and counted in 100X magnification field

Appendix 3

Coprological Findings of *Capillaria* sp., *Ancylostoma* sp., *Trichuris* sp. and *Toxocara* sp. Eggs from Adult Dogs in an Oklahoma Greyhound Kennel (Kennel 2)

Dog #	Date	*Capillaria* pos / neg *	*Ancylostoma* pos / neg *	*Trichuris* pos / neg *	*Toxocara* pos / neg *
1	02/14/91	neg			
2	02/14/91	neg			
3	02/14/91	neg			
4	02/14/91	neg			
5	02/14/91	neg			
6	02/14/91	neg			pos
7	02/14/91	neg			
8	02/14/91	neg	pos		
9	02/14/91	neg			
10	02/14/91	neg			
11	02/14/91	neg			
12	02/14/91	neg			
13	02/14/91	neg			
14	02/14/91	neg			
15	02/14/91	neg			
16	02/14/91	neg			
17	02/14/91	neg			pos
18	02/14/91	neg			
19	02/14/91	neg			
20	02/14/91	neg			
21	02/14/91	neg			pos
22	02/14/91	neg			
23	02/14/91	neg			
24	02/14/91	neg			
25	02/14/91	neg			
26	02/14/91	neg		pos	
27	02/14/91	neg			
28	02/14/91	neg			
29	02/14/91	neg			

* Eggs recovered from two grams of feces and counted in 100X magnification field

continued

Appendix 3 (continued)

Dog #	Date	*Capillaria* eggs/field *	*Ancylostoma* eggs/field *	*Trichuris* eggs/field *	*Toxocara* eggs/field *
30	02/14/91	neg			
31	02/14/91	neg			
32	02/14/91	neg			pos
33	02/14/91	neg		pos	
34	02/14/91	neg			
35	02/14/91	neg			
36	02/14/91	neg			
37	02/14/91	neg			

* Eggs recovered from two grams of feces and counted in 100X magnification field

Appendix 4

Coprological Findings of *C. boehmi* Eggs and Anthelmintic Treatments of a Blue-tick Hound During a Seven Year Period

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments, LAR Records #
06/19/80	positive	
06/19/80		De-wormed
08/10/82		Spotton
12/16/82		DNP 7.5 mls SQ
01/01/83		Nemex 30 mls orally
01/21/83		Nemex 30 mls orally
03/05/83		Strongid T
04/10/83	positive	
05/12/83		Strongid T
06/18/83	positive	
07/25/83	positive	
08/21/83	positive	
09/14/83	positive	
10/16/83	positive	
11/22/83	positive	
12/19/83	positive	
01/09/84	positive	
02/06/84	positive	
02/27/84	positive	
03/19/84	positive	
03/22/84		Nemex
03/26/84	positive	
04/03/84	positive	
04/09/84	positive	
04/30/84	positive	
05/07/84	positive	
05/07/84		Task
05/23/84	7-10 eggs/field	

* Eggs recovered from two grams of feces and counted in 100X magnification field
# Laboratory Animal Resources

continued

Appendix 4 (continued)

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments, LAR Records #
07/16/84	3-5 eggs/field	
07/23/84		Strongid T
08/16/84	0-1 eggs/field	
08/23/84	1-3 eggs/field	
08/30/84	0-1 eggs/field	
09/06/84	0-1 eggs/field	
09/20/84	0-1 eggs/field	
09/28/84	3-4 eggs/field	
10/10/84		Nemex 2
10/11/84	1-3 eggs/field	
10/18/84	1-3 eggs/field	
10/25/84	1-3 eggs/field	
11/08/84	5-7 eggs/field	
11/15/84	5-7 eggs/field	
11/30/84	1-3 eggs/field	
12/06/84	0-1 eggs/field	
12/13/84	5-7 eggs/field	
01/03/85	7-10 eggs/field	
01/04/85	7-10 eggs/field	
01/04/85		DNP 5 cc SQ
01/09/85	5-7 eggs/field	
01/10/85	5-7 eggs/field	
01/11/85	3-5 eggs/field	
01/17/85	7-10 eggs/field	
01/24/85	5-7 eggs/field	
01/31/85	3-5 eggs/field	
02/07/85	1-3 eggs/field	
02/14/85		Nemex-2 25 cc orally
02/14/85	3-5 eggs/field	
02/22/85	5-7 eggs/field	
02/28/85	0-1 eggs/field	

*Eggs recovered from two grams of feces and counted in 100X magnification field
Laboratory Animal Resources

continued

Appendix 4 (continued)

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments, LAR Records #
03/07/85	1-3 eggs/field	
03/14/85	1-3 eggs/field	
03/21/85	3-5 eggs/field	
04/04/85	1-3 eggs/field	
04/16/85	0-1 eggs/field	
07/16/84	3-5 eggs/field	
04/19/85	0-1 eggs/field	
04/25/85	0-1 eggs/field	
05/02/85	0-1 eggs/field	
05/09/85	0-1 eggs/field	
05/17/85	0-1 eggs/field	
05/30/85	0-1 eggs/field	
06/06/85	1-3 eggs/field	
06/13/85	0-1 eggs/field	
06/20/85	1-3 eggs/field	
06/20/85		Task
06/28/85	3-5 eggs/field	
07/05/85	3-4 eggs/field	
07/11/85	0-1 eggs/field	
07/19/85	0-1 eggs/field	
07/25/85	0-1 eggs/field	
08/05/85	0-1 eggs/field	
08/15/85	0-1 eggs/field	
08/22/85	0-1 eggs/field	
08/29/85	0-1 eggs/field	
09/05/85	2-4 eggs/field	
09/12/85	3-5 eggs/field	
09/19/85	1-3 eggs/field	
09/26/85	1-3 eggs/field	
10/03/85	3-5 eggs/field	
10/13/85	1-3 eggs/field	

* Eggs recovered from two grams of feces and counted in 100X magnification field
# Laboratory Animal Resources

continued

Appendix 4 (continued)

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments, LAR Records #
10/17/85	positive	
10/24/85	1-3 eggs/field	
10/31/97	5-7 eggs/field	
11/07/85	1-4 eggs/field	
11/14/85	5-7 eggs/field	
11/21/85	7-10 eggs/field	
11/27/85	0-1 eggs/field	
12/05/85	10-15 eggs/field	
12/12/85	5-7 eggs/field	
12/20/85	3-5 eggs/field	
01/09/86	3-5 eggs/field	
01/16/86	7-10 eggs/field	
01/23/86	0-1 eggs/field	
02/06/86	3-6 eggs/field	
02/13/86	0-1 eggs/field	
02/20/86	1-3 eggs/field	
02/27/86	1-3 eggs/field	
03/06/86	0-1 eggs/field	
03/13/86	0-1 eggs/field	
03/20/86	0-1 eggs/field	
04/03/86	0-1 eggs/field	
04/10/86	negative	
04/11/86	0-1 eggs/field	
04/17/86	1-3 eggs/field	
04/24/86	0-1 eggs/field	
05/01/86	3-5 eggs/field	
05/15/86	1-3 eggs/field	
05/22/86	3-5 eggs/field	
05/29/86	1-3 eggs/field	
06/06/86	1-3 eggs/field	
06/07/86		DNP 0.1 mls/10 lbs SQ

*Eggs recovered from two grams of feces and counted in 100X magnification field
Laboratory Animal Resources

continued

Appendix 4 (continued)

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments, LAR Records #
06/12/86	0-1 eggs/field	
06/26/86	0-1 eggs/field	
07/03/86	0-1 eggs/field	
07/09086	0-1 eggs/field	
07/16/86	0-1 eggs/field	
07/17/86		Nemex-2
07/24/86	0-1 eggs/field	
07/31/86	1-3 eggs/field	
08/07/86	1-3 eggs/field	
08/14/86	0-1 eggs/field	
08/21/86	3-5 eggs/field	
08/29/86	1-3 eggs/field	
09/05/86	3-5 eggs/field	
09/11/86	1-3 eggs/field	
09/18/86	0-1 eggs/field	
09/25/86	1-3 eggs/field	
10/02/86	0-1 eggs/field	
10/08/86	0-1 eggs/field	
10/16/86	3-5 eggs/field	
10/23/86	10-15 eggs/field	
10/30/86	10-15 eggs/field	
11/06/86	1-3 eggs/field	
11/13/86	5-7 eggs/field	
11/20/86	5-7 eggs/field	
11/26/86	3-5 eggs/field	
12/04/86	5-7 eggs/field	
12/11/86	5-7 eggs/field	
12/18/86	3-5 eggs/field	
01/09/87	0-1 eggs/field	
01/15/87	0-1 eggs/field	
01/22/87	0-1 eggs/field	

* Eggs recovered from two grams of feces and counted in 100X magnification field
# Laboratory Animal Resources

continued

Appendix 4 (continued)

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments LAR records #
01/29/87	0-1 eggs/field	
02/05/87	1-3 eggs/field	
02/13/87	1-3 eggs/field	
02/18/87	1-3 eggs/field	
02/19/87	0-1 eggs/field	
02/26/87	0-1 eggs/field	
03/31/87	1-3 eggs/field	
04/09/87	0-1 eggs/field	
04/16/87	3-5 eggs/field	
04/23/87	1-3 eggs/field	
04/30/87	3-5 eggs/field	
05/07/87	5-7 eggs/field	
05/11/87	5-7 eggs/field	
05/14/87	7-10 eggs/field	
05/18/87	1-3 eggs/field	
05/21/87	5-7 eggs/field	
05/25/87	3-5 eggs/field	
05/26/87	3-5 eggs/field	
05/28/87	3-5 eggs/field	
05/29/87	1-3 eggs/field	
06/01/87	3-5 eggs/field	
06/04/87	3-5 eggs/field	
06/08/87	5-7 eggs/field	
06/11/87	1-3 eggs/field	
06/22/87	3-5 eggs/field	
06/25/87	5-7 eggs/field	
06/29/87	2-4 eggs/field	
07/02/87	5-7 eggs/field	
07/06/87	1-3 eggs/field	
07/10/87	1-2 eggs/field	
07/13/87	0-1 eggs/field	

*Eggs recovered from two grams of feces and counted in 100X magnification field
Laboratory Animal Resources

continued

Appendix 4 (continued)

Date	*Capillaria* sp. Pos/Neg or eggs/field *	Anthelmintic Treatments LAR Records #
07/16/87	0-1 eggs/field	
07/30/87	negative	
08/06/87	0-1 eggs/field	
08/10/87	negative	
08/17/87	negative	
08/20/87	0-1 eggs/field	
08/24/87	negative	
09/03/87	0-1 eggs/field	
09/08/87	negative	
09/10/87	negative	
09/14/87	0-1 eggs/field	
09/17/87	2-4 eggs/field	
09/21/87	0-1 eggs/field	
09/24/87	0-1 eggs/field	
09/28/87	0-1 eggs/field	
10/01/87	1-3 eggs/field	
10/05/87	1-3 eggs/field	
10/08/87	1-3 eggs/field	
10/12/87	0-1 eggs/field	
10/15/87	1-3 eggs/field	
10/19/87	0-1 eggs/field	
10/22/87	1-3 eggs/field	
10/26/87	0-1 eggs/field	
10/29/87	3-5 eggs/field	
11/02/87	0-1 eggs/field	
11/05/87	3-5 eggs/field	
11/09/87	0-1 eggs/field	
11/16/87	0-1 eggs/field	
11/19/87	3-5 eggs/field	
11/23/87	2-4 eggs/field	
12/07/87	7-10 eggs/field	
12/22/87	5-7 eggs/field	

* Eggs recovered from two grams of feces and counted in 100X magnification field
# Laboratory Animal Resources

Appendix 5

Daily Egg Counts Following Anthelmintic Treatment with Ivermectin

Days Post Treatment	*Capillaria boehmi* Eggs/gram/feces*	*Ancylostoma* sp. Eggs/gram/feces*
0	60 epg	1188 epg
1	96 epg	2 epg
2	48 epg	0 epg
3	44 epg	0 epg
4	44 epg	0 epg
5	36 epg	0 epg
6	12 epg	0 epg
7	12 epg	0 epg
8	4 epg	0 epg
9	8 epg	0 epg
10	32 epg	0 epg
11	16 epg	0 epg
12	108 epg	0 epg
13	40 epg	0 epg
14	124 epg	0 epg

* Egg counts determined using Modified Wisconsin egg counting technique

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