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STUDIES OF THE QUANTITATION AND POPULATION DYNAMICS OF CYATHOSTOME NEMATODES OF HORSES

The Ohio State University

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STUDIES OF THE QUANTITATION AND POPULATION
DYNAMICS OF CYATHOSTOME NEMATODES OF HORSES

DISSERTATION
Presented in Partial Fulfillment of the Requirements of
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Craig Robert Reinemeyer, D.V.M.

The Ohio State University
1984

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Department of Veterinary
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DEDICATION

To Shirley
ACKNOWLEDGEMENTS

A great many people have helped me to achieve this degree, the latest in a long series of goals. The greatest contributions have come from my wife, Shirley, and daughter, Bree. They have given unselfishly of their time, love and support, and have borne the brunt on the many occasions when I brought frustration home with my briefcase. A special tribute goes to the little unborn Reinemeyer who won't appear for 5 months hence. You provided the necessary motivation to finish this task on schedule. I am eternally grateful to you all.

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Finally, I certainly would be remiss if I did not express my constant gratitude to the Great Parasitologist in the Sky for favoring me with a glance at The Blueprints.
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Ingestion of ova by cyathostome nematodes. Zeitschrift fur Parasitenkunde. 69:547-549.

The prevalence and intensity of internal parasites of horses at necropsy in the U.S.A. Veterinary Parasitology (in press).


Population dynamics of several cyathostome nematodes of horses in Ohio. Veterinary Parasitology (accepted for publication).
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GENERAL INTRODUCTION

Nematodes of the subfamily Cyathostominae, commonly known as small strongyles or cyathostomes, are the major constituents of the parasite burdens of mature horses (Ogbourne, 1978). Despite their recovery in the early 19th century (Mehlis, 1831), the Cyathostominae were not separated from the Strongylinae and recognized as a distinct taxonomic group until 1900 (Looss). Since that time, there have been several taxonomic revisions, and the development and adoption of a uniform classification system has only occurred within the past decade (Lichtenfeld, 1975). The subfamily Cyathostominae currently consists of 8 genera and 41 species world-wide, of which 6 genera and 29 species are known to occur in North America (Lichtenfeld, 1975).

Cyathostomes have been reported to be of minor pathogenicity because they are frequently present in very large numbers in clinically healthy horses (Drudge, 1972). It is difficult to ascribe specific pathologic changes to the Cyathostominae in naturally infected horses, as infection with multiple parasite groups is the rule after natural exposure. Experimental infections with cyathostomes have resulted in eosinophilia, diarrhea, delayed shedding of hair coat and persistent weight loss (Smith, 1976; 1978), anorexia, pyrexia and hemorrhagic typhlitis/colitis (Tiunov, 1951; 1953), and elevated beta globulin levels and hypoalbuminemia (Round,
1970). Subclinical infections may be responsible for failure to achieve full performance potential, and decreased feed efficiency (Ogbourne, 1978).

Mortality in horses has also been attributed to a specific syndrome associated with cyathostominosis. A clinical picture of diarrhea, weight loss and high mortality in the late winter and early spring has been reported from Britain (Blackwell, 1973; Chiejina and Mason, 1977; Jeggo and Sewell, 1977), Europe (Velichkin, 1952; Mirck, 1977) and South America (Tagle, 1948). These signs are due to the simultaneous emergence of large numbers of cyathostome larvae from the mucosa of the large bowel, with consequent physiologic changes.

Large strongyles (Strongylinae) historically have been considered more pathogenic than small strongyles (Round, 1969), and most equine worm control programs are directed against these nematodes. The success of these programs is based on the quantitation of strongyle eggs in the feces, and determination of the interval post-treatment before egg counts reach levels indicative of significant environmental contamination. Cyathostomes produce 95% of the strongyle eggs shed by horses (Levine et al., 1956; Drudge, 1978), and are therefore the primary target of worm control programs based on fecal egg counts. One of the major problems associated with the control of small strongyles is the short egg-reappearance period post-treatment. The duration of this interval is generally on the order of only 4 to 5 weeks (Herd et al., 1981), and is determined by the emergence of larvae from mucosal pools, with subsequent
reproductive activity. The short egg-reappearance period necessitates frequent anthelmintic treatment in order to avoid serious levels of environmental contamination with ova and infective larvae.

Increased frequency of anthelmintic treatment has the undesirable property of increasing drug and labor costs, and exerts strong selection pressure for the development of resistance to anthelmintics (Kelly et al., 1981). Frequency of anthelmintic application has contributed to the severity of the drug resistance problem in certain nematodes of horses and sheep, whereas resistance has not been proven in cattle, which are not treated with such intensity. Donald and Waller (1982) have stated that anthelmintic resistance is the biggest challenge to veterinary parasitology in the 1980's.

To date, 10 species of small strongyles have shown decreased susceptibility to phenothiazine and the benzimidazole carbamates in Britain (Round et al., 1974), Canada (Slocombe and Cote, 1977), Australia (Arundel, 1978; Barger and Lisle, 1979; Kelly et al., 1981) and the U.S.A. (Drudge and Lyons, 1965; Drudge et al., 1977; Wescott et al., 1982; Drudge et al., 1983). Resistance to one benzimidazole drug is unfortunate because it usually is associated with side-resistance, which is the insusceptibility of parasites to several related chemicals with a similar mode of activity (Prichard et al., 1980).

Seven benzimidazole or pro-benzimidazole anthelmintics are currently licensed for use in horses in the U.S.A., and resistance to one may be evidence of resistance to other members of this group.
The only exception is oxibendazole, which still retains good efficacy against benzimidazole-resistant populations of small strongyles (Drudge et al., 1981; Wescott, 1983).

The incomplete activity of many current anthelmintics, and the limited development of new anti-parasitic drugs with unique modes of action, necessitate the development of alternate methods of control for successful management of equine parasitism. Effective control programs must be based on thorough knowledge of the host/parasite/environment relationship. Detailed information about the population dynamics of cyathostome nematodes is deficient.

The purpose of the present research has been to study several aspects of cyathostome population dynamics in horses in Ohio. This project has investigated the prevalence and relative abundance of cyathostome nematodes throughout the year, and the seasonal patterns of their reproductive activity. Additionally, a method for quantifying larval cyathostome populations in horses was developed and compared to a standard quantitative technique for nematode larvae in other domestic species. Finally, this technique was used to determine the distribution of encysted cyathostome larvae within the tissues of the host.
CHAPTER I

THE PREVALENCE AND INTENSITY OF INTERNAL
PARASITES OF OHIO HORSES AT NECROPSY

Introduction

A comprehensive survey of gastrointestinal parasites of the horse has never been performed in North America. Published surveys have been limited to a single species or genus, such as Strongylus vulgaris (Whitlock and Leasure, 1939), Gasterophilus spp. (Schooley et al., 1971; Drudge et al., 1975; Scialdo, 1977), or small groups such as S. vulgaris and Parascaris equorum (Lyons et al., 1981) and cestodes and gastric spirurids (Lyons et al., 1983).

The prevalence and relative abundance of various species of equine parasites has been reported for control horses in anthelmintic trials, but such data is derived from small numbers of animals killed over a short period of time. Currently, the most complete survey of equine internal parasites in the U.S.A is a compilation of drug trial data assembled over several years on 779 horses from 12 different geographical regions (Hass, 1979). In that survey, as in most anthelmintic trials, the cyathostomes were not identified to species, but grouped collectively as 'small strongyles'.

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Checklists of cyathostome species known to occur in North America have been published (Becklund, 1964; Lichtenfels, 1975). The cyathostome fauna of small numbers of equines has been described in North America (Ransom, 1918; Ward, 1947) and the United Kingdom (LeRoux, 1924; Lewis, 1926; Robertson, 1939), but quantitative data necessary to determine the intensity of individual species was not collected. Qualitative and quantitative evaluation of cyathostome populations has been reported from Australia (Mfitilodze, 1982), Czechoslovakia (Barus, 1962), Panama (Foster, 1936), Scotland (Mathieson, 1964), and the United Kingdom (Ogbourne, 1976), but has not been attempted in North America. The importance of establishing such data for the U.S.A. was stressed in the Report on Research Needs of Internal Parasites of Horses by an ad hoc committee of the American Association of Veterinary Parasitologists (Klei et al., 1982).

The absence of basic descriptive data on North American equine parasites fostered the decision to enumerate and identify the worm burdens in horses in the central Ohio area.
Materials and Methods

Animals Horses were selected, on the basis of a positive fecal egg count, from equines presented for necropsy at The Ohio State University College of Veterinary Medicine from May, 1981 to July, 1982. Age, sex, breed and relevant clinical data were recorded, and an attempt was made to determine the anthelmintic history of each animal.

Parasitologic Procedures Horses were necropsied in right lateral recumbency. The abdominal cavity was carefully opened, the rectum transected, mesenteric attachments and diaphragm severed and the abdominal viscera removed en masse. The cecum and colon were ligated and opened separately, the contents removed from each, and the mucosal surface washed with water. Contents and washings of each organ were made up to a convenient volume with water and a 10% aliquot removed. Each aliquot was washed over stacked 35 and 100 mesh screens (0.5 and 0.15 mm apertures, respectively) and the material retained on the screens was preserved in 10% formalin. The entire aliquot, or in some cases a 10% subaliquot if volume was excessive, was examined with an illuminated 3X lens and all parasites removed and counted. One hundred worms were randomly selected from each aliquot and identified to developmental stage, genus and species using the key of Lichtenfels (1975). Photographs of representative cyathostome specimens were taken with an Olympus PM-6 35 mm camera using Kodak Panatomic-X film.
The aorta and major branches of the cranial mesenteric artery were examined grossly for endarteritic lesions associated with larval *Strongylus vulgaris*. The stomach and small intestine were ligated and separated. The small intestine was opened and examined grossly for parasites. The stomach was opened longitudinally and examined for lesions associated with *Draschia megastoma*. *Gasterophilus* larvae were detached from the mucosa, enumerated and speciated, and gastric contents were processed in the same manner as the contents of the large bowel, as described above. The mucosa was scraped from the glandular region of the stomach, the predilection site of gastric nematodes, and digested in 0.4% pepsin/0.725% hydrochloric acid at 37°C for 2 to 6 hours. Digestion was halted by the addition of an equal volume of 10% formalin. Ten percent aliquots of the stomach digests were examined with a dissecting microscope, and all worms were counted and speciated.

Fecal egg counts were performed by the modified McMaster technique (Whitlock, 1948). Fecal samples from each horse were cultured at 27°C for 10 days and third stage larvae recovered to determine the relative contribution of cyathostomes to the strongyle egg counts. Strongylid larvae were differentiated by the key of Russell (1948).

**Analysis** The prevalence and mean intensity of individual cyathostome species were determined. Prevalence was defined as "the number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined" (Margolis *et al.*, 1982). Mean intensity was defined as "the total number of
individuals of a particular parasite species in a sample of host species divided by the number of infected individuals of the host species in a sample" (Margolis et al., 1982).
Results

A total of 55 horses was examined from May, 1981 to July, 1982. An attempt was made to examine 4 horses per month, and this was accomplished with the exceptions of July, 1981 and June, 1982, when 1 and 2 horses were surveyed, respectively. Median age of the horses was 6 years (range 1 to 30 years) and there was a nearly equal ratio of males to females. Several horse breeds were represented, with Standardbreds, Thoroughbreds and Quarterhorses comprising the majority. Anthelmintic histories proved too incomplete to be of any value in assessing the effects of prior treatment.

Fecal egg counts ranged from 24 to 4,325 eggs per gram (epg), with a median of 328 epg. Small strongyles comprised 94.7% of the larvae recovered from fecal cultures.

Cyathostomes were recovered from all horses surveyed, and 21 small strongyle species were found. Species distribution ranged from 2 to 11 species per horse, with a median of 7 species per horse. Total cyathostome burdens ranged from 680 to 663,100, with a mean of 75,566 worms per horse, and 78.2% and 85.5% of horses had worm burdens of less than 100,000 and 200,000 worms, respectively. The colon contained 94.2% of the total lumen population of small strongyles.

The prevalence and mean intensity of individual species of cyathostomes are presented in Figure 1. Those species which were most prevalent also occurred in the highest average numbers. The 5
most prevalent species, *Cyathostomum catinatum* (Plate I), *Cylicocyclus nassatus* (Plate II), *Cylicostephanus longibursatus* (Plate III), *Cyathostomum coronatum* (Plate IV) and *Cylicostephanus goldi* (Plate V), comprised 84% of the total lumen small strongyle population. The 10 most prevalent species, consisting of the previous 5 plus *Cylicostephanus calicatus* (Plate VI), *C. minutus* (Plate VII), *Cylicocyclus leptostomus* (Plate VIII), *C. insigne* (Plate IX) and *Cyathostomum pateratum* (Plate X), comprised 98.9% of the total adult population. The remaining 11 species made up only 1.1% of the total population.

Individual cyathostome species appeared to have a predilection for site location within the host (Figure 2). Most species were more prevalent and numerous in the colon, but the prevalence of *Cyathostomum coronatum* was greater in the cecum (80%) than the colon (41%), and *Cylicostephanus poculatus* was found exclusively in the cecum.

Cyathostome fourth stage larvae (L4) were also recovered from the gut lumen, and the mean intensities determined. Most larval cyathostomes currently cannot be differentiated to species, therefore these results will be discussed under population dynamics in Chapter II.

Data on the non-cyathostome internal parasites of the horse were also recorded (Table 1). The prevalence of various species of Strongylinae was low, averaging 27% for *Strongylus vulgaris*, and 10.9% and 1.8% for *S. edentatus* and *S. equinus*, respectively. Arterial lesions associated with larval *S. vulgaris* were present in 41.8% of horses. The prevalence of cestodes, ascarids and pinworms was low, averaging 18% or less. Gastric parasites, including bots
(10.9-71%), spirurids (47-71%) and associated lesions (40%) were relatively common. Digestion of gastric mucosa revealed Habronema muscae and Draschia megastoma, and in every case, digests recovered the same species found in the contents. Setaria equina was found in small numbers in the peritoneal cavity of one animal. Strongyloides westeri, Habronema majus, Probstmayria vivipara, Anoplocephala magna and Paranoplocephala mammillana were not found.
Discussion

The results show that a small number of species comprise the majority of the total cyathostome population and that the most prevalent species also occurred in the highest average numbers. This finding is consistent with the conclusions of Ogbourne (1978) and with survey results from Panama (Foster, 1936), Czechoslovakia (Barus, 1962), Australia (Mfitilodze, 1982), and Britain (Mathieson, 1964; Ogbourne, 1976). This was a conclusive finding in spite of the fact that the 55 horses examined originated from 54 different farms with various systems of housing, management and anthelmintic exposure. These factors could be expected to give marked variability to the parasite populations examined.

The species comprising the 10 most prevalent cyathostomes in this survey are in close agreement with previous reports. Although the relative ranking varied among surveys, the composition of the 10 major species demonstrated marked similarities. Accordingly, Ogbourne (1976) listed the same 10 major cyathostomes as found in the present report. Foster (1936), Barus (1962) and Mathieson (1964) each included 8 of the same species. The disparate species were a non-cyathostome, Strongylus vulgaris, and Cyathostomum labiatum (Foster, 1936), C. labiatum and Gyalocephalus capitatus (Mathieson, 1964), and G. capitatus and Cylicodontophorus bicoronatus (Barus, 1962). Mfitilodze (1982) reported 9 of the species listed in the
present report and differed only with the inclusion of **Cyllicocyclus radiatus** among the 10 most prevalent cyathostomes in Australia.

The prevalence ranking of the 10 most common cyathostome species worldwide was compiled from the present report and the papers cited above (Table 2). As determined by the median prevalence ranking in these 6 studies, **Cyathostomum catinatum**, **Cyllicocyclus nassatus**, **Cyllicostephanus longibursatus**, **Cyathostomum coronatum** and **Cyllicostephanus calicatus** are the 5 most prevalent species, and **Cyllicostephanus goldi**, **C. minutus**, **Cyllicocyclus leptostomus**, **C. insignis** and **Cyathostomum pateratum** complete the top 10. The strong similarities in these surveys demonstrate the homogeneity of cyathostome populations in different areas of the world over the past 45 years.

Populations of strongyles resistant to phenothiazine were reported in the United States in 1961 (Drudge and Elam), and resistance of small strongyles to thiabendazole was documented in 1965 (Drudge and Lyons). Since that time, resistance to benzimidazoles has been reported in strongyles of horses in Britain (Round et al., 1974), Canada (Slocombe and Cote, 1977), and Australia (Arundel, 1978; Barger and Lisle, 1979; Kelly et al., 1981). Ten species of small strongyles have been reported to be resistant in the U.S.A.: **Cyllicocyclus nassatus**, **C. insignis**, **Cyllicostephanus goldi**, **C. longibursatus**, **Cyathostomum catinatum**, **C. coronatum** and **C. labiatum** (Drudge et al., 1977), **Cyllicocyclus leptostomus** and **C. brevicapsulatus** (Wescott et al., 1982), and **Cyllicostephanus minutus** (Drudge et
Eight of these resistant species are among the 10 most prevalent, as listed above.

The relative prevalence of cyathostome species in surveys conducted prior to the development of phenothiazine (Foster, 1936) and the widespread use of thiabendazole (Barus, 1962; Mathieson, 1964) is similar to that recorded in recent surveys. This suggests that the prevalence of most species has been altered minimally by the advent and utilization of modern equine anthelmintics, and has not changed in the face of anthelmintic resistance.

Anthelmintic resistance appears to have a greater influence, however, on the mean intensity of cyathostome species. *Cyllocyclus leptostomus*, which was recently shown to be resistant to fenbendazole (Wascott et al., 1982) is a case in point. It was ranked 16th in prevalence and 14th in intensity by Foster (1936), 10th in prevalence by Barus (1962), and was not found at all by Mathieson (1964). Ogbourne (1976) listed *C. leptostomus* as the 8th most prevalent small strongyle, and as having the 7th highest mean intensity. In the present survey, *C. leptostomus* was still 8th in prevalence, but was elevated to the third highest mean intensity. This pattern suggests a change from relative obscurity of *C. leptostomus* in the pre-benzimidazole era to a position of significance after several years of benzimidazole availability.

The remaining species of small strongyles that fall outside the top 10 comprised only 1.1% of the total adult population and are therefore of little practical importance. Their total numbers within a host may be limited by factors such as specific nutritional
requirements (Crompton, 1973) or low fecundity. Of the minor species, anthelmintic resistance has been demonstrated in Cyathostomum labiatum (Drudge et al., 1977) and Cylicocyclus brevicapsulatus (Wescott et al., 1982), but it is dubious whether these species would ever establish themselves in significant numbers in a mixed population of small strongyles.

Although no distinction was made between the regions of the colon in the present study, Ogbourne (1976) examined the site preference of various cyathostome species for the cecum, ventral colon or dorsal colon. Each species demonstrated a distinct preference for location within one region of the large bowel. There was a similar numerical distribution between the ventral and dorsal colon, but only 2 species, Cyathostomum coronatum and Cylicostephanus poculatus demonstrated site preference for the cecum. The latter species was found exclusively in the cecum. C. coronatum and C. poculatus had a similar distribution in the present study (Figure 2). The determinants of site preference by cyathostomes remain largely unknown, but physiochemical conditions in the gut lumen, and specificity of feeding habits have been suggested as possible mechanisms (Ogbourne, 1976).

The results of this survey are very similar to those of Ogbourne in the United Kingdom (1976). Twenty-one species of Cyathostominae were observed in each study, although horses in the British survey had larger worm burdens and were infected with more species on the average. Great variability in worm numbers was seen in both studies, with at least a hundred-fold difference between the lowest and
The highest burdens. Ranges of 680-663,100 (present study) and 12,000-1,239,000 (Ogbourne, 1976) worms per horse were recorded. These differences may be attributable to variations in anthelmintic treatment or exposure to infective larvae.

The cecum was shown to be a minor source of adult cyathostomes, containing only 5.8% of the total lumen population. This concurs with the results of Ogbourne (1976) who recovered only 5% of total small strongyle burdens from the cecum. In his review of the Cyathostominae, Ogbourne (1978) states that less than 10% of cyathostomes normally reside in the cecum.

Cyathostomes comprised 94.7% of strongyle larvae derived by fecal culture. This is consistent with other publications that credit 95% of the equine strongyle egg output to the Cyathostominae (Levine et al., 1956; Drudge, 1978). Thus, small strongyles are the major target of control programs based on reduction of fecal egg counts.

The prevalence of non-cyathostome parasites recorded in Table 1 is similar to most previous reports, except that adult *Strongylus vulgaris* were present in only 27% of horses, compared to 100% (Foster and Ortiz, 1937; Mathieson, 1964), 84% (Whitlock and Leasure, 1939), 71.4% (Barus, 1962), and 85.4% (Slocombe and McGraw, 1973). However, a recent report from Kentucky (Lyons et al., 1981) showed only 39% of horses to be infected with *S. vulgaris*. In the present study, 41.8% of horses had arterial lesions due to *S. vulgaris*. This is similar to a report of 40% (Lyons et al., 1981) but less common than reports of 79% (Foster and Clark, 1937), 95.4% (Ottaway and Bingham, 1946),
93% (Poynter, 1960) and 64.4% (Ogbourne, 1975) of horses with arterial lesions. The decreased prevalence in recent North American reports may be due in part to the frequent, repeated use of benzimidazole anthelmintics in the United States. Resistance has drastically reduced the efficacy of these drugs against the cyathostomes, but benzimidazole resistance has only recently been observed in large strongyles (T.R. Klei, personal communication). Therefore, the decreased prevalence of Strongylus vulgaris in the U.S. may be due partially to the selective spectrum of the benzimidazoles in the face of resistance.

*Habronema muscae* was the most common gastric nematode found. It was present in 71% of horses examined and averaged over 500 worms per infection. *H. muscae* was also shown to be the dominant gastric spirurid (74%) by Foster and Ortiz (1937), but burdens averaged only 21 worms per horse. *H. muscae* was also present in 95.8% of equine stomachs examined in Morocco (Pandey et al., 1981). *Draschia megastoma*, was present in 47% of horses examined and frequently was associated with fibrous nodules near the margo plicatus. In contrast to this survey, *D. megastoma* was found in very low numbers in only 8% of horses examined in Panama (Foster and Ortiz, 1937), and was not seen in any of 94 horses examined in Morocco (Pandey et al., 1981). Hass (1979) reported the prevalence of *H. muscae* and *D. megastoma* under the combined designation "stomach worms" as 59% in adult horses from various regions of the United States. *Habronema majus* was not observed in the present study, or by Hass (1979), although it had a
prevalence of 75.6% and 62% in Morocco (Pandey et al., 1981) and Panama (Foster and Ortiz, 1937), respectively.

Trichostrongylus axei was not observed in this study although Herd et al. (1981) reported finding T. axei larvae in 14% of 188 positive fecal cultures from two horse farms in Ohio, and T. axei accounted for 100% of the larvae in 6% of the cultures. T. axei has a reported prevalence of 80.9% in Morocco (Pandey et al., 1981).

Gasterophilus intestinalis was the most common bot, being present in 71% of the stomachs examined. Other North American surveys have also demonstrated a high prevalence: 100% (Schooley et al., 1971), 98.7% (Drudge et al., 1975), 99% (Hass, 1979) and 89.5% (Scialdo, 1977). G. nasalis was found in only 10% of horses. This figure is lower than previously reported values of 65% (Schooley et al., 1971), 80.7% (Drudge et al., 1975), 62% (Hass, 1979) and 65.5% (Scialdo, 1977). Two larvae of Gasterophilus hemorroidalis were found in one horse. This parasite has been reported to be uncommon in the United States (Hass, 1979).

The low prevalences of Anoplocephala perfoliata (18%), Parascaris equorum (18%) and Oxyuris equi (10.9%) were probably related to the mature age of the horses examined. Cestodes (Dunn, 1978; Bello, 1979), Parascaris equorum and Oxyuris equi (Drudge and Lyons, 1977) have been reported more frequently from young animals, and acquired resistance to P. equorum usually develops before the second year of life (Drudge, 1972). The prevalence of ascarids in this survey is lower than the 39% reported from Kentucky Thorough-
breds (Lyons et al., 1981) but the Kentucky horses had a lower mean age and were less likely to have developed immunity to *P. equorum*.

*Strongyloides westeri* was not observed, but no attempt was made to examine the contents or mucosa of the small intestine microscopically. In addition, foals become resistant to *S. westeri* after 6 months of age (Drudge, 1972), and the youngest horse in this study was a yearling. *Probstmayria vivipara* was likewise not seen, but this nematode is very small (Dunn, 1978) and may have passed through the 0.15 mm aperture screen.
Summary

Fifty-five adult horses were necropsied over a 15 month period, and the prevalence and mean intensity of their parasitic fauna determined. Twenty-one species of Cyathostominae were recovered, and species distribution ranged from 2 to 11 species/horse (median 7 species/horse). Total cyathostome burdens ranged from 680-663,100, with a mean of 75,566 worms/horse. The colon contained 94.2% of the total lumen population of cyathostomes.

Ten cyathostome species, Cyathostomum catinatum, Cylicocyclus nassatus, Cylicostephanus longibursatus, Cyathostomum coronatum, Cylicostephanus goldi, C. calicatus, C. minutus, Cylicocyclus leptostomus, C. insigne and Cyathostomum pateratum, comprised 98.9% of the total cyathostome burdens. The remaining 11 species made up only 1.1% of the total population. Most species were more prevalent and numerous in the colon, but C. coronatum was recovered more commonly from the cecum (80%) than the colon (41%), and C. poculatus was found exclusively in the cecum. The results of this survey indicate that the composition of the cyathostome populations has remained fairly stable for over 45 years, and has been altered minimally by the advent and utilization of modern equine anthelminitics.

The prevalences of Strongylus edentatus (10.9%) and S. equinus (1.8%) were low, and the occurrence of S. vulgaris adults (29%) and arterial lesions (41%) were less common than previously reported.
Gastric parasites including Habronema muscae (71%), Draschia megastoma (47%) and associated lesions (40%), and Gasterophilus intestinalis (71%) were common. The low prevalences of Parascaris equorum (18%), Oxyurus equi (10.9%) and Anoplocephala perfoliata (18%) were attributed to the mature age of the horses examined.
Table 1. Prevalence and mean intensity of non-cyathostome internal parasites of Ohio horses at necropsy, 1981-1982.

<table>
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<tr>
<th>Parasite</th>
<th>Prevalence</th>
<th>Mean Intensity</th>
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<tbody>
<tr>
<td>Strongylus vulgaris</td>
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<td>91</td>
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<tr>
<td>Strongylus edentatus</td>
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<td>5</td>
</tr>
<tr>
<td>Strongylus equinus</td>
<td>1.8</td>
<td>600</td>
</tr>
<tr>
<td>Craterostomum acuticaudatum</td>
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<td>1744</td>
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<tr>
<td>Triodontophorus serratus</td>
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<td>20</td>
</tr>
<tr>
<td>Triodontophorus minor</td>
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<tr>
<td>Habronema muscae</td>
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<td>504</td>
</tr>
<tr>
<td>Draschia megastoma</td>
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<td>155</td>
</tr>
<tr>
<td>Anoplocephala perfoliata</td>
<td>18</td>
<td>147</td>
</tr>
<tr>
<td>Gasterophilus intestinalis</td>
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<td>119</td>
</tr>
<tr>
<td>Gasterophilus nasalis</td>
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<td>14.5</td>
</tr>
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<td>Oxyuris equi</td>
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<tr>
<td>Parascaris equorum</td>
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<td>167</td>
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<tr>
<td>Draschia lesions</td>
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</tr>
<tr>
<td>Strongylus vulgaris lesions</td>
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Table 2. Summary of prevalence rankings of major cyathostome species compiled from 6 international surveys

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<td>C. catinatum</td>
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<td>C. longibursatus</td>
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<td>2.17</td>
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<td>C. coronatum</td>
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<td>6</td>
<td>5</td>
<td>5</td>
<td>4</td>
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<td>6</td>
<td>9</td>
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<td>C. goldi</td>
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<td>C. insigne</td>
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<td>7</td>
<td>9</td>
<td>8.0</td>
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<td>6</td>
<td>8</td>
<td>8.0</td>
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<td>C. pateratum</td>
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<td>16</td>
<td>17</td>
<td>9</td>
<td>4</td>
<td>10</td>
<td>10.0</td>
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</tr>
<tr>
<td>C. labiatum</td>
<td>10</td>
<td>10</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>G. capitus</td>
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<td>C. bicoronatus</td>
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<td></td>
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<tr>
<td>C. radiatus</td>
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</table>
Figure 1. Prevalence and mean intensity of cyathostome species from Ohio horses at necropsy, 1981-1982.
Figure 1
Figure 2. Site distribution of adult cyathostomes in the lumen of the large intestine of Ohio horses at necropsy, 1981-1982. Species numbered as in Figure 1.
Figure 2

**MEAN INTENSITY (THOUSANDS)**

- COLON
- CECUM

* PREFERRED SITE
Plate I. Buccal capsule of *Cyathostomum catinatum*. 960X.
Plate II. Buccal capsule of *Cylicocyclus nassatus*. 960X.
Plate III. Buccal capsule of *Cylicostephanus longibursatus*. 960X.
Plate IV. Buccal capsule of *Cyathostomum coronatum*. 960X.
Plate V. Buccal capsule of *Cyllicostephanus goldi*. 960X.
Plate VI. Buccal capsule of *Cyclicostephanus calicatus*. 960X.
Plate VII. Buccal capsule of *Cylicostephanus minutus*. 960X.
Plate VIII. Buccal capsule of *Cylincyclus leptostomus*. 960X.
Plate IX. Buccal capsule of Cylicocyclus insignis. 960X.
Plate X. Buccal capsule of *Cyathostomum pateratum*. 960X.
CHAPTER II

POPULATION DYNAMICS OF FIVE CYATHOSTOME NEMATODES OF HORSES IN OHIO

Introduction

Elucidation of the dynamics of parasite populations is crucial to the development of successful control programs. The seasonal patterns of larval infection, adult recruitment, and reproductive activity are the major determinants of acquisition and perpetuation of parasite populations. Demonstration of the patterns of these events will delineate which may be exploited most effectively by strategic control. The development of strategic equine parasite control programs, especially those which rely on minimal anthelmintic treatment, has timely significance in light of the increasing importance of the anthelmintic resistance problem in small strongyles of horses (Hard et al., 1981; Bennett, 1983).

The epidemiology of equine cyathostomes has been the subject of relatively few research efforts for a variety of reasons. Prospective investigators have balked at the sheer volume of ingesta to be examined in the equine (Becklund, 1964), cyathostomes are very host-specific and laboratory infections in alternate hosts have been unsuccessful (Ogbourne, 1978), and small strongyles erroneously have
been assumed to be non-pathogenic, and therefore unimportant, by practitioners and parasitologists alike. Investigation of the population dynamics of equine cyathostomes presents many inherent difficulties: natural infections usually involve multiple species (Ogbourne, 1976, see Chapter I), cyathostome ova and infective larvae cannot be differentiated to genus and species (Ogbourne, 1975), and identification of fourth stage larvae to species is not completely defined (Rupasinghe, 1975; Ogbourne, 1976). Due to these limitations, most knowledge about cyathostome epidemiology consists of easily measured parameters (e.g., fecal egg counts) or data which can be determined readily within a laboratory setting, such as studies on larval bionomics and environmental development.

Currently, the only method of monitoring the population dynamics of individual cyathostome species in a mixed natural infection involves using seasonal changes in reproductive status of female worms as a marker for the age structure of the populations (Ogbourne, 1975). Ogbourne monitored the reproductive status of female cyathostomes recovered from horses slaughtered over a 16 month period in Great Britain. He utilized this data to describe the course of natural infections with several small strongyle species. It was concluded that Cylicocyclus nassatus develops quickly to the adult stage after infection, whereas Cylicostephanus goldi, Cylicostephanus longibursatus, and Cyathostomum catinatum undergo arrested development and do not mature until the following spring.

The present study describes monthly variations in adult and larval cyathostome burdens as well as seasonal changes in the
reproductive status of female small strongyles observed during a prevalence study of internal parasites of horses at necropsy in Ohio (see Chapter I). The population dynamics of 5 highly prevalent cyathostome species are proposed on the basis of these observations.
Materials and Methods

Fifty-five horses of various breeds were necropsied at The Ohio State University College of Veterinary Medicine from May, 1981 to July, 1982. Four horses were examined monthly with the exceptions of July, 1981 and June, 1982, when 1 and 2 horses were necropsied, respectively. Selection of horses and necropsy procedures have been described in Chapter I.

The cecum and colon were ligated and opened separately, the contents removed from each, and the mucosal surface washed with water. Contents and washings of each organ were made up to a convenient volume with water and a 10% aliquot removed. Each aliquot was washed over stacked 35 and 100 mesh screens (0.5 and 0.15 mm apertures, respectively) and the material retained on the screens was preserved in 10% formalin. The entire aliquot, or in some cases a 10% subaliquot if volume was excessive, was examined with an illuminated 3X lens and all parasites removed and counted. Total worm counts of individual animals were calculated by summation of the cecal and colonic burdens.

One hundred cyathostomes were selected randomly from aliquots of cecal and colonic contents, identified to stage of development, sex, genus and species using the key of Lichtenfels (1975). Adult female worms were further classified as immature, gravid or spent according to the criteria of Ogbourne (1975): "Immature worms were relatively small individuals that had not yet commenced reproduction; gravid
worms were fully reproductive, with numerous eggs in the uteri; spent worms were those that apparently had reached the end of their reproductive life and were about to die; they contained no eggs except perhaps a few in the ejaculatory duct on the way down to the vulva."

The proportion of female worms belonging to each of these three reproductive categories was individually recorded for Cyathostomum catinatum, Cyathostomum coronatum, Cylicocyclus nassatus, Cylicostephanus goldi and Cylicostephanus longibursatus, and monthly means were calculated. Representative specimens of immature, gravid and spent females were photographed with an Olympus PM-6 35 mm camera using Kodak Panatomic-X film (Plates XI-XIII).

After collection of the cecal contents, the mucosal surface was rinsed with water until all adherent particles of ingesta were removed. The mucosa and submucosa of the entire cecum were scraped from the underlying tissues with a glass microscope slide. Mucosal scrapings were digested in a 0.4% pepsin/0.725% hydrochloric acid solution at 37°C for 2-6 hours. Digestion was halted by the addition of 1/5 total volume of 37% formaldehyde. Mucosal digests were either concentrated by sedimentation or washed over a 100 mesh screen (0.15 mm aperture) prior to examination of a 10% aliquot with a 10X dissecting microscope. Total numbers of encysted larvae in the cecal wall were calculated and recorded.
Results

Cyathostomes were recovered from all horses. Total worm burdens ranged from 680 to 663,100 with a mean of 75,566 small strongyles per horse. *Cyathostomum catinatum*, *Cyllcoclyclus nassatus*, *Cylcostephanus longibursatus*, *Cyathostomum coronatum* and *Cylcostephanus goldi* were, in order, the 5 most prevalent species, and comprised 84% of the total adult small strongyle population.

Mean monthly numbers of adult cyathostomes are represented in Figure 3. Monthly means showed a bimodal distribution, with peaks in March and September of 221,300 and 232,551 adult cyathostomes, respectively. Both peaks dropped off rapidly after achieving maximal numbers. The lowest mean worm burdens occurred from November to January, with a monthly average of only 20,190 cyathostomes. Worm burdens were similar during May of 1981 (24,025) and May, 1982 (22,322), but were markedly different in July, 1981 (94,847) compared to July, 1982 (18,404).

Monthly variations in mean numbers of cyathostome fourth stage larvae (L4) in the cecocolic lumen, and mean numbers of 3rd and 4th stage larvae in the cecal mucosa, as determined by tissue digestion, are represented in Figure 4. Lumen larvae were present in greatest numbers in February, March and October, with mean burdens of 6,748, 7,247 and 5,667 worms, respectively. Mucosal larval burdens were extremely variable, ranging from zero to 19,870 per horse, but also exhibited peaks in February, April and October of 5,568, 4,980 and
4,095 larvae, respectively. Changes in mucosal larval numbers were frequently associated with similar variations in the lumen L4 although the two populations alternated in dominance. This pattern was disrupted during the last 2 months of the survey.

Monthly variations in the reproductive status of females of the 5 most prevalent cyathostome species are shown in Figures 5-9. Although there were marked differences between species during certain months, seasonal trends were similar for all species. Immature females were most common from late winter to spring (March to May) and persisted in moderate numbers through summer. Gravid females predominated in late spring and summer (June to September), and spent females prevailed in autumn and winter (October to February).
Discussion

The results demonstrate seasonal patterns of emergence, maturation and decline of larval and adult cyathostome populations. Integration of these separate elements permits a logical scheme of annual events in the population dynamics of the Cyathostominae to be proposed.

The peaks of adult cyathostomes observed in spring (March) and autumn (September) (Figure 3) are comparable to those seen in May and November in a similar study in Great Britain (Ogbourne, 1976). Both spring and fall peaks in the present study occurred 2 months earlier than the respective peak in Britain. Nevertheless, this suggests recurrent, seasonal trends in the magnitude of adult small strongyle populations despite wide geographical separation. The source and significance of these peaks will be discussed later.

The low numbers of adult cyathostomes observed in May, 1981 and May, 1982 are comparable to the low worm burdens reported in July in Britain (Ogbourne 1976) if the pattern of a two month delay holds true. These similar results would indicate a loss of adult small strongyles after the initial peak in spring.

The marked variability of worm burdens in July, 1981 compared to July, 1982 could be due to the small sample size (1 horse in July, 1981), or differences in anthelmintic treatment, management at the farm of origin, or seasonal climatic effects on availability of infective larvae.
Mean numbers of cyathostome fourth stage larvae (L4) in the cecocolic lumen and cecal mucosa (Figure 4) exhibited peaks in spring (February-April) and autumn (October). Although absolute numbers were rather small, the spring larval peak (6,748 worms) began one month (February) earlier than the adult peak (March). The larval peak in autumn (October), however, occurred one month after that of the adults (September). Ogbourne (1976) reported a spring peak of lumen larvae one month (April) prior to a peak of adults (May), but concurrent larval and adult peaks in autumn (November). It is presumed that larvae develop to adults in both countries in spring, but the fate of the autumn larval population is unknown. Since both lumen adult and larval numbers are low through winter, perhaps both are expelled in autumn by a mechanism similar to "self-cure" in other parasitisms. This may be related to an immunostimulatory effect of acquisition of infective L3, which are available in high numbers on autumn pasture (Mirck, 1981; Craig et al., 1983; Eysker et al., 1983).

Lumen fourth stage larvae (L4) attained a maximum of only 12% of the entire worm burden in the present study, whereas Ogbourne (1976) frequently observed larval proportions in excess of 50% in spring and summer. Differences in parasite exposure or ante-mortem management could account for these discrepancies, and it has already been noted (Chapter I) that Ohio horses had lower worm burdens than those in the British study (Ogbourne, 1976). Additionally, some larvae may have been lost through the 0.15 mm aperture of the 100 mesh screen used for washing gut contents. Rupasinghe (1975) measured fourth stage
cyathostome larvae from the gut lumen, and reported body widths from 0.07 to 0.15 mm.

Another possible explanation for lower larval and adult numbers lies in the medical history of the experimental horses. Animals in the British study were obtained from a commercial slaughter house and were assumed to be in good health, whereas horses in the present work frequently were ill and/or hospitalized for several days prior to death. Horses have been reported to spontaneously expel up to 34% of their small strongyle burdens due to stress, physiologic alterations of the host, immune mechanisms or age of the parasitic infection (Colglazier, 1979).

Numbers and patterns of mucosal L4 shown in Figure 4 should be regarded cautiously. The dynamics of encysted larval cyathostomes have not been investigated thoroughly by the present study because populations of L4 within the colonic mucosa were not evaluated. Representative sampling of the mucosa of the colon presents many practical difficulties because of its great volume. The colon has been shown to be an important source of cyathostome larvae (Tiunov, 1951; Mathieson, 1964; Ludwig, 1982). In addition, digestion of cecal mucosa with pepsin/hydrochloric acid may destroy encysted larvae and yield artificially low numbers. It has been suggested that cyathostome larvae, acclimated to the neutral pH of the cecum and colon, are disrupted by acid during digestion, and subsequently are lost (Mathieson, 1964). Data to be presented in Chapter III will verify that quantitative loss of encysted cyathostome larvae occurs during mucosal digestion.
Figures 5-9 demonstrate that each reproductive category of female small strongyles was dominant during only one period per year, and that these periods were similar for the 5 species examined. This suggests that the most common Cyathostominae produce only one generation annually, and tend to have comparable seasonal patterns of reproductive activity. These findings concur with Ogbourne (1975) for Cylicostephanus goldi, C. longibursatus and Cyathostomum catinatum. Ogbourne (1975) suggested that Cylicocyclus nassatus reproduced over a short time span, with maximum activity in late summer. This contention was not supported by the present work, and Figure 7 demonstrates a pattern of maturation for C. nassatus which is similar to the other species examined.

In all 5 species, immature females were most common in late winter and early spring (March to May), a period of time when many horses in Ohio are housed, and there is likely to be decreasing availability of infective larvae (L3) on pasture. Therefore, it is suspected that these immature adults were derived from L4 that had remained hypobiotic within the host during winter. A similar pattern of winter arrested development has been reported for several economically important nematodes, including Haemonchus contortus in sheep and Ostertagia ostertagi in cattle (Michel, 1974).

Immature cyathostomes were consistently present in low to moderate numbers in summer, and subsequently matured to supplement the gravid population. The immature adults could have been derived from 3 possible sources. The first is a population of larvae that had persisted in hypobiosis since the previous grazing season.
Prolonged prepatent periods of up to 2 years, strongly suggestive of arrested development, have been observed by Gibson (1953) and Smith (1976) after repeated treatment of housed horses with phenothiazine and thiabendazole, respectively. The second possible source of immature worms is larvae that were ingested recently and became patent within a short time. Indeed, prepatent periods of 5 to 8 weeks have been reported in helminth-naive foals upon primary infection with cyathostomes (Russell, 1948; Tiunov, 1951; Round, 1969). The third source is larvae ingested earlier in the grazing season which manifested a longer prepatent period as a consequence of host immunity, age resistance or differences in parasite species. Prepatent periods of approximately 18 weeks have been demonstrated in mature, worm-free ponies with prior sensitization to small strongyles (Smith, 1978). All 3 mechanisms for the presence of immature adult cyathostomes during summer may possibly co-exist simultaneously within a worm species in the same host animal.

Gravid worms were most common from late spring to early autumn (June to September), and maintained high levels of egg production throughout the grazing season (May to October). Gravid worms apparently were long-lived because there was no increase in spent females until October, after which senescent adults predominated through autumn and winter (October to February). The spent worms presumably died and were replaced by a succeeding generation of larvae and immature worms in the following spring.

The separate concepts discussed above may be combined to describe the annual population dynamics of cyathostome nematodes.
The spring peak of larval worms is derived from hypobiotic larvae acquired during the previous grazing season. This larval population matures into the peak of adults observed in March, and these adults are the source of the spring rise in fecal egg counts seen in April and May in the northern United States (Todd et al., 1949; Herd, 1984). Gravid cyathostomes persist through the summer and their numbers are supplemented from a modest pool of immature worms which are also present. The gravid adults produce a summer rise in fecal egg counts, which has been reported in August/September in northern U.S. (Herd, 1984). Susceptible horses grazing contaminated pastures in summer may develop patent infections in 5 to 8 weeks, but hypobiosis or delayed patency may also occur in sensitized animals. There is an apparent loss of adults and lumen larvae in the autumn, which is suggestive of a "self-cure" reaction. Overwintering worm burdens in the host consist primarily of hypobiotic larvae in the mucosa and senescent worms in the lumen. In late winter and early spring, spent adults are eliminated and larvae emerge from arrested development. This basic cycle probably is repeated annually, with some modifications introduced by environmental, immunologic and/or management factors.
Summary

Variations in the magnitude of adult and larval cyathostome burdens were observed at monthly intervals in 55 horses necropsied over a 15 month period in the northern U.S.A. There was a bimodal distribution of both adult and larval populations. Peak numbers of adult cyathostomes occurred in spring (March) and autumn (September). Larval cyathostomes attained peak numbers in spring (February-April) and autumn (October), beginning one month earlier than the adult spring peak and one month after the adult autumn peak, respectively.

The reproductive status of females of Cyathostomum cattinatum, Cyathostomum coronatum, Cylicocyclus nassatus, Cylicostephanus goldi and Cylicostephanus longibursatus was classified as immature, gravid or spent, and seasonal changes in these classifications were monitored as a marker for the age structure of these populations. Each reproductive category of female small strongyle was dominant during only one period per year, suggesting that the most common Cyathostominae produce only one generation annually. Immature cyathostomes were most common from late winter to spring (March to May), gravid worms predominated through summer (June to September), and spent worms were most common in winter (October to February).

These observations suggest a logical scheme of the annual population dynamics of cyathostome nematodes. The spring peak of larval worms (L4) is derived from hypobiotic larvae acquired during the previous grazing season, and these larvae mature to form the
adult peak in spring. Gravid worms are predominant through summer and are supplemented by a small population of immature worms. This ensures active reproduction and high egg production through the grazing season. Some adults and lumen larvae apparently are lost in the autumn, which is suggestive of a "self cure" reaction triggered by the ingestion of L3. Overwintering worm burdens in the host consist primarily of hypobiotic larvae in the mucosa and senescent adults in the lumen. Spent adults are eliminated in late winter and early spring. The annual cycle recurs as a new population of larvae emerges from arrested development.
Figure 3. Seasonal variation in mean numbers of adult cyathostomes in Ohio horses at necropsy.
Figure 4. Seasonal variations in mean numbers of cyathostome fourth stage larvae in gut lumen and cecal mucosa.
Figure 4

LUMEN (●●●)
MUCOSA (○○○)
Figure 5. Seasonal variations in reproductive status of female *Cyathostomum catinatum*. 
Figure 5
Figure 6. Seasonal variations in reproductive status of female *Cyathostomum coronatum.*
Figure 6
Figure 7. Seasonal variations in reproductive status of female *Cylicocyclus nassatus*.
Figure 7
Figure 8. Seasonal variations in reproductive status of female *Cylicostephanus goldi*.
Figure 8
Figure 9. Seasonal variations in reproductive status of female *Cyclostephanus longibursatus*.
Figure 9

IMMATURE

GRAVID (● - ●)

SPENT (○ - ○)

MAY  J  JA  SO  ND  FM  AM  MAY  J  J

81 82
Plate XI. Immature female cyathostome exhibiting rudimentary reproductive tract. 960X.
Plate XII. Gravid female cyathostome exhibiting numerous ova in mature reproductive tract. 960X.
Plate XIII. Spent female cyathostome exhibiting one ova near ovjectors in terminal reproductive tract. 960X.
CHAPTER III

COMPARISON OF TECHNIQUES FOR QUANTITATION
OF ENCYSTED CYATHOSTOME LARVAE IN THE HORSE

Introduction

Detailed investigation of the dynamics of larval parasite populations has demonstrated such complex behavior as prenatal infection (Dunn, 1978) and arrested development (Michel, 1974) in a variety of hosts. Virtually no effort has been made to investigate the population dynamics of encysted cyathostome larvae of the horse, primarily because no standard technique has been devised for quantitation of larvae in tissue. Mucosal larval numbers in the horse have been estimated only in a few anthelmintic trials which evaluated larvicidal efficacies (Duncan and Reid, 1978; Duncan et al., 1977; Duncan et al., 1980; Kingsbury and Reid, 1981; Lyons et al., 1983; Hopfer et al., 1984). South African investigators have quantified larvae in small numbers of assorted equids (Reinecke and Brooker, 1972; Reinecke and LeRoux, 1972; Malan et al., 1981; Scialdo et al., 1982; Scialdo-Krecek, 1983).

Most tissue larval determinations in the horse have utilized a pepsin/hydrochloric acid digestion technique developed to quantify nematodes in the abomasal mucosa of ruminants (Herlich, 1956; Ciordia
et al., 1957; McKenna, 1976). This technique is a labor-saving device but may result in artificially low rates of larval recovery (Kendall et al., 1969). Mucosal digestion techniques in the horse have been criticized for low larval yield and damaged worms (see Chapter II; Mathieson, 1964; Reinecke and LeRoux, 1972). It has been suggested that cyathostome larvae, acclimated to the neutral pH of the cecum and colon, are destroyed by the acidity of the pepsin/HCl mixture used for tissue digestion (Mathieson, 1964).

Mathieson (1964) attempted to quantify tissue larval burdens in the cecum and colon by direct observation of 4 cm² areas of gut wall with a magnifying lens. He was unable, however, to estimate larval numbers because the total surface area of the large bowel cannot be determined. The cecum and colon of the horse are plicated by the taeniae coli and the mucous membrane is folded by the muscularis mucosae (Ham, 1969). Malan et al. (1981) quantified the encysted cyathostomes of one mule by excising the taeniae and cutting the remaining gut into 10 cm³ sections. These sections were trans-illuminated on a diamond sorter's scope and larvae counted by direct observation.

This chapter presents the results of 3 experiments to determine the feasibility of a similar mural transillumination technique (MIT) for the horse, and to compare it to a standardized digestion procedure for quantitative and qualitative effects on tissue larvae.
Experimental Design

Experiment 1

This experiment was designed to evaluate the accuracy of the mural transillumination technique (MIT). Numbers of encysted cyathostome larvae in labelled sections of tissue were counted and recorded. The identical tissues were examined again later the same day and larvae re-counted. First and second counts of 80 sections of tissue (8 replicates of 10 each) from 6 horses were compared to test the hypothesis that no significant difference would exist between counts.

Experiment 2

This experiment was designed to compare the quantitation of encysted larvae by mucosal transillumination and mucosal digestion. Eighty sections of gut wall were examined and larval numbers in each recorded. Mucosal tissues were scraped from each section and digested in pepsin/HCl for 3 hours (40 replicates) or 6 hours (40 replicates). Numbers of larvae recovered by mucosal digestion for each time period were compared to the number in the same tissue section as originally determined by MIT.

The quantitative recovery of dissected larvae exposed to various components of the digestion solution or saline was also compared. Individual fourth stage larvae were held in HCl, pepsin, saline or water for 3 or 6 hours (8 replicates of 10 larvae each for both time
periods). Larvae were counted and recovery rates for the various component solutions determined.

Experiment 3

This experiment was designed to characterize morphologic changes in larvae recovered by mucosal digestion or held in various component and control solutions. Each larva was rated by an objective scoring system for somatic damage. Scores were compared for larvae recovered from tissues digested for 3 and 6 hours, respectively, to determine if somatic damage increased with digestion time. The scores of individual larvae held in component solutions were also compared to characterize the element most likely responsible for somatic damage to larvae.
Materials and Methods

Mural transillumination technique

Freshly killed horses, or those dead for less than 12 hours, were opened and the viscera processed as described in Chapter I. Segments of cecum and/or ventral colon were isolated, contents discarded and the mucosal surface washed with water until no adherent particles of ingesta remained (Plate XIV).

Portions of cecal or colonic wall containing taeniae were excised to minimize folding of tissues (Malan et al., 1981) (Plate XV). This isolated hastral portions of gut and facilitated stretching of the tissue on a cork cutting board. Full-thickness sections were harvested from the gut wall using a customized tissue punch with a 25 cm.$^2$ cutting area (5 cm. x 5 cm.) (Plate XVI). Tissue sections were stored at 4°C for not longer than 24 hours.

Preparatory to viewing, each tissue section was spread individually, mucosa uppermost, in a glass petri dish and mucosal folds were smoothed out. Tissues were illuminated from the serosal side with a strong light source and examined with a dissecting microscope at 15X magnification. Encysted larvae were counted with the aid of a wire grid, and numbers recorded (Plate XVII).

Additional sections of gut wall were examined and individual fourth state larvae (L4) dissected from the mucosal and submucosal tissues using a small needle (Muller, 1953). Larvae were stored in 0.9% saline at 4°C for not longer than 24 hours.
Tissue digestion and larval processing

A standard digestion solution, consisting of tap water, hydrochloric acid (1.1%, pH=1) and pepsin (7,000 units of activity/ml.) was formulated. The mucosa and submucosa were scraped from each tissue section with a glass slide (McKenna, 1976) (Plates XVIII and XIX), deposited in a labelled 4 oz. jar, and 30 ml. of standard digestion solution added. Equal numbers of replicates were agitated at 42°C for 3 and 6 hours, respectively. Digestion was halted by the addition of an equal volume of 10% formalin.

Solutions were formulated to represent individual components of the digestion solution (i.e., water, 1.1% HCl, 7,000 units/ml. pepsin) and compared to a control solution (0.9% saline). Eight replicates of 10 individual larvae were held in each solution for 3 and 6 hours, respectively. These times were representative of the duration of digestion in previous reports, for 3 hours (Reinecke and Brooker, 1972; Reinecke and LeRoux, 1972; Scialdo et al., 1982; Scialdo-Krecek, 1983) and 6 hours (Mathieson, 1964; Duncan and Reid, 1978; Duncan et al., 1977; Duncan et al., 1980; Kingsbury and Reid, 1981). After agitation at 42°C for the appropriate period of time, an equal volume of 10% formalin was added to each sample.

Twenty four hours after formalin fixation, each sample was transferred to a conical 60 ml. centrifuge tube and allowed to settle for 1 hour. Supernatant was withdrawn by gentle suction, and sediment washed into another tube and resuspended with 0.9% saline q.s. 60 ml. After centrifugation at 1,000 r.p.m. for 10 minutes, supernatant was again withdrawn and sediment transferred to a
rectangular counting dish. Larvae were counted at 10X magnification and stored in 10% formalin.

Scoring system

An objective scoring system was devised to rate morphologic damage to larvae on a scale of 1 to 5.

The scale was defined as follows:

1. Normal larva (Plate XX)
2. Mild, local damage; characterized by one small area of indistinct or granular somatic cells (Plate XXI)
3. Mild to moderate generalized damage; characterized by shrinkage of cells away from the cuticle; multiple areas of cellular granularity; somatic continuity intact (Plate XXII)
4. Severe, localized damage; characterized by one area of complete loss of somatic cells (Plate XXIII)
5. Severe, generalized damage; characterized by several areas of complete loss of somatic cells; loss of somatic continuity (Plate XXIV).

Statistical analysis

Differences between first and second counts of the mural transillumination technique, and differences in numbers of larvae recovered from tissues digested for 3 and 6 hours were evaluated by a paired t-test. A paired t-test was also used to analyze differences in numbers of larvae recovered after exposure to control and component solutions for 3 and 6 hours. Larval losses among the solutions and between time periods were evaluated by analysis of variance.
Distribution of larvae among the 5 classification groups, for pepsin/HCl and the test solutions (HCl, pepsin, saline, water), was evaluated for each time period by chi-squared analysis for independence.
Results

Experiment 1

A comparison of two separate counts of 80 tissue replicates by the mural transillumination technique is shown in Table 3. There was no significant difference (p<0.05) between first and second counts as determined by a paired t-test for equality of sample means.

Experiment 2

Comparison of larval quantitation by MIT and tissue digestion for 3 and 6 hours is shown in Figure 10. Fifteen percent fewer total larvae were recovered after digestion for 3 hours than were counted by mural transillumination, and 56% fewer worms were recovered after digestion for 6 hours. Both differences were highly significant (p<0.01) as determined by a paired t-test.

In two replicates, digestion for 3 hours yielded higher total numbers of larvae than were counted by direct observation. This inconsistency was not observed in digestions carried out for 6 hours.

Recovery of larvae held in HCl, pepsin, saline or water for 3 and 6 hours is presented in Figure 11. Significantly fewer (p<0.05) larvae were recovered from component and control solutions for both time periods. Analysis of variance revealed no significant differences among the 4 solutions tested, for either time period.
Experiment 3

Larvae recovered by tissue digestion for 6 hours had higher somatic scores than those digested for 3 hours (Table 4). This difference was statistically significant ($p < .01$).

Larvae held in pepsin received higher somatic scores than those kept in HCl, saline or water for both 3 and 6 hours (Tables 5 and 6, respectively). In either time period, this significant deviation disappeared when the pepsin was excluded and a split chi-squared analysis performed with the remaining 3 solutions (Tables 5 and 6).
Discussion

The results indicate that mural transillumination is a consistent technique which yields greater numbers of larvae and causes less morphologic damage than mucosal digestion.

Marked loss of larvae associated with mucosal digestion (Figure 10) would explain the low larval numbers cited in several studies (see Chapter II; Mathieson, 1964; Reinecke and LeRoux, 1972). Larval recovery after digestion for 6 hours was less than 50%, and this would suggest that quantitative results in studies with prolonged digestion times (Duncan and Reid, 1978; Duncan et al., 1977; Duncan et al., 1980; Kingsbury and Reid, 1981; Hopfer et al., 1984) are likely to be inaccurate. Questions therefore are raised concerning subsequent claims of anthelmintic efficacy against encysted larvae. This also may explain the erratic results of controlled trials in which treated animals had higher larval burdens than control animals (Lyons et al., 1983).

Two replicates yielded greater larval numbers after digestion for 3 hours than were originally counted by mucosal transillumination. This suggests that certain sources of error are inherent in the MIT. Larvae may have been obscured by folds of mucosa, or by wires of the grid. The mucosa was manipulated with a probe during viewing to open folds and temporarily distract tissues away from the grid, in an attempt to rectify both situations. Accuracy could be
increased by eliminating mucosal folds, but this would necessitate removal of the mucosa and submucosa from the muscularis and serosa.

Significantly lower numbers of larvae recovered after exposure to HCl, pepsin, saline or water (Figure 11) demonstrate that quantitative loss may occur in any technique in which larvae are removed from the tissues. The source of loss seems to lie in the recovery technique, and not the properties of individual components, because no significant differences existed among the various agents tested.

Morphologic damage increased with time, as manifested by higher somatic scores in larvae exposed to digestion for 6 hours (Table 4). Such damage was observed first by Mathieson (1964) who noted, "Although considerable tissue digestion occurred, the larvae had also undergone digestion in most instances, or if an occasional larva was found it disintegrated with the slightest handling." Mathieson (1964) believed acidity of the digestion solution to be the cause of larval damage, based on the naivete of cyathostomes to acid conditions. However, low somatic scores, indicative of little damage, were observed in larvae held for 3 and 6 hours in 1.1% HCl (Tables 5 and 6), and distribution of somatic scores was shown to be independent of solution when pepsin was excluded. Pepsin is activated only in an acid environment, and reference to a pH-activity profile for pepsin (Lehninger, 1970) shows greatest activity between pH 1 and 4. The pH of pepsin solution (7,000 units/ml.) in this experiment was 3.583, as determined by a pH meter, so damage to larvae apparently was due to the enzymatic activity of pepsin.
Although mural transillumination was superior to mucosal digestion for quantitation of larvae, it has several practical disadvantages. Tissues must be obtained from recently dead horses and examined within 24 hours. Routine examination of reasonable percentages of the total gut would discount processing more than 1 or 2 horses on any given day. Tissues examined must be normal because autolysis, edema and congestion interfere with transmission of light through the gut wall. Frozen and refrigerated tissues acquire the gross appearance of congestion within 24 hours. No effective method of storage for later examination has been developed. Despite these shortcomings, mucosal transillumination is the only technique which affords the recovery of intact larvae for morphologic examination and species identification.
Summary

Uniform 5 cm. X 5 cm. sections of cecal or ventral colonic haustra were transilluminated and encysted cyathostome larvae counted. Two separate counts of 80 replicates revealed no significant difference between sample means.

Numbers of larvae recovered by mucosal digestion were compared to those originally counted by MTT and found to be significantly lower after 3 hours (p<0.05) and 6 hours (p<0.01). Recovery of individual larvae held in components of the digestion solution (HCl, pepsin, water) or saline was also significantly decreased (p<0.05) after 3 and 6 hours.

Larvae recovered by tissue digestion were individually examined and given a score for morphologic damage subsequent to digestion. Distribution of scores was time-dependent, indicating that damage to larvae increased with duration of digestion.

Larvae exposed to pepsin (7,000 units/ml.) for 3 and 6 hours had significantly higher scores for morphologic damage than larvae held in HCl, saline or water. It was concluded that pepsin is the agent most likely responsible for somatic damage to larvae during the digestion process.

It was concluded that mural transillumination is a consistent technique for quantitation of encysted cyathostomes, which yields greater numbers of larvae and causes less morphologic damage than mucosal digestion.
Table 3. Comparison of two counts of tissue replicates by the mural transillumination technique.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>(A-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Means (s.d.)</td>
<td>20.8(12.33)</td>
<td>21.04(12.78)</td>
<td>-0.2625(3.6)</td>
</tr>
<tr>
<td>Variance</td>
<td>152.05</td>
<td>163.48</td>
<td>12.9</td>
</tr>
<tr>
<td>F-statistic</td>
<td>1.075 n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(.05,79,79)</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-statistic</td>
<td></td>
<td>-0.6562 n.s.</td>
<td></td>
</tr>
<tr>
<td>t(.025, 79)</td>
<td></td>
<td>1.96</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Distribution of scores of larvae recovered by tissue digestion for 3 and 6 hours.

<table>
<thead>
<tr>
<th>Class</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Hours</td>
<td>12</td>
<td>42</td>
<td>106</td>
<td>111</td>
<td>427</td>
</tr>
<tr>
<td>6 Hours</td>
<td>2</td>
<td>6</td>
<td>11</td>
<td>14</td>
<td>369</td>
</tr>
<tr>
<td>Chi-squared value</td>
<td>119.79**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X^2(.005, 4)$</td>
<td>14.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Distribution of scores of larvae held in HCl, pepsin, saline and water for 3 hours.

<table>
<thead>
<tr>
<th>Class</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>46</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pepsin</td>
<td>15</td>
<td>5</td>
<td>16</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Saline</td>
<td>50</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Water</td>
<td>44</td>
<td>16</td>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Chi-squared statistic</td>
<td>67.01**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X^2(.01, 12)$</td>
<td>26.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pepsin excluded

| Chi-squared statistic | 8.542 n.s. |
| $X^2(.05, 8)$ | 15.52 |
Table 6. Distribution of scores of larvae held in HCl, pepsin, saline and water for 6 hours.

<table>
<thead>
<tr>
<th>Class</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>35</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pepsin</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>41</td>
</tr>
<tr>
<td>Saline</td>
<td>48</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Water</td>
<td>39</td>
<td>22</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Chi-squared statistic</td>
<td></td>
<td></td>
<td></td>
<td>181.24**</td>
<td></td>
</tr>
<tr>
<td>$X^2(0.01, , 12)$</td>
<td></td>
<td></td>
<td></td>
<td>26.22</td>
<td></td>
</tr>
</tbody>
</table>

Pepsin excluded

| Chi-squared statistic | 13.02 n.s. |
| $X^2(0.05 \, 8)$ | 15.52 |
Figure 10. Recovery of larvae by digestion of tissues in pepsin/HCl for 3 and 6 hours.
MURAL TRANSILLUMINATION

** (p<.01) PEPSIN/HCl

3 HRS.  6 HRS.

NUMBERS OF LARVAE

Figure 10
Figure 11. Recovery of larvae held in HCl, pepsin, saline and water for 3 and 6 hours.
NUMBERS OF LARVAE

PRETREATMENT

HCl

PEPSIN

SALINE

WATER

* (p<.05)

** (p<.01)

3 HRS.

6 HRS.

Figure 11
Plate XIV. Segment of cecal wall with taeniae intact. Note folding of mucous membrane and total length.
Plate XV. Segment of cecal wall with taeniae excised. Note decreased mucosal folding and increased length and translucency.
Plate XV
Plate XVI. 5 cm. X 5 cm. punch applied to stretched cecal wall.
Plate XVII. 25 cm.³ tissue section prepared for examination, with wire grid in place.
Plate XVIII. Histologic section of equine cecum, with intact mucosa, submucosa, muscularis and serosa. 150X.
Plate XIX. Histologic section of equine cecum after scraping with glass slide. Note absence of mucosa and most submucosa. 150X.
Plate XX. Class 1; normal larva. 85X.
Plate XXI. Class 2; small area of indistinct, granular somatic cells. 85X.
Plate XXI
Plate XXII. Class 3; shrinkage of cells away from cuticle, multiple areas of cellular granularity. 85X.
Plate XXIII. Class 4; area of complete loss of somatic cells. 85X.
Plate XXIV. Class 5; several areas of complete loss of somatic cells. 85X.
CHAPTER IV

DISTRIBUTION OF ENCRYPTED CYATHOSTOME LARVAE IN THE HORSE

Introduction

The presence of larval cyathostomes within nodules in the gut wall has been recognized for 150 years (Gurit, 1831; Knox, 1836). Unfortunately, these larvae have received little practical attention other than as developmental stages occurring during the life cycle of small strongyles (Cobbold, 1896; Boulenger, 1921; Schmid and Johannsen, 1937). The morphology of cyathostome larvae has been investigated more thoroughly, and Cuille et al. (1913) and Ihle and van Oordt (1923) described several morphologic types, but could not assign specimens to genus or species. Muller (1953) performed the most exhaustive investigation to date, examining 3,173 larvae and assigning them to 26 type-groups based on morphology of the anterior end.

The identification of larvae to species could not be accomplished until moulting forms were found which exhibited the buccal capsule of the L5 yet still retained the larva buccal capsule of the L4. By comparing these morphologic components, the L4 of the following species have been described: Cylicostephanus longibursatus, Cyathostomum catinatum, Cyathostomum coronatum (Rupasinghe, 1975),
Cyclicocyclus elongatus and Gyaloocephalus capitatus (Barus, 1962), and Cyclicocyclus insigne (Boulenger, 1921).

No reports of the quantitative distribution of larvae in the gut wall of the horse have been published. Several authors have measured numbers of larvae per unit area (cm.²) (Cuille et al., 1913; Tagle, 1948; Velichkin, 1952; Muller, 1953; Mathieson, 1964; Poynter, 1969), but were unable to determine the total larval burdens of individual animals. Transformation of larvae per unit area into total larval burdens is complicated by the anatomical features of the equine discussed in Chapter III. Nevertheless, based on impressions formed by enumeration of larvae per unit area, Tiunov (1951) and Mathieson (1964) suggested the density of cyathostome L4 to be greatest in the cecum and ventral colon, and least in the dorsal colon.

The present study was designed to investigate the quantitative distribution of cyathostome larvae in the equine large intestine. Miscellaneous observations on species distribution are also reported.
Materials and Methods

Six horses which had been dead for less than 12 hours, were necropsied between December, 1983 and February, 1984, and the viscera removed as described in Chapter I. The cecum, ventral colon and dorsal colon were separated from each other by severing the ileocecal and cecocolic junctions, pelvic flexure and the union of the dorsal and small colons.

The lengths of the cecum, ventral colon and dorsal colon were determined, and each organ was cut into 4 segments of equal length. This yielded 12 segments of large intestine which were labelled, proximally to distally, proximal cecum (PC), proximal mid-cecum (PM), distal mid-cecum (DM), distal cecum (DC), right ventral colon-A (RVA), right ventral colon-B (RVB), left ventral colon-A (LVA), left ventral colon-B (LVB), left dorsal colon-A (IDA), left dorsal colon-B (LDB), right dorsal colon-A (RDA) and right dorsal colon-B (RDB) (Figure 12). Ingesta were washed from each segment with water, and tissues were placed in individual, labelled plastic buckets. Excess water was removed from the tissues, and the weight of each segment recorded.

Taeniae were excised from each segment and 25 cm.² sections removed with a tissue punch (Chapter III). (Hereafter, 'sections' refers to 5 cm. X 5 cm. areas of tissue and 'segments' refers to one of the 12 regions of gut described above.) Five percent of the total
segment weight, or 5 sections, whichever was greater, were collected from the haustra of each segment.

Within 24 hours, sections were examined by the mural transillumination technique (Chapter III) and numbers of larvae present were recorded. Total numbers of larvae per segment were calculated using a conversion factor derived by dividing the total segment weight by the weight of the sections examined.

Values were calculated for the mean percentage contribution by each segment to total larval numbers, as well as the mean percentage contribution of each segment to the total weight of the large intestine. Differences between contributions of each segment to total larval burdens and large intestine weight were evaluated by analysis of variance. Multiple comparisons between means were analyzed by Duncan's New Multiple Range test.

During examination of tissues, the first 20 larvae encountered in each segment were dissected free from the mucous membrane with small needles (Muller, 1953) and identified to species by the key of Rupasinghe (1975). No statistical analysis of species data was attempted due to the small sample size of horses examined, and the low number of larvae recovered from each segment.
Results

The majority of cyathostome larvae (98%) were recovered from the anterior 7 segments of large intestine, extending to and including the left ventral colon-A (LVA) (Figure 13). Less than 2% of total encysted larvae were recovered from the left ventral colon-B (LVB) and segments distal. These differences in larval distribution were significantly different (p < .05) (Table 7). Larval burdens of the 6 horses examined ranged from 1,628 to 60,429 (X = 19,913) larvae per horse.

Mean percentages of total large intestine weight contributed by each organ segment were significantly different (p < .01), but multiple comparison of means yielded a confusing pattern. Generally, the tissues comprising the sternal flexure and distal dorsal colon had the greatest weights, and the smallest contributions came from the distal cecum (DC) and left dorsal colon (LDA and LDB).

The cumulative contribution of each organ to total large intestine weight and larval burdens is combined in Figure 14. The cecum comprised only 27% of total weight, yet contributed 57% of total larvae. Weight and larval provision by the ventral colon both approximated 41%, and the dorsal colon contributed 31% of the total mass yet average less than 2% of the larval supply.

No conclusions about the distribution of cyathostome larvae by species could be reached due to the small numbers of larvae (20)
recovered per segment, and the low number of horses examined. Never­
theless, several miscellaneous observations were made. Most larvae
observed belonged to species which demonstrate high prevalences as
adults (Cyathostomum catinatum, Cylicoclyclus nassatus, Cyathostomum
coronatum), and one horse had a virtually monospecific infection with
C. nassatus. Cylicostephanus longibursatus 1A, as well as larvae of
Triodontophorus and indeterminate species occurred with low
frequency. Cylicoclyclus insigne larvae, third stage larvae (L3) and
L3 moulting to L4 were relatively common.
Discussion

The results suggest that the overwhelming majority of encysted cyathostome larvae occur in the anterior 7 sections of the large intestine, and that the posterior 5 sections make a negligible contribution (Figure 12). The cecum, although the smallest of the 3 organs of the large intestine, was shown to provide the greatest cumulative percentage of larvae. Thus, the cecum has the greatest density of larvae per unit mass.

This verifies the contentions of Tiunov (1951) and Mathieson (1964) that the cecum had the highest concentration of larvae. Mathieson (1964) proposed that the cecum demonstrated the greatest larval density because it was the first organ encountered by infective larvae after they had exsheathed in the small intestine. This suggests that location of larvae in the gut is determined by simple opportunity, and contradicts prevailing opinions that specific site preference within the host is determined by local anatomic or physiologic conditions. Although the latter theory is attractive, there is little data to reinforce it (Crompton, 1973).

The distribution of larvae in the various portions of the large intestine differs markedly from the distribution of adult cyathostomes. Despite its high larval concentration, only 5-10% of adult small strongyles are normally found in the cecum (Chapter I; Ogbourne, 1978). Ogbourne (1976) found 50% and 45% of lumen cyathostomes in the ventral and dorsal colons, respectively. This finding
is remarkable because the dorsal colon is the site of minimal larval development.

This pattern suggests that cyathostomes emigrate in the direction of gastrointestinal flow during maturation in the lumen (Crompton, 1973). A similar pattern for the horse has been suggested previously by Boulenger (1921) and Mathieson (1964), based on finding adults of a given species more distally in the tract than the site where larval development in the gut wall occurred. This is best exemplified by *Cylicocculus insigne*. Encysted L4 of this parasite were observed only in the cecum, L4 were found in the lumen of the ventral colon, and adults were observed only in the lumen of the dorsal colon. This clearly demonstrates distal emigration as the worms matured. The phenomenon of a site preference for adults which is distal to the site of larval development has been reported for *Chabertia ovina* (Herd, 1971).

The anterior distribution of larvae in the horse predisposes those areas to damage caused by larval invasion and/or emergence. Indeed, hemorrhagic typhilitis and inflammatory changes of the ventral colon have been associated with naturally-occurring cyathostomiasis in Russia (Velichkin, 1952; Tiumov, 1953), Britain (Chiejina and Mason, 1977) and the Netherlands (Mirck, 1977).

Only one horse exhibited appreciable numbers of cyathostome larvae (6% of total) in the wall of the dorsal colon. This same animal also had the greatest larval burden (60,429) of all horses examined. This suggests that larvae may colonize the posterior portions of the alimentary tract as the level of infection increases.
Similar behavior has been reported in experimental infections of *Chabertia ovina* when the number of infective larvae administered was increased from 400 to 2,000 (Herd, 1971). The distal emigration of *C. ovina* was likely to be a consequence of overcrowding rather than adverse local immune conditions because of the rapidity with which it occurred. Brambell (1965) and Connan (1966) suggested that local changes in the gut were responsible for the emigration of adult *Nippostrongylus brasiliensis* and *Trichostrongylus colubriformis*, respectively, away from the original site of adult habitation, because this occurred one to two weeks after adult habitation.

The small sample size which gave rise to these observations precludes any dogmatic statements about the quantitative distribution of encysted cyathostome larvae. As suggested by other, simpler models (Crompton, 1973) the variables of level of infection, interspecific and intraspecific reactions, host immunity, concurrent disease states and nutritional status, would result in endless permutations for polyspecific cyathostome infections.

The high prevalence of larvae belonging to species which are common as adults is quite logical. The paucity of *Cylicostephanus longibursatus*, however, is probably explained by its small size (Rupasinghe, 1975). Although the first 20 larvae encountered in each segment were to be dissected and preserved, the smaller larvae were often damaged and could not be recovered with reasonably intact morphology. This resulted in an artificial under-representation of the smaller larvae. The phenomenon of larval disruption during dissection was reported previously by Mathieson (1964).
A monospecific infection with *Cylicocyclus nassatus* larvae is difficult to explain because natural infections usually consist of multiple species (Chapter I; Foster, 1936; Mathieson, 1964; Ogbourne, 1976). This is unlikely to be a manifestation of anthelmintic resistance because cyathostome larvae are not susceptible to current equine anthelmintics (Chapter III). Seasonal predominance by *C. nassatus* larvae is improbable (Chapter II), unless the horse had been infected by monospecific third stage larvae. This conceivably could occur if *C. nassatus* were the only resistant cyathostome species in a mixed population, and was selected by benzimidazole treatment. *C. nassatus* then could contaminate pasture unchallenged for a short period of time and a monospecific infective pool result.

*Trlodonophorus* spp. larvae were common in 2 horses, yet this genus was present in only 3.6% of 55 horses recently examined in Ohio (Chapter I). *Cylicocyclus insignis* larvae were likewise fairly prevalent despite their occurrence in only 30% of horses in the above survey. Both of these cases may be attributable to annual or local variation.

Horses in this study were necropsied between 12/13/83 and 2/6/84, a time when many Ohio horses were housed, so the frequent occurrence of L3, and L3 moulting to L4, is interesting. Cyathostome infective larvae (L3) can survive on pasture through winter (Ogbourne, 1973), and may induce infection if ingested during this time. The presence of immature parasitic stages suggests that they had been ingested recently, because usual periods for development of
L3 to L4 after infection are 6 to 12 days for *Cylicostephanus longibursatus*, and durations up to 20 days have been observed in experimental infections (Wetzel, 1963). Accurate grazing histories, unavailable in the present study, are mandatory to properly evaluate the dynamics of larval populations from random observations.

The accuracy of the procedure reported in this study has intrinsic limitations. The larvae counted were sampled only from the haustral regions, and it is possible that the distribution of larvae in the gut wall differs between taenial and haustral areas. Taenial regions may weigh more than haustral areas, and the removal of this tissue could alter the final calculation of total larvae/segment. Additionally, enough variance was observed within segments to question whether intrasegmental distribution may vary with the anatomic position of the gut wall (dorsal, ventral, etc.).

Observation of larger numbers of horses may reveal a constant relationship between total larval burdens and the numbers present in a given segment. This would greatly facilitate the quantitation of whole gut larval burdens by allowing accurate predictions to be made from a localized aliquot. The presence of 98% of encysted larvae in the cecum and ventral colon clearly emphasizes that these organs require thorough quantitative investigation, while the dorsal colon may be all but ignored.
Summary

The large intestines of 6 horses were each divided into 12 segments by length, and at least 5% by weight of each segment was examined by mural transillumination. Encysted cyathostome larvae were counted, and total numbers of larvae in each segment calculated. Ninety-eight percent of encysted larvae occurred in the anterior 7 segments of large intestine, and less than 2% developed in the distal ventral colon and dorsal colon. The cecum was the most important source, harboring 57% of encysted larvae, yet contributing only 27% of the total weight of the large intestine.

Distal emigration apparently occurs during maturation of cyathostominae, as manifested by the disparity between proportions of adult (45%) and larval (2%) worms residing in the dorsal colon. Distal emigration of larvae also may result as the level of infection increases, as observed in one horse with a heavy infection (60,429 larvae), in which 6% of larvae were located in the dorsal colon.

Recovered larvae belonged to species which demonstrated high prevalence as adults, although one animal exhibited a monospecific infection of *Cylicocyclus nassatus*. 
Table 7. Mean percentage contribution of gut segments to total larval burdens of 6 horses.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Mean (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>12.84 (4.1) c</td>
</tr>
<tr>
<td>PM</td>
<td>13.8 (4.99) c, d</td>
</tr>
<tr>
<td>DM</td>
<td>19.72 (7.1) d</td>
</tr>
<tr>
<td>DC</td>
<td>9.86 (4.13) b, c</td>
</tr>
<tr>
<td>RVA</td>
<td>15.3 (4.62) c, d</td>
</tr>
<tr>
<td>RVB</td>
<td>14.1 (2.37) c, d</td>
</tr>
<tr>
<td>LVA</td>
<td>11.97 (10.1) c</td>
</tr>
<tr>
<td>LVB</td>
<td>1.32 (1.27) a, b</td>
</tr>
<tr>
<td>IDA</td>
<td>0.24 (0.35) a</td>
</tr>
<tr>
<td>IDB</td>
<td>0.25 (0.3) a</td>
</tr>
<tr>
<td>RDA</td>
<td>0.35 (0.78) a</td>
</tr>
<tr>
<td>RDB</td>
<td>0.28 (0.63) a</td>
</tr>
</tbody>
</table>

Means bearing different superscripts are significantly different (p < .05).
Figure 12. Equine large intestine partitioned into 12 segments.
Figure 12
Figure 13. Mean percentage contribution of gut segments to encysted larval burdens of 6 horses.
Figure 13
Figure 14. Cumulative percentage contributions of organs to total larval burdens and large intestine weight.
CHAPTER V

INGESTION OF STRONGYLID OVA BY CYATHOSTOME NEMATODES

Introduction

The pathogenicity of various parasites is often directly related to their feeding behavior. *Ancylostoma caninum*, for example, is associated with high morbidity and mortality in pups, whereas *Ancylostoma braziliense* in the same host may induce only transient hypoproteinemia. These intragenic differences may be attributed to feeding behavior. *Ancylostoma caninum* aggressively removes large bits of mucosa with marked, subsequent hemorrhage (Kalkofen, 1970), and has been shown to ingest blood actively via suction (Roche and Martinez, 1960). *A. braziliense*, however, is a plug-feeder (Dunn, 1978) and merely ingests tissue fluids and exfoliated cells without causing significant blood or tissue loss.

Feeding behavior of parasites may also be a major determinant of site specificity within the host. Schad (1963) demonstrated that certain oxyurids of cheloniens fed preferentially on bacteria, which in turn had a very localized distribution within the gut. It has been suggested that many cyathostome species are able to co-exist within one host animal because of highly specific feeding habits of each species (Theiler, 1923; Crompton, 1973).

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No detailed investigation of the feeding behavior of cyathostome nematodes has been performed, and available information consists of sundry observations. The purpose of the present chapter is to report miscellaneous observations on the feeding behavior of cyathostomes recovered from horses in Ohio.
Materials and Methods

Specific identification of individual worms examined in Chapter I was based on morphologic characteristics of the buccal capsule, as described in the key of Lichtenfels (1975). The caudal end of each worm then was examined to determine its gender. Proceeding in this manner, from anterior to posterior, the entire alimentary tract could be observed and the presence of unusual ingestants noted. The number and location of ingestants, as well as the species and sex of the ingesting worm, were recorded. Specimens with unusual alimentary contents were photographed with an Olympus PM-6 35 mm camera using Kodak Ektachrome tungsten film.

Total worm burdens of horses were determined as described in Chapter I, and fecal egg counts were performed by a modified McMaster technique (Whitlock, 1948). Mean worm burdens and mean fecal egg counts were compared between horses with and without ova-ingesting cyathostomes, and differences evaluated by a t-test for approximate significance.
Results

Strongylid ova were observed in the alimentary tracts of 43 of 8112 (0.53%) cyathostomes examined. A total of 139 eggs was observed, with a mean of 3.23 eggs/ova-ingesting cyathostome (range 1-39). Ova were distributed within the buccal capsule (2.9%), esophagus (39.8%) (Plate XXV), intestine (31%) (Plates XXVI and XXVII), and rectum (26.3%) (Plate XXVIII) in male (26/43) and female (17/43) worms. Species ingesting ova and the distribution of this behavior were *Cylicocyclus nassatus* (65%), *Cylicocyclus insigne* (16%), *Cyathostomum coronatum* (14%), *Cyathostomum catinatum* (2.5%) and *Cylicocyclus leptostomus* (2.5%).

Horses with ova-ingesting nematodes had mean egg counts of 1,265 eggs per gram (epg) compared to 512 epg from horses with no ova-ingestors. The former had mean worm burdens of 140,438 worms compared to 38,203 worms in the latter. Differences in egg counts and worm burdens were statistically significant (p < .05).
Discussion

The results indicate that a small proportion of cyathostomes ingest strongyloid ova, and that ingestion of ova was significantly more prevalent in hosts with higher egg counts and larger worm burdens. Increased availability of ova and greater competition for food within a population raise the probability of ingestion, whether accidental or intentional.

The argument for accidental ingestion in the present report is supported by its low prevalence (0.53%) and by the intact condition of the ova within the alimentary tract. These nematodes apparently lack enzymes capable of digesting the shell of strongyloid ova, so there is no nutritional advantage to their ingestion.

The cyathostome species observed to ingest ova reflect both their prevalence and the size of their buccal capsules. Ova-ingesting cyathostomes were among the 10 most prevalent species (Chapter I), and *Cylicocyclus insigne*, *Cylicocyclus nassatus* and *Cyathostomum coronatum* are among the largest of the small strongyles (Lichtenfels, 1975). *Cyathostomum catinatum* and *Cylilocyclus leptostomus* are the smallest of the 5 species, which may account for their lower frequency of ova ingestion. *Cylilocyclus leptostomus*, as its name implies, has a small buccal capsule (60 um diameter), but could accommodate equine strongyloid ova, which range from 40-48 um in diameter (Thienpont et al., 1979).
Early investigators observed plant material within the alimentary tracts of cyathostomes, and considered ingestion of host gut contents to be the primary feeding mode (Looss, 1901; Ihle, 1922; Théiler, 1923). Levine (1949) frequently observed ciliate protozoa (Cyclopoisthium spp.) among the ingesta of small strongyles, and considered them to be the sole diet of the Cyathostominae. The presence of ciliates and vegetable matter within the nematode gut has also been attributed to accidental ingestion (Wetzel, 1930).

The typical feeding mode of cyathostomes is thought to be attachment to the gut wall by the buccal capsule. Adult small strongyles (LeRoux, 1924) and adults and larvae of 15 cyathostome species (Wetzel, 1930) were observed feeding in this manner when the host was examined immediately after death. Wetzel (1930) demonstrated histologically that small strongyles draw a plug of mucosa, and occasionally submucosa, into the buccal capsule. The cyathostome buccal capsule is virtually unarmed, but these tissues are destroyed, apparently by enzymatic secretions, and epithelial erosion of the large bowel results. The necessary enzymes may be produced by the dorsal esophageal gland, and anatomical connections between this gland and structures within the buccal capsule of the Strongyloidea have been demonstrated (Chitwood and Chitwood, 1937; Threlkeld, 1948).

The feeding behavior of large strongyles has been investigated more thoroughly. Mucosal and submucosal tissues are drawn into the buccal capsules of attached large strongyles (Wetzel, 1928; Rahman and Waddell, 1979) and degenerative changes occur in all tissues
involved. Rahman and Waddell (1979) observed erythrocytes, leukocytes and mucosal cells in the alimentary tracts of large strongyles. Rogers (1940) performed chemical and cytologic analysis of gut contents of *Strongylus edentatus* and *S. vulgaris* and detected very small quantities of host blood within the worms. It was concluded that mucosal tissue, and not blood, was the major nutrient for adult large strongyles. This ingestive mode appears to be common to all equine strongylids.

The finding of strongylid ova in cyathostome ingesta was serendipitous and of little biological import, but investigators should take care to identify the sex of cyathostomes by traditional morphologic criteria, and not trust in the presence of ova as strictly a female characteristic.
Summary

Strongyloid ova were observed in the alimentary tracts of 43/8112 (0.53%) cyathostome nematodes of both male (26/43) and female (17/43) worms. Ingestion of ova was significantly (p<.05) more prevalent in worms from horses with higher egg counts and greater worm burdens. Ingestion of ova appears to be accidental, but may be a cause of gender misidentification if morphologic characteristics are not examined.
Plate XXV. Strongylid ova within the esophagus of *Cylicocyclus* nassatus. 960X.
Plate XXVI. Strongylid ova within the intestine of *Cylilocyclus nassatus*. 960X.
Plate XXVII. Numerous strongylid ova within the intestine of *Cyathostomum coronatum*. 960X.
Plate XXVIII. Strongylid ova within the rectum of male *Cylilocyclus nassatus*. 960X.
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