THE EPIDEMIOLOGY OF EQUINE STRONGYLIDOSIS ON PRINCE EDWARD ISLAND

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ABSTRACT

Detailed knowledge of the seasonal patterns of transmission of gastro-intestinal parasites of livestock is important for the design of effective and efficient control programmes. Climatic factors have a major impact on these patterns, hence it is necessary to investigate specific patterns for each unique climatic region. The purpose of this study was to monitor the level of strongyle infection and pasture contamination in pastured horses in Prince Edward Island; investigate the patterns of pasture infectivity during the grazing season; and determine the rates of development, migration and longevity of the free-living stages of horse strongyles on pasture plots at different times of the year.

Three horse herds were monitored for 13 months (April 1993-April 1994) using monthly faecal egg counts. Herds 1 and 2 were standbred brood mare herds; Herd 3 was a mixed breed herd consisting entirely of adult horses. Positive faecal samples were cultured and third stage larvae (L3) recovered and identified. Peak egg counts occurred between August and September in all groups. Minor peaks were observed between January and April. Cultures revealed that the majority of the eggs contributing to the peaks in Herds 1 & 2 were cyathostomes while in Herd 3 all were cyathostomes. These results were consistent with the necropsy findings from 3 tracer/cull horses from 2 of the herds, in which *Strongylus vulgaris* (Herd 1), *Triodontophorus* spp. (Herd 1) and various species of cyathostomes (Herds 1 and 3) were recovered.

Monthly pasture larval counts were performed on the three pastures during the 1993 grazing season. Increasing numbers of L3 were recovered from all pastures as the season progressed, with maximum counts occurring in October to November.

The seasonal patterns of larval parasite development were examined by monitoring the development, migration and longevity of strongyle free-living stages experimentally placed on pasture in faecal samples on 1.5 m^2 plots. The infective third larval stage was reached in 7-15 days (July-September) and 74-100 days (December-March). Infective L3 were first recovered from the grass in the plots 7-21 days post deposition (PD) (July-September) and peak recoveries were 25-42 days PD. Peak total recoveries ranged from 47% in September to 0% in October to December. Longevity of L3 was greatest in samples placed on pasture in August (up to 358 days PD) and September (up to 410 days PD). Very low recoveries were recorded for samples deposited between October and June ($\leq 1.6\%$).

Transmission of horse strongyles on PEI occurs to the greatest degree in the period of the grazing season from July to September. During this time, pasture contamination through egg shedding is at a peak and environmental conditions are conducive to maximal production, migration from the faecal reservoir to grass and survivability. The greatest danger of infection of horses early in the grazing season is larvae from the previous season that overwinter on pasture. Strongyle control programs directed at decreasing the amplitude of this summer peak in egg shedding should provide the greatest control.

DEDICATION

Kiri Kaari na Makena

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

Abbreviation	Term
EPG	Eggs per gram of faeces
ERP	Egg reappearance period
L1-L5	Larval stages 1-5
Pd	Post deposition
Q1	First quartile
Q3	Third quartile
UPEI	University of Prince Edward Island

1. INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

1.1.1 Distribution and economic importance

Strongylidosis is a disease caused by helminth parasites of the order Strongylida. Members of this order are commonly referred to as "strongyles" or "strongylids". Equine strongyles are cosmopolitan in distribution (Ogbourne, 1978; Ogbourne & Duncan, 1985). Strongyles are important for several reasons. Throughout the world, wherever horses are reared, control of the parasite is expensive. A conservative estimate in USA in 1987 showed that $178 million was used in deworming programmes (Reinemeyer & Rohrbach, 1990). The cost of control in other countries with high horse populations is likely similar. Most control programmes are aimed at strongyles, which, together with ascarids, are the most important gastrointestinal parasites of horses (Ogbourne, 1978; Herd 1990a). Indirect costs due to strongyles are due to the discrete disease entities they cause in the horse population. *Strongylus vulgaris* causes formation of anterior mesenteric artery aneurysms and thrombi that may cause infarction of the intestines and death of the animal (Ogbourne & Duncan, 1985). Small strongyles (cyathostomes) are responsible for the syndrome of "larval cyathostominosis", a disease characterized by massive emergence of larvae from the intestinal mucosa (Herd, 1990a). Overall, strongyles are considered the most important cause of colic in horses (Klei et al, 1984a). In addition, subclinical strongyle infections can cause growth retardation and failure to achieve full performance in young horses (Frerichs et al, 1976, Herd, 1990a).

Small strongyles have recently emerged as the most important cause of verminous colic in horses (Herd, 1990a, 1990b, Love & Duncan, 1991). Faecal egg cultures reveal that this group contributes most of the strongyle eggs passed in horse faeces (Herd, 1986a). This emergence to prominence of the small strongyles has been attributed to the widespread use of highly efficacious anthelmintics including benzimidazoles and ivermectin (Herd, 1990b). While benzimidazole efficacy against the large strongyles has been retained, small strongyle strains have developed that are resistant to these anthelmintics. Significant losses can occur when horse owners continue to use anthelmintics whose efficacy is either low or nil. Frequent dosing, especially with anthelmintics of high efficacy, increases selection pressure for development of drug resistance within a parasite population (Klei et al, 1984a; Reinemeyer & Rohrbach, 1990; Love & Duncan, 1991). Therefore there is a need for the development of control protocols that rely less on frequent dosing with anthelmintics and more on the epidemiology of the parasites (Herd, 1993).

1.1.2 Aetiology

A description of the characteristics of the order Strongylida can be found in most standard parasitology text books. Males possess a well developed copulatory bursa consisting of cuticular projections or alae. Alae are further divided into dorsal, lateral and ventral rays. The dorsal ray is variously divided. The mouth contains important distinctive characteristics which are similar in both males and females. Female strongylids lay typical "strongylid" eggs which are smooth shelled and contain an embryo

in the morula stage when passed in faeces. Members of this order are parasites of humans and both wild and domesticated animals. Horse strongyles belong to the superfamily Strongyloidea, family Strongylidae. Lichtenfels (1975), classified the family into two subfamilies; Strongylinae (large strongyles) and Cyathostominae (small strongyles).

Large strongyles, subfamily Strongylinae, are parasites of equines and elephants (Soulsby, 1982; Georgi & Georgi, 1990). One genus has been described infecting the rhinoceros in East Africa (Soulsby, 1982). Strongylinae or strongylins are parasites of the large intestine. Large strongyles have a well developed, globoid buccal capsule, whose anterior margin is surrounded by a cuticular leaf-like structure or "corona radiata" (Soulsby, 1982). They exhibit long migratory tendencies through various tissues in the abdominal cavity during their larval stages; have long prepatent periods; and the adults attach on the caecal and colonic mucosae, where they feed by ingestion of a "plug" of the mucosa (Giles et al, 1985). Genera in this subfamily include *Strongylus, Triodontophorus, Oesophagodontus* and *Craterostomum.*

The small strongyles (Cyathostominae), parasitize a wide range of animals including equines, elephants, marsupials and turtles (Georgi & Georgi, 1990). Equine cyathostomes comprise a group of over 40 species and mixed infections of 15-20 species can be found in one host simultaneously (Ogbourne, 1976; 1978; Giles et al 1985; Torbert et al, 1986; Georgi & Georgi, 1990). Intensity of infection varies according to

geographic region and history of anthelmintic treatment. Worm counts of over one million (1,239,000) have been recovered from a single animal (Reinemeyer et al, 1984). Cyathostomes generally have a shorter life cycle than the strongylins with limited migration of larvae (Giles et al, 1985). The buccal capsule of cyathostomes is smaller than that of the strongylins and is cylindrical or annular (Soulsby, 1982). Cyathostomes have both inner and outer leaf crowns (Georgi & Georgi, 1990). Common genera in this group are *Cyathostomum, Cylicocylus, Cyiicodontophorus* and *Cylicostehpanus*. Other genera include *Gyalocephalus* and *Poteriostomum.*

1.2 Life cycle

All strongylid worms have a direct life cycle (Ogbourne & Duncan, 1985; Ogbourne, 1978). Adult females lay their eggs in the caecum and large colon and the eggs pass out with the faeces. Eggs of large strongyles cannot be differentiated from those of small strongyles (Reinemeyer, 1986). When conditions are favourable, eggs develop through two free-living larval stages (L1, L2), to become infective third-stage larvae (L3) (Ogbourne, 1978; Ogbourne & Duncan, 1985; Reinemeyer, 1986). First- and second-stage larvae feed on bacteria in faeces. The L3 retain the cuticle of the second larval stage and do not feed; subsisting on food reserves accumulated in their intestinal cells. Infective larvae gain access to a suitable host by migration from faeces to surrounding grass (Ogbourne, 1978; Ogbourne & Duncan, 1985). Ingestion of the third stage larva by a horse ends the pre-parasitic or free-living stage and marks the beginning of the parasitic stage. After entering the small intestine the L3 shed their cuticles in response

to stimuli resulting from the physico-chemical conditions inside the intestinal lumen (Ogbourne & Duncan, 1985). After exsheathment L3 penetrate the mucous membrane of the caecum and ventral colon. Further development depends upon the species of the parasite (see Figure 1).

1.2.1 Strongylinae

The developmental pattern for *Strongylus vulgaris* infections was first postulated by Kikuchi in 1928 and confirmed by Enigk in 1951 by experimentally infecting young foals (Georgi, 1973). Third-stage larvae penetrate into the submucosa, where they become encapsulated and moult to the fourth larval stage (L4). The L4 penetrate arterioles in the vicinity of the area where exsheathment occurs and they move against the blood flow into small and finally large arteries. Apparently larvae can sense the curvature of the larger arterial walls, restricting of their movement to main arteries (Aref, 1982). Destruction of endothelium and cell layers in the path of the larvae causes deposition of fibrin and thrombus formation begins. *L4* remain in thrombi and aneurysms in the cranial mesenteric artery and its branches, moulting to 5th stage. When thrombi resolve, the 5th stage and some later L4 are swept out gradually and carried with the blood flow to the intestinal wall. Here they become encapsulated in the subserosa, forming nodules of approximately 5-8 mm in diameter (Jubb et al, 1993). Immature adults break into the lumen and reach full maturity after a further 6-8 weeks. The prepatent period is approximately 6-7 months.

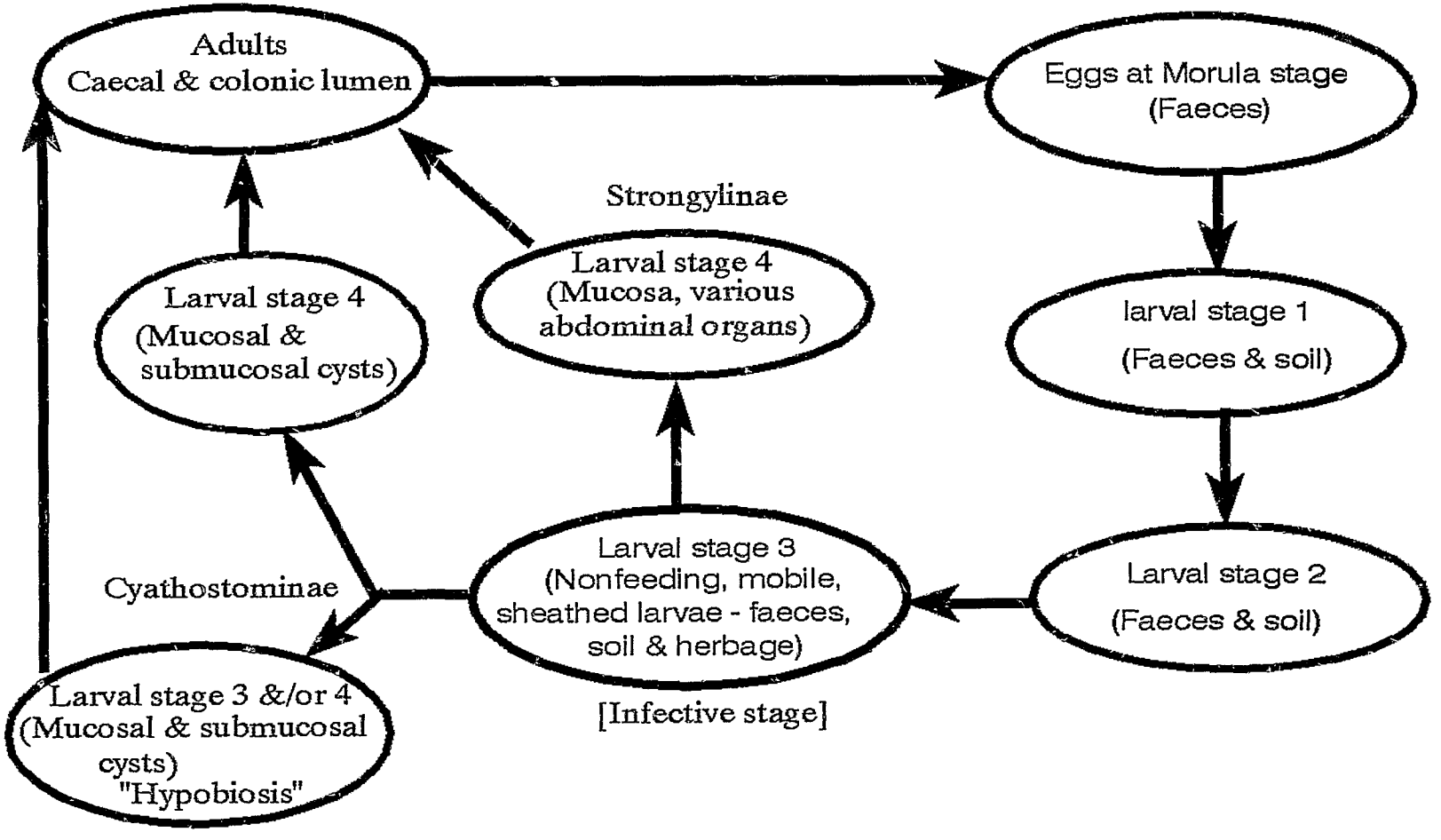

Figure 1. Simplified life cycle of equine strongyles. L5 stage (not indicated in this diagram) is considered to be an immature adult.

In *Strongylus edentatus*, L4 move through the portal system to the liver, where they undergo extensive migration (Slocombe, 1985; Soulsby, 1982; Austin, 1994). From the liver they exit through the hepatorenal ligament to the right flank (Soulsby, 1982; McCraw & Slocombe, 1978; Slocombe, 1985), where they remain in oedematous, thickened masses and moult to the 5th stage (Austin, 1994). Further migration occurs through the caecal ligaments to the caecal and colonic walls and into the lumen (Jubb et al, 1993). Maturity to egg-laying adults requires 10- 12 months. Sometimes, aberrant migration occurs in the wall of the intestine, diaphragm, lungs and omentum (McCraw & Slocombe, 1978; Slocombe, 1985; Jubb et al, 1993).

Strongylus equinus larvae migrate from the caecal and colonic mucosae to the sub-serosa where nodules are formed and the moult to the L4 takes place. The L4 emerge from nodules and migrate into the peritoneal cavity and then to the liver. There they migrate for 6-8 weeks, after which they exit through the hepatic ligament and pancreas to the peritoneal cavity where the L4-L5 moult takes place. The 5th stage returns to the caecum and colon via an unknown route (Soulsby, 1982), probably by direct migration from the peritoneal cavity and pancreas to the adjacent base of caecum and colon (McCraw & Slocombe, 1985; Jubb et al, 1993). Like *S. edentatus*, *S. equinus* larvae may undergo aberrant migration to the flanks, perirenal fat, diaphragm, and omentum (McCraw & Slocombe, 1985; Slocombe, 1985; Jubb et al, 1993).

The site of development for the genera *Triodontophorus* and *Craterostomum* is not known. Ortlepp (1925) postulated that *Triodontophorus* develops solely in the lumen of the colon based on observations that adults and fourth larval stages of *T. tenuicollis* could only be found in the lumen, not in mucosal cysts. Their development probably involves local migration into the mucosa and submucosa (Jubb et al, 1993).

1.2.2 Cyathostomes

Development of cyathostomes occurs locally in the mucosa and submucosa of the caecum and colon (Ogbourne, 1978). Apparently the patterns of development are similar within the group. After exsheathment, L3 enter the glands of Lieberkuhn in caecal and colonic mucosae and become enclosed in fibrous tissue capsules (Ogbourne, 1978; Reinemeyer, 1986). The moult to the fourth stage takes place in these nodules, after which the L4 emerge into the lumen and the final moult takes place. The prepatent period is 38 days in naturally infected ponies (Love & Duncan, 1992a), but can be as long as 126 days post infection in experimentally infected animals that have recovered from prior infection (Reinemeyer, 1986). The prepatent period can be prolonged, up to two years, following arrested development (Smith, 1976a, 1978). Arrested development has been reported at the third and fourth stages (Eysker & Jansen, 1984; Eysker & Mirck, 1986, Eysker et al, 1986a), but it is not clear whether different species are arrested at different stages or whether all cyathostomes can be arrested at both stages (Reinemeyer & Herd, 1986). Larvae arrested in development are also referred to as hypobiotic larvae. The stimulus for arrested development is not known, but may be connected to the weather. Arrested

development has only been reported from temperate areas characterized by cold winters and warm summers. Arrested development is thought to be an adaptation to enable the worms to survive adverse winter conditions (Soulsby, 1982). In tropical countries, cyathostomes survive the dry season predominantly as adults in the lumen of the caecum and colon (Eysker & Pandey, 1987, 1989). There is evidence for the distal migration of worms as they mature, with the larval stages being found predominantly in the caecum and most adults in the colon (Ogbourne, 1975; 1976, Reinemeyer & Herd, 1986; Reinemeyer et al, 1988; Love & Duncan, 1992b).

1.3 Pathology and clinical signs

1.3.1 Large strongyles

1.3.1.1 Larvae

Experimental infections with *S. vulgaris* in young animals produce an acute syndrome characterized by pyrexia, anorexia, weight loss, dullness, colic, and occasionally death (Georgi, 1973; Duncan & Pirie, 1975; Slocombe, 1985; Klei et al, 1982a, 1990). The severity of clinical signs varies with the number of infective larvae used, period of exposure, number and size of arteries involved; and the age and immune status of the host (Klei et al, 1982a; Slocombe, 1985; Ogbourne & Duncan, 1985; Austin, 1994).

In natural infections the syndrome is difficult to diagnose in non-fatal cases. In addition, there are many other causes of equine colic and this makes it difficult to determine the incidence of *S. vulgaris*-induced colic in the horse population. In chronic cases,

however, the most frequent sign of infection is colic (Drudge, 1979; Slocombe, 1985). The frequency of colic associated with *S. vulgaris* has decreased over the years due to the development of more effective anti-strongyle compounds and better control strategies (Drudge & Lyons, 1986).

Clinical signs are attributed to lesions associated with extensive migration of larvae in the mesenteric arteries and its branches. Damage to the endothelium, platelet adhesion and the initiation of the coagulation cascade, cause deposition of fibrin and initiation of thrombus formation (White et al, 1983; Slocombe, 1985; Austin, 1994). The thrombus, or a portion of a thrombus, may dislodge, and result in vascular blockage, producing infarction of the intestines (Jubb et al, 1993; Austin, 1994;). Marked depletion of smooth muscle cells and accumulation of collagen apparently causes aneurysms in *S. vulgaris* infections (Morgan et al, 1991). There is inflammatory thickening of the arterial wall and progressive dilatation of the artery via degeneration of elastic and muscle fibres of the intima (Soulsby, 1982; Jubb et al, 1993). Inflammation is followed by a productive connective tissue response initiated by the smooth muscle cells of the media (Jubb et al, 1993). Proliferating connective tissue of the intima and adventitia may replace the normal structure of the arterial wall which becomes rigid and thick. Affected arteries may be up to 20 cm in diameter. Weakening of the arterial wall may result in saccular or fusiform aneurysms.

Arteriographic examination of experimental foals has been used to study the verminous arteritis lesions in live animals, revealing enlargement of the mesenteric artery and some of its branches, right renal artery and segments of the aorta (Slocombe et al 1977; Holmes et al, 1990). Lesions resolve after the larvae return to the intestines (Duncan & Pirie, 1975; Georgi & Georgi, 1990; Jubb et al, 1993). Resorption is also noticed after treatment with larvicidal doses of albendazole (Rendano et al, 1979) and ivermectin (Klei et al, 1990). Aberrant migration can occur in the brain, heart, aorta, coronary arteries, spinal cord and iliac arteries (Drudge, 1986; Slocombe, 1985; Austin 1994), spermatic or ovarian artery (Wright, 1972) and right colic branch (White et al, 1983). Clinical signs of aberrant migration depend on the site and may include paraplegia (Mayhew et al, 1984).

Lesions due to *S. edentatus* and *S. equinus* larvae include migratory tracts in the liver, serosal haemorrhages in the ileum and ventral colon, disruption of omental architecture, granulomas on the diaphragm and especially with *S. equinus*, damage of pancreatic tissue with loss of conformation (McCraw & Slocombe, 1978, 1985; Petty et al, 1992). Clinical signs of *S. edentatus* and *S. equinus* infection include diarrhoea, peritonitis, colic and death (Slocombe, 1985). Depression has been associated with *S. equinus* infection (McCraw & Slocombe, 1985), while experimental infection of foals with *S. edentatus* resulted in no clinical signs (McCraw & Slocombe, 1978).

During the initial penetration and migration, large strongyle larvae cause the formation of slightly elevated haemorrhagic plaques along the antimesenteric border of the small intestines, and to a lesser extent, the large intestines (Jubb et al, 1993). These lesions, which are 1-2 cm by 3-4 cm in size, are referred to as "hemomelasma ilei". They are associated with trauma by migrating larvae and comprise oedema, haemorrhage, a mixed population of ieucocytes and macrophages ingesting erythrocytes. The lesions are sometimes associated with clinical, nonfatal colic.

1.3.1.2 Adults

Adult large strongyles attach their buccal capsules to the glandular membrane of the large intestines, mainly the caecum, and feed by withdrawing and digesting plugs of mucosa. This causes formation of ulcers and/or erosions of the mucosa. As parasites relocate to new sites of attachment, craterlike lesions are left behind. Lesions can extend to the muscularis and damage to the blood vessels can cause haemorrhage (Ogbourne & Duncan, 1985; Austin, 1994). There is a higher rate of albumin catabolism and reduced red blood cell survival in infected animals (Duncan & Pirie, 1975). Adult *Strongylus vulgaris* are sometimes associated with normocytic, normochromic anaemia (Soulsby, 1982; Jubb et al, 1993).

1.3.2 Cyathostomes

Disease caused by cyathostomes is becoming widely recognised globally (Blackwell, 1973; Cheijina & Mason, 1977; Jeggo & Sewell, 1977; Jasko & Roth, 1984; Harmon

et al, 1986; Ch[illegible]al, 1986; Kelly & Fogarty, 1993; Mair et al, 1993; Reilly et al, 1993; Mair, 199[illegible], Lyons et al, 1994a). The disease is usually associated with acute onset of diarrhoea that becomes chronic. There is rapid weight loss, subcutaneous oedema and in some cases, death. This syndrome most often affects young adult horses and usually occurs in winter and early spring. The condition is thought to be due to massive emergence of previously inhibited fourth-stage larvae through the caecal and colonic mucosae. Larval cyathostominosis has also been reported in aged ponies where it is associated with recurrent diarrhoea (Mair, 1993). A syndrome with rapid weight loss and subcutaneous oedema without diarrhoea has been reported (Mair, 1994). Other signs include low grade colic (Uhlinger, 1990) and chronic weight loss (Ogbourne, 1978).

Lesions in larval cyathostominosis include nodules of different sizes and shapes in the mucosa and submucosa (Ogbourne, 1978). The nodules contain larvae and may be up to 6 mm in diameter. Emergence of larvae from the mucosa results in ulceration (Kelly & Fogarty, 1993, Reilly et al, 1993). In addition there is mucosal oedema (Reilly et al, 1993). Histologically, nodules comprise fibrous tissue surrounding inflammatory cells including mononuclear cells and eosinophils (Ogbourne, 1978; Slocombe, 1985). Other lesions include hypertrophy and hyperplasia of goblet cells (Slocombe, 1985).

Adult cyathostomes apparently cause little or no damage and very heavy loads can occur in clinically normal animals (Georgi & Georgi, 1990). However, they are sometimes

associated with catarrhal enteritis with loss of epithelial layers and thickening of the intestinal wall (Ogbourne, 1978).

1.4 Biology of the free-living stages

Both laboratory and field experiments have been performed to elucidate the behaviour of the free-living stages of equine strongyles. Laboratory studies on species from different areas of the world have indicated similar development and survival requirements for either different species or for species from different areas (Ogbourne, 1972; Mfitilodze & Hutchinson, 1987). A summary of the behaviour and requirements follows. The main factors affecting the development of the free-living stages are temperature and moisture. Eggs develop and hatch only at 7.5-39 °C. Infective stage is attained at 10-35 °C. At 4-6.5 °C, there is only slow development without hatching. Below 4 °C there is no development. Development is very slow at 10 °C. Infective stage is reached faster (2 days) at 35 °C than at 20 °C (3-4 days). At 37-39 °C there is rapid hatching but the larvae die before reaching infectivity.

By culturing 5 g and 10 g faecal samples in open and lightly capped jars between 25-39 °C, Mfitilodze and Hutchinson (1987), demonstrated that the yield of infective larvae at a certain temperature is dependent on relative humidity. In samples cultured in open jars at the same temperature, 5 g samples lost their moisture content faster than 10 g samples. The percent yield of infective larvae was higher in 10 g samples than in 5 g samples. The yield from closed jars was always higher than from open jars in both 5 g and 10 g

samples. This suggested that there were higher yields of larvae from samples with higher moisture content. The relative humidity in closed jars varied between 60 and 70%, while in open jars, the relative humidity reached 18-21% in one month. There was a marked reduction in the number of larvae surviving in closed jars at 37 °C, while in open jars a few larvae were alive after 3 months. At temperatures of 20-28 °C large numbers were alive in both open and closed jars. This suggests that the long term survival of infective larvae is dependent on both temperature and moisture. The critical moisture content for development is 15- 20%. Similar results were obtained by Ogbourne (1972) by allowing faecal cultures to dry rapidly (by pumping dry air through a tube connecting the culture jars) or slowly (by pumping non-dry air through the apparatus containing the samples). When faeces dried quickly, many larvae died before reaching the infective stage; but those that survived desiccation developed to the infective stage when faeces were moistened. If faeces dried slowly, most larvae developed to the second stage and those that survived desiccation at the second stage resumed development after faeces were moistened.

Experiments involving prolonged exposure of eggs to cold conditions were carried out by Lucker (1941). In temperatures of between -10 to -6 °C for 7-8 weeks, very few or none of the unembryonated eggs survive. At a temperature of about 2 °C a large proportion of the eggs die but a small proportion survives for up to 195 days. In the same temperature range, embryonated eggs die more readily than unembryonated ones. Rapid freezing and thawing does not reduce the resistance of eggs to temperatures of

about -10 °C so long as the thaw is not long enough for development to take place. However, if the cultures are allowed to stand at room temperature long enough for some development to take place, most larvae die during the next freezing period. At -10 to -6 °C, pre-infective larvae die rapidly, while at 2 °C 90% of larvae die in 80 days. Larvae that have recently attained infective stage are susceptible to freezing.

Infective larvae are very resistant to most environmental conditions and have been shown to withstand temperatures of -196 °C for one hour and once thawed are capable of exsheathment and motility (Bemrick, 1978). Desiccation of larvae before exposure to low temperatures can also protect them from the adverse effects of freezing.

1.4.1 Outdoor experiments

Outdoor experiments on the development, survival and migration of the free-living stages of equine strongyles have been completed under various environmental conditions. In northern temperate lands, studies have been performed in Britain (Ogbourne, 1972, 1973), Saskatchewan (Polley, 1986) and the USA (Lucker, 1941). In tropical and subtropical environments, such studies have been carried out in Australia (English, 1979a, 1979b; Mfitilodze & Hutchinson, 1988; Hutchinson et al, 1989).

These studies suggest that temperature and moisture are the most important factors influencing development, survival and migration of free-living stages of equine strongyles. However, there are major differences in behaviour depending on climatic

region. In Britain hatching and subsequent development to the third stage takes place fastest in the warm summer months. Development to the third stage only takes place during March to October and the proportion of eggs that attain infective stage varies, with lower numbers in March and higher proportions in April to October. Development is delayed during warm, dry conditions, but is completed after faeces are moistened by rain. Eggs deposited on British pastures during winter do not produce infective larvae. Winter temperatures in this region are not low enough to inhibit development of the eggs but are too low for the pre-infective larvae hatching from the eggs.

Migration of infective larvae from faeces to pasture is gradual and depends on the amount and frequency of rain. Very few larvae were recovered from herbage during persistent dry conditions and heavy rain led to a sharp increase in numbers. By seeding plots with infective larvae, Ogbourne (1973) was able to show that movement to pasture during the warm months was in a few weeks of deposition, with a decline to very low levels within 6-8 weeks. Amount of rain influenced the rate of migration and there were sharp increases of larvae recovered following rain after a long dry spell. There were very high and prolonged recoveries during winter, and larvae persisted up to the following spring.

The study in the USA was conducted in the winter, when temperatures were 7.7-13.3 °C. Under these conditions the eggs developed but the greater proportion of the resulting larvae died before attaining the infective stage. As temperatures became warmer, more

larvae developed to infectivity. Lack of prolonged observation made it impossible to deduce anything about the longevity of these larvae. Similar results were obtained in the Canadian prairie (Polley, 1986). Winter experiments yielded very low levels of infective larvae and although decreasing numbers of eggs were recovered through to spring, most were not viable. Persistent low temperatures in the winter also killed most pre-infective larvae but the few that survived had the potential to develop to infective stage when temperatures became favourable in the spring. Fastest development occurred in warm summer months, with May, June and July being the most favourable months. Development to infective stage took place throughout the summer. Small numbers of larvae from samples deposited in May survived until the following May. In samples deposited during July and August, small numbers of larvae survived till the following summer and fall.

In tropical and subtropical lands, development takes place all year round with the most important factor being moisture. In the warmest months when the mean maximum temperatures are 30-35 °C and mean minimum temperatures are 28-33 °C, development is rapid although resulting larvae survive on pasture for relatively short periods. In cooler months when mean maximum temperatures are 27-30 °C and mean minimum temperatures are 15-20 °C, development is much slower and larvae survive for up to 11 weeks. In S.E. Queensland, highest numbers of larvae in pasture are recovered in spring and early summer and in autumn and early spring (English, 1979a, 1979b). Migration of larvae to herbage takes place following 25 mm of rain. In north east Queensland,

where drier conditions prevail, highest recoveries of larvae are in winter while lowest recoveries are in spring. Longest survival is in samples deposited just before the onset of the cool period (Mfitilodze & Hutchinson, 1988). During hot wet periods, low yields of infective larvae are accompanied by translation (movement of larvae from faeces to herbage) of large numbers to pasture and survival of a maximum of 56 days. During the hot dry periods, there are low yields of larvae and translation is rare (Hutchinson et al, 1989).

1.4.2 Egg counts

Faecal egg counts can indicate patterns of pasture contamination with strongyle eggs. Patterns of egg shedding by strongyle worms have been studied in northern temperate lands indicate there is seasonal variation in the egg counts of strongyle worms. Poynter, (1954) noticed this phenomenon in Britain by studying a group of horses for a prolonged period of time. Similar patterns have been observed in Britain (Duncan, 1974, Herd, 1986a), northern USA, (Herd & Willardson, 1985; Herd et al, 1985) and southern USA, (Craig et al, 1983; Courtney & Asquith, 1985; Kivipelto & Asquith, 1994) and Ontario (Slocombe et al, 1987). There are no studies relating the pasture contamination with strongyle eggs to the seasonality in other regions in Canada.

In general there is a spring/summer rise in egg counts from all areas studied. However, peak counts are reached at different times in different areas. In Britain, maximum faecal egg counts are observed in summer (Poynter, 1954, Duncan, 1974). Poynter noted that

the egg counts were at a minimum during the winter in pony mares. After this there was a gradual increase, with a maximum count being attained in August or September, followed by a decline in the winter months. Duncan, (1974) noticed a similar pattern with maximum counts being attained between August and October for the mares and in November for the foals. In northern USA, a second peak in egg counts has been noticed in the spring (Herd et al, 1985). A spring peak has also been reported in New Market, England (Herd, 1986a). In Ontario, Slocombe et al (1987), reported an increase in egg counts through spring and early summer with a small peak in late September to early October. In the southern USA, where the climate approaches tropical, a summer rise in egg counts has also been noticed with maximum counts in the summer and early fall (Craig et al, 1983). Seasonal changes do not appear to be related to parturition or lactation (Duncan, 1974, Herd et al, 1985). The spring rise also takes place even in horses still indoors. This suggests that the rise is not due to ingestion of new infective larvae but is probably due to maturation of immature worms emerging from tissues (Herd et al, 1985).

Differential counts of larvae from faecal cultures reveal that in all the regions studied, cyathostomes are the main contributors to the egg counts with a range from 94 to 100% (Craig et al, 1983; Herd & Willardson, 1985; Herd, 1986a; Herd et al, 1985; Boersema et al, 1995). Additionally a seasonal pattern in the occurrence of *Strongylus* species also occurs (Poynter, 1954; Duncan, 1974). A higher proportion of *S. vulgaris* occurs in

mares in summer while in foals the highest proportion of *S. vulgaris* occurs in winter when foals are 9-11 months old (Duncan, 1974).

A seasonal pattern in the profiles of adult gravid female worms has also been reported (Ogbourne, 1971a, 1975; Reinemeyer et al, 1986; Dunsmore & Jue Sue, 1985). During spring and summer the greatest proportion of female worms recovered from the lumen of the large intestines of horses in Britain contained viable eggs. This proportion decreased during late autumn and remained at a low level throughout the winter to rise again the following spring (Ogbourne, 1971a, 1975). A similar pattern is also present in the northern USA, although the cycle is clearly ahead of the British cycle (Reinemeyer et al, 1986). This may suggest that similar patterns of strongyle worm maturation occur in the northern temperate lands, with the differences being the particular month of the year that the peaks are attained in different geographical regions. In western Australia, peak numbers of adult *S. vulgaris* occur in the summer and the lowest levels in the winter. There are no indications of clear trends of infection levels with the small strongyles in this region, although there appear to be minor peaks in midsummer and midwinter (Dunsmore & Jue Sue, 1985). In northern temperate lands there is an annual turnover of worms with single generation yearly cycles (Ogbourne, 1975; Reinemeyer et al, 1986).

1.4.3 Pasture infectivity

Although faecal egg counts are a good indicator of the levels of pasture contamination, the actual pasture infectivity as estimated by the number of strongyle L3 per kilogram dry matter is a more useful parameter in determining exposure to infection of pastured horses (Herd, 1986a). Observational studies estimating the number of L3 per kilogram dry matter indicate that levels are seasonal and closely follow patterns of egg shedding in different regions. In Scotland, farms without a control programme exhibit peak larval counts from July to October. Overwintered larvae persist on pasture until April of the following year and die off by late May to early June (Duncan, 1974). In England maximum numbers are attained in late summer and early fall (Herd, 1986a). Similar patterns exist in other northern temperate regions. Craig et al, (1983) reported an increase in pasture larval counts in October in Southern USA. Peak egg counts were recorded in the same place between August and October and there was a rapid increase in larval counts following rain. In northern USA, pasture larval counts are maximal in late summer and fall (Herd & Willardson, 1985, Herd et al, 1985). Overwintered larvae are found on pasture the following spring and reach a minimum in midsummer. Levels of larvae are lower in lawns (areas of low grass where horses graze) than in the whole pasture and the roughs (areas of tall grass where horses defecate) (Herd & Willardson, 1985). However, similar patterns exist in the whole pasture, the roughs and lawns. Peak larval counts are recorded 2-4 weeks after peak egg counts (Herd et al, 1985; Herd, 1986a).

Horses grazing on Ontario pastures are likely to be exposed to some larvae during spring turnout (Slocombe et al, 1987). Levels decrease to very few or no larvae by the end of June. There is an increase in July and high levels are maintained until mid October.

1.5 Prevalence and relative abundance

Surveys from different regions of the world, beginning in the 1920's to present day have indicated a striking similarity in the species of small strongyles infecting equids over vast regions. Apart from changes in nomenclature, little has changed over more than 50 years. Boulenger (1921), described 21 species of strongyles in the Punjab, India. The same species were recovered in Great Britain (Le Roux, 1924) and similar results were obtained in Panama (Foster, 1936; Foster & Ortiz, 1937). Recently, studies have been carried out in Australia (Dunsmore & Jue Sue, 1985; Mfitilodze & Hutchinson; 1990), Britain (Ogbourne, 1976) and the USA (Reinemeyer et al, 1984; Torbert et al, 1986; Lyons et al, 1991). The prevalence of cyathostomes is very high in all the regions studied, with a range of 89% (Mfitilodze & Hutchinson, 1984) to 100% (Ogbourne, 1976; Reinemeyer et al 1984). However, Reinemeyer *et al* (1984) only performed recoveries from horses that were positive by egg count and this may not reflect the prevalence in the general horse population in Ohio, USA, where the study was carried out. Cyathostomes contribute the higher proportion of eggs to faecal egg counts than the large strongyles (Duncan, 1974; Craig et al, 1983; Herd & Willardson, 1985; Herd et al, 1985; Herd, 1986a; Herd & Gabel, 1990; Yadav et al, 1993; Boersema et al, 1995).

The most prevalent cyathostome species account for up to 98.9% of the worm burden (Reinemeyer et al, 1984).

The prevalence of large strongyles, unlike that of small strongyles, has decreased. *Strongylus vulgaris* had a prevalence of 90 to 100 % and 90% of colics were attributed to this parasite before the advent of the benzimidazole era (Kerster, 1975; Herd, 1990b). Recently years this situation has changed and surveys for both the worms and colics indicate reduced occurrence (Herd, 1990b). Studies involving examination for adult worms in the intestines and parasitic larvae in the mesenteric arteries indicate that prevalence of adult worms is 22.5-39%, while that of parasitic larvae and their lesions is 25-62.9% (Lyons et al, 1981; Reinemeyer et al, 1984; Dunsmore & Jue Sue, 1985; Mfitilodze & Hutchinson, 1990). In farms where anthelmintic use is either nonexistent or irregular, prevalence can be up to 83.3% (Torbert et al, 1986). Low grade infections may also persist, even in horses on regular deworming programmes (Austin, 1994). Histological examination of anterior mesenteric arteries for lesions revealed that as many as 98% of horses had chronic lesions (Morgan et al, 1991). This may indicate that most horses still get exposed to *S. vulgaris*.

Strongylus vulgaris and other large strongyles contribute only a small percentage of the eggs passed in faeces (Duncan, 1974; Craig et al, 1983; Herd & Willardson, 1985; Herd et al, 1985; Herd, 1986a; Kivipelto & Asquith, 1994) and larvae on pasture (Duncan 1974; Herd & Willardson, 1985; Herd, 1986a; Slocombe et al 1986). This contribution

may depend on the season of the study; levels of adult worms in the large intestines and larvae in the mesenteric arteries is seasonal (Ogbourne, 1971a, 1975; English, 1979c; Eysker & Wimmenhove, 1987; Lyons et al 1991; Morgan et al, 1991; Lyons et al, 1994b).

1.6 Diagnosis

Clinical diagnosis of strongyle infection is difficult; there are no pathognomonic signs (Herd, 1986b). History of high stocking densities, poor nutrition and irregular or infrequent deworming may be helpful although disease may occur despite frequent use of anthelmintics if resistance occurs (Drudge & Lyons,1986). Faecal examination to for ova remains the only method of confirming patent infections. The limitations of this method are due to the fact that pathogenicity is due mostly to larvae, and during disease few or no ova are passed (Giles et al, 1985); hence there is no indication of immature, migratory or hypobiotic worms (Herd, 1986b). Also it is not possible to differentiate the eggs of one species from any other or from those of *Trichostrongylus axei*. Furthermore the level of infection cannot be estimated from the level of egg counts since direct correlation between the two is very weak.

In larval cyathostominosis, faecal examination can be used to demonstrate larvae in faeces (Giles et al 1985). Caecal or colonic biopsy or rectal mucosal scrapings can also be diagnostic (Jasko & Roth, 1984; Uhlinger, 1991). Immunological and haematological tests still remain unreliable and can be misleading because they lack specificity (Wright,

1972; Herd, 1986b). ELISA tests give cross-reaction between strongyle infections and those of other nematodes (Nichol & Masterson, 1987). Elevated ß1-globulins are only useful as possible indicators of disease; low values do not indicate its absence due to the large variability in normal values (Mair et al, 1993). Also high ß- globulins in a non-symptomatic horse is inconclusive (Schulze et al 1983). A preliminary study measuring fibrinogen concentration in foals indicated that this parameter is of no value in detecting early strongyle infection (Jensen et al, 1993).

1.7 Treatment and control

1.7.1 Treatment

Treatment for strongyle infections depends on highly efficacious broad spectrum drugs. Large strongyles are susceptible to most of the anthelmintics presently available. Adult large strongyles can be removed easily by ivermectin (Piche et al, 1990, 1991; Maqbool, 1993), and benzimidazoles (Austin, 1994). Ivermectin has also been reported to be effective against migratory larvae (Slocombe & Cote, 1984; Slocombe et al, 1986; Love, 1992), in preventing lesions (Klei et al, 1984b, 1990, 1993) and eliminating lesions (Drudge et al, 1984a; Turk & Klei, 1984b; Holmes et al, 1990). Some benzimidazoles i.e fenbendazole, oxfendazole and thiabendazole at elevated doses are also effective against migratory stages (Kingsbury & Reid, 1981; Lyons et al, 1983, 1986, 1988; Slocombe et al, 1983, 1986; Hopfer et al, 1984; Love, 1992).

Adult small strongyles are treated successfully using ivermectin (Slocombe & Cote, 1984, Dipietro et al, 1986; Burger & Bauer, 1987; Berry II et al, 1993). In farms where benzimidazole resistance already exists oxibendazole (Drudge et al, 1984a, 1985; Slocombe et al, 1989; Herd & Gabel, 1990; Lyons et al, 1994c) is effective but this activity decreases with continued use. Larval cyathostominosis is difficult problem to treat. Elevated doses of fenbendazole (Jeggo & Sewell, 1977; Lyons et al, 1983) oxfendazole and thiabendazole (cited by Klei et al, 1993) are effective. Ivermectin is effective against the L4 in the lumen (Klei & Tobert, 1980; Burger & Bauer, 1987; Dipietro et al, 1986; Klei et al, 1993) but is ineffective against encysted larvae (Klei et al, 1993). Treatment is more successful when supportive therapy and anti-inflammatory drugs such as dexamethasone are included in the regimen (Uhlinger, 1991).

Other broad spectrum drugs include closantel (Guerrero et al, 1985), pyrantel (Drudge et al, 1982, 1984b; Burger & Bauer, 1987; Austin, 1994; Boersema et al, 1995), thiophanate (Yadav et al, 1993) and moxidectin, a member of the milbemycin family (Dipietro et al, 1992). Trichlorfon has been mixed with several benzimidazoles, resulting in efficacy against both large and small strongyles (Seibert et al, 1986; Presson et al, 1984). Morantel and trichlorfon are active against large strongyles (Drudge et al, 1983, 1984c). A mixture of febantel and piperazine has satisfactory activity against cyathostomes (Drudge et al, 1984b; Dipietro et al, 1985). A mixture of piperazine and levamisole has a wider spectrum than either drug used alone (Dipietro and Todd, 1987).

1.7.2 Control

Effective strongyle control is aimed at prevention of pasture contamination and prevention of infection in horses. Traditional methods of control emphasize reduced contamination of the environment through removal of egg-bearing adults from the intestines (Herd, 1986b). Previously deworming was performed at fixed intervals, usually 6 to 10 weeks, using either one drug repeatedly, or in rotation with others (Ewert et al, 1991). The use of one drug throughout is very common (Herd, 1993). It has however been realised that this method of control is deficient in that the intervals between deworming are too long to prevent pasture contamination yet short enough to select for drug resistance (Reinemeyer & Rohrbach, 1990). Shorter treatment intervals of 30-60 days can eliminate pasture contamination and infectivity completely. However, shorter treatment intervals are not desirable as they do not allow development of immunity in young animals, and increase the occurrence of drug related problems (Herd, 1993). Due to these disadvantages, other methods of control that do not rely heavily on anthelmintics, have been suggested and some of them have been tried and found to be successful. Among these are methods based on the biology of parasites. Strategic control with oxibendazole every four weeks during the summer only was found to be effective in reducing pasture infectivity in English pastures (Herd, 1986a). In the Netherlands, three treatments with ivermectin during the summer reduced both egg counts and pasture infectivity (Eysker et al, 1991). In northern USA, two treatments, one in spring and one in summer were very effective in reducing pasture larval counts (Herd et al, 1985). Subsequently, Herd (1986b, 1993) formulated a control programme

for northern USA recommending that horses be treated at 8-week intervals with ivermectin or 4-week intervals with other anthelmintics. The anthelmintics should be used in slow rotation every one to two years. In the south, s similar regime can be used but only in the autumn and winter. This regimen has been tried successfully in southern USA (Reinemeyer & Henton, 1987). Integration of anthelmintic use with climatic features was also used in Australia resulting in a reduced availability of *S. vulgaris* infective larvae on pasture (Dunsmore, 1985).

Strategic treatment can be combined with faecal egg counts to decide when to treat (Herd, 1990a) and to monitor success of the programme (Ewert et al, 1991; Herd, 1993; Uhlinger, 1993). High faecal egg counts following anthelmintic treatment may indicate the beginning of drug resistance (Bennett, 1983, Herd, 1993). Faecal egg count data has been used to design control programmes whereby only horses shedding high numbers of eggs were dewormed (Duncan & Love, 1991, Gomez & Georgi, 1991). In one study, the programme was successful in reducing pasture contamination and was economical (Duncan & Love, 1991). Other methods include alternate grazing of horses and sheep (Eysker et al, 1983, 1986b). However, this may enhance the transmission of *Trichostrongylus axei*. *T. Axei* causes gastritis in horses (Eysker et al, 1986b)An alternative control method is pasture sweeping or vacuuming (Herd, 1986c, 1986d) and twice weekly removal of manure from pasture (Herd, 1986a). This also increases the area available for grazing. Treatment of mares once with ivermectin at parturition has been found to protect their foals for the first two months of life (Ludwig et al, 1984).

In farms where new horses are introduced at various times, treatment with ivermectin and isolation for 2-3 days can prevent contamination of pastures by new arrivals (Berry II et al, 1993). Recently, daily therapy using anthelmintics at a lower than therapeutic dose during the period of highest exposure has been advocated. It has been suggested that this, in addition to removing adult parasites, prevents infection and migration (Dipietro, 1992). Strategic daily use of pyrantel tartarate in mares reduced both pasture contamination and infectivity; and when used in foaling mares it protected their foals until weaning (Herd & Majewski, 1994). Integrated control involving strategic use of anthelmintics and pasture management is the most beneficial. This is especially important when the fact that anthelmintics are less efficacious in young as compared to adult animals is taken into consideration (Herd, 1986a; Herd & Gabel, 1990; Herd & Majewski, 1994). Successful strongyle control has been associated with reduction of the incidence of colics (Uhlinger, 1990).

1.7.3 Anthelmintic resistance

Anthelmintic resistance is defined as "a significant increase in the ability of individuals within a strain to tolerate doses of a compound which would prove lethal to the majority of individuals in a normal population of the same species" (Prichard et al, 1980). A further condition for drug resistance to occur was added by an Australian party on anthelmintic resistance (Bjorn et al, 1991). There should be less than 95% reduction in faecal egg counts among resistant strains and the lower 95% confidence limit of the percent reduction should be less than 90%. Drug resistance is a heritable trait (Prichard

et al, 1980). Anthelmintic resistance in strongylid nematodes of the digestive tract of domestic animals occurs globally (Bjorn, 1994; Prichard, 1994). Among horse nematodes benzimidazole resistance is common in cyathostomes in Britain (Ryan et al, 1987; Mair & Cripps, 1991; Fisher et al, 1992), the Netherlands (Eysker et al, 1988, Boersema et al, 1991), Denmark (Bjorn et al, 1991), Germany (Bauer et al, 1986) and Australia (Kelly et al, 1981). In North America benzimidazole resistance has been reported in the USA (Drudge et al, 1982; Uhlinger & Johnstone, 1984, 1985) and Canada (Slocombe et al, 1989). Benzimidazole resistance in small strongyles has been attributed to widespread use of the benzimidazoles (Herd & Gabel, 1990). This has resulted in a strong selection for resistance in the cyathostomes while efficacy against the Strongylinae has been retained. The end result has been an increase in the prevalence of cyathostomes together with the disease attributed to them.

Benzimidazole resistance is very widespread in regions where prevalence surveys have been carried out (Uhlinger & Johnstone, 1985; Bauer et al, 1986, Bjorn et al, 1991; Boersema et al, 1991). In one study, drug resistance has been associated with clinical disease (Fisher et al, 1992). Drug resistance is more prevalent on farms which use the benzimidazole class of anthelmintics exclusively (Fisher et al, 1992), although Uhlinger & Johnstone (1985) found no relationship between resistance and management in the farms they surveyed. They attributed this to possible widespread use of the benzimidazoles on these farms prior to their study. Resistance can be spread from farms where it exists to farms with susceptible small strongyle populations by mixed grazing

of horses and purchase of animals harbouring resistant strains (Bjorn et al, 1991) or by importation of animals from one country to another (Slocombe et al, 1989). Once resistance occurs to one member of the class, there is side resistance between that compound and other members of the same group, including the probenzimidazoles. The only benzimidazoles effective against cyathostomes with resistance to the benzimidazoles (and probenzimidazoles) are oxibendazole (Drudge et al, 1984b, 1985; Bauer et al, 1986; Lyons et al, 1994c) and fenbendazole. Resistance develops quickly with continued use (Drudge et al, 1985, Lyons et al, 1994c). Once resistance has developed, reversion does not occur even after 2-3 years of use of non-benzimidazole anthelmintics (Uhlinger & Johnstone, 1984). The most common species are also the ones reported to be resistant (Reinemeyer et al, 1984; Eysker et al, 1985; Tobert et al, 1986). The mechanism of resistance to the benzimidazoles is not understood properly, but in *Haemonchus contortus*, a strongylid parasite of sheep, it has been shown to be associated with a mutation in the gene coding for ß-tubulin (Kwa et al, 1994; Lubega et al, 1994).

Widespread anthelmintic resistance has not been noted for any other drug class in equines. However, pyrantel resistance has been reported in one stud in England and cases of resistance have also been reported in USA (Bjorn, 1994). Piperazine resistance has been reported after prolonged use in Kentucky, USA (Drudge et al, 1988). Ivermectin still remains very effective although cases of resistance in *Haemonchus contortus*, *Trichostrongylus* and *Teladorsagia* species in both sheep and goats have been reported (Shoop, 1993; Prichard, 1994); with side resistance to moxidectin (Shoop,

1993). There is no widespread benzimidazole resistance in large strongyles, although one case of *S. vulgaris* resistance to albendazole has been reported (Bjorn, 1994).

It is doubtful that any new drugs, with novel modes of action, will be available in the market in the next few decades. A need to preserve the efficacy of the ones already available is therefore great (Bjorn, 1994). In areas where resistance already exists, use of anthelmintics in slow rotation is a viable alternative. In one study, there was no evidence that this serves to delay the onset of resistance as far as number of times of exposure is concerned; but neither does it enhance its onset (Uhlinger & Kristula, 1992). Use of anthelmintics sparingly where anthelmintic resistance does not exist should delay development of resistance. This can be achieved by utilising methods that rely less on chemicals and more on the epidemiology of the parasites.

1.8 Immunity

There is no report of absolute immunity to strongyle infection. Prior infection fails to protect the animal from either reinfection or establishment of considerable worm burdens, but it seems to delay patency (Smith, 1976b, 1978;). Ingestion of large numbers of infective larvae is more immunogenic than ingestion of small numbers at regular intervals (Klei et al, 1982b). *In vitro,* that extracts from L4, L5 and adults cause lymphocyte proliferation (Bailey et al, 1984) and this was thought to be the mechanism of development of hyper ß-globulinaemia in strongyle-infected animals. Ponies experimentally infected with *S. vulgaris*, develop the ability to induce lymphocyte

responsiveness, followed by development of complement fixing antibodies and finally eosinophilia (Bailey et al, 1989). However, continued exposure leads to decreased responsiveness and there is no antibody response to adults in the intestinal lumen. Vaccination with irradiated larvae failed to protect animals against infection with *S. vulgaris*, although disease and lesions were less serious than in non-vaccinated controls (Klei et al, 1982b). *In vitro* studies have shown that eosinophil adherence to L3 is antibody dependent (Klei et al, 1992). The antigens stimulating this response are species specific and epitopes are shared between adults and L3. In addition there is a non-specific leucocyte adherence factor in both immune and non-immune animals, probably complement. Recently a cytokine responsible for chemotactic attraction of eosinophils and neutrophil chemokinesis was described (Dennis et al, 1993). Eosinophils accumulate and degranulate at sites of infection with parasites (Tizard, 1992). Degranulation leads to the release of products of the respiratory burst including superoxide ions, hydrogen peroxide and other free radicals. In addition, there is release of eosinophil major basic protein, which causes damage to the cuticle (Tizard, 1992). Eosinophils immobilization and death of L3 *in vitro* (Klei et al, 1992). However the role of eosinophils in resistance *in vivo* is not known.

Age is not a factor in resistance to infection, although disease is more serious in young animals than in older animals. Foals and yearlings have shorter egg reappearance periods after treatment (Drudge & Lyons, 1986). This is thought to be due to a relative

lack of immunity in the younger animals leading to accumulation of encysted larvae in the intestinal wall (Herd & Gabel, 1990; Mair, 1993).

1.9 Objectives of the study

Horses in northern temperate climates show a peak in strongyle egg production in spring and summer. However, regional differences with regard to peak egg production exist, depending on climatic factors (Herd, 1986b). In 1984, the Equine Subcommittee of the American Association of Veterinary Parasitologists Research Committee recommended that studies be performed to identify seasonal patterns and ecological factors involved in the transmission of equine parasites on a regional basis (Klei et al, 1984a). Up to now no such study has been carried out in P.E.I or elsewhere in the Maritime Provinces of Canada. It is anticipated that the results of this study will be useful in the formulation of a control programme that is specific for this region.

The broad objective of the study was to establish the patterns of pasture contamination, infectivity, rates of development and survival of equine strongyles in the Maritime region. The specific objectives of the study were:

1. (a) To follow three herds of horses using monthly egg counts for one year to establish the patterns of pasture contamination with strongyle eggs on P.E.I.

   (b) To culture positive faecal samples used in egg count data and identify the resulting third-stage larvae to determine which subfamilies of strongyles are responsible for the egg counts in the three herds.

(c) Carry out postmortem examination of three tracer horses that grazed the same pastures with the three herds for one grazing season to recover and identify the adult worms found in the large intestines.

2. To sample pastures monthly during the grazing season to establish the patterns of pasture infectivity.

3. To place faecal samples of known levels of infection on pasture monthly for 12 months and to monitor them for larval recoveries to establish the rates of development, survival and migration of the infective larvae to surrounding herbage during different times of the year in the climatic conditions of the Maritime region.

2. MATERIALS AND METHODS

2.1 Faecal egg counts, faecal cultures and necropsy of tracers

2.1.1 Faecal egg counts

Three horse herds (Tables I and II) in P.E.I were followed with monthly faecal egg counts for thirteen months from April 1993 to April 1994. Herds 1 and 2 consisted of mares, foals and yearlings, while Herd 3 initially consisted of ten mares. Three horses were introduced into Herd 3 in August, but their egg count data was not included in the herd data until November following the October treatment. All animals grazed natural pastures during the summer and fall. Herd 3 was turned out to pasture in June, moved to an adjacent pasture in the middle of July and back to the original one in the first week of August. It was stabled at the end of September and treated with 200μg/kg body weight ivermectin (Eqvalan®, Merck AgVet, Pointe Claire-Dorval, Quebec) after the October sample was taken. This herd was normally used as the University teaching herd and it was normally treated once a year at the time of stabling. The other two herds which were combined at the beginning, were treated with pyrantel pamoate (Strongid-P®, Rogar/STB, London, Ontario) at a dose rate of 6.6mg/kg body weight in April 1993 before the spring turn out. Before this they were under no particular anthelmintic control programme. The two herds were used as breeding stock for racing horses and there was considerable movement both between the two herds and in and out of the herds. Yearlings and foals were stabled in October. Herd 1 mares were housed in early

Table I. Herd composition and pasture size.

Herd	Composition	Pasture size (m^2)	Age where known	Breed	Remark
1	6-10 mares, 5-12 yearlings	103,522	2-9 years	Standard-breds	foaling, 2 in 1993; 4 in 1994
2	30-40 mares	53,312	3-12 years	Standard-breds	foaling; 11 in 1993; 10 in 1994
3	10-12 mares	31,403	2-25 years	10 Standard-breds; 1 Appaloosa; 1 Morgan	Used for teaching in winter; rested on pasture June to September; treated annually in fall.

Table II. Management practices for Herds 1,2 and 3 during the period of the study.

Herd	1	2	3
Month			
April '93	Housed together; Pyrantel treatment		In barn
May	Out to pasture		In barn
June	Separated	Yearlings moved to 1	Out to pasture
July	At pasture	At pasture	Moved to adjacent pasture
August	At pasture	At pasture	back to original pasture
September	At pasture	At pasture	housed; treated-ivermectin
October	Yearlings back to 2 and housed; some foals moved to 2	Foals housed; mares moved to adjacent pasture.	In barn
November	Mares housed; treated with levamisole	Some mares housed; foals treated with ivermectin	In barn
December	In barn	Some mares, all yearlings and all foals in barn	In barn
January	Remaining foals treated - ivermectin	As above	In barn
February	In barn	As above	In barn
March	In barn	As above	In barn
April '94	In barn	As above	In barn

November, while most Herd 2 mares stayed out all winter where they were fed hay. Herd 2 foals were treated with ivermectin in the middle of November, while those in Herd 1 were treated twice, once in November and again in January. Herd 1 mares were also dewormed in the middle of November using a levamisole cattle preparation at an unknown dosage. The three herds were chosen because of owner compliance, an absence of anthelmintic prevention protocol and their close location to Atlantic Veterinary College.

Faecal samples were obtained per rectum or as freshly voided faeces. Samples were collected in air tight, screw-top, plastic, 120 ml sample containers (Corning Glass Works, New York) and were labelled with the name of the horse, date of collection and the herd. Effort was made to collect samples from the whole Herd but when this was not possible as many samples as possible were collected. The aim was to sample at least 70% of the horses.

After collection samples were transported to the laboratory and stored at 4 °C. Faecal egg counts were done within 4 days of sample collection, using the modified Cornell-MacMaster technique (Georgi & Georgi, 1990) with a slight modification. The counting chambers were made by cementing together two microscope slides separated by two thickness of slides cut into narrow strips. The upper and lower slides were offset slightly to facilitate filling of the chamber. Ten grams of faeces were weighed in a plastic cup and 150 ml of distilled water was added. Samples were mixed thoroughly for

approximately 5 minutes by stirring with a magnetic stirring bar. Counting chambers were loaded with two 300 μl samples of concentrated sucrose solution (Sheathers sugar - 5000 g sugar, 4000 ml water and 32 g phenol). Equal volumes of the faecal-water mixture were added to each of the sucrose solution pools. The pools were mixed thoroughly with a wire loop and allowed to stand for 15 minutes, after which all the eggs in each pool were counted. Care was taken to include the eggs found in the darkened edges. The eggs per gram (EPG) of faeces was calculated by multiplying the raw count (total number of eggs counted in both pools) by 25. This method can detect at least 25 EPG. Egg count data were expressed as median EPG for the different groups because some individual horses passed more eggs than others.

2.1.2 Faecal culture and larval identification

All samples positive for strongyle eggs were cultured for L3 recovery and identification (Georgi and Georgi, 1990). The samples were cultured in the sample collection containers. If the container was almost full, some faeces was discarded so that there was room in the container for free movement if the container was shaken. Containers were capped loosely and stored in a dark shelf at room temperature (18-20°C) for seven to ten days, after which the larvae were recovered for identification.

Initially, larval recovery was carried out using the Baermann technique (Georgi and Georgi, 1990). Funnels were set up on a stand and the rubber hoses at the end of the funnels were clamped. The cultured faecal samples were wrapped in cheese cloth and

placed in the funnel. Luke-warm water was poured into the funnel using culture containers. The containers were rinsed thoroughly with the washings being put into the apparatus. The apparatus was left to stand overnight at room temperature after which water was drawn from each hose into 15 ml centrifuge tubes. The tubes were left to stand at 4 °C overnight.

The Baermann technique was abandoned due to space requirements and inconvenience of processing the large number of samples involved. All samples from June 1993 onwards were recovered following the jar-over-petri-dish method (Ministry of Agriculture Fisheries and Food, 1986). Water was added to the culture until the container was half full. Faeces were loosened using a tongue depressor and the container was filled to the brim with water. A petri dish was placed over the mouth of the container which was then inverted with the petri dish serving as a base. About 15 ml of water was poured into the petri dish and the whole apparatus allowed to stand overnight. The fluid in the petri dish, which contained the larvae was pipetted into 15 ml centrifuge tubes and allowed to stand at 4 °C overnight. Larvae from this technique, if harvested with care were usually as clean as those from the Baermann apparatus and further examination was carried out as below. Both techniques also depend on active movement of the infective larvae (Mfitilodze & Hutchinson, 1988) and yield similar results.

Larvae were killed and stained by adding 0.15 ml Lugol's iodine and 0.15 ml formalin to 4.5 ml of water containing larvae in a 15 ml centrifuge tube. Water was added to

make up the volume to 5 ml. The mixture was left to stand for 10 min. Larvae were dispersed by bubbling air through the mixture several times from a pipette. A small amount of the mixture was pipetted and transferred to a microscope slide. A 22 X 40 mm coverslip was added. The slide was examined using a compound microscope with a 10 X objective. Occasionally a larva was examined with the 25 X objective when the intestinal cells could not be seen properly at the 10 X objective. The first 100 larvae encountered were examined and classified according to Russell (1948) and Georgi and Georgi (1990). When less than 100 larvae were recovered from a sample, all larvae were characterised. Larvae having 8 to 12 intestinal cells were classified in the subfamily Cyathostominae. Those of *Strongylus vulgaris* were easily recognised due to their large size and the presence of 28 to 32 clearly defined intestinal cells. All other larvae were classified in another group designated 'others' which included larvae with 16 to 20 intestinal cells.

2.1.3 Necropsy of tracers and worm identification.

Three horses were necropsied as tracer horses for two of the Herds. One horse was a yearling colt (Herd 1), one was a cull mare from Herd 3 (University Herd) and the other was a gelding from Herd 3 that died accidentally during the trial months of the study. The colt was purchased in the spring and was given one dose of ivermectin at the standard dose of 200 μg/kg body weight and 5 daily doses of oxibendazole (Anthelcide® Smithkline Beecham, Missassauga, Ontario) paste at a rate of 20 mg/kg body weight. This was to ensure that it was parasite-free before turnout. A faecal egg count during

turn out yielded a count of zero. The colt was grazed together with Herd 1 throughout the grazing period (between June and November). It was necropsied on December 8, 1993. The cull mare grazed together with the other members of Herd 3 but was not dewormed before euthanasia on November 18, 1993. Although the two horses from Herd 3 were not real tracers, it was decided that they could serve the purpose of indicating the species of strongyle to be found in this herd since the herd was completely closed at the beginning and records indicated that it had remained so for a long time.

Necropsy was carried out following a routine procedure. The caecum, ventral colon and dorsal colon were identified and freed from the mesentery. Each section was ligated at both ends and separated from the next section. Each section was opened longitudinally along the dorsal aspect and the contents poured into graduated 10 litre buckets, depending on the amount. The mucosal surface was washed carefully into the buckets. Water was added to a final volume of 10 litres. The contents were mixed by stirring for 2 minutes and a 10% aliquot was taken from each bucket. All aliquots for each section were put in the same bucket and preserved overnight by addition of sufficient formalin to give a final concentration of 10%.

The next morning, the aliquots were examined in 10% subaliquots. Each subaliquot was washed through a 0.15 mm sieve and contents of the sieve were transferred to a tray. Water was added to the tray and the contents examined under an illuminated lens. All parasites were removed, counted and stored in 70% ethyl alcohol containing 5%

glycerine until identification almost a year later. Samples thus preserved can be stored for a long time without losing the detail. The worms were cleared in lactophenol solution and temporary mounts were prepared in lactophenol. The worms were examined under a compound microscope and identified to species using the key of Lichtenfels (1975).

2.2. Pasture larval counts.

2.2.1 Sampling

Pastures grazed by the three Herds used for the egg count data were sampled monthly for larval counts during the grazing season of 1993. Pasture counts were only carried out during the time that the horses were on pasture. Sampling was carried out using the technique of Taylor (1939). Sampling was done about the same time of day throughout the study period, before the dew was off the grass. Each pasture was paced off to determine the number of strides to be taken before each halt. Herbage was collected from 400 different places on the area being sampled. A "W" shaped predetermined route was followed and 25 sampling stops were made on each transect. Grass was collected from four points on each stop, one immediately in front, two sideways and one behind. At each point grass was plucked between the thumb and forefinger. The grass was plucked as close to the ground as possible, but care was taken to avoid including soil. If a faecal deposit was found at the predetermined area, one extra stride was made to avoid sampling areas of possibly much higher larval concentration than would normally be encountered by the feeding horses. The size of sample taken from each spot was

approximately the same over the whole field. About one kilogram of herbage was collected from the pasture at each sampling.

2.2.2 Larval recovery

Larvae were recovered according to the technique described in the "Manual of Veterinary Parasitological Techniques" (Ministry of Agriculture Fisheries and Food, 1986), with a slight modification. The grass was weighed and transferred to a large bucket. Water was added and the grass was agitated for five min, and allowed to stand for at least six hours. Grass was removed in handfuls, squeezed to remove excess water and placed in a tray. The dry weight of the grass was determined by placing the sample in a hot air oven at 100 °C until it was completely dry, removed from the oven and weighed.

The water was passed through a series of sieves (USA standard testing sieves, W.S Tyler, USA), the first with an aperture of 150 μm and the next two of 38 μm each. The top sieve was washed thoroughly by first passing a gentle stream of water then a strong jet, with the lower two sieves still in place. Coarse material on the upper sieve was discarded and the lower sieves were each held at a 45 degree angle to a measuring cylinder and washed thoroughly. The measuring cylinder was stored in the refrigerator for at least three hours after which the water was siphoned off. The sediment was transferred to 50 ml centrifuge tubes (Nalge Co, Rochester NY). Tubes were centrifuged at 500 X G for ten min in a TJ 6 centrifuge (Beckman, USA), and the supernatant was discarded. About 5 ml of magnesium sulphate, specific gravity 1.29, was added to the

sediment and mixed. More magnesium sulphate was added to the tubes to reach the 50 ml mark. Contents of the tubes were mixed thoroughly and then centrifuged at 500 X G for 10 min. The upper 5-10 ml were poured into a measuring cylinder, the tubes were filled and the centrifugation process repeated. The second centrifugation process was introduced to improve larval recovery (Young and Trajstman, 1980). The supernatant was then added to the contents of the measuring cylinder. Water was poured into the measuring cylinder to the 1 litre mark. Cylinders were stored at 4 °C for at least 3 hours after which the water was siphoned off and the sediment containing the larvae was transferred to a petri dish for counting. The cylinders were rinsed thoroughly, and the washings were put into the petri dish. The petri dishes were examined with a dissecting microscope using a magnification of 25 X. Larvae were counted and transferred to 15 ml centrifuge tubes using a fine pipette. Infective strongyle larvae could be differentiated from free-living nematodes by their long tails (the retained cuticle of the second stage).

Weather data, including temperature and precipitation, were obtained from the Environment Canada station at Charlottetown during the study.

2.3 Pasture plots

2.3.1 Sample preparation

Either freshly deposited or rectal samples of faeces were obtained from naturally infected horses each month for twelve months from July 1993 and June 1994. Enough faeces was obtained to make 12-15 samples, each weighing approximately 110 g. A 10 g sub-

sample was taken from each sample and a faecal egg count was carried out using a modified Cornell-MacMaster technique. The 10 g sample was usually obtained from as many different pellets as possible while taking care to conserve the pelleted nature of the samples as much as possible, so that when deposited on the ground samples would be similar to those deposited by horses. Eggs were floated up from the remainder of the Cornell-MacMaster suspension using a method similar to that of Bello and Gordon (1970). The suspension was thoroughly mixed and passed through a tea strainer into a paper cup. Material in the strainer was pressed to express all the fluid and then discarded. The fluid was transferred into a 50 ml centrifuge tube and centrifuged at 500 X G for 10 min after which the supernatant was discarded. Sheather's sugar solution was added to the resulting pellet and the contents were thoroughly mixed and centrifuged at 500 X G for 10 min. The tubes were then filled with sugar solution to form a positive meniscus and a 50 X 70 mm coverslip applied to the mouth of the tubes. The mixture was left to stand for 2 hours after which the cover slips were removed and the eggs were washed into a petri dish. The eggs were pipetted out of the petri dish into 15 ml centrifuge tubes. Excess Sheather's sugar solution was removed by two washes with centrifugation at 500 X G for 5 min. Duplicate samples of the eggs were taken and cultured by incubation at 28 °C for 24 hours in multiwell plates (Falcon, Becton Dickinson labware, Lincoln Park NJ). Culture plates were examined using an inverted microscope and the viability of the eggs was found by counting 200 eggs and determining how many were either embryonated or had hatched. Percent viability was calculated by dividing the total number of eggs with evidence of development by 200 and multiplying

the result by 100. The average between the two samples was considered to be the percent viability for the sample.

2.3.2 Experimental plots

An ungrazed part of the pasture at the UPEI farm at Winsloe was used for the experiment. Twelve to fifteen plots, each 1.5 m^2, were prepared each month. The plots for each month were set up in a straight line and each plot was marked by putting a stick at each corner. A faecal sample was placed in the middle of each plot such that the distance from one sample to the next was 1.5 m. Samples were deposited in heaps to approximate the manner in which faecal samples normally occur on pasture. During the months that grass was overgrown the grass was trimmed to a level of approximately 10 cm above the ground. During the winter when the ground was covered by snow, samples were wrapped in cheese cloth, labelled and placed on pasture. When the snow melted (in April) plots were prepared as previously described and the samples were recovered from the cheese cloth and placed in their respective plots.

2.3.3 Sample recovery

Each month, faecal samples were collected 4 days post deposition (pd) for egg counts. Subsequently samples were recovered on the seventh day pd and then weekly for 5 weeks. Later samples were recovered monthly until no, or, consistently low levels of larvae were recovered. At each collection time the faecal sample, when present was collected and put in a plastic container. Grass around the area occupied by the faeces

for a diameter of 30 cm was clipped as close to the soil as possible without including any soil. The grass was put into a plastic bag. Grass from the remaining area of the plot was clipped in the same manner and put into a separate plastic bag. When faecal samples were dispersed by rain, the grass in the area formerly occupied by the sample was clipped in the same manner.

2.3.4 Processing

2.3.4.1 Faeces

Faecal samples were weighed, mixed and a 10 g portion used for egg counts. If eggs were present, another portion was cultured while a portion was used for larval recovery. Ten grams of faeces was used for the egg count and the portions used for larval recovery and culture usually depended on the weight of the remaining faecal sample. When more than 50 g was available, 40 g was used for larval recovery while 10 g was cultured. When eggs were present they were floated and cultured for viability determination as described in section 2.3.1. Culturing of the faecal sample was done by putting the sample in a screw top container, capping it lightly and storing in a dark shelf for 7-10 days. The portion used for larval recovery was usually divided into two equal parts. Larvae were recovered from the faecal samples by a modification of the Baermann technique. Samples were left in the apparatus for 48 hours after which all water was drained into a conical measuring cylinder. Both funnel nd tube were rinsed twice and the washings added to the contents of the measuring cy arvae were sedimented overnight in a refrigerator at 4 °C. The water was siphoned off and the larvae

transferred to a 15 ml centrifuge tube and further sedimented for at least 3 hours. Water was removed up to the 1 ml level and the larvae were fixed as described in section 2.1.2. Counting was done by bubbling air through the suspension to mix it thoroughly and drawing out 0.5 ml of the suspension in 0.1 ml aliquots. Each aliquot was transferred to a microscope slide and a 22 X 40 mm coverslip was applied. All larvae in the 0.5 ml were examined using the 10 X objective and counted. The result was multiplied by two to obtain the number of larvae in the sub sample. Sometimes preinfective larvae were encountered. These were differentiated from infective larvae and free living nematodes using the key of Ogbourne (1971b). The presence of preinfective larvae was noted but the numbers were not counted since the methods used for larval recovery depended on active movement of larvae. Such methods underestimate numbers of the pre-infective stages (Mfitilodze and Hutchinson, 1988). When the number of larvae present was very high a dilution technique was adopted. A 1:10 or 1:5 dilution was used, depending on the concentration. In diluted samples, the number of larvae counted in the 0.5 ml sample was multiplied by 2 to obtain the total number of larvae in 1 ml. This number was multiplied by the dilution factor to obtain the total number of larvae in the sub sample. The average between the two duplicate sub samples for each plot was divided by the weight of each sub sample and multiplied by the weight of the whole faecal sample at the time of recovery to obtain the total number of larvae in the faeces.

2.3.4.2 Grass

Grass from the two regions (<30 cm from faeces and >30 cm from faeces) was treated separately. Larvae were recovered from the grass as described for section 2.2.2. However, the grass was not weighed and dried, but was discarded. Also the amount of sediment exceeded 7.5-12.5 ml after centrifugation. The volume of sediment after overnight sedimentation was therefore estimated and the water removed until the ratio of the volume of settled sediment to total volume was less than 1:4. The sediment was thoroughly suspended in the remaining liquid before being transferred to 50 ml centrifuge tubes to yield a packed sediment of between 7.5 and 12.5 ml (Young and Trajstman, 1980). Further processing and counting was as described for section 2.2.2. Larval counts from grass and faeces were expressed as a percentage of the expected recovery assuming, that all viable eggs present hatched and developed to the third stage.

Meteorological data available included maximum, minimum and mean temperatures on a daily basis, and monthly basis and a 30 year average of the mean monthly temperatures. Data for precipitation was also available on a similar basis. The amount of snow cover on the ground during each winter day was available as well. Temperature data was summarised in graphs indicating the mean minimum and maximum temperature for each month, the overall mean monthly temperature; and the 30 year average. Precipitation data was plotted in a graph indicating the total monthly precipitation and the corresponding 30 year average.

The theoretical number of larvae that would be available on pasture during the grazing season from 100 g of faeces deposited on pasture during different times of the year was computed. The larvae from each month (or time period) were calculated by the percent recovery of larvae from grass for samples deposited during the particular month multiplied by the number of larvae that would have been available from 100 g of faeces for that month based on the average EPG for the three herds during that month. The percent recovery was calculated by dividing the actual number of larvae recovered by the product of the EPG and the weight of the sample at the time of deposition times 100.

3. RESULTS

3.1 Egg counts, faecal cultures and tracers

3.1.1 Faecal egg counts

Results of the faecal egg counts are presented in Figs. 2-6. Detailed statistics for each month are given in Appendix 1. Herds 1 and 2 were initially combined and median egg counts for both mares and yearlings decreased from an April reading of 350 and 937 eggs per gram (EPG) respectively to zero in May following treatment with pyrantel pamoate. This was followed by an increase in egg counts, with the mares attaining pretreatment levels by July. The yearlings did not attain pretreatment levels until September. All groups demonstrated seasonal variations in median egg counts, with peak levels in summer. Herd 1 mares had a peak count in October, while Herds 2 and 3 mares had peak counts in August. In yearlings, strongyle egg output had two peaks, one in September and another in January. Herd 2 foals had similar peaks to the yearlings. Herd 1 foals had a single peak in October, that coincided with the peak in mare egg counts. All groups, except Herd 1 foals and Herd 3 mares, had an additional small peak in egg production in March. Herd 3 mares had negative counts for three months from November to January following treatment with ivermectin. However, counts rose rapidly in subsequent months and by April the counts had already reached pretreatment levels. The median EPG for April 1994 was higher than that of one year earlier. In Herd 2 mares, counts remained relatively high, although there was a decline from September till February, after which a small peak was recorded in March. Minimum egg counts were

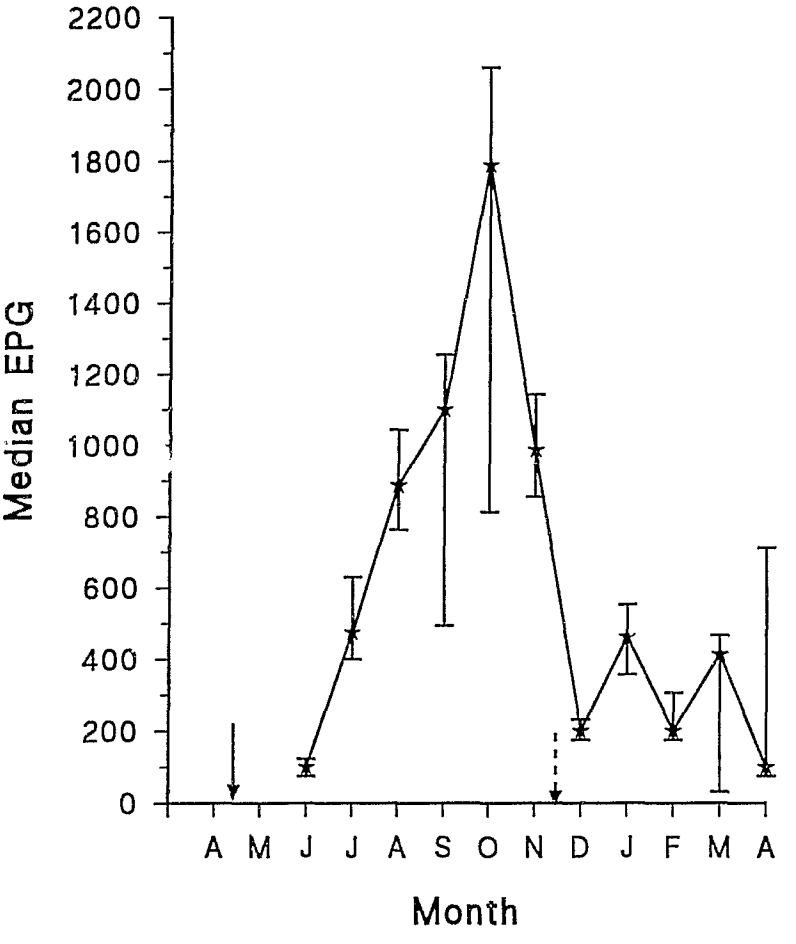

Figure 2. Seasonal fluctuations in median EPG for Herd 1 mares. Positive range bars indicate third quartile (Q3)X10^{-1}. Negative range bars indicate first quartile (Q1). The third quartlie was divided by 10 in order to preserve the scale. Arrows indicate time of deworming with solid arrow, pyrantel pamoate; dashed arrow, a levamisole cattle preparation.

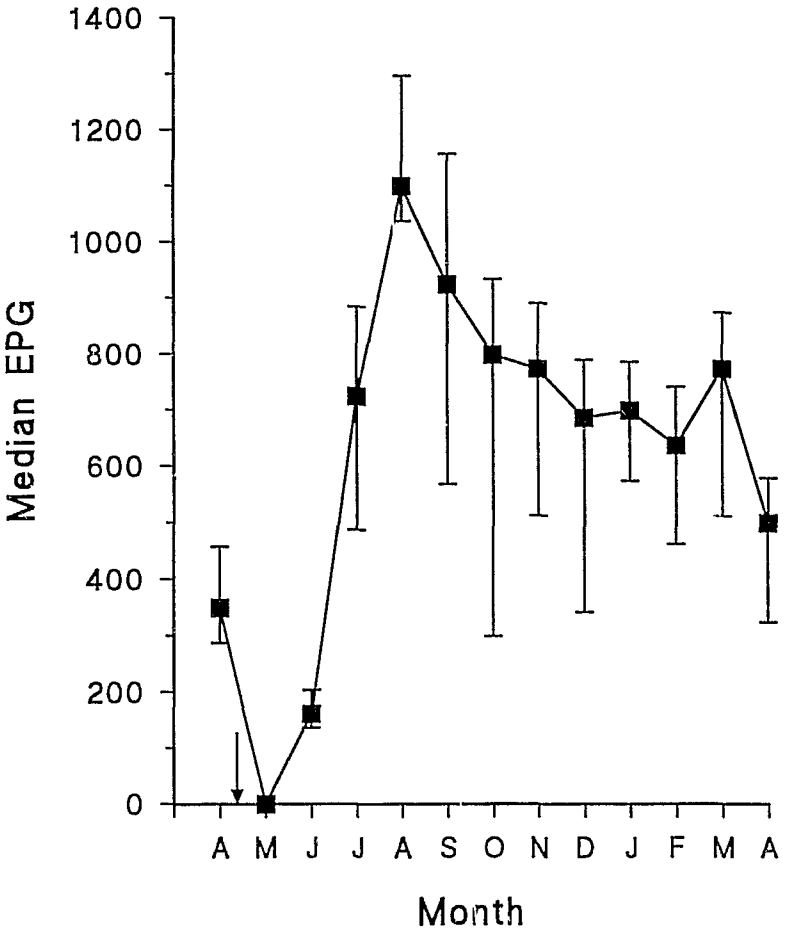

Figure 3. Seasonal fluctuations in median EPG for Herd 2 mares. Positive range bars = Q3 X 10^{-1}. Negative range bars = Q1. The third quartile was divided by 10 to preserve the scale. Arrow indicates the time of deworming with pyrantel pamoate.

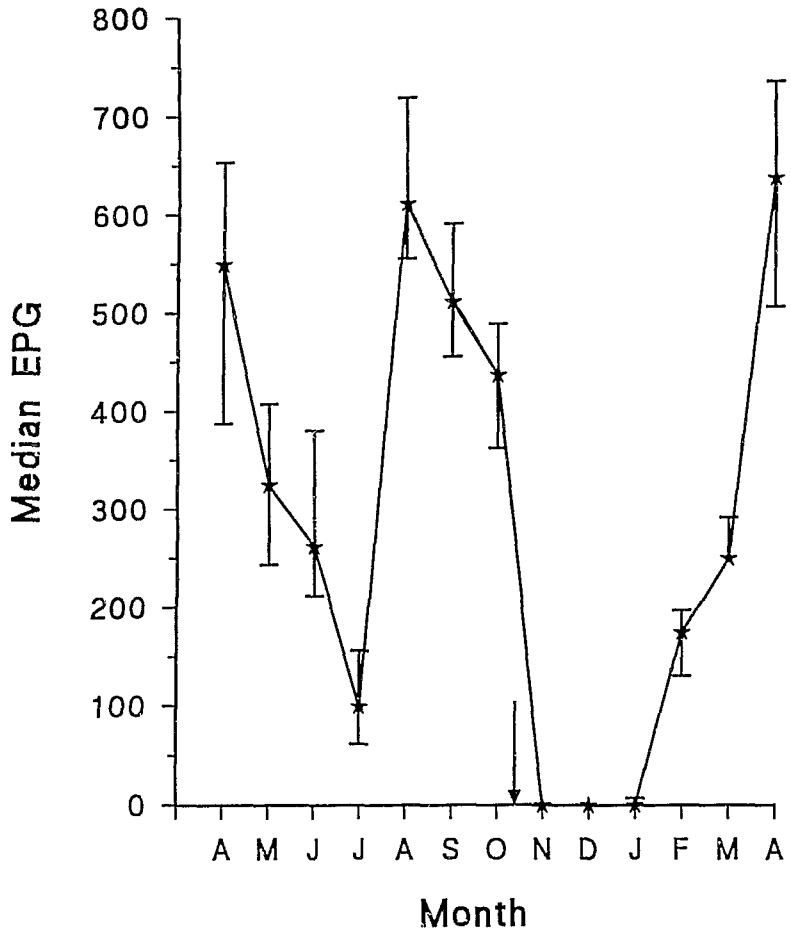

Figure 4. Seasonal fluctuations in median EPG for Herd 3 mares. Positive range bars = Q3 X 10^{-1}. Negative range bars = Q1. The third quartile was divided by 10 to preserve the scale. Arrow indicates the time of deworming with ivermectin.

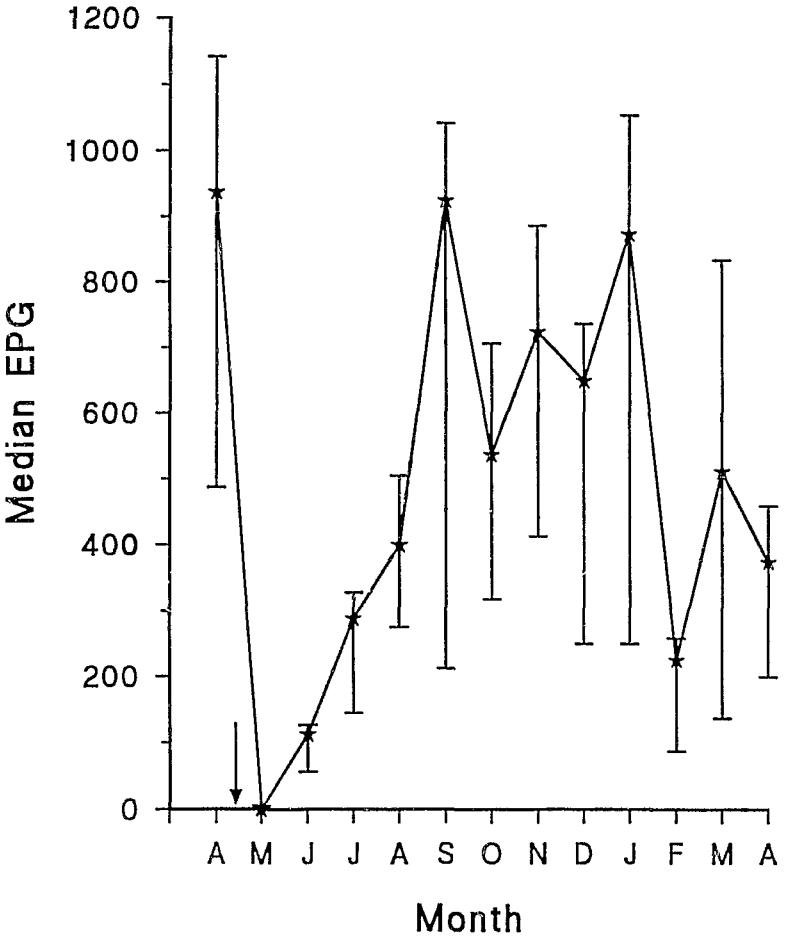

Figure 5. Seasonal fluctuations in median EPG for Herd 1 and 2 yearlings. Positive range bars = Q3 X 10^{-1}. The third quartile was divided by 10 to preserve the scale. Negative range bars = Q1. Arrow indicates time of deworming.

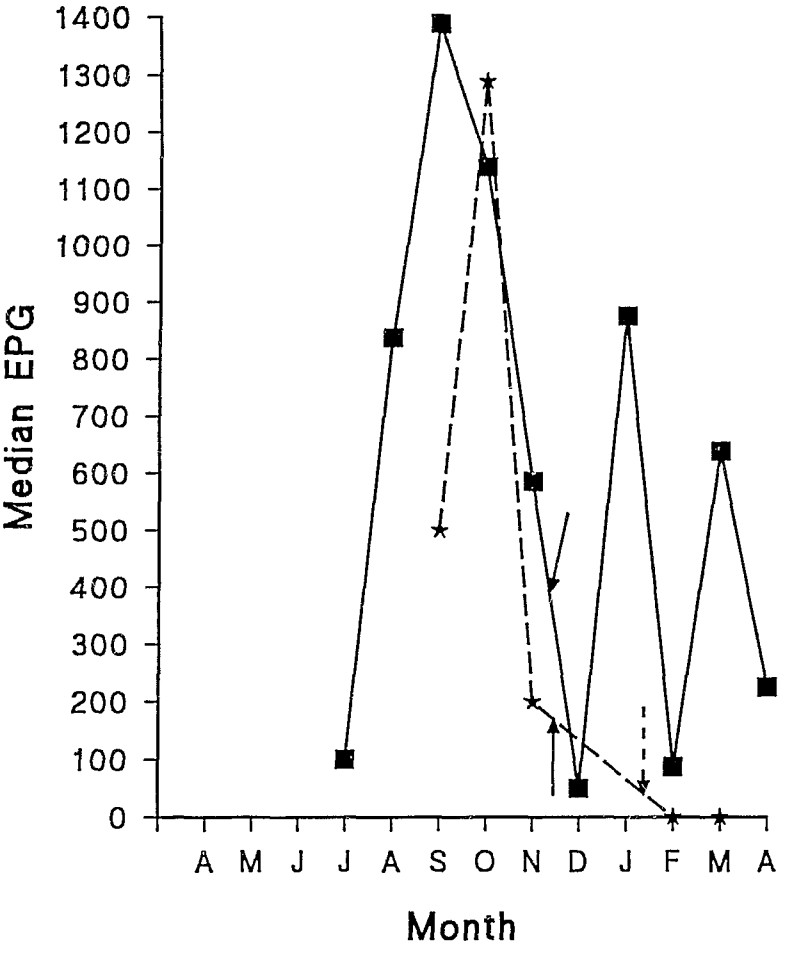

Figure 6. Seasonal fluctuations in median EPG for foals. (*) Herd 1; (■) Herd 2. Solid arrows indicate time of deworming with ivermectin for both herds. Dashed arrow indicates second deworming with ivermectin for Herd 1 only.

recorded in February in the two groups which were not given a second treatment.

3.1.2 Faecal cultures

Most of the eggs contributing to the egg counts in Herds 1 and 2 and all eggs in Herd 3 were from cyathostomes. Overall, in all horses and all months the contribution by cyathostomes was 80.5-100.0% (Appendix 2). *Strongylus vulgaris* contributed 0.0-8.8%, while the "other" group contributed 0.0-13.8%. In individual horses during each month, the percentage of cyathostome eggs was 43.0-100.0%. *Strongylus vulgaris* constituted 0-26% of the eggs in faecal cultures from individual horses. In Herd 2, where no further deworming occurred, cyathostomes contributed 93.4-99.8% of all larvae in mares, and 80.5-100.0% in yearlings (Figs. 7 and 8). Foals did not start shedding large strongyle eggs until February 1994. In both mares and yearlings the percentage of *Strongylus vulgaris* eggs increased in winter and the highest percentage was recorded in yearlings in April 1994. The proportion of individual horse samples positive for *S. vulgaris* in Herd 2 ranged from 0 to 60.9% (Table III). *S. vulgaris* occurred most commonly in March 1994, when 60.9% of all cultures had ≥ 1 larvae of this species.

3.1.3 Tracer horses

Identification of adult worms from the tracer horses revealed similar results as those obtained by faecal culture. The two horses from Herd 2 had only cyathostomes in the

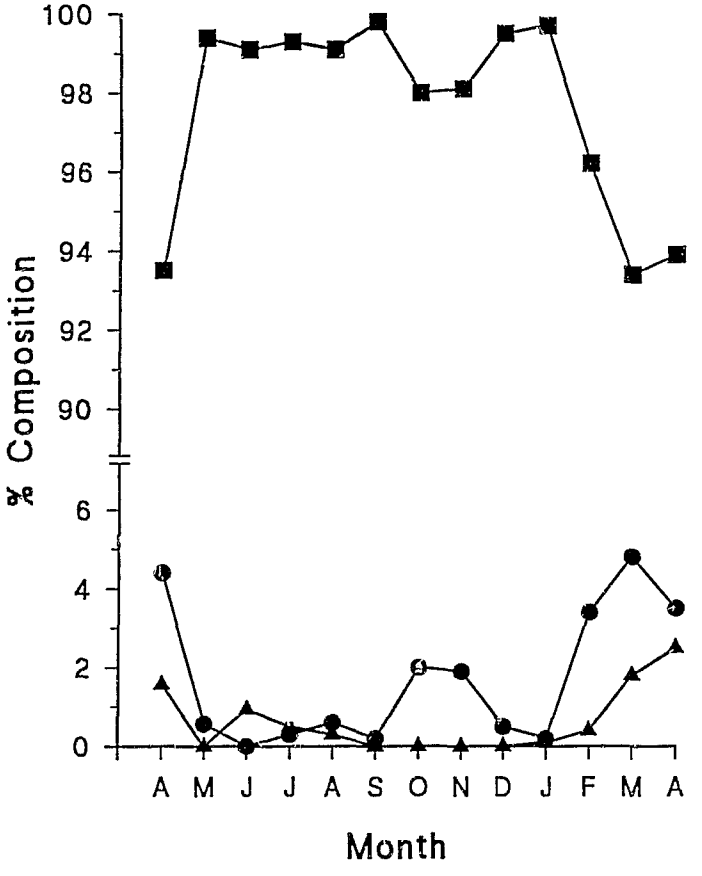

Figure 7. Monthly changes in percentage contribution by the different strongyle groups to the total larvae identified in cultures from Herd 2 mares. (■) cyathostomes; (▲) *Strongylus vulgaris*; (●) others.

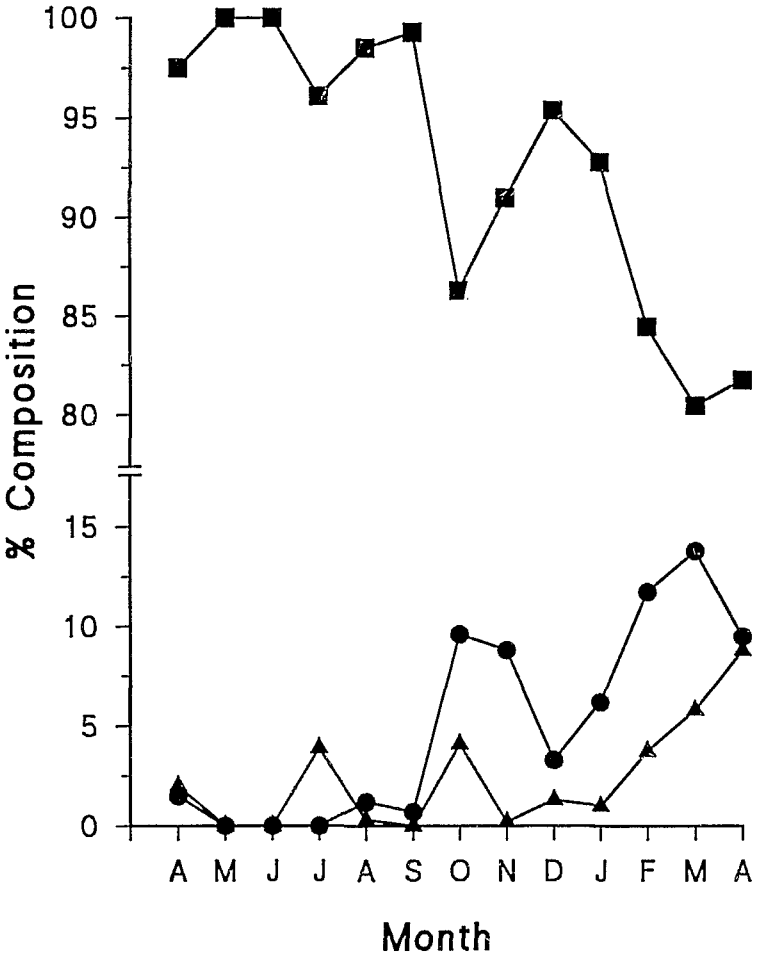

Figure 8. Monthly changes in percentage contribution by the different strongyle groups to the total larvae identified in cultures from Herd 2 yearlings. (■) cyathostomes; (▲) *Strongylus vulgaris*; (●) others.

Table III. Proportion of all individual horses (mares, yearlings and foals) from Herd 2 positive for each of the different strongyle groups during the study period.

Month	Number of samples				Total number positive and (%)		
	Mares	Yearlings	Foals	Total	Cyathostomes	*S. vulgaris*	Others
April	24	6	-	30	20 (70.0)	10 (33.3)	14 (46.7)
May	33	7	-	41	13 (31.7)	0 (0.0)	2 (4.9)
June	28	5	1	34	20 (58.8)	0 (0.0)	4 (11.8)
July	21	4	1	26	17 (65.4)	4 (15.3)	3 (11.5)
August	16	-	4	20	17 (85.0)	3 (15.0)	9 (45.0)
September	16	-	2	18	18 (100.0)	0 (0.0)	3 (16.7)
October	19	6	2	27	24 (88.9)	3 (11.1)	15 (55.5)
November	28	4	9	41	33 (80.5)	1 (2.4)	22 (53.7)
December	27	5	5	37	30 (81.7)	4 (10.8)	9 (24.3)
January	17	5	4	26	24 (92.3)	4 (15.4)	7 (26.9)
February	20	5	4	29	26 (89.6)	6 (20.7)	18 (62.1)
March	17	4	2	23	22 (95.6)	14 (60.9)	13 (56.5)
April	19	5	4	28	20 (71.4)	11 (39.3)	14 (50.0)

large intestines. The Herd 1 horse had one large strongyle species in the caecum. In addition there were *S. vulgaris* larvae in the mesenteric arteries. Thirteen species from six genera were recovered (Table IV). The most common genera in the three animals were *Cylicostephanus*, *Cyathostomum* and *Cylicocylus*. All worms recovered from Herd 3 horses belonged to these three genera. Herd 1 horse had, in addition to the above listed three, *Poteriostomum imparidentatum*, *Cylicodontophorus mettami* and one *Triodontophorus* sp. L4 that could not be identified to species. No single species dominated the same region of the large intestines in all three animals. However, each intestinal segment in each animal was dominated by one species. In the Herd 1 horse *Cylicostephanus calicatus* dominated the caecum (64%). In the ventral colon, *Cylicocylus nassatus* was most common (34%), followed by *Cyathostomum catinatum* (25%) and *Cylicostephanus calicatus* (22%). The dorsal colon was dominated by *Cylicostephanus longibursatus*. In Horse 2 only one strongyle species (*Cylicocylus nassatus*) was recovered from the caecum. The ventral colon of this horse was occupied almost equally by *Cyathostomum catinatum* (39%) and *Cylicostephanus longibursatus* (35%). *Cylicostephanus longibursatus* dominated the dorsal colon of this horse. Horse 3 had *C. catinatum* dominating both the caecum and dorsal colon, (98% and 94.4 % respectively), while the ventral colon was dominated by *Cylicostephanus minutus* which contributed 59% of the adult worms identified in this region.

3.2 Pasture counts

The results of the pasture counts are given in Table V. Pastures 1 and 3 were sampled

Table IV. Distribution of different strongyle species in the large intestines of three tracer horses.

Horse	1			2			3		
Region	C	Vc	Dc	C	Vc	Dc	C	Vc	Dc
Cylicostephanus calicatus	63	22	1	0	0	0	0	0	0
C. longibursatus	4	4	83	0	35	94	0	25	0
C. goldi	0	0	8	0	0	6	0	0	0
C. minutus	0	7	0	0	0	0	1	59	3
C. poculatus	1	0	0	0	0	0	0	0	0
Cyathostomum coronatum	30	0	0	0	0	0	0	0	0
C. catinatum	0	25	0	0	39	0	98	8	94
C. labratum	0	4	0	0	0	0	0	0	0
C. pateratum	0	0	0	0	0	0	0	8	0
Cylicocylus nassatus	1	38	0	100	25	0	1	0	3
Poteriostomum imparidentatum	0	0	7	0	0	0	0	0	0
Cylicodontophorus mettami	0	0	2	0	0	0	0	0	0
Triodontophorus	1	0	0	0	0	0	0	0	0

C = caecum; Vc = ventral colon; Dc = dorsal colon. Figures are percentages of total number of worms recovered from each region. Horse 1 was from Herd 1, Horses 2 and 3 from Herd 3.

Table V. Pasture strongyle larval counts (L3/kg dry matter) for the three pastures during the 1993 grazing season.

Month	June	July	Aug	Sept	Oct	Nov
Pasture 1 (Herd 1)	-	0	605	178	1229	2050
Pasture 2 (Herd 2)	271	201	801	364	1567	-
Pasture 3 (Herd 3)	-	39	101	230	-	-

Pastures were sampled only when horses were grazing.
- indicates not sampled

for the first time in July. The first sampling for pasture 1 revealed no strongyle larvae were available initially. An infectivity of 271 L3/ kg dry matter was recorded in pasture 2 in June. Subsequently, this fell to 201 L3/kg dry matter in July. All pastures had a rise in infectivity in August compared to July. A decrease occurred in pastures 1 and 2 during the September sampling. A high number of infective larvae was recovered from both pastures 1 and 2 in October. Pasture 2 was sampled for the last time in October, while pasture 3 was sampled for the last time in September. All pastures showed highest pasture infectivity at the end of the grazing season. Meterological data (Figures 9 and 10), show that temperatures during the grazing period were similar to those recorded over a 30 year period. However, August was drier than usual, with a total rainfall of 28.4 mm as compared to an average of 88.6 during a 30 year period.

3.3 Experimental plots

Faecal cultures indicated that the viability of eggs in all samples used in the experiment was 100%. The results of the experimental plots are summarised in Table VI. The percent recovery from $\geq$ 30 cm around the sample was negligible, hence recovery from the whole plot was considered as one reading. No eggs were recovered from the plots set up from July to September during the first sampling (4 days pd). Viable eggs were recovered from the November plots up to the time of the last sampling for 1993 (30 days pd), but not during subsequent sampling in the spring. Viable eggs were also recovered from the January, February and March plots during the first spring sampling in April. Eggs were recovered from December plots in April, but the viability was very low

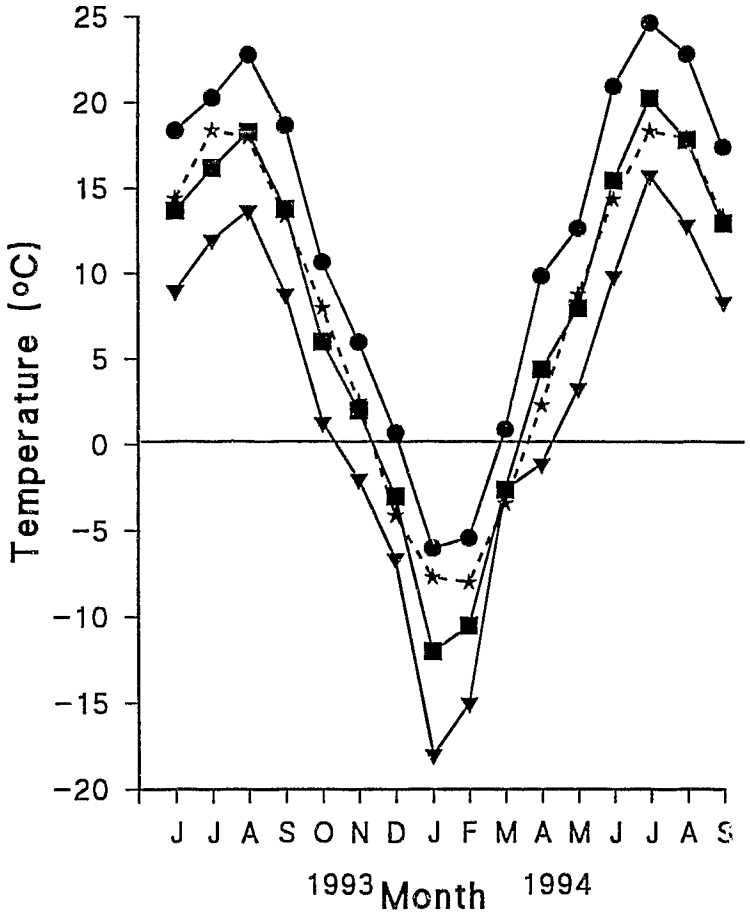

Figure 9. Monthly air temperature readings during the study period (July 1993-September 1994). (▼) minimum; (●) maximum; (■) mean; (*) 30 year average.

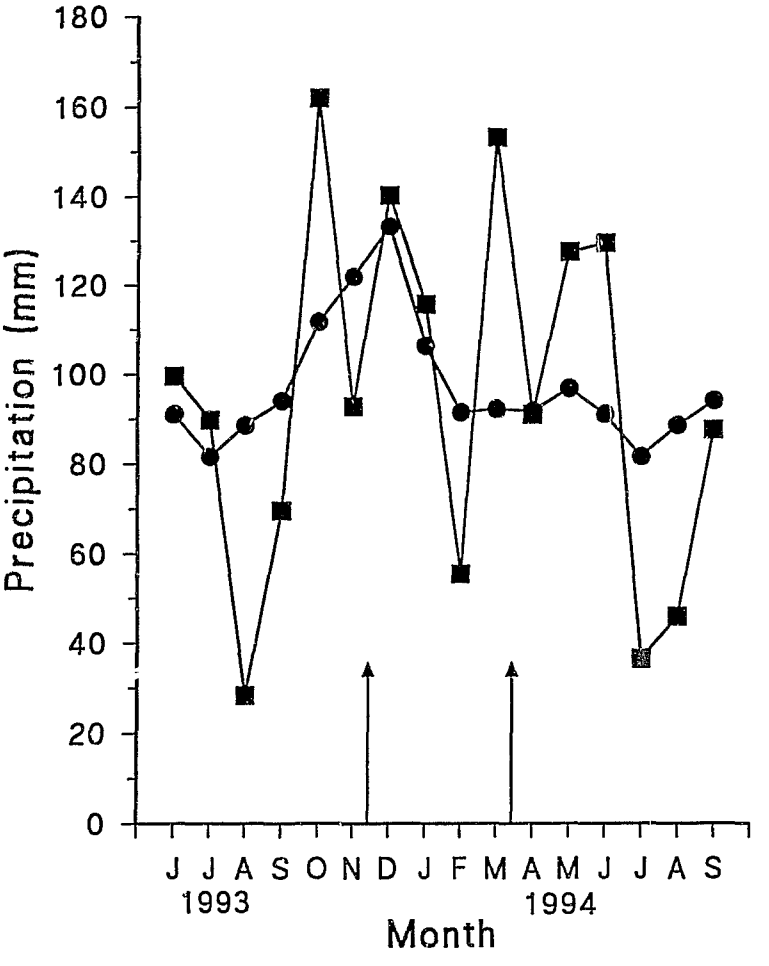

Figure 10. Monthly precipitation during the July 1993-September 1994 period. (■) total precipitation (mm); (●) 30 year average. Precipitation within the period between the arrows was mainly snow.

Table VI. First appearance, peak recovery and last recovery of strongyle L3 from faecal samples and grass surrounding the samples.

Month	Date deposited	First appearance		Peak recovery		Last recovery
		Faeces	Grass	Faeces	Grass	
July 93	6/7/93	7	7	28	35	123
August	3/8/93	15	21	35	42	358
September	2/9/93	ND	ND	11	25	410
October	6/10/93	D	34	-	LR	254
November	3/11/93	-	207	LR	LR	266
December	22/12/93	-	144	LR	LR	217
January	19/10/94	100	100	LR	LR	241
February	18/2/94	82	82	-	185	241
March	3/3/94	74	74	103	103	226
April	13/4/94	D	27	-	LR	147
May	10/5/94	D	31	-	LR	121
June	3/6/94	D	18	-	67	138

All figures are in days post deposition.
ND - not determined due to late sampling.
D - sample dispersed by rain.
LR - very low recoveries, less than 1% of expected recovery.
- Too low for accurate determination.

(19.2%). Only a single infective larva was recovered after culture of a 20 g sample. Viability had decreased in eggs recovered from January samples during the May sampling. No eggs were recovered from February samples in May and 358 larvae (0.002% of the eggs originally present in the sample) were recovered from cultured faeces. Samples deposited in April, May, June and October were dispersed by heavy rain that fell during the first week after deposition, making it impossible to recover eggs from these samples. Preinfective larvae were recovered from the remains of the June sample during the first sampling; 7 days pd. A few infective larvae were recovered from the surrounding grass 18 days pd. Low numbers of infective larvae were subsequently recovered from grass samples until the last sampling date.

Egg hatching was completed within four days in samples deposited between July and September. Infective larvae were first detected at 7 and 15 respectively for July and August samples. At 7 days pd most larvae in July samples were preinfective. Only a few infective larvae were recovered from these samples during this time. At day 7 pd, August samples contained only preinfective larvae. Subsequent samples had increasing numbers of infective larvae with preinfective larvae being recovered up to 35 days pd. The infective third stage was reached in between 74 and 100 days pd in the samples deposited between January and March. It took 14 days, 35 days and 21 days respectively for all larvae to become infective in July, August and September samples. The period to infective stage could not be determined for October, April, May and June samples due to loss of the faecal mass. No infective larvae were recovered from samples deposited

in November and December, although low numbers were recovered from grass around these samples.

The peak recovery from the faecal samples was 28 days pd and 35 days pd for July and August samples respectively. Migration was very fast for July samples, with the first recovery from grass around samples coinciding with the first recovery from the faeces. In August, migration was slower and infective larvae were recovered from the grass 7 days after first appearance in the faeces. First sampling for September samples was at 11 days pd and at this time third stage larvae were recovered from faeces and grass surrounding the samples. There was a gradual build-up of L3 on the surrounding herbage. Figures 11 and 12 demonstrate the number of larvae recovered from faeces and surrounding grass, as a percentage of total number of eggs available in the sample at the time of deposition for samples deposited in July and August. Peak recoveries from the grass were attained 35 days pd for July and 42 days pd for August (1 week after peak recovery from faeces). Larval recovery from all samples deposited in winter months (October to March) was very low, with slightly higher levels being recovered from February and March plots. In addition, very few larvae were recovered from samples that experienced rain during the first week after deposition. These samples were dispersed completely in 1-4 weeks. In contrast, samples that experienced dry weather during their first week on pasture retained their pelleted form and were not completely dispersed until the following summer. Samples deposited during the winter also retained their pelleted nature, but without the firm outer crust noticed on the summer samples.

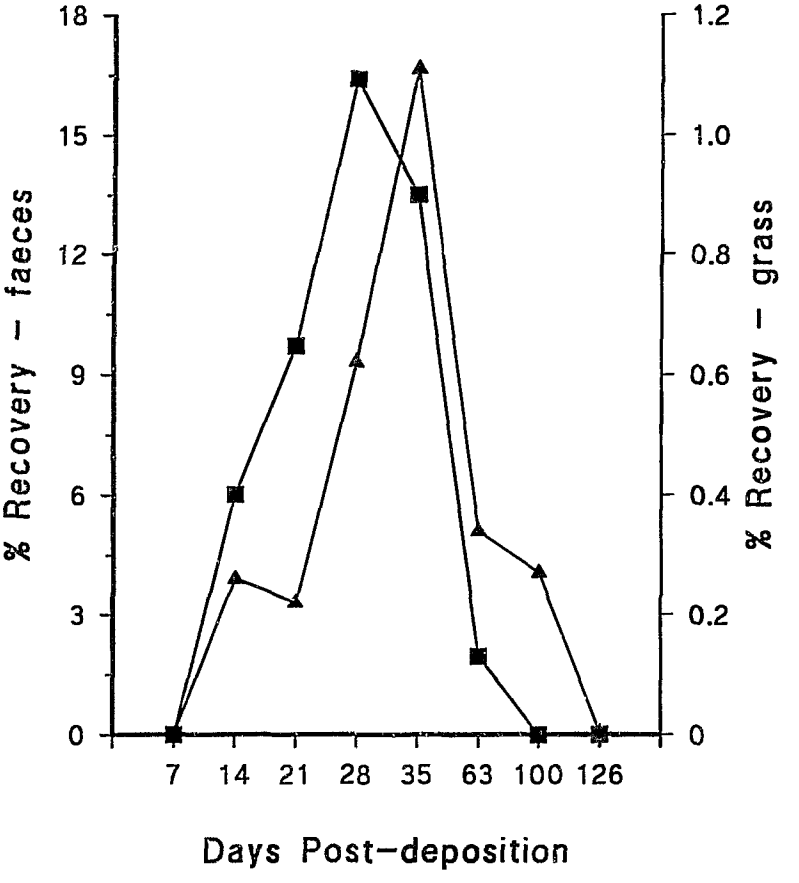

Figure 11. Percent recovery of strongyle L3 from faeces and grass surrounding samples deposited in July. (■) Faeces; (▲) grass.

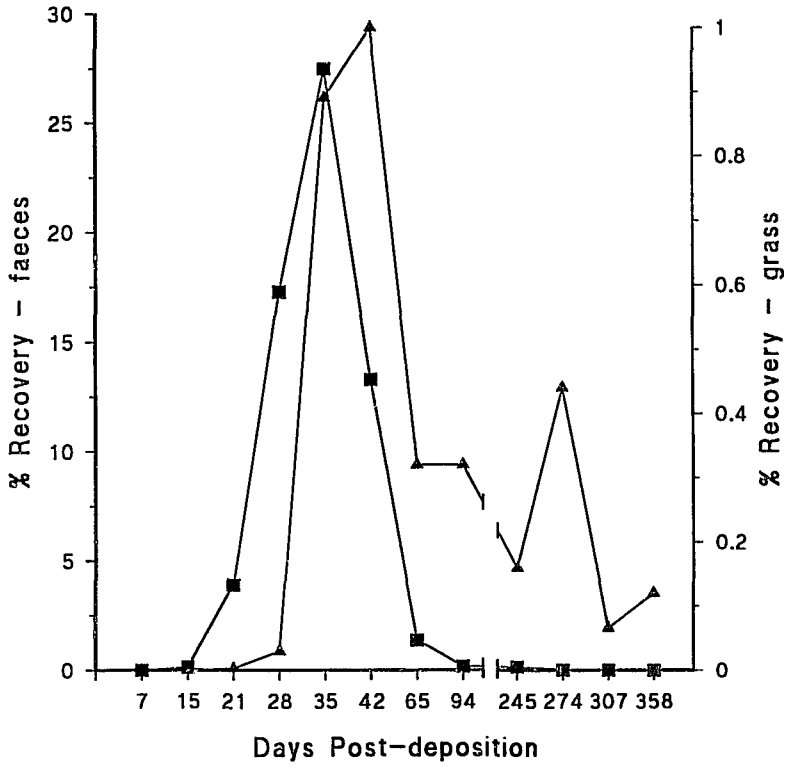

Figure 12. Percent recovery of strongyle L3 from faeces and grass surrounding samples deposited in August. (■) Faeces; (▲) grass.

Grass generally grew through them during the summer. Infective larvae were recovered from the samples deposited in July in appreciable numbers only through October. Larvae were recovered from the samples deposited in August and September until the following summer and in low levels (< 0.1% of eggs originally found in the faeces) until fall. Table VII indicates that most larvae available on pasture for each month during the grazing season arose from the previous month. In addition, larvae resulting from the previous summer are available on grass in appreciable numbers for 1 year. The winter months and June contributed the least number of larvae on pasture. Meteorological data indicated that the mean temperatures during the period of the study were similar to the 30 year average (Fig. 9). Snowfall was similar to the 30 year average. Less than average rainfall was recorded in August 1993, July 1994 and August 1994. Above average rainfall was recorded in October 1993 and April, May and June 1994 (Fig. 10).

Table VII. Expected number of strongyle larvae on pasture during the grazing season (May to November) per 100 g of faeces deposited during the previous 13 months.

Grazing season (C)	Time of deposition						
	July-Sept (P)	Oct-May (P/C)	June (C)	July (C)	Aug (C)	Sept (C)	Total
May	1972	29					2001
June	1355	328	10				1693
July	2630	666	29	303			3628
Aug	760	325	214	834	21		2154
Sept	56	55	14	429	1191	1432	3178
Oct	20	27	0	341	343	1540	2271
Nov	0	0	0	3	413	4000	4416

Number of larvae for each source month is based on the average EPG for the three herds during that month and the percent recovery of larvae on grass from samples set up during that month. P denotes previous year. C denotes current year.

4. DISCUSSION

4.1 Faecal egg counts, cultures and tracer horses

4.1.1 Faecal egg counts

The egg count data were summarised using the median versus the mean which is the more commonly used measure of central tendency. The median was considered to be a better measure because the individual values varied widely with a few animals having very low or zero readings and others having very high readings. The mean would normally be affected by these extreme values while the median is independent of the extreme values. Some individuals in a horse herd usually show higher egg count compared to the remainder (Love & Duncan, 1991; Gomez & Georgi, 1991; Warnick, 1992). Similar distributions of parasitic eggs in populations have been described in sheep and humans (cited by Warnick, 1992). Furthermore, the distribution of egg counts among horses in a herd rarely follows a Gaussian or normal distribution whereby the individual values are evenly distributed around the mean (Warnick, 1992).

4.1.1.1 Seasonal rise in egg counts

The egg count data revealed patterns consistent with studies elsewhere (Duncan, 1974; Herd et al, 1985; Herd, 1986a; Slocombe et al, 1987). There was an increase in egg production in all groups through the summer with peak counts between August and October. In addition, smaller peaks were observed for all groups in Herds 1 and 2 in March while the yearlings and Herd 2 foals had an additional peak in January. Herd 3

mares demonstrated a rapid increase from zero in January to over 500 EPG in April. Results from the previous year indicated that a peak may be present for this Herd in April. As discussed earlier (Section 1.4.2) spring/summer rise in egg production by horse strongyles is common throughout northern temperate lands. It would appear from this study that a smaller peak in egg production occurs in this region between March and April. Herd et al (1985) reported an increase in faecal egg counts through March and April with a peak in May in northern USA. Spring and summer rise in egg counts have also been reported in Newmarket, England (Herd, 1986a). A single anthelmintic treatment of horses in the spring does not eliminate the summer peak, but it reduces the size of the peak (Poynter, 1954; Ludwig et al, 1984; Herd, 1986a). Inclusion of different horses during different months may also affect the patterns observed. If most of the horses included one month ha : low egg counts this can lower the median count, and vice versa.

4.1.1.2 Possible sources of summer rise

The spring/summer rise in egg production is considered an adaptation of strongyles for survival in an hostile environment. Increased egg production during warmer months guarantees egg survival and development to the infective stage in large numbers. It has been hypothesized that this rise may be due to a basic increase in egg laying of already established adults; an increase in number of worms due to establishment of new worms that mature and start egg laying; or due to some innate capability of female worms to produce eggs at particular times of the year due to some particular stimulus (Poynter,

1954). Ogbourne (1971a) attributed the spring rise to differences in the numbers of larvae ingested and the length of parasitic development. Species that have long prepatent periods would complete their development in the winter and contribute to the spring rise. Parasites with short prepatent periods would complete their development in one grazing season and result in the summer rise. This may explain the increased recovery of large strongyle larvae in faecal cultures in late winter and spring. However, most of the eggs passed in the faeces of all groups in Herds 1 and 2 and all of those recovered from Herd 3 were cyathostomes.

An increase in egg counts in Herd 3 mares was observed in the spring. These mares had been treated with ivermectin, which is very effective against all lumenal stages of small strongyles. They were then housed in conditions allowing little further infection. This indicates that the egg-laying females responsible for the spring rise may be from migratory rather than newly ingested larvae. Egg reappearance in horses previously treated before being housed in helminth-free conditions has been reported (Gibson, 1953, Smith, 1976b). Herd et al (1985) reported similar results in northern USA, whereby mares exhibited a spring rise in egg counts while still indoors.

Studies indicate seasonal changes in the population of adult worms in the large intestines of horses with peaks in spring and summer (Ogbourne, 1976; Reinemeyer et al, 1986). The proportion of female worms containing eggs rises in spring and early summer (Ogbourne, 1971a). In northern USA, this rise persists until fall (Reinemeyer et al,

1986). This indicates that worms that survive the winter as encysted larvae may be a significant source for both the spring and summer rise in egg counts. The initial spring rise in egg counts probably results from emergence from tissues and maturity of larvae ingested during the previous late summer and fall. As conditions become favourable for development of eggs to infective larvae, larvae ingested during the grazing season mature and contribute to egg production, resulting in the summer peak. The decrease in the number of eggs passed during the fall may be due to death of some of the adult worms as suggested by Poynter (1954). This fall decline in egg production takes place even though most infective larvae are ingested late in the grazing season. The newly ingested cyathostome larvae probably become arrested in development as suggested by Herd et al (1985). In the Netherlands, most of the larvae ingested between the beginning of July and the end of September are inhibited in development (Eysker et al, 1990).

4.1.2 Faecal cultures

Identification of infective larvae from the cultures revealed that cyathostomes contributed the highest percentage of eggs to the counts in Herds 1 and 2 and all eggs in Herd 3. Across horses and months, *Strongylus vulgaris* eggs constituted 0.0-8.0% of all eggs. In individual horses during each month, *S. vulgaris* contributed 0-26% of eggs to the egg counts. This contribution by *S. vulgaris* appears higher than that reported elsewhere where this species contributed only between 0 and 6% of larvae in individual cultures. These variations can be attributed to differences in management. Herds 1 and 2 did not have a specific helminth control programme. Herd 1 mares were treated (with a cattle

preparation) in November and apparently this removed *Strongylus vulgaris* and other large strongyles from this Herd for the rest of the study period. In Herd 2, only the occasional horse was dewormed and when this happened the horse was excluded from the study as long as the egg count was zero. There was considerable movement in and out of Herds 1 and 2. There was minimal movement in and out of Herd 3 and was considered a closed Herd. All horses in Herd 3 were treated yearly with ivermectin. This probably resulted in the absence of large strongyles in this Herd. Ivermectin very efficacious against all stages of large strongyles (Slocombe & Cote, 1984; Love, 1992) and lumenal stages of the small strongyles (Eysker et al, 1992; Klei et al, 1993; Lyons et al, 1994a; Love et al, 1995). The efficacy of ivermectin against inhibited late third stages and encapsulated mucosal *L4* is generally low (Eysker et al, 1992; Klei et al, 1993; Lyons et al, 1994a), although Love et al (1995) reported an efficacy of 76.8%. The small strongyles contributing to egg counts in Herd 3 must therefore have survived treatment as hypobiotic and encapsulated larvae, which are not normally affected by treatment.

Strongylus vulgaris larvae were not recovered from faecal cultures of foals until February 1994. This is consistent with the life cycle of this species, which has a prepatent period of six to seven months (Ogbourne & Duncan, 1985; Jubb et al, 1993). Foals were born during the spring and went out with their dams during the beginning of the grazing season. They developed patent infections by August and larvae recovered from their cultures were those of cyathostomes. Among the "others" group, only 16-celled larvae

were recovered and these are likely to belong to *Poteriostomum*, a member of the subfamily Cyathostominae. Herds 1 and 2 had been treated with pyrantel pamoate before turnout. Although pyrantel pamoate is very effective against the adults of all strongyle species, there has been no demonstration of efficacy against the migratory and encysted stages. The egg reappearance period (ERP) of 4 weeks following this treatment is consistent with that reported in other studies. This anthelmintic has been reported to have an ERP of 4-6 weeks (Piche et al, 1990, Kivipelto & Asquith, 1994, Boersema et al, 1995). The ERP following treatment with ivermectin is 8 weeks (Kivipelto & Asquith, 1994). Herd 1 mares had an ERP of 9 weeks following ivermectin treatment.

4.1.3 Tracer horses

The results of identification of adult worms, although from a limited number of horses, indicate that the species of small strongyles in this region are similar to those obtained elsewhere. Of the 12 species of small strongyles identified in this study, all have been reported in Panama (Foster, 1936; Foster & Ortiz, 1937), Ohio (Reinemeyer et al, 1986), Australia (Mfitilodze & Hutchinson, 1985, 1990) and Kentucky, (Lyons et al, 1991). In Louisiana, similar species were found in ponies with little or no exposure to anthelmintics (Torbert et al, 1986). Apparently the occurrence of common small strongyle species is not reduced significantly by anthelmintic therapy. Although *Cylicodontophorus mettami* is among the species considered to be rare, it was recorded in one horse in this study and had been reported in five of the above studies. *Gyalocephalus capitatus* eggs were passed intermittently in the faeces of Herd 1 and 2

horses, but adult worms were not identified in the Herd 1 tracer. Ten Cyathostome species are considered most common from various surveys (Reinemeyer et al, 1984). These common species are *Cylicostephanus longibursatus*, *Cylicostephanus calicatus*, *Cylicostephanus goldi*, *Cylicostephanus minutus*, *Cyathostomum catinatum*, *Cyathostomum coronatum*, *Cyathostomum pateratum*, *Cylicocylus nassatus*, *Cylicocylus insigne* and *Cylicocylus leptostomus*. Of these ten species only *Cylicocylus insigne* and *Cylicocylus leptostomus* were absent from this study. Of these species, *Cyathostomum catinatum*, *Cylicostephanus longibursatus*, *Cylicostephanus goldi*. *Cylicostephanus minutus*, *Cylicostephanus calicatus* and possibly *Cyathostomum labratum* have developed benzimidazole resistance (Eysker et al, 1988).

The most common species contribute the highest percentage to the total lumen populations of cyathostomes. Sometimes the five most common species may represent up to 90% of all worms recovered (Ogbourne, 1976). The small numbers of the less common species, together with the fact that only three horses were used as tracers, may account for the fact that none of these species were recovered. Since only 100 worms were identified in each specific large intestinal location, it is possible to overlook those species not represented in high numbers. Studies from Australia (Mfitilodze & Hutchinson, 1985) and Britain (Ogbourne, 1976) show that no single species occurs exclusively in one intestinal location. However, there is a tendency for one or a few species to dominate each location. Most species prefer the ventral colon. In contrast, the caecum is occupied by only a few species.

There was no common species intestinal site preference in the three horses. However in each horse, each location was almost always dominated by one species. *Cylicostephanus goldi* was found only in the colon and was the most common species in the dorsal colons of horses 1 and 2. *Cylicostephanus longibursatus* was found in all three intestinal locations but was the most common species in the dorsal colon of Horse 1. *Cylicostephanus calicatus* was found in all three regions in Horse 1, and was the most common species in the caecum. The distribution of these three species is in agreement with the observations of Ogbourne (1976) and Mfitilodze & Hutchinson (1985). The former author reported a distribution of *Cyathostomum catinatum* similar to that observed in this study, where this species was found in all regions in Horse 3, but was more concentrated in the caecum and dorsal colon. According to Mfitilodze & Hutchinson, (1985), *Cyathostomum coronatum* normally occupies the caecum. In this study *Cyathostomum coronatum* was recovered from one horse only, in which it occupied the caecum. *Poteriostomum imparidentatum* and *Cylicodontophorus mettami* were recovered only from the dorsal colon of Horse 1.

The only large strongyle recovered from the large intestines was a *Triodontophorus* L4 that could not be identified to species. In addition, there were *S. vulgaris* larvae in the anterior mesenteric artery of the Herd 1 tracer. This was expected because the faecal cultures revealed that horses in Herd 1 were shedding low levels of *S. vulgaris* eggs in their faeces. However, the interval between the first time that this horse went out to Herd 1 and the necropsy (June to December) was insufficient for the adults to be found

in the intestinal lumen. Immature cyathostomes were also encountered in all three regions of the large intestines of the Herd 1 tracer horse. Absence of large strongyles in Herd 3 has been discussed earlier in connection with the larvae recovered from the faecal cultures.

4.2 Development, survival and migration of the free-living stages

To survive as a species, strongyles must shed numerous eggs, and have the eggs develop to the infective stage. Horses avoid feeding on heavily contaminated areas where they normally defecate (Herd & Willardson, 1985). The L3 must migrate from the faecal pat to the surrounding herbage where they can be ingested by a suitable host. Long-term survival of large numbers of larvae on pasture is important in increasing the likelihood of finding a host. The development, survival and longevity on pasture are influenced by environmental factors, particularly temperature and moisture. The development of eggs in samples deposited on pasture plots during this study varied depending on weather conditions.

4.2.1 Development

4.2.1.1 July to September

Rapid development was observed in summer especially, July and September. July temperatures ranged from minima of 6.4-18.8 °C and maxima of 12.6-26.8 °C with a monthly mean of 16.2 °C. In September temperatures ranged from minima of 2.9-15.8 °C and maxima of 12.5 to 23.6 °C, with a monthly mean of 13.8 °C. The warmer

temperatures were experienced at the beginning of the month when the plots were set up. Slow development in the August samples can be attributed to the lower than normal rainfall experienced during this month. Ogbourne (1973) reported delayed development of larvae in samples put out in June and July on English pastures. In the present study, although August weather was warm (mean 18.3 °C), there was little rain and the faeces dried resulting in delay of development to infective stage. However, the dry conditions were not severe enough to kill pre-infective larvae. When faeces dries slowly larvae undergo partial development before excessive moisture is lost (Ogbourne, 1972). A small percentage of larvae become infective. Most survive and develop to infectivity upon receiving adequate moisture. Similar observations were reported by English (1979a), who reported that egg hatching can take place in faeces on which no rain has fallen. Free-living stages can develop under low moisture conditions in temperature conditions similar to those that existed during August (Mfitilodze & Hutchinson, 1988). Warm, dry weather following deposition caused a dry crust to be formed on the surface of the faecal pat, which could protect the inner layers from drying. Faeces that retain the pelleted form conserve moisture better, hence providing a suitable environment for the developing stages (Mfitilodze & Hutchinson, 1988). More rapid larval development can be expected in the large heaps of faeces normally deposited by horses. Outer layers of faeces often dry out before development is completed, but the bottom layers remain moist enough for larvae to reach infectivity (Shumakovich & Pukhov, 1941, cited by Ogbourne, 1972). Faster development can also be expected in a normal year with normal amounts of rainfall.

4.2.1.2 October to June

It was not possible to determine how long eggs took to hatch in samples deposited on pasture in April, May, June and October, because samples were dispersed by heavy rain during the first week following deposition. However, from the temperature data, it can be assumed that June samples would have developed at a rate comparable to those of July or slightly slower. The mean temperature for June was 15.5 °C, within the range for both egg development and hatching. May samples would be expected to develop but not hatch until warmer weather arrived in June. May temperatures averaged 4.4 °C with a mean Maximum of 9.9 °C. In this range only very slow development is expected. October temperatures were below those in which egg development with hatching is expected. The eggs most likely embryonated and then were killed by the prolonged cold winter weather. The same fate must have befallen the November samples in which eggs with mobile larvae were recovered until the first week of December after which snow prevented further sampling until the following spring. During spring sampling, no eggs were recovered and only very few infective larvae were recovered in the summer. Samples deposited between December and March experienced temperatures below freezing for most of the time. Hence there was no appreciable development of the eggs. Eggs recovered at the first spring sampling in April were viable. However, one month later, very few eggs were recovered, most of which were not viable. Infective larvae were recovered from the grass surrounding these samples in the summer although numbers were very low.

4.2.2 Survival of the free-living stages

4.2.2.1 July to September

The results of this study show that better survival of pre-infective stages occurs from July to September on P.E.I. Infective larvae were recovered from samples deposited in these months for nine weeks for the July samples and until the following spring for the August and September samples. Larvae were recovered in high numbers from grass surrounding July samples for 11 weeks. Larvae were recovered from grass surrounding August and September samples for 52 weeks. Low levels of infective larvae were recovered from the grass around the area formerly occupied by September samples at of termination of this experiment in October. Shorter survival for samples deposited during warm, wet periods has been reported previously (English, 1979a, 1979b; Ogbourne, 1973; Mfitilodze & Hutchinson, 1988; Hutchinson et al, 1989). In contrast to the July samples, larvae from experiments initiated in August and September were still present in large numbers when cooler temperatures arrived in fall and were subsequently "refrigerated" by the ensuing low winter low temperatures. This ensured their survival over winter. In England, larvae seeded on pasture were shown to survive for short periods during summer when temperatures averaged 20 °C, while recoveries from plots seeded during cooler months remained high for longer periods (Ogbourne, 1973). Long survival of larvae until the following fall in the present study, can probably be attributed to a drier than normal summer in 1994. Infective larvae remain near the soil surface during dry weather, where they are protected from desiccation (Ogbourne, 1972, 1973). Tall grass also offers some protection for larvae, where they can remain for a long time

(English, 1979b). Death of larvae during warm wet weather has been attributed to increased migration of non-feeding larvae, leading to the depletion of food reserves in their intestinal cells (Ogbourne, 1973, English, 1979b, Hutchinson et al, 1989) or from heat stress (Mfitilodze & Hutchinson, 1988). Absolute survival of larvae in pastures is difficult to determine.

Information on the survival of individual cyathostome species is lacking because studies utilised samples from horses with mixed strongyle infections. However, results from one study tend to indicate that in the Netherlands, *Strongylus vulgaris* larvae last longer on pasture than those of the cyathostomes (Eysker & Wemmenhove, 1987). Winter survival of larvae occurs in England (Ogbourne, 1973), Scotland (Duncan, 1974), the Netherlands (Mirck, 1981, Eysker et al, 1983, 1986b, Eysker & Wemmenhove, 1987), Saskatchewan (Polley, 1986) and Ontario (Slocombe et al, 1987). However, in England, the Netherlands and Ontario, overwintering infective larvae are available on pasture for only a short period during spring and are recovered in very low levels by the third week of June (Ogbourne, 1972; Mirck, 1981; Slocombe et al, 1987).

4.2.2.2 October to June

The lowest survival was for samples deposited in October and November. Rain during the first week of deposition caused dispersion of the samples and made it impossible for eggs to be recovered from the October samples. However, when considering the weather data, it can be deduced that the eggs must have developed without hatching because of

low prevailing temperatures (mean, 6 °C). Temperatures in this range are known to allow the eggs to develop without hatching (Ogbourne, 1973; Mfitilodze & Hutchinson, 1987). In addition November samples showed mobile larvae in all eggs until the last sampling in December. However, at the first sampling in spring no eggs were recovered and subsequently the number of infective larvae recovered from grass surrounding the samples in these two months was negligible. The embryonated eggs must have died during prolonged cold temperatures in winter as reported by Lucker (1941).

December samples experienced low temperatures with little snow cover. This may have been the reason why very few eggs recovered in April were viable. In contrast January and February samples were deposited when the temperatures were too low to allow any development until May. All the eggs recovered from the February samples in April were viable while those for January had viability of 95%. No eggs were recovered from the February samples in May while those recovered from the January samples had a viability of only 36.7%. This indicates that prolonged low temperatures eventually lower the viability of unembryonated strongyle eggs. A similar effect of low temperature on unembryonated eggs was reported by Lucker (1941) who demonstrated that although unembryonated eggs are somewhat more resistant to subfreezing temperatures, the number of surviving eggs decreases with continued exposure to subfreezing temperatures. Of the winter experiments only February and March samples yielded an appreciable number of larvae. However, when converted to percentage yield these numbers are 1.60% or less of eggs originally found in samples. Larvae representing less

than 0.01% of the eggs originally present found their way to the surrounding grass during these months.

The pattern of survival of strongyle eggs through the winter in this study is similar to that reported by Lucker (1941) and Polley (1986). Eggs deposited between December and April survive in low numbers and are capable of developing to infectivity with the arrival of warm weather. In contrast, samples deposited on English pastures between November and February do not develop to infective stage (Ogbourne, 1972). This is due to winter temperatures in that region that allow larval development only to pre-infective stages. Resulting larvae die in the subsequent cold weather. During December there were low temperatures with very little snow cover. Death of most October and November eggs and probably some December ones may have taken place during this time. In January low temperatures and adequate snow cover protected unembryonated eggs. In February temperatures fluctuated from above freezing to below zero. Although the temperature fluctuations were marked there was a thick blanket of snow on the ground throughout; hence no development was expected and any eggs that were not already embryonated must have survived this month. According to Lucker (1941) in the presence of an appreciable snow cover, temperatures at the soil surface remain stable around 0 °C despite changes in air temperature. This protects organisms at the soil surface against direct effects of changes in air temperature.

In March low temperatures and little or no snow cover was detrimental for any pre-infective larvae and embryonated eggs. The month of April experienced air temperatures above 7.5 °C for 6 days after the second week. During this time it can be expected that any embryonated eggs hatched, while those that were not embryonated had some development. Some pre-infective larvae present at this time might have developed to infective stage. Subsequently air temperatures were 5 °C or below and these conditions existed to the beginning of May, arresting development to the infective stage of free-living stages present. Hatching of most of the eggs during the warm period in April must have accounted for the low recovery of eggs during the May sampling. However, apparently most resulting larvae did not survive. Very few infective larvae were recovered from faecal samples and surrounding grass them in subsequent sampling during the summer.

4.2.2.3 Effect of heavy rain on yield of infective larvae

The daily minimum and maximum temperatures for June 1994 were not markedly different from those of July 1993. The rates of development and survival patterns during these months would be expected to be comparable. Some pre-infective larvae were recovered from what remained of the June faecal sample 7 days pd. However, yields from this month were very low. This may be attributed to the heavy rain that fell during the first week of deposition, causing samples to be dispersed. Very low recoveries were recorded for April and May samples which were also exposed to heavy rain during the first week of deposition. The temperature conditions existing in May cannot be expected

to be responsible for the death of pre-infective larvae resulting from samples deposited in May as they were generally 7.5 °C or higher. All samples that experienced heavy rain during their first week were dispersed completely within 1-4 weeks. This contrasts the observations of English (1979a) who reported that even the heaviest rain was not able to disperse samples used in his experiment. However, the observations may confirm the theory of Hutchinson et al (1989), that excess rain may damage the faecal pats and in so doing reduce larval availability. If this is true, then the cause of low recoveries from the October and to a lesser extent, April could be twofold. First heavy rain washing the eggs from the faecal sample before any development has taken place, and then low temperatures further retarding the development and/or causing death of the pre-infective stages. If these two factors are in combination for samples in one month it would not be surprising to find low or even negligible recoveries as observed in October and April. It is difficult to explain what happens to eggs that are washed down into the soil before any development has taken place. Some eggs develop as shown by low numbers of infective larvae recovered from samples deposited in April to June. Infective larvae can migrate through soil in faeces buried at depths of 20 cm (Houston et al, 1984a). However only a very small percentage of the eggs initially buried is able to reach herbage from such a depth.

Other factors, including organisms using the faecal pat as habitat, eg. free-living nematodes, fly larvae, beetles, ants and annelids (Ogbourne, 1972) may affect survival and yield of strongyle infective larvae from faecal pats . Although no effort was made

to identify those organisms present in faecal samples, many were observed, the most notable of which were earthworms. The activity of dung beetles has been shown to result in aeration of the faeces causing desiccation and death of pre-infective larvae of cattle trichostrongylids (Bryan, 1973). Burying of eggs in the soil with faecal balls results in death of larvae, if there is insufficient rain for migration of larvae out of the faecal balls. In addition dung beetles ingest eggs and larvae, reducing the yield (English, 1979d). However, even in warm areas where dung beetle activity is marked, the number of infective strongyle larvae moving onto pasture is not reduced significantly (English, 1979b, 1979d; Mfitilodze & Hutchinson, 1988; Hutchinson et al, 1989). In one study, dung beetle activity apparently enhanced development of larvae to infectivity stage (Houston et al, 1984b).

4.2.3 Migration of L3 to surrounding herbage

Migration of larvae from faeces to surrounding herbage began as early as the first week of deposition during July and August. There was a slow build-up of infective larvae on the herbage and peak recovery was 35 days pd or longer for all months. The patterns of migration are consistent with those reported elsewhere (Ogbourne, 1972, 1973; English, 1979a, 1979b; Mfitilodze & Hutchinson, 1988). Larval migration from soil and faeces to herbage depends on both frequency and amount of rain. Very few larvae are recovered from grass during dry weather; and there is a sharp increase of larvae on herbage if rain falls after prolonged dry weather. The minimum amount of rain required for migration is not well established. August 1993, and July and August 1994

were relatively dry months with the few wet days being far apart. This may have influenced the numbers of larvae recovered during these months. However, some migration still occurred. Hutchinson et al (1989), failed to demonstrate significant correlations between the amount of rainfall and the number of larvae recovered from herbage despite good correspondence between the two. They attributed this lack of correlation to other sources of moisture, such as dew, causing larval migration.

Both soil and faeces act as reservoirs from which larvae emerge periodically and gradually (Ogbourne, 1972, 1973; English, 1979a, 1979b). During dry periods larvae remain in the soil. The result of the gradual migration of infective larvae is a slow build up of larvae in the pasture. This can be demonstrated by considering the total number of larvae available on pasture in one particular month during the grazing season and relating these numbers with the average EPG for the three Herds during the month of deposition of the different samples (Table VII -page 76). For example, in the month of August larvae were available on pasture from the previous summer and winter as well as June to July. The gradual build-up was probably responsible for the relative pasture larval counts. There was a gradual increase of larvae recovered from the pasture in all three Herds, with the highest numbers in the fall. This late summer/fall build up of infective larvae on pasture has been reported in Scotland (Duncan, 1974), England (Herd, 1986a), the Netherlands (Mirck, 1981; Eysker et al, 1983, 1986a; Eysker & Wemmenhove, 1987), northern USA (Herd & Willardson, 1985; Herd et al 1985),

southern USA (Craig et al, 1983; Ludwig et al, 1984) and Ontario (Slocombe et al, 1987).

In southern USA, another peak in pasture larval counts occurs in spring (Courtney & Asquith, 1985). The sources of the fall peak larval recovery in this study and the other studies from northern temperate lands are different from the source of similar peaks in the southern temperate lands. Under conditions prevailing in northern temperate regions, fast development of eggs in faeces deposited on pasture during the summer months coupled with high rainfall in late summer and fall leads to accumulation of larvae on grass as larvae migrate from faecal pats. In the southern temperate lands, there is fast development in the summer but translation is negligible (Mfitilodze & Hutchinson, 1988, Hutchinson et al, 1989). Larvae die very fast during this period (English, 1979a, Mfitilodze & Hutchinson, 1988; Hutchinson et al, 1989); hence few larvae from the summer contamination are available on pasture in fall. Only larvae developing in fall, are responsible for the fall peak in pasture larval counts in these regions.

4.3 Implications of the study

The results of this study indicate that the patterns of development, survival and longevity on pasture for equine strongyles on Prince Edward Island are similar to those reported in Saskatchewan (Polley, 1986), with larvae surviving the winter and being recovered from the pasture in low levels up to the following fall. However, there appear to be differences in the specifics of the patterns. The peak period of L3 production occurred

in the months of May, June and July in Saskatchewan and July, August and September on Prince Edward Island. Numbers of larvae recovered from grass in Saskatchewan were much lower for all months than the numbers of larvae in this study. This may be an indication of a more favourable environment for parasite survival and transmission on PEI. It is probably a reflection of the marked differences in summer temperatures and amounts of yearly precipitation between the two regions. Small numbers of eggs from samples deposited in winter in both regions survive and give rise to low levels of infective larvae the following spring and summer. The patterns of egg survival are similar to those reported by Lucker (1941) in northern USA and Parnell in Quebec (Cited by Polley, 1986). The significance of the low levels of eggs and infective larvae resulting from the winter months, and from the summer experiments surviving to the following fall, is not known.

No studies to date relate pasture infectivity to the actual number of worms acquired by grazing horses. However, this should be viewed in conjunction with the levels of contamination. If the number of eggs being shed by the horses is particularly high, then these larvae can be a significant source of infection. A horse passing 1000 EPG in faeces may pass 30 million eggs per day (Soulsby, 1982). Overwintered larvae may present a danger for foals that are going out to pasture for the first time. According to Duncan (1974), the two main sources of strongyle infection for foals on spring and summer pasture are overwintered larvae, and larvae resulting from contamination by their dams, which is considered most important. In Herds 1 and 2, the foals went out to

pasture with their dams early in the grazing season, overwintered larvae were still on pasture. By the time that these levels decreased considerably, larvae from the new contamination from the dams had already reached infectivity and were migrating to herbage. In northern USA, heavily infected summer pastures are a source of infection for grazing horses until the L3 die off from pasture the following spring (Herd, 1986b). A similar situation exists in Britain (Ogbourne, 1973; Duncan, 1974) and Ontario (Slocombe et al, 1987). This study indicates that on PEI, once the pastures are heavily contaminated, the danger of infection of grazing horses does not decrease as larvae from the previous grazing season are supplemented by those from contamination during the current grazing season. Control of strongyles in this region can effectively be achieved by eliminating contamination of pastures between June and September. Eggs in faeces deposited on pasture during the cooler months from October onwards are not likely to pose a big problem, since very few larvae from this contamination survive and contribute to pasture infectivity during the subsequent grazing season.

4.4 Summary and conclusions

Horse strongyles on PEI have a seasonal increase in egg production with peaks between August and October, and minor peaks between February and April. Eggs of small strongyles contribute most to these egg counts. On farms in which no strongyle control programme exists, *Strongylus vulgaris* may contribute up to 26% of the strongyle eggs shed in faeces of individual horses. Small strongyles occur in the large intestines of infected horses more commonly than large strongyles. The species of small strongyles

infecting horses in this region are similar to those identified in other studies from various parts of the world. The implications of these results are that any strongyle control programmes on PEI should be directed at the cyathostomes. Although *Strongylus vulgaris* is more pathogenic, cyathostomes are more numerous and have a shorter egg reappearance period.

Contamination of horse pastures on PEI causes highest pasture infectivity between July and September. During this period high egg counts are accompanied by rapid development to infectivity and increased movement of larvae to the surrounding herbage. Gradual migration of larvae to pasture causes the increase in pasture infectivity as the grazing season progresses. The highest levels of pasture infectivity are attained in November. Contamination of pastures between October and May does not pose as great a danger to grazing horses on PEI, because very low pasture infectivity results from this contamination. Few eggs develop to the infective stage in faecal material exposed to heavy rainfall during the first week of deposition. There may be no need of antistrongyle treatment early in the spring when heavy rainfall is expected in this region. The first treatment can safely be delayed to late June. However, horses that are turned out to pasture early in the grazing season, may be in danger of infection by overwintered larvae from the previous grazing season if the pastures were grazed by horses shedding high levels of strongyle eggs in August and September of the previous year.

4.5 Recommendations

Effective strongyle control on PEI should try to eliminate the late spring/early summer rise in egg production. This should reduce the pasture contamination before July when conditions are most suitable for development of eggs to the infective stage. Pasture contamination should be kept low throughout the grazing season. If this is achieved, then the late summer/fall peak in pasture infectivity will be eliminated. Since larvae that are ingested late in the grazing season are inhibited in development, reducing the level of pasture infectivity at the end of the grazing season should reduce the number of potential hypobiotic larvae and in this way reduce the danger of larval cyathostominosis during the following winter and spring. This should also reduce the level of pasture contamination in the next grazing season by lowering the level of spring rise in egg production. The following recommendations for control of strongyles on PEI are based on the results from this study.

1. If possible, foals going out to pasture for the first time should be grazed on pasture that was not grazed the previous year. The mares can then be treated to maintain the average herd faecal egg output at 100 EPG, or less. This will allow the foals to gradually ingest enough larvae them to develop some immunity, without compromising their health status.

2. Treatment of the herd:

(a) All horses in each herd can be treated in late June. The treatment should be repeated every 8 weeks if ivermectin is used or every 4 weeks if any other anthelmintic is used. Treatments should continue until the end of October and this would translate to a maximum of three treatments with ivermectin. The last treatment ensures that cyathostome larvae ingested late in the grazing season fail to encyst in the mucosa, hence eliminating the spring rise in egg counts.

or,

(b) Performing faecal egg counts on all the horses in the herd throughout the grazing season and treating the adults when a herd average of $\geq$ 100 EPG is attained. The treatment can be limited to between late June and September, since contamination of pastures from October onwards does not result in any appreciable pasture infectivity.

or,

(c) Performing faecal egg counts once a month from late June to September and treating any individual horses that pass high levels of strongyle eggs in their faeces. The cost of performing the faecal egg counts would probably be offset by the lowered costs in not treating all the horses.

If benzimidazole anthelmintics are used, pre-treatment and post-treatment egg counts should be performed to determine the faecal egg count reduction. Other studies have shown that the species of small strongyles recovered in this study commonly develop

benzimidazole resistance. The post-treatment faecal egg count should be performed two weeks following treatment. If faecal egg count reduction is less than 95%, then benzimidazole resistance already occurs and the use of this class of anthelmintics should be discontinued. In addition, the different drug classes available should be used in slow rotation to avoid the emergence of anthelmintic resistance to any of the classes. If the faecal egg count reduction test demonstrates that the population of strongyles in the herd are all benzimidazole susceptible, benzimidazoles can be included in this slow rotation.

3. Pasture hygiene practices such as sweeping or vacuuming can be carried out between late June and September. This may be practical where the pasture size is small, or by mechanising the vacuuming. The added advantage of this method is an increase in pasture available for grazing.

4. Economic analysis of the various methods or combinations of methods should be carried out to determine which one is best suited for each farm. Once a good level of control is achieved, the programme should be monitored for sustained success by performing faecal egg counts periodically from June to September.

APPENDIX 1

Appendix 1.1: Summary of egg count statistics for herd 1 mares

Month	N	Mean	Median	Stdev	Min	Max	Q1	Q3
June	9	269	100	526	0	1650	25	225
July	9	786	475	843	0	2375	75	1550
Aug	10	1040	888	1069	25	3550	125	1556
Sept	8	1100	1100	618	225	2150	606	1537
Oct	6	1829	1788	1012	525	3200	975	2713
Nov	10	912	987	671	25	1700	131	1556
Dec	9	175	200	156	0	425	25	313
Jan	10	510	462	451	0	1275	106	906
Feb	3	425	200	548	25	1050	25	1050
March	4	444	413	90	375	575	381	538
April	9	306	100	357	25	975	25	612

N - number of horses sampled
Stdev - standard deviation
Min - minimum egg count
Max - maximum egg count
Q1 - first quartile egg count
Q3 - third quartile egg count

Appendix 1.2: Summary of egg count statistics for herd 2 mares

Month	N	Mean	Median	Stdev	Min	Max	Q1	Q3
April	24	701	350	890	0	3925	62	1081
May	33	112	0	316	0	1725	0	87.5
June	28	342	162	577	0	2375	25	425
July	21	933	725	774	0	2375	237	1600
Aug	16	1123	1100	999	0	2925	62	1975
Sept	16	1322	925	1120	150	3575	356	2331
Oct	19	1009	800	769	0	3100	500	1350
Nov	28	804	775	572	0	2000	262	1169
Dec	28	747	687	572	0	2100	344	1038
Jan	15	635	700	478	0	1700	125	875
Feb	20	616	638	488	0	1550	175	1044
Mar	17	654	775	398	0	1175	263	1000
April	19	524	500	359	0	1225	175	800

N - number of horses sampled
Stdev - standard deviation
Min - minimum egg count
Max - maximum egg count
Q1 - first quartile egg count
Q3 - third quartile egg count

Appendix 1.3: Summary of egg count statistics for yearlings

Month	N	Mean	Median	Stdev	Min	Max	Q1	Q3
April	6	1262	937	1115	225	3325	450	2050
May	7	21	0	30.4	0	75	0	50
June	8	119	113	84.3	25	300	56	144
July	6	300	288	179	125	625	144	400
Aug	7	671	400	742	50	2125	125	1050
Sept	5	940	925	255	600	1275	713	1175
Oct	8	1000	537	987	125	2925	219	1700
Nov	5	920	725	700	175	1900	312	1625
Dec	5	614	650	125	1025	700	400	875
Jan	5	1145	875	623	450	1900	625	1800
Feb	5	235	225	118	75	400	138	338
March	4	1375	512	1821	375	4100	375	3237
April	5	485	375	441	0	1200	175	850

N - number of horses sampled
Stdev - standard deviation
Min - minimum egg count
Max - maximum egg count
Q1 - first quartile egg count
Q3 - third quartile egg count

Appendix 1.4: Summary of egg count statistics for herd 3 mares.

Month	N	Mean	Median	Stdev	Min	Max	Q1	Q3
April	10	605	550	506	0	1475	162	1044
May	10	517	325	561	0	1800	81	831
June	10	583	262	633	0	1675	50	1187
July	10	282	100	311	0	775	38	569
August	10	583	612	531	0	1375	56	1075
Sept	10	525	512	471	0	1500	56	794
Oct	10	360	438	230	0	650	75	519
Nov	12	125	0	292	0	775	0	0
Dec	11	64	0	212	0	703	0	0
Jan	12	110	0	306	0	1075	0	75
Feb	12	346	175	552	0	1900	44	225
March	10	315	250	350	0	1175	0	419
April	12	802	638	797	0	2425	131	981

N - number of horses sampled
Stdev - standard deviation
Min - minimum egg count
Max - maximum egg count
Q1 - first quartile egg count
Q3 - third quartile egg count

APPENDIX 2

Appendix 2.1: Percent composition of all larvae identified in faecal cultures from each group in herd 2 during each month.

Mo	Mares			Yearlings			Foals		
	Cy	Sv	Others	Cy	Sv	Others	Cy	Sv	Others
April	93.5	1.6	4.4	97.5	2.0	1.5			
May	99.4	0.0	0.6	100.0	0.0	0.0			
June	99.1	0.9	0.0	100.0	0.0	0.0			
July	99.3	0.5	0.3	96.1	3.9	0.0			
Aug	99.1	0.3	0.6	98.5	0.3	1.2	96.7	0.0	0.3
Sept	99.8	0.0	0.2	99.3	0.0	0.7	94.5	0.0	4.5
Oct	98.0	0.0	2.0	86.3	4.1	9.6	94.0	0.0	6.0
Nov	98.1	0.0	1.9	91.0	0.2	8.8	97.3	0.0	2.7
Dec	99.5	0.0	0.5	95.4	1.3	3.3	100.0	0.0	0.0
Jan	99.7	0.1	0.2	92.8	1.0	6.2	100.0	0.0	0.0
Feb	96.2	0.4	3.4	84.5	3.8	11.8	99.3	0.3	0.4
Mar	93.4	1.8	4.8	80.5	5.8	13.8	95.5	0.5	0.0
April	93.9	2.5	3.5	81.8	8.8	9.5			

Cy - cyathostomes; Sv- *Strongylus vulgaris.*

Appendix 2.2: Percent composition of all larvae identified in faecal cultures from mares in herds 1 and 3 during each month

Month	Herd 1			Herd 3
	Cy	Sv	Others	Cy
April				100.00
May				100.00
June	99.1	0.9	0.0	100.00
July	100.0	0.0	0.0	100.00
August	99.6	0.3	0.1	100.00
September	98.3	0.1	1.6	100.00
October	98.2	0.0	1.8	100.00
November	99.2	0.0	0.8	100.00
December	100.0	0.0	0.0	100.00
January	100.0	0.0	0.0	100.00
February	100.0	0.0	0.0	100.00
March	100.0	0.0	0.0	100.00
April	100.0	0.0	0.0	100.00

APPENDIX 3

Appendix 3.1: Number of larvae recovered from faecal samples, grass around the samples; and total number recovered from samples deposited in July to September 1993.

Interval	July			August			September		
(days)	Sample	Grass	Total	Sample	Grass	Total	Sample	Grass	Total
0-7	336	3	339	0	0	0	M	M	M
8-14	5223	218	5441	15	0	15	74083	921	75004
15-21	8133	184	8317	4092	3	4095	21620	930	22550
22-28	10895	275	11170	23263	42	23305	26460	2081	28541
29-35	11035	911	11946	52173	1683	53856	20052	1278	21330
36-65	1423	247	1670	10723	1166	11889	870	2755	3625
66-95	103	92	195	278	511	789	NS	NS	NS
95-125	0	2	2	NS	NS	NS	NS	NS	NS
186-215	-	-	-	NS	NS	NS	NS	NS	NS
216-245	-	-	-	NS	NS	NS	81	1878	1959
246-275	-	-	-	0	176	176	42	1150	1192
276-305	-	-	-	36	857	893	165	989	1154
306-335	-	-	-	6	85	91	67	1315	1382
336-365	-	-	-	0	115	115	0	520	520
366-395	-	-	-	-	-	-	0	44	44
396-425	-	-	-	-	-	-	0	12	12

Interval refers to sampling interval in days post deposition.
NS indicates not sampled due to snow.
M indicates missed sampling.
- indicates sampling had been completed

Apendix 3.2: Numbe. ·•t vae recovered from faecal samples, grass around the samples and total numb· ..vae recovered for samples deposited in January to March, 1994.

Interval	January			February			March		
(days)	Sample	Grass	Total	Sample	Grass	Total	Sample	Grass	Total
0-35	NS	NS	NS	NS	NS	NS	0	0	0
36-65	NS	NS	NS	0	0	0	M	M	M
66-95	0	0	0	33	27	60	1834	12	1846
96-125	85	95	180	0	21	21	549	462	1011
126-155	0	16	16	8	303	311	6	189	195
156-185	0	10	10	0	698	698	0	9	9
186-215	0	10	10	0	0	0	0	50	50
216-245	0	10	10	0	42	42	0	16	16
246-275	0	2	2	-	-	-	-	-	-

Interval refers to sampling interval in days post deposition.
NS indicates not sampled due to snow.
- indicates sampling had beed completed.

Appendix 3.3: Total number of larvae recovered from the grass surrounding samples deposited in April to June 1994.

Interval (days)	April	May	June
0-7	M	M	0
8-14	M	0	0
15-21	M	0	16
22-28	2	0	M
29-35	11	5	M
36-65	5	19	71
66-95	14	M	458
96-125	M	88	28
126-155	8	33	1
156-185	2	0	-
186-215	0	-	-

Interval refers to sampling interval in days post deposition.
M indicates times when sampling was missed.
- indicates that sampling had been completed.

Appendix 3.4: Total number of larvae recovered each month as a percentage of theoretical recovery.

Interval (days)	J	A	S	O	N	D	J	F	M	A	M	J
0-7	0.45	0.00	M	0.00	0.00	NS	NS	NS	NS	M	M	0.00
8-14	6.30	0.01	47.00	0.00	0.00	NS	NS	NS	NS	M	0.00	0.00
15-21	9.90	3.90	14.00	0.00	0.00	NS	NS	NS	NS	M	0.00	0.01
22-28	16.80	17.30	20.60	0.00	0.00	NS	NS	NS	NS	*	0.00	M
29-35	14.60	28.40	13.90	0.01	0.00	NS	NS	NS	0.00	0.02	*	M
36-65	2.30	8.00	2.60	0.00	NS	NS	NS	0.00	0.00	0.01	0.01	0.03
66-95	0.30	0.50	NS	0.00	NS	NS	0.00	0.04	1.60	0.02	M	0.22
96-125	*	NS	NS	NS	NS	0.00	0.14	0.01	1.00	M	0.05	0.02
126-155	-	NS	NS	NS	NS	*	0.01	0.30	0.18	0.01	0.02	*
156-185	-	NS	NS	NS	0.00	*	0.01	0.50	0.01	*	0.00	-
186-215	-	NS	NS	*	0.02	0.01	0.01	0.00	0.06	0.00	-	-
216-245	-	NS	1.00	*	*	0.00	0.01	0.03	0.13	-	-	-
246-275	-	0.16	0.71	*	*	-	*	-	-	-	-	-
276-305	-	0.44	0.75	0.00	-	-	-	-	-	-	-	-
306-335	-	0.07	1.30	-	-	-	-	-	-	-	-	-
336-365	-	0.12	0.38	-	-	-	-	-	-	-	-	-
366-395	-	-	0.03	-	-	-	-	-	-	-	-	-
396-425	-	-	0.01	-	-	-	-	-	-	-	-	-

Theoretical recovery is based on EPG of faecal samples at time of deposition.
Interval refers to sampling interval in days post deposition.
* indicates less than 0.01%.
NS indicates no sampling due to snow.
M indicates missed sampling for any other reason.
- indicates sampling had been completed.

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