STUDIES ON THE PATHOGENESIS OF CYATHOSTOME INFECTIONS IN THE HORSE

by

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August, 1990.

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ACKNOWLEDGEMENTS

This work was funded entirely by a Research Project Grant from the Horserace Betting Levy Board from whom I also received a Veterinary Research Training Scholarship during the first year of the study.

Whilst undertaking the work for this thesis many people generously gave time, advice and assistance. I wish to accord my sincere thanks to: John Armstrong, Ken Bairden, Stuart Brown, Ronnie Barron, Mary Findlay, Linda Forrest, Richard Irvine, Arlene Macrae, Christine Marshall, Pauline Mollison, Jimmy Murphy, Diana McKechnie, Stuart Reid, Gerald Roarty, Tim Watson, Brian Wright and, most particularly, to Celia Marr.

I wish to thank Professor G.M. Urquhart for his help and for providing facilities within the Department of Veterinary Parasitology during this study. Professor P.H. Holmes and Dr. J.M. MacLean gave much invaluable advice in addition to the use of equipment in the Department of Veterinary Physiology. I am also grateful to Professor W.F.H. Jarrett and colleagues in the Department of Veterinary Pathology for readily providing the facilities of the post-mortem room and for performing pathological examinations on some of the clinical cases. Dr. H. Thompson also assisted with photography of pathological specimens.

Throughout this study I have been very fortunate to have had the good humoured assistance of James McGoldrick for which I am

most appreciative. I am also grateful for the dedicated care given to the experimental ponies and clinical cases by Mary Gatherer and Elspeth Ralston. This thesis has been typed by Julie Nybo with skill and tolerance for which I also owe thanks.

I am greatly indebted to Professor J.L. Duncan who initiated my interest in this subject and subsequently he has willingly provided advice and encouragement.

Finally, I wish to acknowledge the support given to me during this study by Professor Max Murray.

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SUMMARY

The aim of these studies was to investigate some aspects of the pathogenesis of cyathostome infections in the horse.

A clinical study on 16 cases of diarrhoea was undertaken; three animals were diagnosed as larval cyathostomiasis, all of which had similar signs of sudden onset diarrhoea and pronounced weight loss in late winter. Two of the cases of cyathostomiasis were diagnosed on the basis of the clinical findings together with the presence of cyathostome larvae in faeces but the other was only diagnosed post-mortem. Although haematological and blood biochemical analyses were useful in the clinical management of diarrhoeic horses, in general laboratory data were not useful in the differential diagnosis of the condition in adult animals in this study.

In studies on the epidemiology of naturally acquired infections in ponies, groups of animals of different ages and experience of previous parasite exposure were allowed to graze a single pasture in autumn. There was evidence that previous exposure modified cyathostome development in that acquired burdens in foals which had previously grazed were smaller and developed more slowly than those of helminth-naive animals of the same age. The burdens acquired by yearling and adult ponies were of a similar size to the previously grazed foals but the incidence of arrested development was notably higher in the younger groups of foals and yearlings, compared with adults. A further manifestation of an effect of age on cyathostome development was that the level of faecal egg output of adult

ponies was lower than in the groups of young animals.

Experimental infections of ponies were performed with the objective of investigating factors which might contribute to arrested larval development. A comparison of single infections of either 50,000 or 500,000 third-stage larvae indicated that the occurrence of arrested larval development was not apparently affected by dose size but that establishment rates were lower in the animals given the larger doses. Similarly, laboratory chilling of infective larvae at $4^{o}C$ for six weeks was shown to reduce establishment rate but did not affect the incidence of arrested larval development. There was also a reduction in establishment rate of infective larvae when these were administered as repeated, or 'trickle', doses compared with establishment following single doses of the same size but there was no difference in the absolute number of larvae which became arrested in development.

The pathogenic effects of cyathostome infections in ponies were studied by use of radioisotope tracer techniques in order to investigate plasma protein and red blood cell metabolism. In these studies only moderate infections were established and there were only minor differences between control or infected groups of ponies with either naturally acquired burdens of mainly adult worms or experimental infections of exclusively immature mucosal worms.

GENERAL INTRODUCTION

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The classification of the family Strongylidae of horses has been a source of taxonomical debate for parasitologists for much of this century. However the system proposed by Lichtenfels (1975) has now achieved almost universal acceptance. He has, like previous authors, classified the equine Strongylidae into two separate subfamilies: Strongylinae (large strongyles) and Cyathostominae (cyathostomes, small strongyles). Within the Cyathostominae there are eight genera, Cyathostomum, Cylicocyclus, Cylicodontophorus, Cylicostephanus, Poteriostomum, Gyalocephalus, Caballonema, Cylindropharynx of which the first four are sometimes referred to collectively as Cyathostomum sensu lato and which were previously considered under the genus Trichonema.

Over one hundred and fifty years ago, parasites within the mucosa of the large intestine of horses were associated with a severe diarrhoeic condition (Knox, 1836). Despite further sporadic accounts of a syndrome of diarrhoea, weight loss and sometimes death in horses (Cuillé, Marotel and Roquet, 1913; Raillet, 1923; Marotel, 1931, Houdemer, 1935; Tiunov, 1951; Velichkin, 1952) veterinarians have tended to regard cyathostomes as having low or limited pathogenicity. Perhaps the adoption of this view has been mainly the result of wide recognition of the blatant, severe pathology associated with the large strongyles, especially Strongylus vulgaris, the parasitic bête noir of horses. The paucity of information on basic aspects of cyathostome biology together with the evidence of the presence of

large burdens of cyathostomes in apparently healthy horses has also fostered this general opinion.

It can be concluded from reports of parasitological data obtained post-mortem that there is an extremely high prevalence of cyathostome infection in horses in Europe (Ogbourne, 1976; Mirck, 1982) and the U.S.A. (Hass, 1979; Reinemeyer, Smith, Gabel and Herd, 1984). The results of similar studies in other countries were summarised by Ogbourne (1976) which suggested that there was a similarity between different geographical regions in the relative importance of individual cyathostome species. Although there were between-study differences in rank orders the most prevalent species have usually been: Cylicostephanus longibursatus, C. goldi, C. minutus, C. calicatus, Cyathostomum catinatum, C. coronatum, C. pateratum, Cylicocyclus nassatus, C. insigne and C. leptostomus (Ogbourne, 1976; Reinemeyer and others, 1984).

Enormous cyathostome burdens are not uncommonly reported in necropsy studies. Ogbourne (1976) recorded lumenal burdens of 86 adult horses in which there was a range of 12,000 - 1,239,000 worms, although 80% of these animals had lumen populations of less than 200,000; seasonal variations were also observed. Hass (1979) reported a national survey in U.S.A. in which small strongyle burdens in 629 adult horses ranged from 1,000 - 2,220,000 with a mean of 68,000; in 150 animals under one year of age the range was 10,000 - 1,035,000, mean 37,000. Also in the U.S.A. the total cyathostome burdens in 55 adult horses studied

by Reinemeyer and others (1984) ranged from 680 - 663,100 worms per horse.

In a series of studies of parasite control using yearling Shetland ponies in The Netherlands, Eysker and his colleagues have frequently observed naturally acquired total cyathostome burdens of several hundred thousand with a maximum of 2,911,140 in one individual (Eysker and Mirck, 1986; Eysker, Jansen and Mirck, 1986a; Eysker, Jansen, Kooyman, Mirck and Wensing, 1986b; Eysker, Boersema, Kooyman and Berghen, 1988; Eysker, Boersema and Kooyman, 1989a; b). Although in some of these studies the heavily parasitised ponies exhibited clinical signs of emaciation, poor coat quality and colic, in many cases animals showed little adverse effect of these large burdens.

In 1978, ignorance of the biology of small strongyles was redressed to a large extent by publication of Ogbourne's review on the pathogenesis of cyathostome infections in the horse. In this account the collation of information from diverse sources gave a detailed overview of the development and behaviour of cyathostomes within their hosts. Following publication of this treatise there was a dramatic increase in veterinary interest in cyathostomes with considerable advances in knowledge of these parasites. Reinemeyer (1986) has further reviewed the information available on small strongyles to create a composite view of their biology.

Although no details are available the life cycle of cyathostomes is presumed direct and non-migratory. Since single species infections have not been carried out it has been assumed

that all species follow the same basic cycle although Tiunov, (1951; 1953) has shown between-species variations in the duration of parasitic development.

Basically cyathostome eggs are expelled in faeces from infected hosts onto pasture where they develop through two free-living stages to become infective third-stage (L_3) larvae. When L_3 are ingested they exsheath in the small intestine prior to entering the mucosa of the caecum and colon via the crypts of Lieberkuhn (Tiunov, 1953). It has been shown that individual species appear to have preferential sites within the mucosal epithelium, the lamina propria or the submucosa (Tiunov, 1951; Muller, 1953; Mathieson, 1964; Poynter, 1970). Although there are anatomical differences in distribution of tissue larval stages in general, such that the majority occur in the caecum and ventral colon with very few in the more distal, dorsal colon (Tiunov, 1953; Mathieson, 1964; Reinemeyer and Herd, 1986b) it is not known if certain species have distinct regions for larval development. In a recent study, fourth-stage mucosal larvae of Cyathostomum catinatum, C. coronatum and Cylicocyclus nassatus were found at various levels of the large intestine (Reinemeyer and Herd, 1986b) but in an early report there was evidence that the caecum was the specific site for development of Cylicocyclus insigne (Boulenger, 1921).

Larvae within the mucosa undergo a moult to the fourth larval stage which subsequently emerge into the lumen of the bowel and establish as adults at a site more distal to that of

larval development. The anatomical distribution of adult cyathostomes is thus different from that of larval stages. In a recent quantitative study 57% of encysted cyathostome larvae were in the wall of the caecum, 41% were in the ventral colonic wall with only 2% in the dorsal colon (Reinemeyer and Herd, 1986b) whereas post mortem studies of the distribution of adults have recorded 1-10% within the caecum, 50-55% in the ventral colon and approximately 45% in the dorsal colon (Ogbourne, 1976; Reinemeyer and others, 1984; Mfitilodze and Hutchinson, 1985).

Although few species occur exclusively in one anatomical region several authors have observed a distinct distribution pattern of adult cyathostomes such that each region of the large colon is dominated by only a small number of species. According to Ogbourne (1978) there is consensus between reports that Cylicocyclus nassatus, Cylicostephanus minutus, C. calicatus and Cyathostomum catinatum predominate in the ventral colon whereas Cylicostephanus longibursatus, C. goldi and Cylicocyclus insigne show preference for the dorsal colon and that the relatively small adult population of the caecum is not dominated by any particular species although it is the preferred site of Cyathostomum coronatum and Cylicostephanus poculatus.

In experimental infections of helminth-naive foals prepatent periods have been between 35 and 70 days (Round, 1969; Reinemeyer, Herd and Gabel, 1988) which suggest that the larvae remained in the mucosa for 4-8 weeks. When Smith (1976b; 1978) reinfected adult ponies which were considered to be virtually parasite-free, he observed that patency occurred at 12 to 18

weeks after infection which implies that host immunity may lead to lengthening of the prepatent period.

Gibson (1953) was the first to suggest that cyathostome larvae could undergo inhibited development. This was based on the results of a three-year experiment in which repeated doses of phenothiazine only temporarily reduced faecal egg counts to zero and adult lumenal strongyles were identified post mortem. In a similar study using intermittent thiabendazole treatments, Smith (1976a) obtained comparable results with progressively lengthening intervals to recurrence of positive faecal egg counts.

Traditionally it has been considered that arrested cyathostome larval development occurs at the early fourth larval stage but recently arrested third-stage larvae have been reported (Eysker, Jansen and Mirck, 1984; Reinemeyer and Herd, 1986b). It is unknown whether arrested development is a feature of all cyathostome species or if there is inter-species variation both in occurrence and the stage at which arrestment occurs. In discussion, Reinemeyer and Herd (1986b) have speculated that arrested development could occur at both third and fourth larval stages in the same parasites which could account for the exceptionally prolonged tissue phases reported by Gibson (1953) and Smith (1976a).

To date the factors which contribute to the occurrence of arrested cyathostome development have not been elucidated. However it has been likened by various authors (Ogbourne, 1975;

Eysker and others, 1984) to hypobiosis as seen in several gastrointestinal nematodes, in particular Ostertagia spp in ruminants, in which environmental conditioning, host immunity and the size of the worm burden are considered important (Michel, 1974; Armour and Duncan, 1987). The stimuli for resumption of cyathostome development are also not known but it has been suggested that the removal of adults from the lumen, either by natural ageing or following therapy, may remove an inhibitory feedback mechanism affecting the tissue stages (Gibson, 1953; Smith, 1976a).

Few specific studies of cyathostome infections have been undertaken and most of the knowledge of the host-parasite relationship has been derived from observations in horses with naturally acquired mixed strongyle species infections. It is not possible to differentiate cyathostome ova or infective larvae to genus and species level or to identify many of the immature parasitic larval stages although morphologically distinct groups have been described (Soulsby, 1965). Consequently there are difficulties in studying the population dynamics of small strongyles and in interpreting host responses to infection and how these may modify parasite behaviour.

Ogbourne (1975) and Reinemeyer, Smith, Gabel and Herd (1986) have attempted to study the population dynamics of several individual cyathostome species by monitoring seasonal changes in the age profile of the population as determined by changes in the reproductive status of the adult female worms. Similar results were obtained in these studies which were conducted in temperate

climates in the U.K. (Ogbourne, 1975) and Ohio, U.S.A. (Reinemeyer and others, 1986). In the latter study total counts of mucosal larvae were also made and these were found to show seasonal trends with peak numbers immediately preceding the peaks of immature lumenal adults. By integrating the seasonal patterns of equine strongyle egg production (Poynter, 1954; Genchi, Malnati and Carrara, 1978; Mirck, 1981; Craig, Bowen and Ludwig, 1983; Duncan, 1974; Herd, Willardson and Gabel, 1985) with the changes in age structure of the small strongyle population, Reinemeyer and others (1986) suggested an annual cycle of development of cyathostome nematodes. During winter the worm burdens are composed predominantly of senescent lumenal adults and immature, mucosal larvae - consequently faecal egg counts are low. When the mucosal stages emerge into the lumen and mature to adults in spring there is an associated rise in faecal egg output. This newly established adult population appear to persist through the summer with cumulative additions of young adult worms such that there is a peak in adult population numbers in late summer associated with a second rise in faecal egg counts (Herd and others, 1985; Slocombe, Valenzuela and Lake, 1987). Subsequently there is an ageing and loss of lumenal stages in the autumn.

In areas where the climate is cool and wet, spring rises in equine strongyle faecal egg counts have frequently been observed to lead to rises in pasture larval counts which peak in late summer or autumn (Duncan, 1974; Genchi and others, 1978; Mirck,

1981; Hasslinger and Bittner, 1984). Herd and others (1985) detected increases in pasture infectivity following both spring and summer peaks of egg output in a study in Ohio, U.S.A. but although a similar biphasic pattern in egg output was detected in a separate study in southern England (Herd, 1986) the spring peak was not associated with a subsequent increase in pasture larvae which was attributed to prevailing unseasonable dry weather conditions. The effects of environmental factors on the transmission potential of equine cyathostomes are apparent from epidemiological studies performed in regions with warm climates. In central Texas (Craig and others, 1983) and north central Florida (Courtney and Asquith, 1985) pasture larval counts were low during the summer months when temperatures were high and they were first noticed to rise with the advent of cooler weather in October and November respectively.

In their monograph on the biology and veterinary importance of Strongylus vulgaris in the horse, Ogbourne and Duncan (1984) have comprehensively reviewed the details of experimental and field studies of the free-living larval stages of mixed strongyle populations.

In general terms strongyle egg hatching, larval development and larval survival rates are all affected by temperature and, to a lesser extent, moisture.

More specifically, optimal egg hatching occurs between 25°C and 30°C and little hatching occurs below 8°C or above 35°C. Whereas unhatched eggs can survive freezing, very low temperatures are considered especially lethal to pre-infective

larval stages and temperatures greater than the optimum are also detrimental to larval development. Although there is a minimal moisture requirement of 15-20% for pre-infective larvae to complete development, those which have reached the second developmental stage may survive in dry conditions and resume development if moistened.

The rate of larval development is mainly affected by temperature, occurring very rapidly (2-3 days) at 30°C but taking two to three weeks at around 12°C although inadequate moisture can also prolong the time taken to reach the infective stage. Dryness may prevent the infective larvae from migrating onto herbage from the faeces which act as a reservoir from which the herbage larval population is periodically replenished when moisture levels increase.

The survival rates of infective larvae on herbage are also affected by climatic conditions : there is a higher larval mortality with increasing temperature but neither prolonged freezing nor desiccation markedly affect longevity. Activity, and therefore metabolic rate of infective larvae is proportional to temperature and above 25°C the larval food reserves are quickly exhausted which results in death. Thus, the conditions which are optimal for larval development are much less favourable for larval survival.

The practical implications of these observations are that infective larvae on pasture can survive for long periods during cold, wet conditions but they die within a few weeks during warm,

dry weather. Consequently there are considerable seasonal and regional differences in equine strongyle ecology : for example, in cold, continental Canada there is a comparatively short period when climatic conditions favour egg hatching and larval development but larvae may survive for many months (Polley, 1986; Slocombe and others, 1987) whereas in subtropical and tropical Australia there is year-round hatching and rapid development of free-living stages but survival times are relatively short (English, 1979a; 1979b; Mfitilodze and Hutchinson, 1988; Hutchinson, Abba and Mfitilodze, 1989).

Variations in pasture infectivity are important both in contributing to the seasonal occurrence of strongyle related diseases in horses (including acute larval cyathostomiasis) and also as a consideration when formulating parasite control programmes.

Since Drudge and Lyons (1966) first advocated control of equine parasites by interval worming to reduce pasture contamination with infective larvae, there has been marked decrease in the prevalence of Strongylus vulgaris infection detected by either post-mortem examination for adults and parasitic larvae (Reinemeyer and others, 1984; Dunsmore and Jue Sue, 1985; Lyons, Tolliver, Drudge, Swerczek and Crowe, 1987) or identification of third-stage larvae in faecal cultures and pasture larval recoveries (Uhlinger and Johnstone, 1985; Herd, 1986; Nilsson, Lindholm and Christensson, 1989; King, Love and Duncan, 1990). As a result of the retreat of S. vulgaris from predominance in equine clinical parasitism and the more frequent

reports of cyathostome-related disease, there has been an increasing recognition of the need to concentrate anthelmintic prophylaxis on the control of small strongyles. Since most anthelmintic compounds used in horses have good efficacy against adult cyathostomes (Westcott, 1986) it is possible to minimise pasture infectivity by repeated dosing of all the animals grazing the same pasture (Duncan, 1974). However, since anthelmintic resistance of cyathostomes was first reported to phenothiazine 29 years ago (Drudge and Elam, 1961) resistance has become a widespread problem in the U.S.A. (reviewed by Bennett, 1983; Westcott, 1986) and although it was reported in the U.K. in the early seventies (Round, Simpson, Haselden, Glendinning and Baskerville, 1974) it is only recently that it has been frequently reported from various European countries including the U.K. (Herd, 1986; Ryan, Lumsden, Smith and Taylor, 1987; Britt and Clarkson, 1988; Love, McKellar and Duncan, 1989; Lumsden, Quan-Taylor, Smith, Washbrooke, 1989; King and others, 1990), the Netherlands (Eysker and others 1988; Eysker and others, 1989b), Belgium (Geerts, Guffens, Brandt, Kumar and Eysker, 1988), Germany (Bauer, Gandras, Stoye and Burger, 1983; Bauer, Merkt, Janke-Grimm and Burger, 1986) and Sweden (Nilson and others, 1989). With the exception of one instance of small strongyle resistance to piperazine (Drudge, Lyons, Tolliver, Lowry and Fallon, 1988) all of these recent studies have reported development of resistance to benzimidazole compounds. Benzimidazole-resistance has been documented in 12 cyathostome

species to date, namely Cyliocyclus nassatus, C. leptostomus, C. insigne, C. brevicapsulatus, Cylicostephanus longibursatus, C. goldi, C. minutus, C. poculatus, Cyathostomum catinatum, C. coronatum, C. labiatum and C. labratum which includes the five common species which collectively constituted 89% and 84% of cyathostome burdens in slaughter surveys in the U.K. (Ogbourne, 1976) and U.S. (Reinemeyer and others, 1984) respectively. Thus, benzimidazole resistance has become a major consideration in equine strongyle control and it has prompted parasitologists to examine approaches which place less reliance on frequent dosing with anthelmintics. For example, Herd and others (1985) have applied an epidemiological approach to the control of equine strongyles in Ohio and they reported some advantages in the concentrated use of anthelmintics to control the rise in faecal egg output during the spring season over conventional methods of regular treatments throughout the entire grazing period. In a subsequent study in Newmarket (Herd, 1986), a non-chemical approach, based on bi-weekly removal of faeces from the pasture, was a more effective prophylactic regimen against parasites than repeated anthelmintic dosing.

In a series of investigations into alternate grazing of ponies and sheep combined with strategic anthelmintic treatments Eysker and others (1986a) demonstrated some beneficial effect in this approach but the same group have also reported failure to prevent acquisition of large cyathostome burdens by a system of removal to clean pasture following treatment with albendazole (Eysker and others, 1986b).

Although there has been concurrence of the documentation of widespread anthelmintic resistance and an increase in the incidence of reports of acute larval cyathostomiasis, at present their correlation is speculative. In recent reports the clinical accounts of larval cyathostomiasis consistently describe sudden onset diarrhoea which becomes chronic and is associated with marked, rapidly progressive weight loss leading to emaciation, and possibly to death (Blackwell, 1973; Chiejina and Mason, 1977; Jeggo and Sewell, 1977; Mirck, 1977; Jasko and Roth, 1984; Giles, Urquhart and Longstaffe, 1985; Church, Kelly and Obwolo, 1986; Harmon, Ruoff and Huoy, 1986). Affected animals are generally first presented in late winter or early spring, usually they are young adults and often additional clinical signs such as colic or subcutaneous oedema may be evident although none of these criteria apply to every case. This syndrome had first been described in detail by Russian authors (Tiunov, 1951; 1953; Velichkin, 1952) although Ogbourne (1978) cites several earlier accounts of disease associated with cyathostome infection from France (Cuillé and others, 1913; Raillet, 1923; Marotel, 1931; Houdemer, 1935).

Mirck (1977) reported that cyathostomiasis cases often shed large numbers of fourth or early fifth stage larvae in their faeces and in subsequent studies this has been regarded as a useful differentiating clinical feature (Giles and others, 1985; Mair, De Westerlaken, Cripps and Love, 1990). Most of the acute larval cyathostomiasis cases described in contemporary reports

were found to have neutrophilia and hypoalbuminaemia (Blackwell, 1973; Chiejina and Mason, 1977; Jeggo and Sewell, 1977; Mirck, 1977; Jasko and Roth, 1984; Giles and others, 1985; Church and others, 1986; Harmon and others, 1986) but the hyperglobulinaemia - especially betaglobulinaemia - described as characteristic of this condition by Giles and others (1985) was an inconsistent finding in most other reports and is not considered to be pathognomonic. Velichkin (1952) reported that the cyathostomiasis cases in his study often developed anaemia and/or eosinophilia and/or lymphocytosis but similar changes have been infrequently observed in more recent reports.

The Russian workers Velichkin (1952) and Tiunov (1953) classified the condition of nodular trichonematosis (acute larval cyathostomiasis) on the basis of pathology as a catarrhal and fibrinous inflammation of the caecum and colon accompanied by diffuse haemorrhagic foci in the mucosa. Velichkin (1952) considered the appearance of the large intestinal mucosa "as if it had been dusted with poppy seeds" to be characteristic and he recognised that this appearance was due to coiled cyathostome larvae in the mucosa at densities of 20-50 per cm^2. Other pathological observations included: mucosal oedema; larval cyathostomes on the mucosal surface, in the intestinal contents and faeces; necrotic nodules in the mucosa as a sequel of larval emergence and enlargement of mesenteric lymph nodes. Velichkin (1952) observed histopathological changes of generalised mucosal disruption along with cellular infiltration. The authors of subsequent case reports (Chiejina and Mason, 1977; Jasko and

Roth, 1984; Harmon and others, 1986) and case series (Mirck, 1977; Giles and others, 1985; Reinemeyer and Powell, 1986) describe very similar gross pathological findings. Larval species identified post mortem in clinical cases were Cylicostephanus longibursatus and Cyathostomum catinatum (Chiejina and Mason, 1977), Cylicocyclus radiatus (Harmon and others, 1986) and C. insigne (Reinemeyer and Powell, 1986).

The histopathological changes associated with cyathostome larvae in the gut wall of clinically normal horses have been extensively reviewed by Ogbourne (1978) in his well illustrated review on the pathogenesis of cyathostome infections. Following penetration of the basement membrane of the epithelial cells of the tubular glands, the larvae in the lamina propria initially provoked a fibroblastic reaction which increased as the larvae grow resulting in distortion of the architecture of the glands in which goblet cell hyperplasia and hypertrophy occur. Modest cellular infiltration occurs in the area below and around encapsulated larvae which is predominantly lymphocytic with some plasma cells and in some cases large numbers of eosinophils. On occasion this tissue eosinophilia was intense around the periphery of the larval capsule and was considered to be associated with larval emergence. Larvae located in the submucosa were recorded to induce formation of a dense capsule of fibroblasts and collagen together with marked infiltration of plasma cells, eosinophils and mast cells.

Further information on the histopathological features in horses with clinical cyathostomiasis have been described in recent reports. There was a marked cellular response in the wall of the large intestine which was either diffuse in the mucosal lamina propria and/or submucosa (Jasko and Roth, 1984; Church and others, 1986) or more focally intense around submucosal larvae (Giles and others, 1985; Harmon and others, 1986; Reinemeyer and Powell, 1986). Mixed populations of mononuclear cells, eosinophils and epithelioid cells were recorded. Other microscopic findings included: fibrous capsule formation around larvae; mucosal and submucosal oedema; disseminated focal areas of submucosal haemorrhages with eosinophilic infiltrates; dilated submucosal lymphatics and variable epithelial shedding. Interestingly, in one of the cases investigated by Church and others (1986), there was an apparent marked infiltrate of mononuclear cells and eosinophils in jejunal lamina propria and submucosa as well as oedema in the rectal lamina propria.

Successful treatments of cases of acute larval cyathostomiasis using larvicidal doses of anthelmintics (50-60 mg/kg fenbendazole on one or several occasions, as well as 0.2 mg/kg ivermectin in one case) in conjunction with supportive and symptomatic treatment have been recorded (Jeggo and Sewell, 1977; Giles and others, 1985). However in many cases it appeared that cyathostome tissue stages were insusceptible to anthelmintics as evidenced by their presence post mortem in cases which were unresponsive to treatment (Chiejina and Mason, 1977; Jasko and Roth, 1984; Giles and others, 1985; Harmon and others,

1986). Two cases confirmed by intestinal biopsy made immediate clinical improvement following treatment with corticosteroids which were considered to modulate the host response while frequent anthelmintic dosing was maintained to eliminate any maturing parasites (Church and others, 1986).

In two separate papers Tiunov (1951; 1953) published the first reports of the clinical, parasitological, pathological and haematological effects of experimental infections of horses with cyathostomes. There are only eight subsequently recorded studies of experimental cyathostome infections which address different aspects of infection: Round (1969; 1970) determined prepatent periods and investigated changes in serum proteins; Smith (1976b; 1978) recorded clinical, haematological and parasitological effects of reinfection; Bueno, Ruckebusch and Dorchies (1979) investigated gut motility patterns; Burger and Bauer (1987) and Geerts and others (1988) undertook studies of chemotherapy of benzimidazole-resistant small strongyles and Reinemeyer and others (1988) examined the anatomical distributions of larval and adult stages of a field isolate of benzimidazole-resistant species.

The clinical signs of experimental infection were described by Tiunov (1951) following infection of four worm-free foals with an unspecified dose of small strongyle larvae said to be of the genera Trichonema, Cylicocyclus and Gyalocephalus. He observed a range of severity of signs which he categorised as mild or severe. In the mild form there was a transitory pyrexia (39.5 -

40°C) between days 4 and 12 following infection. A second phase of slight fever (39.5 - 39.8°C) was detected about one month after larval challenge which lasted for four to five days. Other signs which were recorded were general depression and loss of body condition. The severe form of the experimental disease was associated with a rise in body temperature to 40 - 41.5°C and an increase in heart rate. Specific gastrointestinal signs of watery diarrhoea, sometimes containing blood and mucus, and increased borborygmi were detected in the severely affected animals which became anorexic, weak and grew poorly.

In the experimental infections conducted by Smith (1976b; 1978), adult ponies whose strongyle worm burdens had been removed or markedly reduced by repeated thiabendazole treatments were given doses of between 100,000 and 500,000 infective cyathostome larvae as a single or divided dose and on both occasions uninfected control animals were also monitored. In these experiments the reinfected ponies which received 200,000 or more larvae began to pass soft faeces three to seven days after single infections and about one month after trickle infections. Some ponies continued to pass soft faeces for two to three weeks. About half of the ponies were observed to have a reduced appetite for a few days following single infections: body temperatures were not recorded. There were marked differences in body weight between the uninfected control and the reinfected animals. The reinfected animals began to lose weight within two weeks of larval administration and by two months there were differences in group mean body weights of 18 - 25 kg which then persisted for

1 - 2 years.

Smith (1976b) also made the observation that in the first spring following reinfection, ponies retained their winter haircoats for several weeks longer than uninfected control animals, which also showed a greater sheen in their summer coat. This difference was not observed in subsequent years or after further reinfection (Smith, 1978).

In studies of digestive motility in horses (Bueno and others, 1979), experimental infections of strongyle larvae were administered to three foals which had grazed strongyle-infected pasture for six months. Three similar animals had been treated every three weeks with mebendazole at 10 mg per kg body weight and on the basis of negative weekly faecal worm egg counts they were considered to be clean controls. The infective larval dose contained 85% cyathostome larvae and 15% large strongyle larvae. One of the three infected foals was anorexic following administration of 300,000 larvae and this animal had transient diarrhoea on the 39th day of the infection. No other clinical signs were recorded. In each of the three experimentally infected ponies there were significant decreases in ileo-caeco-colic motility on the first day after infection and decreases in caeco-colic activity for about three days were also recorded from day 40 of the infection. The changes in motility patterns coincided with the periods of overt clinical signs in the animal which was anorexic and diarrhoeic.

Both cellular and serological responses in peripheral blood occur following experimental cyathostome infection. The changes reported by Tiunov (1951) were of an anaemia and a leucocytosis. By contrast, in the older animals which Smith (1976b; 1978) reinfected with cyathostomes there were no changes in red blood cell parameters but there was an immediate marked eosinophilia which then persisted for two years. In his studies on yearlings, Round (1970) monitored changes in serum proteins following initial infections and on reinfection. Increases in betaglobulins were detected about six weeks after a first infection of worm-free yearlings and somewhat earlier - present by one week and maximal at four weeks - in the same animals when reinfected. No changes in the amounts of the other serum globulins were recorded in these experiments but a reduction in serum albumin levels occurred in three out of four ponies receiving an initial infection of 105,000 larvae. The same three animals exhibited a transitory decrease in serum albumin levels within two weeks of a second infective dose of 110,000 larvae with minimum levels between four and seven weeks after infection.

Only limited observations on parasitic development were made in these experimental studies. Tiunov (1951; 1953) studied cyathostome development in four sucking foals which were necropsied at 10, 28, 30 and 60 days post infection. He suggested that the duration of the tissue phase of Cylicostephanus longibursatus, C. minutus, C. calicatus, Cyathostomum catinatum, C. coronatum and Gyalocephalus spp was 30 days whereas Cylicocyclus spp had a minimum period of submucosal

development of two months. These observations were made by examination and identification of mucosal and immature lumenal larvae which has been regarded as imprecise at species level (Soulsby, 1965).

In studies using helminth-naive foals the proportions of the infective dose present as mucosal stages at post-mortem three to four months post infection were small which suggests that cyathostome larvae developed progressively in these animals (Burger and Bauer, 1987; Reinemeyer and others, 1988).

In contrast, the results of experimental reinfection studies of adult ponies reported by Smith (1976b; 1978) were indicative of delayed or inhibited development: prepatent periods were prolonged to 12-15 weeks (1976b) or 17-18 weeks (1978) and shedding of faecal eggs resumed following thiabendazole treatment.

The observations from these experimental studies provide some baseline data on the host responses to cyathostome infection and together with clinical and pathological reports of severe enteritis ensuing from naturally acquired small strongyle infections are evidence that larval cyathostomes are pathogenic in horses. The three main objectives of the work reported in this thesis were: to establish the incidence of larval cyathostomiasis as a cause of diarrhoea in horses; to investigate factors which affect the host-parasite relationship in cyathostome infections with particular reference to those contributing to arrested larval development; to investigate the

pathogenic effects of cyathostome infections in horses.

These objectives were addressed by a multidisciplinary approach.

First, a clinical survey of diarrhoeic horses was completed.

Secondly, parasitological studies of host and larval factors on development of cyathostome infections were undertaken.

Finally, pathophysiological studies of protein and red blood cell metabolism using radioisotope tracer techniques were performed in ponies.

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

PARASITOLOGICAL AND PATHOLOGICAL TECHNIQUES

Faecal Worm Egg Counting

Faecal samples were collected per rectum and stored at $4^{o}C$ until examined, within 24 hours, by a modified McMaster technique (Gordon and Whitlock, 1939). Forty-two millilitres (ml)of water were added to three grams of faeces which were homogenised to form a suspension which was passed through a 250 micron sieve and the filtrate collected into a plastic bowl. A 15 ml sample of the filtrate was taken into a flat bottomed tube and centrifuged at 2000 revolutions per minute for three minutes after which the supernatant which had formed was poured off. The pellet of faeces within the tube was agitated (Vortex Junior Mixer, Scientific Industries Inc., New York) and then resuspended in a saturated solution of sodium chloride. A plastic pipette was used to fill both chambers of a McMaster worm egg counting slide (Gelman Hawksley Ltd., Northampton) with this final suspension. The worm eggs under both grids of the slide were counted by use of a dissecting microscope (Wild M5 stereomicroscope) and the number multiplied by 50 to give the number of eggs per gram (e.p.g.).

Larval Culture

Cyathostome larvae for experimental infections were obtained from naturally infected horses. Freshly voided faeces were collected and placed into plastic pots to occupy two thirds of their 500 ml volume. The pots were covered with loosely fitting

lids and incubated at 23°C for 10-14 days. Recovery of larvae was performed in four stages. First, the culture pots were filled with lukewarm water and left for 2-3 hours. Secondly, the fluid from the pots was passed through a coarse sieve and collected as a pooled sample. Thirdly, the pooled sample was passed through two milk filter pads (Maxa Milk Filters, A. McCaskie Ltd., Stirling) in a Buchner funnel. Finally, the filters containing the larvae, were placed onto a Baermann apparatus which was filled with lukewarm tap water. Larvae migrated out of the filter papers and down into the clamped neck of the funnel from where they were collected after six hours.

The suspension was examined microscopically in order to confirm that only larvae of cyathostome species were present and to calculate the larval concentration. A total of 1 ml of the suspension was examined, in 0.025 ml aliquots, and the number of larvae per ml was then used to calculate the volume required for the individual larval doses. Immediately prior to administration, individual doses were pipetted from the well-mixed larval suspension into glass universal bottles and made up to a standard volume of 20 ml with tap water.

Necropsy and Sampling

Experimental ponies were shot using either a captive bolt or a free bullet from a pistol. Immediately after death the carcases and viscera were examined for gross pathological changes, which were recorded, prior to separation of the gastrointestinal tract. In each animal the stomach, small intestine, caecum, ventral colon, dorsal colon and small colon

were ligated with string at both ends and then separated with a knife. The contents from each opened intestinal segment were washed with water into separate buckets and then made up to standardised volumes as follows: stomach - 5 litres (L); small intestine - 10 L; caecum - 10 L; ventral colon - 30 L; dorsal colon - 20 L; small colon - 10 L. The diluted contents were thoroughly mixed before samples of 10% volume were taken into plastic containers to which formalin was added as a preservative.

The caecum, ventral colon and dorsal colon were weighed separately and samples of 10% by weight were cut as longitudinal strips from the haustra between taenial bands; taking approximately equal amounts from proximal, middle and distal parts of each organ. These tissue samples were examined by a transillumination technique (vide infra) within 24 hours of collection.

Worm Counting

In order to count lumenal worms 5 ml aliquots of the intestinal contents were pipetted into Petri dishes. Each aliquot was stained for a few minutes with a few drops of 45% iodine solution, then decolourised with 5% sodium thiosulphate solution and the worms counted under a dissecting microscope. The contents of the large intestine were extremely thick such that they required to be further diluted with tap water for counting. For each animal, 10% subsamples of the 10% samples of the contents of the separate intestinal segments was examined i.e. the worms in one per cent of the intestinal contents were

counted and the total values obtained by multiplying by a factor of one hundred.

A method of mural transillumination similar to that described by Reinemeyer and Herd (1986a) was used to count mucosal cyathostome larval stages. Mucosa of the 10% samples of caeca, ventral and dorsal colons was manually stripped from the serosa and placed in small pieces into grid-marked Petri dishes. The tissue was examined at x 15 magnification with a dissecting microscope and the mucosal cyathostome larvae within the whole sample were counted.

Worm Identification

About one hundred worms were randomly selected from each sample of intestinal contents and placed into 30% alcohol in universal bottles. Subsequently these worms were mounted in Berlése Fluid on glass microscope slides and identified as far as possible according to the key of Lichtenfels (1975). For this purpose the morphological appearances of the worms were studied by use of a compound light microscope (Leitz) connected to a video camera (Panasonic F15 CCD) from which an image was projected directly onto a high resolution TV monitor (Sony KX 14CDI).

BIOCHEMICAL TECHNIQUES

Serum samples were stored at -20^{o}C and analysed in batches from each experiment. Samples taken for serum iron estimation were collected and stored in iron-free tubes.

Continuous flow analysis (Technicon Auto Analyser) by standard biuret and bromcresol green methods was used for

measurement of total serum protein and serum albumin respectively. The level of serum globulin was calculated as the difference between the values of total protein and albumin.

Electrophoretic separation of the serum proteins was performed in an integrated system (Paragon Electrophoresis System, Beckman Instruments Inc.) on thin-layer, buffered agarose gels (Paragon SPE Kit, PN 655900, Beckman Instruments Inc.) and the protein patterns were quantitated by desitometry (Appraise Junior Densitometer System, Beckman Instruments Inc.)

A commercial test kit (Boehringer-Mannheim) was used to measure serum iron levels.

HAMEATOLOGICAL TECHNIQUES

Haematological analyses were performed on blood samples collected into vacuum tubes containing ethylenediamine tetra-acetic acid. Total white and red blood cell counts were obtained by using an automated Coulter-counter (Coulter ZF6 plus C100 Channelyzer, Coulter Electronics Ltd.). Differential cell counts were performed on blood films which had been stained by the May-Grunwald Giemsa staining technique (Dacie and Lewis, 1975).

PATHOPHYSIOLOGICAL (RADIOISOTOPE) TECHNIQUES

Isotope-Labelled Preparations

^{125}I-Labelled Albumin

The iodine monochloride method of McFarlane (1958) was used to label commercial horse albumin (Fraction V, Sigma Chemical Company Ltd.) with ^{125}Iodine (Amersham International plc). After addition of 'carrier protein' to reduce the specific activity to

less than 0.185 MBq/mg, the solution containing the labelled protein was dialysed for 48 hours against two changes of 20 litres of 0.9% NaCl. Individual doses were accurately weighed and injected intravenously.

^{51} Chromium Chloride ($^{51}CrCl_3$)

An appropriate amount of $^{51}CrCl_3$ (Amersham International plc) was diluted with isotonic saline and then divided into individual doses which were injected intravenously.

^{59}Ferric Citrate (^{59}Fe citrate)

Accurately weighed doses of ^{59}Fe citrate (Amersham International plc) were injected intravenously.

^{51}Chromium Ethylenetetraacetate (^{51}Cr EDTA)

Animals were dosed via stomach tube with accurately weighed doses of ^{51}Cr EDTA (Amersham International plc).

Radioactivity Measurements

Standard Solutions

Standard solutions were made up by adding dilute NaOH to accurately weighed samples of approximately 1 ml of each isotope-labelled preparation to give total volumes of 100 ml. Several 1 ml samples of each standard solution were then pipetted into separate counting vials and weighed before adding 9 ml dilute NaOH to each. The standard solution samples were used to calculate both the injected doses of isotope for each animal and also the crossover factors between preparations by measuring the count rates of each isotope within each specific energy spectrum. In addition the activities within the standard solutions were monitored to detect variations in counting sensitivity of the

gamma counter and they also served as correction factors for radioactive decay.

Blood, Plasma, Faeces and Urine Samples

Whole blood samples were thoroughly mixed prior to pipetting one ml from each into counting vials. The blood samples were then centrifuged at 3,500 revolutions per minute for five minutes and one ml of plasma from each was pipetted into counting vials. Both blood and plasma samples were made up to 10 ml with dilute NaOH.

The total 24-hour urine collections from individual animals were thoroughly mixed in plastic buckets before two samples of 1 ml volume were taken with a syringe into counting vials. Each sample was accurately weighed and then made up to 10 ml with dilute NaOH.

The faeces collected from each animal in 24-hour periods were well mixed in plastic buckets. Five samples of approximately 10 g from each collection were packed into a volume of 10 ml and then accurately weighed.

Radioactivity Counting

Radioactivity counting of blood, plasma, faeces and urine samples was carried out in an automatic gamma counter (Minaxi Auto-Gamma Counter 5000 Series, Canberra-Packard).

Calculation of Results

Packed Cell Volumes, Plasma Volumes and Blood Volumes

(i) Packed Cell Volume (PCV)

Two capillary tubes of whole blood in lithium heparin were

spun at 10,000 revolutions per minute (Hawksley Microhaematocrit Centrifuge) and the PCV measured on a Hawksley microhaematocrit reader. Results were expressed as a percentage of the whole sample volume.

(ii) Plasma Volume (Vp)

Plasma volumes were calculated by use of the dilution principle from the equation:

$$Vp\ (ml) = \frac{\text{total } ^{125}\text{I-activity injected (counts/min)}}{^{125}\text{I-activity of 1 ml plasma at 10 mins post-infection (counts/min)}}$$

and results expressed as ml per kilogram body weight (ml/kg).

(iii) Blood Volume (V_b)

Blood volumes were calculated from the equation:

$$V_{b(ml)} = \frac{Vp\ (ml) \times 100}{PCV\ (\%)}$$

Albumin Metabolism

(i) Intravascular Albumin (CA)

Intravascular albumin pools were calculated from the equation:

$$CA\ (g) = Vp\ (ml/kg) \times \text{serum albumin (mg/ml)}$$

and the results expressed as grams per kilogram body weight (g/kg.bwt).

(ii) Total Body Albumin (TA)

Total body albumin values were calculated by the extrapolation method of Sterling (1951) and/or by the

"equilibrium time" method of Campbell, Cuthbertson, Matthews and McFarlane (1956). In the extrapolation method the TA was obtained by extrapolating the linear part of the plasma activity curve to time zero and noting the intercept value (C_1) which was then used in the equation:

$$\text{TA (g/kg/bwt.)} = \frac{\text{CA (g/kg.bwt.)}}{C_1}$$

The equilibrium time method involved calculation of the plasma activity (Qp), retained activity (Qr) and extravascular activity (Qe). The definitions of Qp, Qr and Qe were as follows: Qp was the activity in plasma samples expressed as a percentage of the ten minute post injection sample; Qr was the activity retained in the body at the end of each 24-hour period obtained by subtracting the daily excreted activity (urine and faeces) from the total injected activity and expressed as a percentage of the injected activity; Qe was the activity within the extravascular compartment which was calculated as the difference between the Qr and Qp values at the end of each 24-hour period.

The maximal values of Qe together with the Qp values from the start of the same collection period (the equilibrium-time) were used to calculate the TA values from the equation:

$$\text{TA (g/kg.bwt.)} = \frac{\text{CA (Qp + Qe)}}{\text{Qp}}$$

(iii) Extravascular Albumin (EA)

Extravascular albumin pools were calculated as the difference between TA and CA, expressed as g/kg.bwt.

(iv) Albumin half-life (T 1/2)

Albumin half-lifes were calculated by analysis of the linear part of the ^{125}I plasma disappearance and expressed in minutes.

(v) Fractional Catabolic Rate - F(CA)

Fractional catabolic rates, defined as the fraction of CA pools broken down during each 24-hour period, were calculated from the equation:

$$F(CA) = \frac{\text{Total activity excreted (urine + faeces) in 24-hour period}}{\text{Total plasma activity at beginning of 24-hour period (counts/min/ml x Vp)}}$$

(vi) Absolute Albumin Catabolism

The absolute amounts of albumin degraded during each 24-hour period were calculated as the product of mean F(CA) and CA, expressed as g/kg.bwt./day.

Faecal Clearance of Plasma and Blood

Faecal clearances of plasma or blood are defined as the amounts of plasma or blood which would have to leak into the gastrointestinal tract in order to account for the faecal radioactivities present.

Two separate estimations of faecal plasma clearances were made dividing the separate total ^{125}I and ^{51}Cr activities in the faeces passed in each 24-hour period by plasma activities (counts/ml) of those isotopes at the beginning of each period.

Faecal clearances of blood were obtained by dividing the 24-hour faecal activities of ^{59}Fe by the activity of ^{59}Fe in blood at the beginning of each 24-hour period.

Red Blood Cell Metabolism

(i) ^{59}Fe Plasma half-life (T 1/2)

The plasma half-life of ^{59}Fe was calculated by analysis of the plasma activity curve.

(ii) Plasma Iron Turnover Rate (PITR)

Plasma iron turnover rate, defined as the amount or iron (complexed with transferrin) passing through the plasma per unit time, was calculated from the equation:

$$\text{PITR (mg/kg.bwt./day)} = \frac{\text{serum iron (mg/ml)} \times 0.693 \times 1440 \times Vp}{^{59}Fe \text{ T 1/2 (min)}}$$

(iii) Percentage Iron Utilisation

The percentage iron utilisation by red blood cells (r.b.c.) were calculated from the equation:

$$\text{\% Iron utilisation} = \frac{100 \times \text{r.b.c. volume} \times \text{max.}\ ^{59}Fe \text{ activity (counts/min/ml r.b.c.'s)}}{\text{total injected activity}}$$

(iv) Red Cell Iron Incorporation Rate (RCIIR)

The red cell iron incorporation rate was calculated as the product of percentage iron utilisation and PITR, expressed as mg/kg.bwt./day.

Intestinal Permeability

The percentages of administered ^{51}Cr-EDTA recovered in urine and faeces collected during 24-hour periods were calculated by dividing the ^{51}Cr activities within the daily collections by the total administered activity, expressed as percentages.

STATISTICAL METHODS

Statistical Analysis

In Experiment 3 the results were statistically tested by one-way analysis of variance and then differences at a 5% significance level were investigated by use of the Newman-Keuls Range Test.

The Mann-Witney test was used to analyse the results in Experiments 4, 5 and 6 and probability values of $P < 0.05$ were considered significant.

CHAPTER ONE

STUDIES ON NATURALLY OCCURRING LARVAL CYATHOSTOMIASIS

CHAPTER ONE

STUDIES ON NATURALLY OCCURRING LARVAL CYATHOSTOMIASIS

INTRODUCTION

Chronic diarrhoea of adult horses presents considerable diagnostic and therapeutic difficulties to equine clinicians. Although a diverse range of conditions including large strongyle larval migration (Greatorex, 1975; Merritt, Bolton and Cimprich, 1975), granulomatous enteritis, alimentary neoplasia, salmonellosis, chronic liver disease (Merritt and others, 1975), peritonitis (Dyson, 1983; Mair, Hillyer and Taylor, 1990) and sand enteropathy (Scrutchfield, 1987) have been recorded as causes of chronic diarrhoea, it is often difficult to reach a specific diagnosis in such cases and frequently treatment is, at best, symptomatic.

The syndrome of larval cyathostomiasis typically has an acute onset of diarrhoea but cases often progress to chronic diarrhoea, emaciation and eventually death. During the last twenty years larval cyathostomiasis has been increasingly recognised as a cause of chronic diarrhoea (Blackwell, 1973; Chiejina and Mason, 1977; Jeggo and Sewell, 1977; Mirck, 1977; Jasko and Roth 1984; Giles and others, 1985; Church and others, 1986; Harmon, Ruoff and Huey, 1986) but there are few published reports on the incidence of this condition. In a recent survey of diarrhoea encountered in 66 adult horses in general practice 15% of the cases were diagnosed as parasitic colitis, in particular larval cyathostomiasis (Mair and others, 1990).

The objectives of this study were to determine the incidence of larval cyathostomiasis in horses which were referred with diarrhoea to the Department of Veterinary Medicine, University of Glasgow Veterinary School and to conduct detailed investigations on these animals with a view to identifying any differentiating features of diarrhoea caused by cyathostome infection.

MATERIALS AND METHODS

When cases referred by general practitioners were admitted to the clinic, detailed histories of the animals were obtained from the owners and their veterinary surgeons. All animals were given full clinical examinations on admission and then at least daily thereafter and from these complete clinical records were compiled.

In all cases blood samples were submitted for haematological and biochemical analysis by routine methods in the diagnostic laboratories of the veterinary school.

On one or several occasions faecal samples from each case were cultured under aerobic conditions at 37^{o}C on sheep blood agar and MacConkey agar and also microaerophilically at 37^{o}C on a campylobacter selection medium. In each case tetrathionate broth was inoculated with faeces which were then subcultured onto salmonella-shigella agar and deoxycholate citrate agar plates.

Faeces from all animals were examined for worm eggs by a modified McMaster technique and, by scanning samples diluted in water with magnification (x 12), for any cyathostome larvae which might be present.

When indicated on clinical grounds additional diagnostic procedures such as oral glucose tolerance tests (by the method of Roberts and Hill, 1973), rectal biopsy (as described by Traver and Thacker, 1978) or abdominocentesis (using the technique of Ricketts, 1983) were performed.

Animals were given treatment as considered appropriate and these most commonly included oral ivermectin, 0.2 mg/kg, oral codeine phosphate, 1-3 mg/kg daily, oral prednisolone, 1 mg/kg daily and intravenous fluid therapy. The cases were either discharged following clinical recovery or they were destroyed on humane grounds, poor prognosis or at the owner's request. Post mortem examinations were performed in the Department of Veterinary Pathology.

RESULTS

During a three-year period, equine medical cases referred to the clinic included 16 adult animals which were hospitalised for investigation and treatment of diarrhoea. Most of the diarrhoeic cases were pony breeds or types with an age range from 2 to 36 years. The minimum duration of signs of diarrhoea was one week and some persisted for several months. The breed/type, age, sex, clinical findings, laboratory results, diagnosis and outcome of these cases are summarised in Tables 1.1, 1.2 and 1.3.

The most common diagnoses were larval cyathostomiasis, alimentary lymphosarcoma and chronic colitis of unknown aetiology of which there were three cases each. Intralumenal obstruction of the colon was found in two cases. The two undiagnosed cases

Table 1.1 - Clinical Features of 16 Adult Horses with Diarrhoea

Case no.	Breed	Age (yrs)	Sex	Duration	Faeces	Month of Onset	Wt. Loss	Colic	Other	Codeine Respon-sive	Outcome	Diagnosis
1	Highland	2	M	2 wks	Watery, Sloppy, Normal	March	+++	-	Preputial Oedema	ND	Lived	Cyathostomiasis
2	Riding Pony	6	MN	1 wk	Watery, Sloppy, Normal	March	++	Mild, Inter-mittent	Anorexia	Yes	Lived	Cyathostomiasis
3	Riding Horse	15	MN	6 wks	Sloppy, Normal	Feb.	+++	-	-	ND	Euth.	Cyathostomiasis
4	Riding Pony	13	MN	4 mnths	Watery	April	+++	-	Preputial Oedema	Partial	Euth.	Alimentary Lymphosarcoma
5	Riding Pony	15	F	3.5 mnths	Sloppy	April	+	-	-	No	Euth.	Alimentary Lymphosarcoma
6	Riding Pony	15	F	9 wks	Sloppy, Watery	Dec.	++	-	-	No	Euth.	Alimentary Lymphosarcoma
7	Riding Pony	20	F	2 wks	Watery	Sept.	+	-	Laminitis Arthritis	No	Euth.	Chronic Colitis
8	Riding Pony	8	F	5 wks	Watery	Nov.	+++	-	-	No	Euth.	Chronic Colitis
9	Riding Pony	12	F	5 wks	Sloppy, Watery	June	+	Mild Inter-mittent	Dullness Anorexia	Partial	Euth.	Chronic Colitis
10	Riding Pony	20	F	6 mnth	Sloppy	May	-	-	-	Yes	Euth.	Undiagnosed
11	Riding Pony	36	MN	4 wks	Watery	Oct.	+	-	-	ND	Died	Sand Impaction Ventral Colon
12	Riding Horse	27	MN	1 wk	Sloppy, Low Volume	Oct.	+	Chronic Low Grade	'Mass' Palpable Per Rectum	ND	Lived	Impaction Right Dorsal Colon at Laparotomy
13	Irish Draught	4	MN	9 wks	Watery	Nov.	+	-	-	Yes	Lived	Undiagnosed

Table 1.1 (Cont'd)

Case no.	Breed	Age (yrs)	Sex	Duration	Faeces	Month of Onset	Wt. Loss	Colic	Other	Codeine Respon-sive	Outcome	Diagnosis
14	Shetland	7	F	2 wks	Sloppy	April	+	-	Pregnant Anorexia Dullness	ND	Euth.	Hyperlipaemia
15	TB X	27	MN	4 wks	Watery	Sept.	-	-	Polydypsia Oral Ulcers Distended Abdomen	ND	Euth.	Cushing's Syndrome
16	Riding Pony	15	F	2 wks	Sloppy	March	+	Chronic, Moderate	Pregnant Dullness Anorexia	ND	Euth.	Hyperlipaemia

Note:

M	-	Male	+	mild
MN	-	Male-neutered	++	moderate
F	-	Female	+++	marked
TBX	-	Thoroughbred cross		
ND	-	Not Done		
Euth.	-	Euthanased		

Table 1.2 - Laboratory Findings in 16 Adult Horses with Diarrhoea

Case no.	Breed	Age (yrs)	Sex	↑AP	↓Alb	↓OGTT	↑Neut	FWEC	Rectal Biopsy	Other	Outcome	Diagnosis
1	Highland	2	M	-	✓	ND	✓	N	ND	Hyperglobulinaemia Anaemia	Lived	Cyathostomiasis
2	Riding Pony	6	MN	✓	✓	ND	✓	N	ND	Hypochloraemia Hyponatraemia Hypokalaemia	Lived	Cyathostomiasis
3	Riding Horse	15	MN	✓	✓	Partial	✓	N	NAD	Hyperglobulinaemia Basophilia	Euth.	Cyathostomiasis
4	Riding Pony	13	MN	-	✓		✓	100 st	Enter-opathy	Hypoalbuminaemia	Euth.	Alimentary Lymphosarcoma
5	Riding Pony	15	F	✓	✓	-	✓	N	NAD	Hyperglobulinaemia Raised GT	Euth.	Alimentary Lymphosarcoma
6	Riding Pony	15	F	-	✓	Partial	✓	N	NAD	Anaemia	Euth.	Alimentary Lymphosarcoma
7	Riding Pony	20	F	-	-	-	-	N	ND	-	Euth.	Chronic Colitis
8	Riding Pony	8	F	-	✓	ND	✓	N	NAD	Hypochloraemia Hypokalaemia	Euth.	Chronic Colitis
9	Riding Pony	12	F	✓	✓	ND	✓	1250 st	Chronic Enteritis	Raised GT and SDH	Euth.	Chronic Colitis
10	Riding Pony	20	F	✓	-	-	-	N	NAD	-	Euth.	Undiagnosed
11	Riding Pony	36	MN	✓	-	ND	-	N	ND	-	Died	Sand Impaction Ventral Colon
12	Riding Horse	27	MN	-	-	-	-	N	ND	-	Lived	Impaction of Right Dorsal Colon at Laparotomy
13	Irish Draught	4	MN	✓	✓	-	-	N	Chronic Enter-itis	Hyperglobulinaemia	Lived	Undiagnosed

Table 1.2 (Cont'd)

Case no.	Breed	Age (yrs)	Sex	↑AP	↓Alb	↑OGTT	↑Neut	FWEC	Rectal Biopsy	Other	Outcome	Diagnosis
14	Shetland	7	F	✓	-	ND	-	N	ND	-	Euth.	Hyperlipaemia
15	TB X	27	MN	-	-	ND	✓	N	ND	Lymphopaenia Hyperglycaemia Glucosuria Raised GT	Euth.	Cushing's Syndrome
16	Riding Pony	15	F	✓	-	ND	✓	200 st	ND	Raised: SDH, GT Cholesterol Triglyceride	Euth.	Hyperlipaemia

Note:

AP - Alkaline Phosphatase
Alb - Albumin
OGTT - Oral Glucose Tolerance Test
Neut - Neutrophil
FWEC - Faecal Worm Egg Count
GT - Gamma glutamyltransferase
M - Male
MN - Male-Neutered
F - Female
Euth. - Euthanased
ND - Not Done
SDH - Sorbitol Dehydrogenase
N - Negative
St - Strongyle worm eggs
TBX - Thoroughbred cross
yrs - years

✓ - present
\- - absent

Table 1.3

Diagnosis and Outcome of 16 Cases of Diarrhoea of Adult Horses

Diagnosis	No. of Cases	Outcome		Diagnosis	
		Survived	Euthanasia/ Died	Pre mortem	Post mortem
Larval Cyathostomiasis	3	2	1	2	1
Alimentary Lymphosarcoma	3	0	3	0	3
Chronic Colitis	3	0	3	0	3
Colonic Impaction					
- Sand	1	0	1	1	0
- Ingesta	1	1	0	1	0
Hyperlipaemia	2	0	2	2	0
Pituitary Adenoma	1	0	1	1	0
Undiagnosed	2	1	1	-	-
Totals	16	4	12	7	7

were thought to be of alimentary origin and in both these animals the diarrhoea could be controlled with daily oral codeine phosphate at a dose of 1 mg/kg body weight. However, diarrhoea recurred in both if treatment was stopped and the owner of one of these animals (Case 10) requested that it be euthanased. Three of the animals which were presented to the clinic as cases of diarrhoea were found to have primary diseases not associated with the alimentary tract: two were ponies with hyperlipaemia and one horse was diagnosed as a case of Cushing's disease.

Of the four animals which were successfully treated it was possible to make a definitive diagnosis in three: two were larval cyathostomiasis (Case Nos. 1 and 2) and one was an impaction of the right dorsal colon (Case No. 12). A specific diagnosis was made in life for seven animals which included all of the cases with non-alimentary diseases. At the time of euthanasia eight animals were undiagnosed but in all but one of these cases it was possible to reach a specific diagnosis post mortem.

The most common abnormalities detected in blood samples were hypoalbuminaemia, neutrophilia and raised serum alkaline phosphatase. There were no enteric pathogens cultured from any animal and only three animals had positive faecal strongyle egg counts. Rectal biopsies were performed in eight animals and histopathological abnormalities were found in two (Case Nos. 4 and 13).

Larval Cyathostomiasis Case Reports

The following reports of the three cases of larval cyathostomiasis include accounts of the history, clinical findings, abnormal laboratory findings, case management and progression, and, in one case, pathological findings. The sampling of these animals for laboratory investigations was performed on the basis of clinical indications rather than a standardised pre-arranged protocol. Full details of all laboratory data of the three cases is given in Appendices 1.1, 1.2 and 1.3 together with normal value ranges for the laboratory in which the samples were analysed.

Case 1

2 year-old unbroken Highland Pony colt

History

This pony had been recently purchased with a group of 12 similar ponies and they were kept on rough, hill pasture. They received supplementary feeding of hay and turnips and all of the animals had been dosed with fenbendazole in early March.

About one week after the anthelmintic treatment the owner noticed that the pony had lost a lot of weight and it was stabled for observation. The pony was bright and had a good appetite for hay, oats and sugar beet pulp but was noticed to have severe diarrhoea. No treatment was given at this time but referral for investigation and treatment was arranged when the diarrhoea had persisted for one week.

Clinical Findings

The pony was bright, alert, responsive, had a good appetite but it was in an emaciated condition and the prepuce was swollen and oedematous (Figure 1.1). The rectal temperature was 37.5°C. The oral and conjunctival mucous membranes were of normal colour and well perfused. The heart rate was 48 beats per minute and a soft, Grade I systolic murmur was detected.

In all four abdominal quadrants loud, watery borborygmi were heard which were more frequent than normal. On rectal examination the colonic and caecal contents were of very fluid consistency. Following rectal examination huge numbers of 5-7 mm long, bright red small strongyle larvae were noticed on the rectal glove and also in the faeces which were watery.

Laboratory Findings

The serum albumin level was extremely low (9 g/l), the alkaline phosphatase level was slightly raised (440 iu/l) and the neutrophil count was raised (9.83×10^9/l). On analysis of serum proteins by electrophoresis there was evidence of an increase in the betaglobulin fraction. There were no other blood biochemical or haematological abnormalities. Faecal samples were negative for worm eggs and bacterial enteropathogens.

Clinical Diagnosis

Larval cyathostomiasis.

Clinical Management and Progression

The animal was treated with oral ivermectin (0.2 mg/kg) on Days 1, 8, 15 and 38 of hospitalisation. Cyathostome larvae were passed in the faeces in large numbers for two days after the

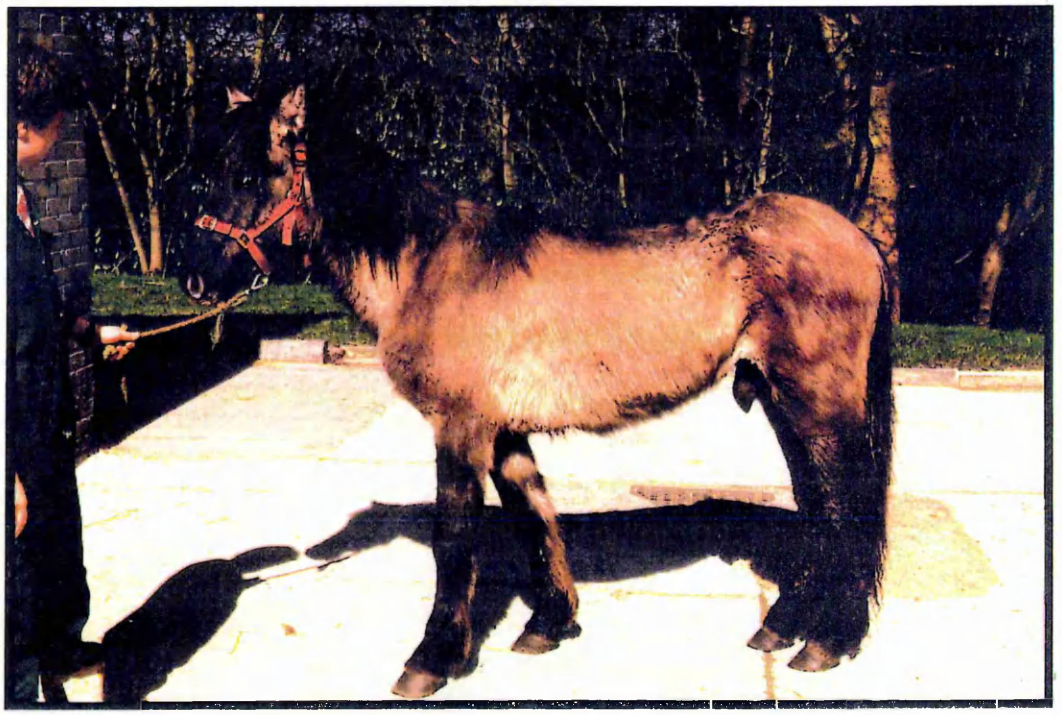

Figure 1.1

Case 1, 2 year-old Highland Pony colt, at admission. The animal was emaciated, preputial oedema was present and diarrhoeic faeces were coated to the tail and hind legs

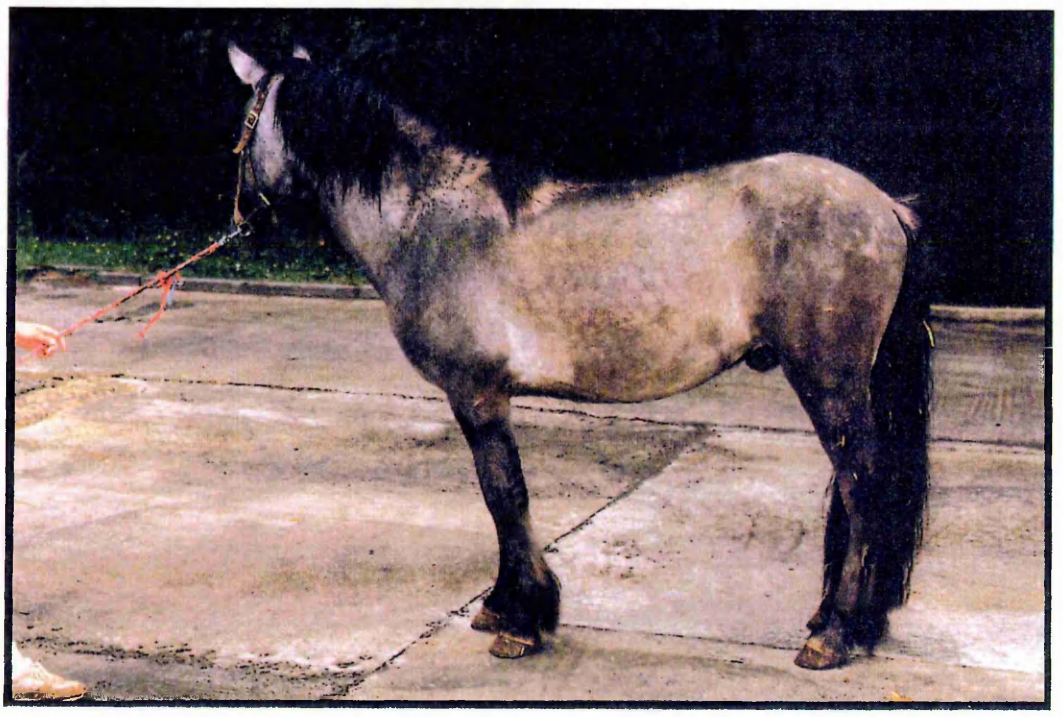

Figure 1.2

Case 1, 2 year-old Highland Pony colt, 77 days after hospitalisation. The animal gained 94 kg in body weight, the preputial oedema reduced and faeces were of normal consistency

first treatment and were detected in smaller numbers for a further week. The nature of the diarrhoea changed from watery to cowpat consistency within the first three days of hospitalisation and then gradually became normal over the following fortnight. The preputial oedema began to slowly reduce two weeks after admission. The pony was very bright in demeanour, it had a ravenous appetite and it gained 94 kg in bodyweight in 77 days (Figure 1.2).

On sequential blood samples the neutrophilia quickly resolved but the pony became slightly anaemic three weeks after it was hospitalised. The serum albumin levels were still moderately low at 21 g/l (normal 25-35 g/l) and the hyperbetaglobulinaemia, although less marked, was still evident by electrophoresis at time of discharge, 11 weeks after first treatment.

Case 2

6 year-old riding pony gelding

History

This pony was kept at a large livery stable and apart from having 'strangles' eighteen months earlier it was not known to have been affected by any previous illness. The pony grazed for part of each day in a four acre paddock with seven other horses. All of the animals at the stable were dosed five times per year with fenbendazole and once in the autumn with oral ivermectin. The last anthelmintic dose given to Case 2 had been six weeks prior to the onset of illness.

In mid-March the pony was noticed to be dull and anorexic for three days prior to developing mild colic. Soon after the onset of abdominal pain the pony developed severe watery, blood-stained diarrhoea. The pony was afebrile but the owner's veterinary surgeon treated it with a broad spectrum antibiotic together with an intestinal spasmolytic compound for two days; this resulted in some improvement in demeanour and appetite although the diarrhoea and low grade colic persisted. This treatment was continued for a further three days but the animal's condition deteriorated and it was referred to the Department of Veterinary Medicine at Glasgow for further investigation and treatment, nine days after it was first noticed to be ill.

Clinical Findings

The pony was thin, dull and weak. The rectal temperature was 37^{o}C, the oral mucous membranes were congested, the heart rate was 56 beats per minute and the pulse quality was poor.

Very loud, fluid borborygmi were heard in all abdominal quadrants and these were more frequent than normal. On rectal examination the contents of the large intestine were found to be very fluid in consistency and cyathostome larvae were grossly visible in faeces.

Laboratory Findings

The animal was found to be haemoconcentrated (PCV - 50%), hypochloraemic (90 mmol/l), hyponatraemic (124 mmol/l), hypokalaemic (2.1 mmol/l), hypoproteinaemic (55 g/l), and the level of serum alkaline phosphatase was raised (1170 iu/l).

Faecal samples were negative for helminth eggs and no enteric bacterial pathogens were cultured.

Clinical Diagnosis

Larval Cyathostomiasis.

Clinical Management and Progression

The animal was treated with intravenous Hartmann's solution to which potassium chloride was added. The plasma electrolyte profile was closely monitored to assess the status of the pony and the fluid therapy regimen was adapted as necessary. The animal was given flunixin meglumine at 0.25 mg/kg body weight four times daily for 48 hours. Oral ivermectin was given on the day of admission at a dose rate of 0.2 mg/kg.bwt. and codeine phosphate was given orally at 1 mg/kg.bwt for three days and then the dose rate was gradually reduced over the next week.

Within 72 hours of being admitted to the clinic the pony was brighter in demeanour and had developed reasonable appetite. The vital signs were all within normal limits, the intestinal borborygmi were of normal character and semi-formed faeces were passed in small quantities. The animal continued to intermittently show signs of dull, low-grade abdominal pain for three further days. The intravenous fluid therapy was discontinued after one week. The pony continued to improve and had made a complete clinical recovery one month after it was first noticed to be ill.

During the period of hospitalisation sequential blood and faecal samples were analysed. The animal had been stabilised by Day 8 when the abnormal laboratory findings were neutrophilia

(7.74×10^9), hypoalbuminaemia (22 g/l), and raised serum alkaline phosphatase (1200 iu/l). These variables progressed to normal levels more slowly than the clinical improvement. No bacterial enteropathogens were cultured and after the second day of treatment no further cyathostome larvae were found in faeces.

Case 3

15 year-old riding horse gelding

History

The horse had been in the owner's possession for four years during which time it had no illnesses. The animal was kept at grass where it received supplementary feeding with hay and oats. The grazing, which was shared with several other horses, had previously been used for cattle rearing and had only been grazed by horses during the previous year. All of the horses were treated with anthelmintic (ivermectin or fenbendazole) three or four times annually.

During February the owner noticed that the animal suddenly showed marked weight loss which was progressive despite housing the horse and increasing the level of feeding. When the owner's veterinary surgeon examined the animal at this time, there were no specific clinical findings and he rasped the cheek teeth of the horse and dosed it with oral ivermectin. Two weeks following this treatment the horse became anorexic and diarrhoeic with faeces of cowpat consistency for four days. After this the horse had a ravenous appetite and the faeces were only intermittently soft.

The horse was referred to the Department of Veterinary Medicine two months after it was first noticed to be ill.

Clinical Findings

The horse was bright, responsive and appetant. It had an unkempt appearance with a shaggy haircoat and was in very poor body condition. The rectal temperature was 37.5^{o}C and the oral and conjunctival mucous membranes had normal colour and perfusion. The heart rate was 36 beats per minute and a grade II systolic murmur was detected, which was loudest over the mitral valve.

The mandible was undershot and there was uneven wear of the central incisors but the cheek teeth were normal. On abdominal auscultation normal borborygmi were heard in all quadrants. There were no abnormal findings on examination of the abdomen per rectum and the faeces were only slightly soft.

No abnormalities were detected on examination of respiratory lymphatic, urogenital, musculoskeletal or neurological systems.

Laboratory Findings

Abnormalities were present in haematological and blood biochemical profiles, namely neutrophilia (11.47 x 10^{9}/l), basophilia (0.67 x 10^{9}/l), hypoalbuminaemia (23 g/l), raised plasma levels of alkaline phosphatase (580 iu/l) and hyperglobulinaemia (49 g/l), which was shown to be due to an increase in the betaglobulin fraction by serum electrophoresis. Glucose tolerance was reduced: following oral administration of 1.0 g/kg of 20% glucose solution plasma levels were 146% of

resting level at two hours (normal 200%).

Faeces were negative for worm eggs and larvae and no enteric bacterial pathogens were cultured. Peritoneal fluid had a normal cellular content and it was sterile on culture. The microscopic appearance of a rectal biopsy was reported to be essentially normal, containing some globule leucocytes in the epithelium and plasma cells in the lamina propria.

Clinical Diagnosis

A malabsorption syndrome and protein losing enteropathy of undefined origin.

Clinical Management and Progression

The animal remained bright during the succeeding three week period of hospitalisation and diarrhoea was not observed after the second day although the faeces were occasionally slightly soft. There were no additional clinical findings on daily examinations. A dose of ivermectin at 0.2 mg/kg was given by mouth on the fifth day of hospitalisation. Despite consuming 7 kg of proprietary, high-protein concentrate and about 8 kg of good quality hay daily the horse gained only 4 kg in bodyweight. Serum albumin levels were decreased (16-18 g/l) on samples taken at two and three weeks after admission. On the basis of malabsorption and a progressive hypoalbuminaemia the prognosis was considered to be poor and although no definitive diagnosis was reached the animal was destroyed.

Pathological Findings

Gross

The only abnormal findings at necropsy were in the large intestine: the caecum and the ventral colon were grossly thickened due to mucosal oedema. Large numbers (20-30 per cm^2) of mucosal cyathostome larvae were present throughout the caecum and ventral colon and the mucosal surface had a gritty, rough texture with irregular reddening around the parasitic nodules (Figure 1.3). There was no evidence of mucosal ulceration and the parasites were considered to be immature.

Microscopic

In sections from various levels of the caecum and ventral colon there was mucosal oedema and a marked cellular infiltration of lymphocytes and plasma cells. Many larvae were seen within the mucosa and some of the larger parasites were surrounded by a granulomatous reaction (Figure 1.4).

DISCUSSION

In this study, larval cyathostomiasis was diagnosed in 19% of adult horses referred to the clinic with diarrhoea: this was similar to the incidence of this condition in a survey of diarrhoea in horses in general practice (Mair and others, 1990). From both observations it would appear that larval cyathostomiasis is a common cause of equine diarrhoea.

In the three cases of larval cyathostomiasis in this study there was a range of severity of the disease but they had common clinical features of sudden onset, chronic diarrhoea in late

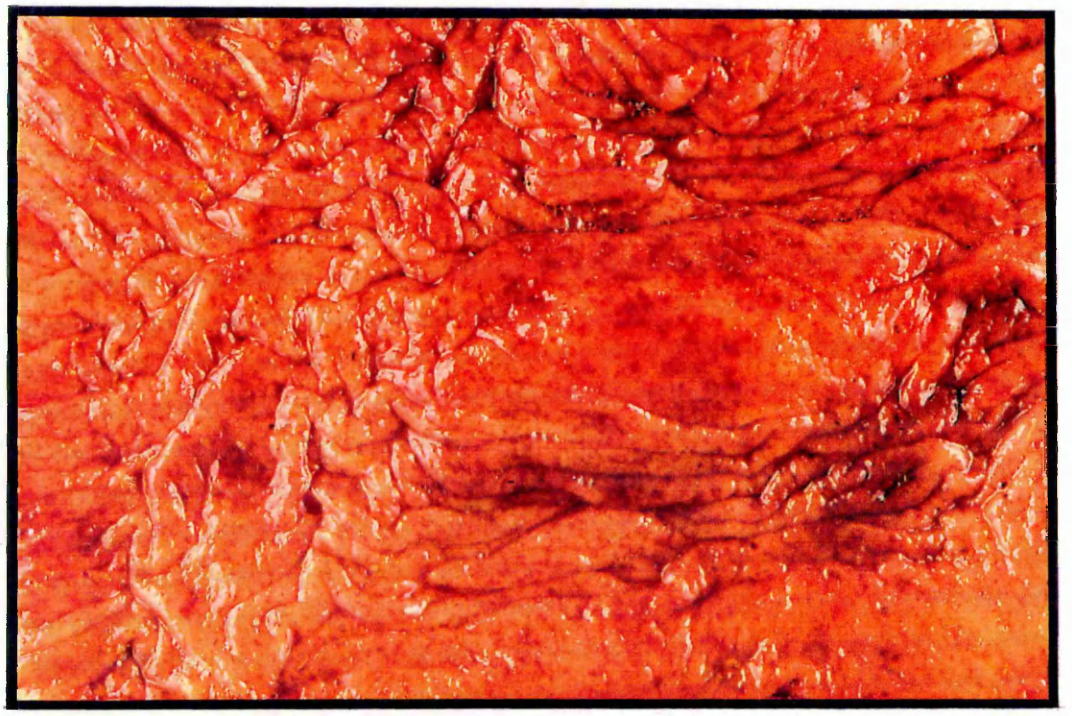

Figure 1.3

Case 3, gross appearance of the caecum. There is widespread, irregular areas of reddening around mucosal cyathostomes

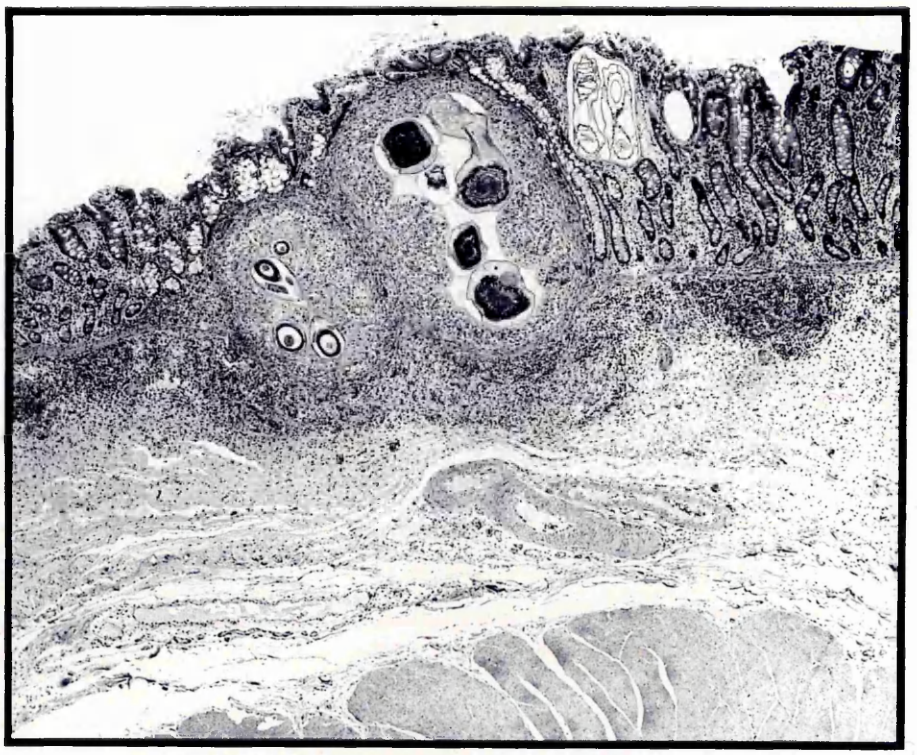

Figure 1.4

Case 3, Photomicrograph of the caecal wall. There is a severe reaction within the lamina propria with oedema and congestion accompanied by a diffuse infiltrate of lymphocytes and plasma cells. The cellular reaction is most marked around larvae within the mucosa and is also present in adjacent submucosa (Haematoxylin and Eosin x 90)

winter associated with marked, rapid weight loss. These signs were first described in detail by Velichkin (1952) and together with the seasonality of the condition they have been considered by subsequent authors to be typical, and therefore they are useful, distinguishing features (Blackwell, 1973; Chiejina and Mason, 1977; Mirck, 1977; Jasko and Roth, 1984; Giles and others, 1985; Church and others, 1986). Although most reported cases have been in young animals the cases in this study were two, six and 16 years old, which indicates that larval cyathostomiasis should be considered in the differential diagnosis of diarrhoea in animals of all ages.

Mair and others (1990) reported that blood analyses of PCV, total white blood cell count, neutrophil count, serum albumin and serum alkaline phosphatase (AP) were of prognostic value to the outcome of their cases (which included animals with acute or chronic diarrhoea) although they considered that these haematological and biochemical tests did not provide any precise diagnostic information. In the present study there was a similar lack of specifity of laboratory investigations with the exception of pathognomonic biochemical findings in the three cases which were diarrhoeic as a result of non-alimentary diseases i.e. the two ponies with hyperlipaemia and the horse with Cushing's Syndrome. In the other animals hypoalbuminaemia, neutrophilia and raised serum alkaline phosphatase (AP) were commonly detected but neither appeared to relate to particular types of disease nor correlate with the outcome.

In Cases 1 and 2, serum albumin levels progressively increased during clinical recovery i.e. the hypoalbuminaemia was reversible. Similar observations have been made in cyathostomiasis cases by previous authors (Jeggo and Sewell, 1977; Giles and others, 1985; Church and others, 1986). In the three cases of larval cyathostomiasis reported here there was evidence of raised betaglobulins on serum protein electrophoresis. In Case No. 1 the serum protein profile returned towards normal during treatment but at a slower rate than the clinical improvement. Giles and others (1985) considered that such changes in serum protein chemistry were characteristic of cyathostomiasis. However in the present case series hyperbetaglobulinaemia was also detected in animals with other diseases and so was not of particular diagnostic significance.

In both Cases 1 and 2 clinical recovery occurred more quickly than the improvement in blood profiles which was also observed in the single case reported by Jeggo and Sewell (1977). Giles and others (1985) reported that a circulating eosinophilia was definitely not a feature of their series of cyathostomiasis cases which was also the finding in this study.

Examination of faeces for parasitic eggs was not useful in differential diagnosis and, similarly, bacteriological examinations of faeces were not helpful, although it has been recommended that at least five sequential faecal cultures should be performed for Salmonella spp since they are inconsistently shed (Palmer and Benson, 1984) and this was not performed in

every case. However, faecal shedding of Salmonella spp may occur in clinically normal horses or as a result of another primary alimentary tract disease, including larval cyathostomiasis (Giles and others, 1985), and so their presence would not necessarily be diagnostic. The actual significance of most bacterial isolates from equine faeces is unknown. Al-Mashat and Taylor (1986) identified potential bacterial enteropathogens from the intestinal tract of horses with signs of gastrointestinal diseases and similar post mortem studies in the animals in this study may have yielded information on the pathogenesis of, for example, the chronic colitis in Cases 4, 5 and 6.

Of the two cases of larval cyathostomiasis which were successfully treated in this study, Case 1 was managed very simply with repeated anthelmintic therapy and nutritional support whereas Case 2 received more intensive care due to the presence of fluid and electrolyte imbalances. Conceivably earlier diagnosis and initiation of anthelmintic treatment of Case 2 may have reduced the severity of the clinical effects and therefore the need for aggressive therapy may have been avoided. Giles and others (1985) observed that the horses in their series which responded to treatment were those which were given anthelmintic within three weeks of the onset of diarrhoea. The number of treated cases of cyathostomiasis in the literature is small, such that it is not possible to judge one particular treatment protocol to be more effective than any other.

Although larval cyathostomiasis may be considered to have characteristic presentation and clinical signs the condition may present a diagnostic challenge, especially in chronic stages when differentiation from other enteropathies may be difficult. In Case 3 no specific diagnosis was made in life but at post mortem examination the findings were considered typical of larval cyathostomiasis. Church and others (1986) carried out intestinal biopsy, under general anaesthesia, and they considered that of two horses with chronic diarrhoea and wasting, the presence of immature small strongyles within the mucosa of the caecum and colon were confirmation of a diagnosis of larval cyathostomiasis. These two animals were successfully treated by Church and her co-workers (1986) which illustrates merit in exhaustive clinical investigations in order to obtain definitive diagnoses even in cases of chronic protein-losing enteropathy.

In this study, Case 1 first showed clinical signs shortly after treatment with an anthelmintic and the diarrhoea in Case 3 was first noticed after dosing with anthelmintic although it had previously lost a lot of weight. It could be speculated that these treatments initiated emergence of mucosal cyathostomes - possibly by removal of an inhibitory feedbeck effect of lumenal worms, a hypothesis first proposed by Gibson (1953). The parasite control programmes which were used on the farms of origin of Cases 2 and 3 failed to prevent these animals accumulating pathogenic cyathostome burdens although it is not possible to identify the reasons for this from the information available.

The pathological findings in Case 3 were of chronic typhlitis and colitis which were confirmed microscopically to be an inflammatory response to mucosal cyathostomes. Accounts of the pathology of cyathostomiasis in the literature describe a varying severity of caecal and colonic hyperaemia, haemorrhage, congestion, ulceration or necrosis (Velichkin, 1952; Tiunov, 1953; Blackwell, 1973; Chiejina and Mason, 1977; Jasko and Roth, 1984; Giles and others, 1985; Harmon and others, 1986; Reinemeyer and Powell, 1986) in addition to the presence of parasitic nodules or cysts; in general the severity of the gross pathology was inversely related to the chronicity of the cases. The findings in Case 3 were typical of those described in animals with protracted cyathostomiasis. Interestingly, mucosal ulceration was not a feature of Case 3 which would infer that there were functional rather than simply physical mechanisms of protein leak and diarrhoea. Absence of gross or histological ulceration in hypoalbuminaemic animals with cyathostomiasis has been observed previously (Giles and others, 1985; Church and others, 1986).

The post mortem finding of large numbers of viable cyathostome larvae in Case 3 which had received two oral doses of ivermectin and which had no opportunity for reinfection support the view that the large intestinal mucosa can provide refuge from anthelmintic action as has been observed in experimental (Gibson, 1953; Smith, 1976a) and clinical studies (Chiejina and Mason, 1977; Jasko and Roth, 1984; Giles and others, 1985; Church and

others, 1986; Harmon and others, 1986).

In conclusion, larval cyathostomiasis was a common cause of diarrhoea within a group of cases referred for investigation of diarrhoea. The best differentiating feature of this disease was the clinical finding of cyathostome larvae in faeces or on rectal mucosa. Supportive evidence of this diagnosis could be gathered from the season of occurrence, a history of sudden onset of signs which became chronic and an associated marked, progressive weight loss. In general laboratory data were not useful in the differential diagnosis of diarrhoea in adult horses in this study.

CHAPTER TWO

STUDIES ON NATURAL AND EXPERIMENTAL CYATHOSTOME INFECTIONS IN PONIES

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STUDIES ON NATURAL AND EXPERIMENTAL CYATHOSTOME INFECTIONS IN PONIES

INTRODUCTION

The results of the few detailed post-mortem surveys of the naturally occurring gastrointestinal cyathostome burdens of horses indicated that virtually all grazing horses were infected although great variability in adult small strongyle burdens was observed. In southwest England, Ogbourne (1976) found between 12,000 and 1,239,000 worms per horse; in Ohio, U.S.A., Reinemeyer and others (1984) recorded a range of 680 - 663,100 worms per horse; and in a smaller study in South Africa counts of between 415 and 190,316 per horse were obtained (Krecceck, Reinecke and Horak, 1989). Seasonal trends in the age structure of small strongyle burdens as recognised by Ogbourne (1976) and Reinemeyer and others (1986) may in part explain the wide range of burdens although it seems probable that host, management and indeed parasite factors may also contribute to the wide variation in worm burdens seen in individual animals.

Although increasing host age was associated with lower cyathostome burdens in early reports (Foster, 1937; Ward, 1948) there has been little data in subsequent studies to support this finding. The majority of reports of clinical cyathostomiasis describe disease in young adult animals but it is not clear whether older animals are less commonly affected due to resistance to acquiring large cyathostome burdens or because they

are less susceptible to the effects of heavy infection.

Since most animals are repeatedly exposed to cyathostome infection throughout their lives it is very difficult to investigate independently any effect of age of the host on the development of small strongyle infection. In the experimental studies conducted by Smith (1976a, 1976b) there were longer prepatent periods and lower egg counts when ponies were infected with 300,000 cyathostome larvae at seven to eight years of age than when they had been given a similar infection three years earlier. This was interpreted as a degree of resistance to reinfection but whether this affected the proportion of the larval dose which established in the ponies was not known since the animals were not necropsied.

Gibson (1953) made the initial observation that there was repopulation of the gut lumen with immature cyathostomes from the intestinal mucosa following treatments with phenothiazine and he suggested that the intralumenal worms were exhibiting an effect on the mucosal stages such that their development was inhibited. It was subsequently shown by Smith (1976b) that cyathostome larvae could remain viable in the horse intestine for at least two and a half years. The phenomenon of arrested larval development, also termed hypobiosis or inhibited larval development, is well recognised as an important factor in the pathogenesis of gastrointestinal trichostrongyle infections in cattle, sheep and goats. In ruminants there is generally a seasonal pattern of inhibited larval development (Michel, 1974; Armour and Duncan, 1989) and a similar pattern has been observed

in cyathostome infections of horses (Ogbourne, 1975; Reinemeyer and others, 1986). Various factors may contribute to the occurrence of arrested larval development such as climatic conditioning of larvae, size of the parasite population or host immunity. It is difficult to consider each of these factors separately since each is dynamic and to some extent they are likely to be interdependent.

There are major implications of arrested development of cyathostome larvae. First, synchronous emergence of larvae from the intestinal mucosa is associated with a severe clinical syndrome of weight loss and diarrhoea which is frequently fatal (Tiunov, 1951; Velichkin, 1952; Tiunov, 1953; Blackwell, 1973; Chiejina and Mason, 1977; Jeggo and Sewell, 1977; Mirck, 1977; Jasko and Roth, 1984; Giles and others, 1985; Church and others, 1986; Harmon and others, 1986). Secondly, anthelmintic efficacy against inhibited cyathostome larval stages is generally low such that treatment of clinical cases is difficult and control programmes for these parasites may be compromised (Eysker and others, 1986a, 1986b, 1989b; Herd and Gabel, 1990). Thirdly, the reservoir of mucosal larvae may be a factor in the development of populations of anthelmintic resistant parasites.

The responses of the host animal to cyathostomes cannot be studied readily under conditions of natural infection since animals usually harbour a burden of mixed strongyle and other parasites. Some general observations on serological and haematological responses have been described in accounts of

clinical cases of cyathostomiasis and of experimental cyathostome infections (Tiunov, 1951; Round, 1970; Smith, 1976b, 1978). The changes which were detected in peripheral blood were primarily neutrophilia, hypoalbuminaemia and hyperbetaglobulinaemia but Smith (1976b, 1978) also recorded a notable eosinophilia. Neutrophilia appears to be a result of the inflammatory changes in the typhlitis and/or colitis associated with heavy infections; this damage may also result in protein losing gastroenteropathy with consequent markedly low serum albumin levels.

Changes in serum proteins - particularly raised levels of betaglobulins and IgG(T) together with lowered levels of albumin - have also been observed in helminth-naive animals which showed no clinical signs following either experimental (Round, 1970) or natural infections (Round, 1971) and in young ponies grazing pasture contaminated with cyathostome and Trichostrongylus axei larvae (Herd and Kent, 1986). However, there have been certain limitations in the usefulness of serum protein analysis in the study of equine helminth infections. For example they were non-specific with similar changes being detected in ponies experimentally infected with Strongylus vulgaris (reviewed by Bailey, 1987) and Strongyloides westeri (Greer, Bello and Amborski, 1974); also serum protein responses were much less evident in mature ponies when compared with yearling animals (Herd and Kent, 1986) nor could they be correlated quantitatively with size of worm burden (Bailey, Kent, Martin, Lloyd and Soulsby, 1984). To date, no studies of specific antibody responses in cyathostome infections have been reported.

It is increasingly recognised that cyathostomes as a group have become the principal parasitic pathogens of grazing horses and so it is remarkable still how little is known of the details of their host-parasite relationship. The recognition of the occurrence of prolonged periods of arrested larval development were landmark observations in the understanding of both the epidemiology and pathogenesis of cyathostome infections, however, very little research of the factors which control arrested cyathostome larval development has been completed. The studies recorded in this chapter were undertaken with a view to investigating some fundamental aspects of the interaction of cyathostomes with their hosts with particular reference to the aetiology of arrested larval development.

EXPERIMENT 1

Epidemiology of Naturally Acquired Cyathostome Infection in Ponies.

INTRODUCTION

The seasonal occurrence of the clinical syndrome of acute larval cyathostomiasis has prompted the comparison of that condition with Type II ostertagiasis of cattle in the United Kingdom (Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart, 1965). There is strong evidence that acquired immunity by the host is of major significance in arrested larval development of nematodes in cattle (Michel, Lancaster and Hong, 1979) but experiments in calves have clearly demonstrated a role of environmental chilling of the infective larvae in the

induction of hypobiosis of Ostertagia ostertagi larvae within the host (Anderson, Armour, Jennings, Ritchie and Urquhart, 1969; Armour, Jennings and Urquhart, 1969a, 1969b; Armour and Bruce, 1974).

The objectives of this experiment were to investigate the possible influences of animal age, previous exposure to cyathostomes and climate on the incidence of arrested cyathostome larval development using naturally acquired infections in ponies grazing heavily contaminated pasture during autumn on a farm situated in West Central Scotland.

MATERIALS AND METHODS

Experimental Design

The design of Experiment 1 is summarised in Table 2.1. Twenty-three ponies were allocated to four groups as follows: one group of three helminth-naive foals and three groups of six foals, six yearlings and eight adult ponies which had previously been exposed to natural infections. All of the ponies were turned out to graze the same paddock for five weeks in early autumn (7th October - 11th November, 1986). Two adult ponies were designated as tracer animals and they were killed seven weeks after turnout. The three helminth-naive foals along with three animals from each of the other groups were killed 13 weeks after turnout. Measured samples of the mucosa and contents of caecum, ventral colon, dorsal colon and small colon were collected post mortem to enable calculation of total worm burdens and the anatomical site distribution of parasites. The remaining

Table 2.1

Experiment 1 - Design

	Group A	Group B	Group C	Group D
	Pony No.			
Pre-Turnout FBZ 5 x 7.5 mg/kg	11,13, 34	14,15,16, 17,30,31	1, 2, 3, 4, 5, 35	21,22,23,24 25,26,27,28
Week 0	---------- Turnout ----------			
Week 5	---------- Housing ----------			
Week 7 Necropsy	-	-	-	24,26
Week 13 Necropsy	11,13, 34	15,16,31	4,35,2	28,22,23
Week 26 Pyrantel	-	17,30,14	5, 3, 1	27,25,21
Week 38 Necropsy	-	17,30,14	5, 3, 1	27,25,21

Group A - 3 helminth-naive foals

Group B - 6 foals

Group C - 6 yearlings

Group D - 6 adults

animals were treated 26 weeks after turnout with pyrantel embonate to remove lumenal cyathostomes and they were killed after a further 12 weeks when their total and differential worm burdens together with the anatomical site distribution of parasites were recorded.

Experimental Animals

The animals used in this experiment were either Shetland or Welsh-crossbred ponies. Details of their breeds, age and sex together with their grazing and anthelmintic histories are given in Appendix 2.1. The helminth-naive foals were reared indoors with their dams which were repeatedly treated with ivermectin.

Animal Management

The yearling and adult ponies were housed for four and five months respectively prior to the beginning of the experiment whereas the grazed foals were only off pasture for one week before the experiment. All of the animals were dosed with fenbendazole at 7.5 mg/kg for five consecutive days immediately prior to turnout and the yearling and adults had previously been treated with ivermectin (Appendix 2.1). On 7th October all the ponies were turned out onto a paddock of about eight hectares in size which had been grazed for many successive seasons by horses and which was known to be heavily contaminated with equine strongyle larvae - pasture larval count of 6,014 L_3/kg herbage on 2nd October. All of the animals were housed after five weeks' grazing. They were fed hay ad libitum, bedded on straw which was cleaned out daily and they were allowed to exercise in a concrete yard.

Measurements and Sampling

No sampling was performed during the five week grazing period. Once housed, bi-weekly faecal samples were analysed for worm egg counts using a modified McMaster method.

RESULTS

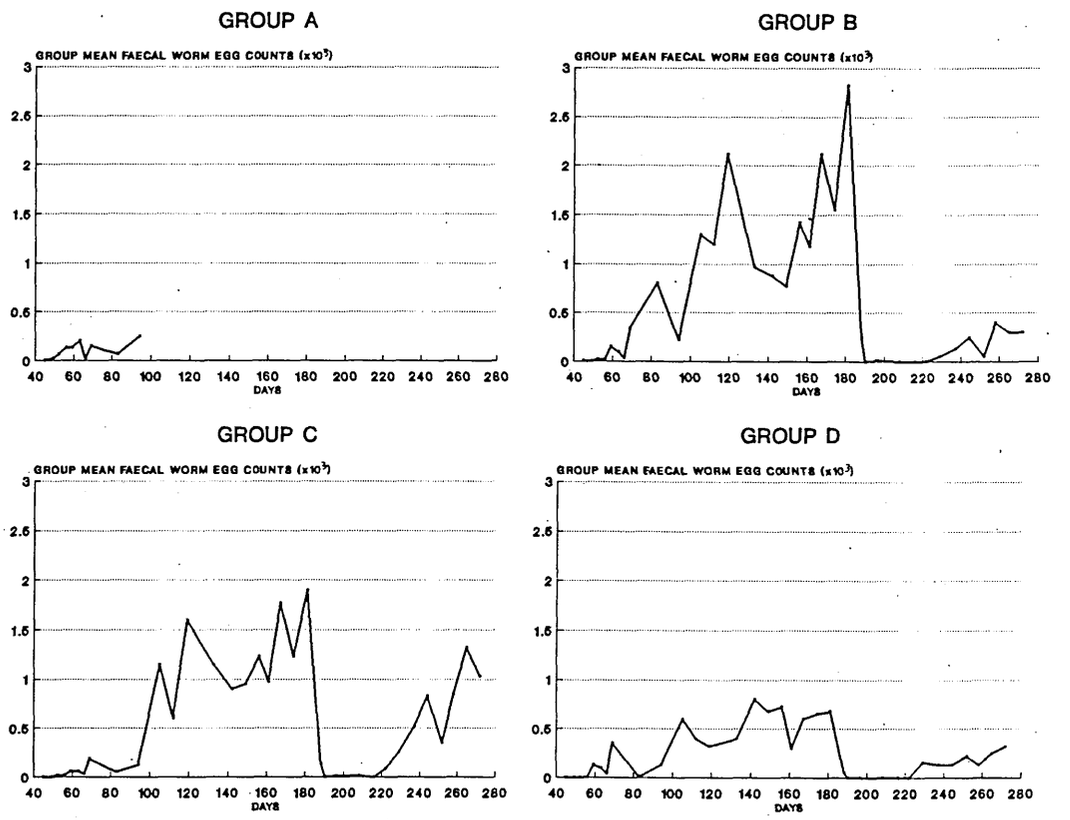
Prepatent Periods (Table 2.2)

The first day on which a positive faecal strongyle egg count was detected in each pony together with the average pre-patent period for each group are listed in Table 2.2. The shortest and longest times to patency were 38 and 83 days in Pony No. 16 and Pony No. 1 respectively. The average pre-patent period for both foal groups (Group A - 56 days; Group B - 54 days) was about one week less than that of the yearling (63 days) and adult (62 days) groups.

Faecal Worm Egg Counts (Figure 2.1)

The group mean faecal worm egg counts (FWEC) are depicted in Figure 2.1. In all groups the level of faecal egg output was low during the first three months following housing and at this time some individuals were intermittently negative for faecal worm eggs. Around Day 100 there was a sharp increase in the mean FWEC's of Groups B, C and D which persisted at a relatively constant level until all the animals were treated with pyrantel embonate on Day 186. The adult ponies (Group D) consistently had a lower mean FWEC than that of the grazed foals (Group B) and yearlings (Group C). Following treatment the FWEC of all nine ponies fell to zero. Two animals (Pony No. 30 and Pony No. 5) transiently had low-positive FWEC's ten days post treatment.

Figure 2.1
Experiment 1: Group Mean Faecal Worm Egg Counts



Group A: Helminth-naive foals
Group B: Foals
Group C: Yearlings
Group D: Adults

Table 2.2

Experiment 1 - Prepatent Periods

	Pony No.	1st Day of Positive Faecal Egg Count	Group Average (Day)
Group A	11	56	
	13	63	56
	34	49	
Group B	16	38	
	15	52	
	31	59	54
	17	56	
	30	59	
	14	59	
Group C	4	63	
	35	56	
	2	52	63
	5	59	
	3	63	
	1	83	
Group D	28	56	
	22	69	
	23	63	
	27	59	62
	25	63	
	21	59	
	24	-	
	26	-	

Five weeks following treatment all animals had positive FWEC's which persisted for the remaining six weeks of the study although the group mean values were lower than the pre-treatment levels, particularly in the foals (Group B) and adult ponies (Group D).

Cyathostome Worm Burdens (Table 2.3)

The two tracer animals had acquired burdens of 9,850 and 36,350 cyathostome worms of which 31% and 51% were within the mucosa of the intestine. Of the animals killed 13 weeks after turnout the helminth-naive foals had the highest mean cyathostome burdens (60,167 worms) and the grazed foals had the lowest mean burden (12,337 worms). In the yearlings (Group C) there was a wide range of total cyathostome worm burdens with both the lowest and the highest individual values (5,560 and 104,190) occurring in this group. One adult animal had a noticeably higher total cyathostome worm burden of 65,250 worms, compared with those of the other animals in the same group (11,020 worms and 20,020 worms).

The numbers of mucosal worms in animals at the 13-week necropsy varied considerably within all groups and represented only 3% of the total cyathostome burden in Pony No. 11, a helminth-naive foal, but constituted 28% of the total in Pony No. 35, a yearling. The mean mucosal proportion of the total count was lowest in Group A (6%) and highest in Group D (15%) with values of 12% and 10% in Groups B and C respectively. During counting of the mucosal larvae at the 13 week necropsy it was observed that many of these worms appeared as red, half

Table 2.3

Experiment 1 - Cyathostome Worm Burdens

	Pony No.	Total Count	Mean	Mucosal Count	Mean	Mean Mucosal/ Mean Total (%)
			Week 7 (Necropsy)			
Group D (Tracers)	24	36,350	23,100	18,650 (51%)	10,850	47
	26	9,850		3,050 (31%)		
			Week 13 (Necropsy)			
Group A	11	44,540	60,167	1,340 (3%)	3,500	6
	13	51,740		4,340 (8%)		
	34	84,220		4,820 (6%)		
Group B	16	9,540	12,337	2,240 (23%)	1,537	12
	15	12,390		1,490 (12%)		
	31	15,080		880 (6%)		
Group C	4	5,560	45,978	960 (17%)	4,670	10
	35	28,185		7,960 (28%)		
	2	104,190		5,090 (5%)		
Group D	28	65,250	32,097	12,700 (19%)	4,920	15
	22	11,020		540 (5%)		
	23	20,020		1,520 (8%)		
			Week 38 (Necropsy)			
Group B	17	2,480	6,433	440 (18%)	1,687	26
	30	11,690		2,390 (20%)		
	14	5,130		2,230 (43%)		
Group C	5	48,230	78,940	19,830 (41%)	17,613	22
	3	173,060		30,160 (17%)		
	1	15,530		2,850 (18%)		
Group D	27	24,450	8,950	1,710 (7%)	700	8
	25	1,890		160 (12%)		
	21	510		230 (45%)		

Note: Figures in brackets are individual mucosal counts as percentage of total count
Group A - helminth-naive foals; Group B - foals
Group C - yearlings; Group D - adults

centimetre hoops (Figure 2.2) which represented fourth stage larvae about to emerge into the intestinal lumen. Due to technical constraints a quantitative differentiation of mucosal worms to third or fourth larval stages was not performed at this time.

Of the animals killed 38 weeks after turnout the yearlings had considerably greater mean values of both total (78,940 worms) and mucosal (17,613 worms) cyathostome burdens compared with both grazed foals (Total - 6,433 worms; mucosal - 1,687 worms) and adult ponies (Total - 8,950 worms; mucosal - 700 worms). There was a wide range of size of individual total burdens with only 510 worms in Pony No. 21, an adult animal, but as many as 173,060 worms in Pony No. 3, a yearling. At the second necropsy the mean percentages of the total count which were present as mucosal worms were noticeably higher at 26% and 22% in Groups B and C respectively, compared with 8% in Group D.

Details of the anatomical site distribution of the cyathostomes at the 13 and 38 week necropsies are given in Appendices 2.2 and 2.3 respectively. Mucosal worms were found mainly in the caecum and ventral colon but several animals had appreciable numbers of mucosal stages in their dorsal colons, notably Pony No. 14, Pony No. 1 and Pony No. 27 which were from foal, yearling and adult groups respectively. Lumenal worms were recovered predominantly from the ventral and dorsal colons. At the first necropsy the average proportion of the total lumenal burden in the ventral colon was higher, at 58%, than that in the dorsal colon, which was 34% but at the second necropsy there was

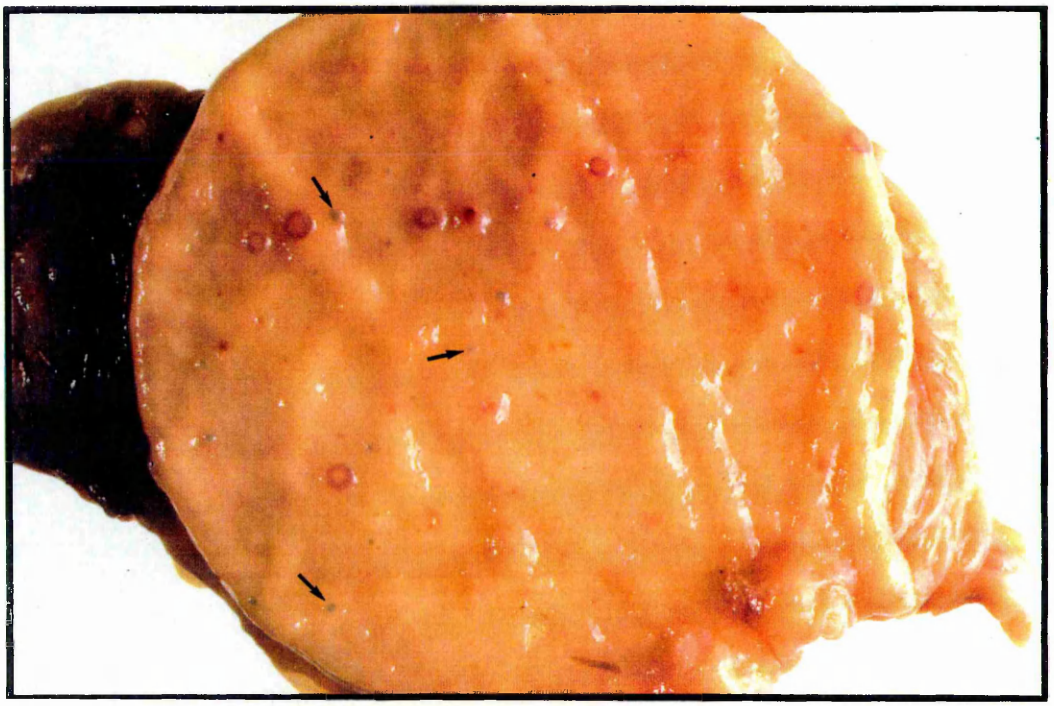


Figure 2.2

Experiment 1 - Gross appearance of caecal mucosa of Pony No.16 at Week 13 necropsy. Mucosal larvae can be visualised as grey 1 mm marks (→) and also as 5 mm red hoops

an average of 52% lumenal stages in the dorsal colon compared with 44% in the ventral colon.

Generally there were small numbers of lumenal worms in the caecum and only four individuals had more than 2,000 worms in the caecal contents. At the second necropsy the proportion of lumenal worms within the caecal contents was very low in all animals (average 3.7%) with the exception of Pony No. 5, a yearling, in which 24% of the total lumenal burden was recovered from the caecum. Small numbers of worms were found within the lumen of the small colon in a few animals.

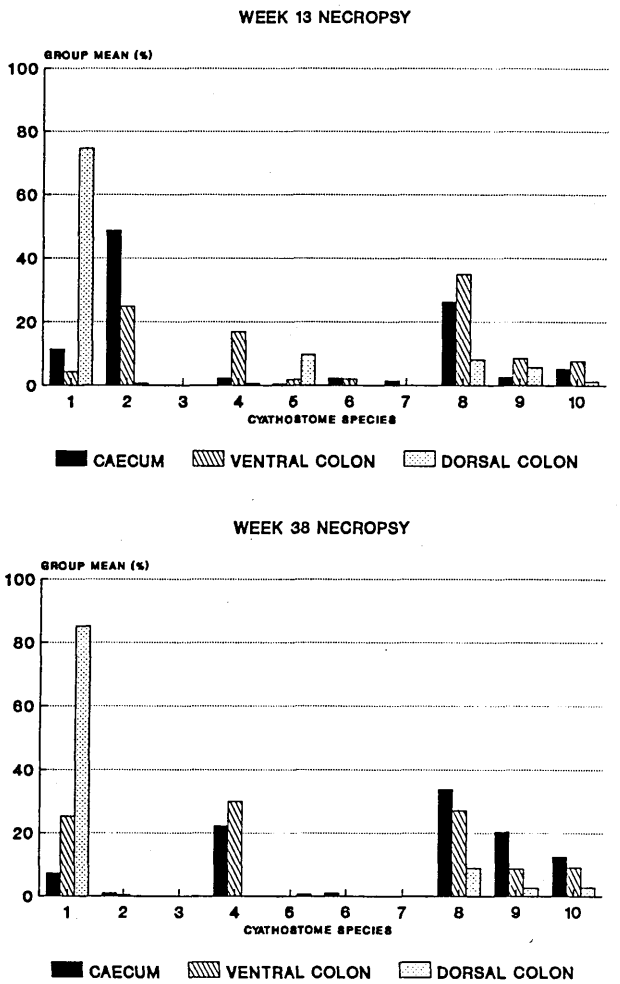
The cyathostome species present within the intestinal contents are given in Figure 2.3. Nine different species were identified, namely: Cylicostephanus longibursatus, C. minutus, C. poculatus, Cylicocyclus nassatus, C. insigne, C. brevicapsulatus, C. radiatus, Cyathostomum catinatum and C. coronatum, of which seven occurred commonly but Cylicostephanus poculatus and Cylicocyclus radiatus were found in only one and two animals respectively. There was a difference in species prevalence between the 13 and 38 week necropsies in that Cylicostephanus minutus and Cyclicocyclus insigne were recovered from almost all animals at Week 13 but they were identified in only a few animals at Week 38.

DISCUSSION

The results of the epidemiological study suggested that cyathostome development was modified by age and/or previous exposure to parasites in that there were differences between

Figure 2.3

Experiment 1: Anatomical Distribution of Individual Cyathostome Species



Species Key to Figure 2.3
and Relative Prevalence of each species

Cyathostome Species	Relative Prevalence	
	Week 13	Week 38
1 *Cylicostephanus longibursatus*	12/12	7/9
2 *Cylicostephanus minutus*	11/12	3/9
3 *Cylicostephanus poculatus*	0/12	1/9
4 *Cylicocyclus nassatus*	11/12	6/9
5 *Cylicocyclus insigne*	12/12	2/9
6 *Cylicocyclus brevicapsulatus*	6/12	1/9
7 *Cylicocyclus radiatus*	2/12	0/9
8 *Cyathostomum catinatum*	11/12	8/9
9 *Cyathostomum coronatum*	8/12	6/9
10 Lumenal larvae (unspeciated)	11/12	5/9

certain groups. Some evidence of an influence of these factors could be derived from the patterns of faecal worm egg output which were lower in adult ponies than in the groups of grazed foals or yearling. There was further indication of modified cyathostome development from analysis of two aspects of the worm counts at the 13 week necropsies; total cyathostome burdens were highest in the helminth-naive foal group and lowest in the grazed foal group and the proportions of the total burden present as mucosal stages were lower in helminth-naive foals than in grazed foals, yearlings or adult ponies. There were also differences in the profiles of the cyathostome burdens of the animals necropsied at Week 38 which were additional evidence of modification of parasite development; the yearling group had higher total worm burdens than the grazed foal or adult groups and also compared with the group of adult ponies, the yearlings and grazed foals were found to have higher numbers of larvae in arrested development.

The shortest pre-patent period of 38 days observed in one of the grazed foals in the present study is comparable to those of 42 and 35 days reported in experimental infections of six and two month-old foals respectively (Tiunov, 1951; Reinemeyer and others, 1988). Prepatent periods recorded by Round (1969) following experimental or natural infection of animals six to 17 months old were between 49 and 66 days and all animals in the present study also showed prepatent periods within that range with the exceptions of the foal mentioned above (38 days) and one of the yearlings at 83 days. In experimental reinfections of a

group of adult ponies whose strongyle worm burdens had been removed by multiple treatments with thiabendazole the prepatent periods recorded when the animals were four to five years old were 12 to 15 weeks and notably longer, i.e. 17 to 18 weeks, in two out of three of the same animals following dosing with 300,000 infective larvae when they were approximately eight years of age (Smith, 1976b, 1978). In this experiment there were no differences in the apparent prepatent periods in adult ponies (mean 62 days - Group D) when compared with yearling animals (mean 63 days - Group C) and time to patency in the foals was only about one week shorter (56 and 54 days - Groups A and B respectively). All adult ponies in this study were between eight and 15 years of age with experience of multiple grazing seasons except for Pony No. 28 which was two and a half years old and had grazed for only one previous season when a yearling. It appears that age and previous exposure to cyathostomes did not markedly affect the time for naturally acquired infections to develop to patency but it is possible that the worm egg counts which were detected were from worms which had repopulated the intestinal lumen from inhibited mucosal stages. All animals had received anthelmintic treatments prior to the beginning of the experiment, including two or three doses of ivermectin in Groups C and D respectively, but it is well recognised that drug efficacy against mucosal stages which are arrested in development is variable (Gibson, 1953; Smith, 1976a, 1976b, 1978; Herd, 1986; Eysker and others 1989a, 1989b; Herd and Gabel, 1990).

The general patterns of faecal worm egg output were similar in all groups with a sharp increase in counts evident at about 100 days after first grazing which then remained fairly constant until treatment on Day 186. Although there were variations in values in successive samples from individual animals there was no evidence of a cyclic pattern of mean egg counts as reported by Smith (1976b, 1978). The level of mean worm egg output in the adult ponies was in the range of 300 to 800 e.p.g. which was lower than those detected in yearlings and grazed foals which generally had similar counts of between 600 and 2,825 e.p.g. There was no apparent correlation between faecal strongyle worm egg output and post mortem cyathostome worm burdens (vide infra). These observations of lower faecal worm egg counts in older animals were interpreted as a manifestation of modified cyathostome development as a consequence of host age and/or previous exposure to parasites. Smith (1978) recorded significantly lower faecal worm egg counts in his ponies when they were reinfected for a second time which he considered to be an indication of resistance to cyathostome infection when the animals were older.

The differences in the sizes of cyathostome worm burdens which were present between groups of animals at the Week 13 necropsies were considered to be the result of previous parasite exposure on the development of cyathostome infections. The three helminth-naive foals of Group A which were killed at this time had individual burdens of 44,540, 51,740 and 84,220 worms with a mean of 60,167; these were considerably higher than those in the

three grazed foals from Group B killed at the same time which were 9,540, 12,390 and 15,080 worms with a mean of 12,337. An additional difference between these two groups was that the proportions of the total counts which were present as larvae within the intestinal mucosa were 3%, 6% and 8% with a mean of 6% in Group A compared with values of 6%, 12% and 23% giving a mean of 12% in Group B. Both groups of foals were of a similar age and it was concluded that previous exposure to parasites resulted in both reduced susceptibility to infection, reflected in lower acquired burdens, and also a slower rate of development of cyathostomes with higher proportional mucosal burdens at 13 weeks after first infection.

The profile of cyathostome worm burdens present in the yearlings of Group C were similar to those of the grazed foals; two of the three animals had total burdens of 5,560 and 28,185 with proportions of mucosal stages of 17% and 28% respectively. However the third yearling, Pony No. 2, had a much higher total count of 104,190 worms of which only 5% were within the mucosa; this was similar to the results in the helminth-naive foals, and could mean that this animal had either experienced a limited previous parasite exposure or that it had a lower response to previous infection.

Within Group D, the adult ponies, the burdens at 13 weeks were also similar to those of the grazed foals and yearlings in two animals which had 11,020 and 20,020 worms of which 5% and 8% respectively were mucosal stages. The remaining adult animal,

Pony No. 28, had a total count of 65,250 of which 19% were within the mucosa. This pony, as mentioned previously, had a different background to the other adult ponies in that it was much younger at only two and a half years old and it had only grazed for a six month period as a yearling animal one year prior to the beginning of the experiment. Thus it had a parasitic experience equivalent to that of the grazed foals but without any recent exposure to infective larvae. It is perhaps not surprising therefore that this animal acquired a similar worm burden to helminth-naive foals suggesting that it had little resistance to cyathostome infection. Interestingly the proportion of the total burden present within the mucosa at 13 weeks post-infection was 19% suggesting that the rate of larval development was slower than in naive animals.

During the five-week autumn grazing period there were moderate ground frosts but the low values of 3%, 6% and 8% for mucosal worms relative to the total burden in the helminth-naive foals was evidence of a limited or negligible effect of climatic conditioning of larvae on pasture on the subsequent occurrence of arrested larval development in the host. In epidemiological studies on bovine ostertagiasis in West Central Scotland during October and November the proportion of arrested larval stages found at necropsy of tracer calves has been more than 50% (Anderson and others, 1969) and similar levels of inhibited development were obtained when calves were experimentally infected with Ostertagia ostertagi which had been cold conditioned (reviewed by Armour and Duncan, 1987). In a series

of experiments in The Netherlands using naturally infected yearling Shetland ponies which were killed in late autumn or early winter the percentages of the total burdens present as immature stages have commonly been of the order of 70% to 90% (Eysker and others, 1984; Eysker and Mirck, 1986; Eysker and others, 1986a, 1986b, 1989b); in general the adult cyathostome burdens were of a similar order to those found in the present study at around 10 - 70,000 worms but often several hundred thousand larvae were present following grazing periods of six months. These findings could be a manifestation of the feedback mechanism proposed by Gibson (1953) who hypothesised that mature worms within the intestinal lumen could influence the inhibition of larval development. It is possible that in the Dutch studies the larvae ingested early in the season developed unimpeded but that these then exerted feedback repressive effects on subsequently ingested larvae causing an accumulation of inhibited stages. In the experiment described here the animals only grazed for five weeks and, because of anthelmintic treatment prior to turnout, there was no superimposition of larvae on an established mature infection; this might account for the comparatively low levels of arrested larval development in the present study. The profile of the worm burdens in two tracer animals used by Eysker and others (1986b) however, was of an exclusively immature cyathostome burden when animals were examined at necropsy three months after first infection. This suggested to the authors that inhibition of cyathostome larvae was not induced by host exposure

or resistance but that it was a seasonal event. The tracer animals were yearlings which had been reared helminth-free and which grazed strongyle infected pasture for two months so although they were slightly older than the parasite-naive foals used in this study it might have been expected that their cyathostome infections would have developed in a similar way. Although the results of these studies were so strikingly different, there was no obvious explanation for these differences other than the fact that the levels of larval intake were unknown which may have had some effect. In two studies in which necropsy data were reported following experimental infections of helminth-naive foals with either 20,000 (Reinemeyer and others, 1988) or 130,000 larvae (Burger and Bauer, 1987) there was apparently progressive development of larvae with minimal inhibition similar to the findings in the helminth-naive foals in the present study.

There were also differences in the sizes and profiles of the cyathostome burdens in the ponies included in the Week 38 necropsies. Of these animals the grazed foals, Group B, had total cyathostome burdens of 2,480, 11,690 and 5,130 worms with a mean of 6,433 which was similar to the mean of 8,950 in the adult ponies of Group D although the individual burdens were more variable (510, 1,890 and 24,450 worms). In contrast the yearling animals in Group C had burdens of 15,530, 48,230 and 173,060 with a much higher mean of 78,940. These values suggested that the yearlings had acquired and/or maintained higher burdens than the grazed foals and adult ponies. With regard to mucosal stages of cyathostomes in these animals the

three yearlings had the highest numbers of 19,830, 30,160 and 2,850 (mean 17,613) which represented 41%, 17% and 18% of total worm burdens respectively. Although the mucosal burdens were lower in the grazed foals at 440, 2,390 and 2,230 (mean 1,687) they represented high proportions of the total burdens at 18%, 20% and 43%. The adult ponies had very low mucosal counts of 160, 230 and 1,710 which were 12%, 45% and 7% of the total burdens respectively.

Due to the prolonged period of 38 weeks between infection and necropsy of these animals the mucosal larvae were considered to have definitely been arrested in their development. The differences between groups in this experiment suggested that host age had an effect on arrested larval development in that it was more common in yearlings and foals than in older, adult animals. The clinical syndrome of acute larval cyathostomiasis certainly occurs more commonly in young animals (Giles and others, 1985) and the finding in Group D of lower burdens of larvae in arrested development suggested that older animals may be less prone to accumulate large mucosal burdens.

In discussion of studies of arrested larval development, Michel and others (1979) warned that misinterpretation of data on proportions of total worm burdens present as immature stages could arise if a marked loss of adult parasites occurred prior to the time of slaughter. Colglazier (1979) has recorded spontaneous elimination of about 34% of the total worm burdens of a group of 29 ponies in a period of three days. Although

parasite losses could not be determined in the animals at the 38 week necropsy they were likely to have been high because they had been treated 12 weeks earlier with an anthelmintic. It is possible that parasite losses, either spontaneous or as a result of treatment, varied between animals in this study such that definitive conclusions could not be made on the factors affecting arrested development. Similar limitations also apply to the interpretation of results of previous field studies due to the practical difficulties of monitoring the dynamics of cyathostome infections.

The anatomical distribution of cyathostomes were generally similar in all of the animals necropsied at both 13 and 38 weeks: mucosal stages were predominantly in the caecum and ventral colon whereas lumenal stages were mainly present in the ventral and dorsal colons with only small numbers within the caecal contents. There were slight differences in the distribution of lumenal worms between the two kills in that at Week 13 the caecum, ventral colon and dorsal colon contained on average 7.5%, 58% and 34% of lumenal worms respectively whereas the corresponding values at Week 38 were 3.7%, 44.1% and 52%. At the second necropsy the two adult animals with low absolute burdens had particularly high proportions of 97% and 86% of lumenal worms within the dorsal colon. The anatomical distributions of both mucosal and lumenal stages in the present study are similar to those recorded previously (Ogbourne, 1976; Reinemeyer and others, 1984; Mfitilodze and Hutchinson, 1985). The greater proportions of lumenal worms in the dorsal colons at the second kill together

with the lower caecal lumenal populations were presumed to reflect seasonal changes in site distribution of cyathostomes with a posterior shift of ageing parasites similar to that reported from post-mortem studies in Britain (Ogbourne, 1976) and tropical Australia (Mfitilodze and Hutchinson, 1985). In helminth-naive foals given experimental cyathostome infections only very small numbers of adult worms were recorded within the dorsal colon which was attributed to the small size of the infective inocula (Reinemeyer and others, 1988). The helminth-free foals in this study had distributions of lumenal population comparable with adult animals.

An interesting observation in the present study was that several ponies had counts of more than one hundred larvae in the mucosa of their dorsal colons and one of the grazed foals (Pony No. 14) had 1,280 mucosal worms at this site, which was 57% of the total mucosal burden. Generally, the dorsal colon has been regarded as an atypical site for mucosal cyathostome development although Reinemeyer and Herd (1986) considered that the more distal portions of the alimentary tract may become colonised as the level of infection increases. Although Pony No. 14 had a low burden at the time of necropsy it had received anthelmintic treatment some months earlier and so the initial level of infection could not be be ascertained.

The range of species of cyathostome worms recovered from ponies in Experiment 1, and also their relative prevalence, were comparable with those of previous reports (Ogbourne, 1976;

Reinemeyer and others, 1984). Several species, namely Cylicostephanus goldi, C. calicatus, Cyathostomum pateratum and Cylicocyclus leptostomus, which occurred commonly in horses surveyed by those authors were not detected in the animals in the present experiment which were all infected from a single pasture. The site distributions of the most abundant cyathostome species in Experiment 1 were similar to those described by Ogbourne (1978) and Mfitilodze and Hutchinson (1985) in that Cylicostephanus minutus, Cyathostomum catinatum and C. coronatum were present in highest numbers in the caecum and ventral colon whereas Cylicostephanus longibursatus showed preference for the dorsal colon. The notably lower prevalence of both Cylicostephanus minutus and Cylicocyclus insigne at Week 38 compared with Week 13 was an interesting observation in that it suggested that the population dynamics of those species may be different from those of Cylicostephanus longibursatus, Cylicocyclus nassatus, Cyathostomum catinatum and C. coronatum which occurred in most animals at both time periods. For example those worms present at the second necropsy had survived anthelmintic dosing at Week 26, presumably because they were immature mucosal larval stages at time of treatment. The relative decrease in prevalence of Cylicostephanus minutus and Cylicocyclus insigne at the second necropsy indicated that these species might have developed more rapidly than the other species identified and existed predominantly as susceptible lumenal stages by time of treatment.

In conclusion, the results of Experiment 1 suggested that previous exposure to parasites affected the epidemiology of naturally acquired cyathostome infection in ponies by reducing the size of acquired burdens and slowing the rate of parasite development. Although host age *per se* was not shown to affect the size of the worm burdens there was a lower level of faecal egg output in adult ponies than in younger animals. There was also some evidence that the incidence of arrested larval development was affected by host age in that higher levels of inhibited larvae occurred in foals and yearlings compared with adult animals. In this study there was no apparent effect of climate on the occurrence of arrested larval development.

EXPERIMENT 2

Development of Experimental Cyathostome Infection in Yearling Ponies

INTRODUCTION

There are only two published accounts of experimental cyathostome infections in which detailed post-mortem differential worm counts were undertaken (Burger and Bauer, 1987; Reinemeyer and others, 1988). In both studies there was apparently progressive development of larvae in helminth-naive foals given single doses of 20,000 or 130,000 larvae. In Experiment 1, (vide supra) although the group sizes were small, there was also a low incidence of arrested larval development following natural infections of helminth-naive foals. However, there appeared to be evidence of a predisposition for hypobiosis in previously grazed foals and yearlings after limited exposure to field infections. An important limitation of the design of this study however, was that it was not possible to ascertain the size of the infective dose acquired by individual animals during grazing.

The objectives of Experiment 2 were to investigate the effects of moderate and large doses of larvae, given either singly or as repeated infections, on the population dynamics of cyathostome infections especially in relation to the occurrence of arrested larval development.

MATERIALS AND METHODS

Experimental Design

The design of Experiment 2 is summarised in Table 2.4. Six yearling ponies were infected with cyathostome larvae in doses of

Table 2.4

Experiment 2 - Design

	Pony No.	Infective Dose L_3	Necropsy
Group A	1)		
	)	50,000	Week 15
	6)		
	8	10 x 5,000	Week 16
Group B	3)		
	)	500,000	Week 15
	5)		
	7	10 x 50,000	Week 16
Control	4	-	Week 16

either 50,000 or 500,000 L_3 given as either a single or trickle infection. An additional pony was kept uninfected as a control. The animals were killed 16 or 17 weeks post infection when their total worm burdens together with the anatomical site distribution of parasites were recorded.

Experimental Animals

Seven Welsh-crossbred colt foals were purchased when newly weaned at about five months of age. They were approximately one year old at the time of experimental infection.

Animal Management

The ponies were housed for seven months prior to the beginning of the experiment and they remained in the same accommodation until necropsy. They were bedded on straw, fed hay ad libitum and they had free access to a concrete exercise yard. Three months prior to experimental infection they were dosed with ivermectin at 0.2 mg/kg and they were given five daily doses of 7.5 mg/kg fenbendazole between 12 and eight days pre-infection. Infective larvae were administered orally.

Measurements and Sampling

The animals were examined daily from two weeks before infection. Twice weekly faeces samples were analysed for worm egg counts by a modified McMaster technique.

RESULTS

Clinical Observations

One animal which was given 500,000 L_3 in a single dose developed diarrhoea on Day 47 post-infection which persisted until the end of the experiment although the faeces were of semi-formed consistency from Day 75 onwards and the pony remained bright, appetant and well thriven. Another animal which was given ten daily doses of 50,000 L_3 passed soft faeces between Day 16 and Day 23 following the first infection dose but it was subsequently considered to be clinically normal. The remaining ponies were clinically unaffected by experimental cyathostome infection.

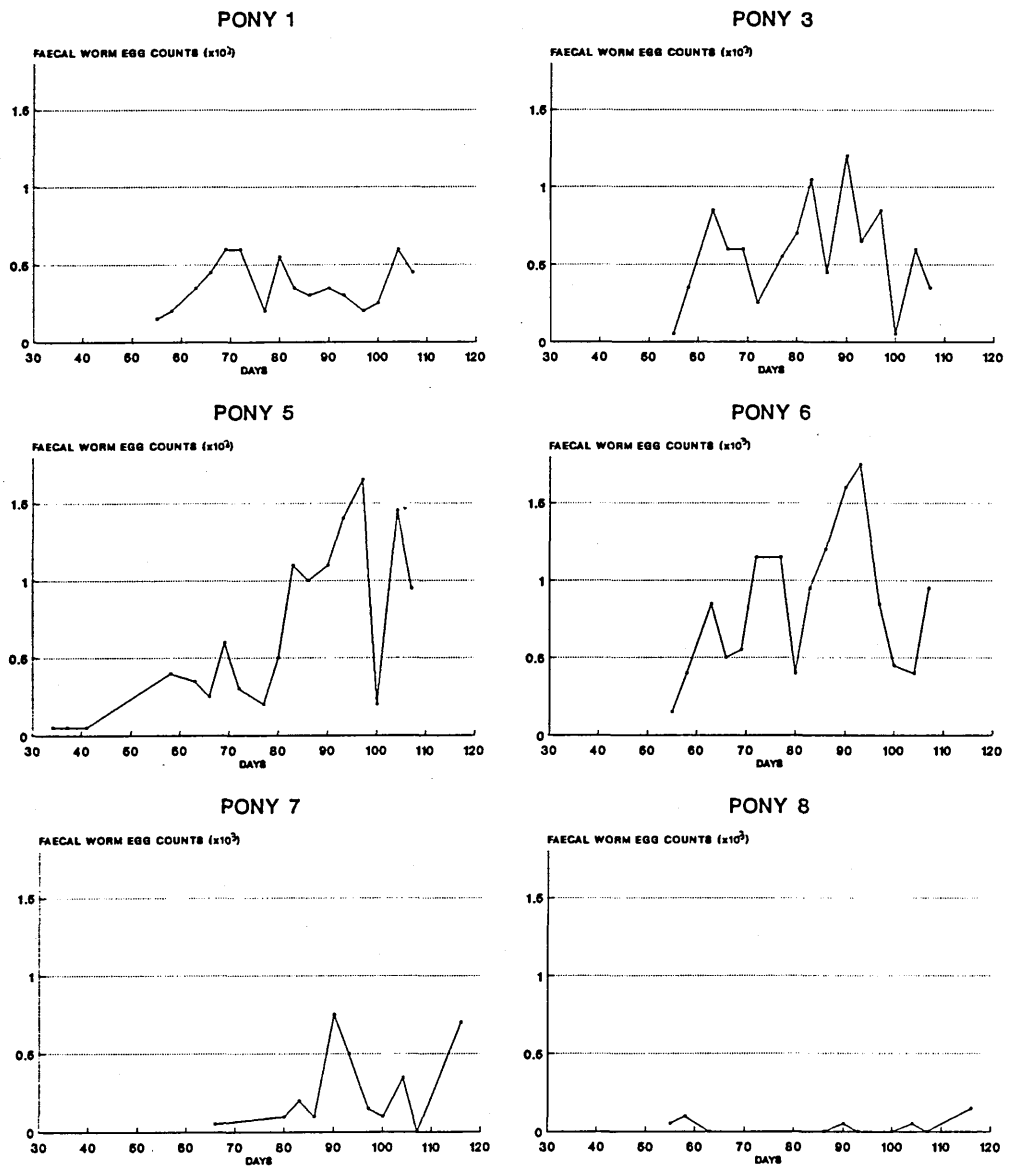
Prepatent Periods

Positive faecal strongyle egg counts were first detected in ponies given single infections around Day 55 post-infection with the exception of one animal (Pony No. 5) which had low positive strongyle counts on three occasions between Weeks 5 and 6 post-infection. Of the two animals given trickle infections, the one given the high dose (Pony No. 7) was consistently positive for strongyle eggs from Day 80 onwards although it had a single positive count of 50 epg on Day 63 while the other given the low dose (Pony No. 8) was first positive on Day 55 but only infrequently had positive counts thereafter.

Faecal Worm Egg Counts (Figure 2.4)

The faecal worm egg output of individual animals followed similar trends in all ponies given single infections with sharp rises in egg counts immediately following patency to values which

Figure 2.4
Experiment 2: Individual Faecal Worm Egg Counts



Pony Nos. 1 & 6: 50,000 L3, single dose
Pony No. 8: 50,000 L3, trickle dose
Pony Nos. 3 & 5: 500,000 L3, single dose
Pony No. 7: 500,000 L3, trickle dose

were then maintained until the end of the study. The level of egg output in Pony No. 1 was lower, at between 200 and 600 epg, than those of Pony No. 3, 5 and 6 which were mainly between 500 and 1,200 epg. Both animals which were infected by trickle dosing had patterns of faecal egg counts which were markedly different from the single-dose animals in that in Pony No. 7 the onset of egg output was delayed by about one month and Pony No. 8 was only occasionally positive for faecal strongyle eggs throughout the period of study.

Despite the previous anthelmintic treatments the control animal had low positive faecal worm egg counts of 50 epg on two occasions.

Cyathostome Worm Burdens (Table 2.5)

The control animal had a total of 440 worms at post mortem examination which were mainly lumenal worms.

The total burdens in Group A animals were 3,880, 7,080 and 13,350 worms which constituted 11%, 14% and 27% of the infective doses of 50,000 L_3. The lowest burden was in the pony which was infected with ten serial doses and in this animal 32% of the total burden was within the intestinal mucosa compared with 2.5% and 2.9% in the ponies which received single doses.

Group B animals had total burdens of 29,680, 33,880 and 71,270 which represented 6%, 7% and 14% of the infective doses of 500,000 L_3. The pony which was infected with ten trickle doses had the highest proportion of mucosal stages within the group at 13% compared with values of 6.1% and 1.2% in the animals given a single infection.

Table 2.5

Experiment 2 - Cyathostome Worm Burdens

	Pony No.	Total Worm Count	% Establishment of Infective Dose	Mucosal Larval Count	% Total Worm Count in Mucosa
Group A	1	7,080	14	180	2.5
	6	13,350	27	390	2.9
	8	3,880	11	1,830	32
Group B	3	71,270	14	830	1.2
	5	29,680	6	1,820	6.1
	7	33,880	7	4,380	13
Control	4	440	-	40	9

Note: (i) % Establishment of infective dose is the total worm count at 15-16 week necropsy expressed as a percentage of the infective dose of larvae.

(ii) Group A - 50,000 L_3 Group B - 500,000 L_3

Details of the anatomical distribution of the cyathostome worm burdens are given in Appendix 2.4. Mucosal larvae were mainly found in the caecum and ventral colon with only two animals (Pony No. 3 and Pony No. 8) having cyathostomes in the mucosa of the dorsal colon. In all animals the ventral colon harboured the majority of lumenal stages except for one animal (Pony No. 7) in which 72% of the total number of lumenal worms were contained within the dorsal colon. The cyathostome species present within the intestinal contents are shown in Figure 2.5; nine different species were identified of which two, Cylicostephanus longibursatus and Cyathostomum catinatum, were found in all six animals.

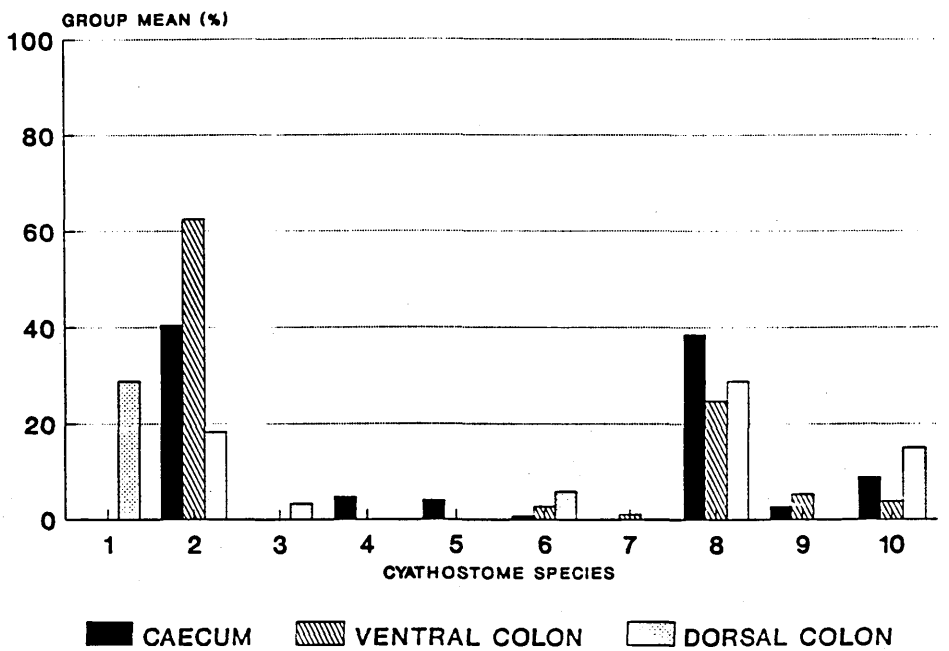
DISCUSSION

In Experiment 2 both ponies given trickle infections were found to have longer prepatent periods and higher levels of arrested development than animals given single infections. There were generally lower establishment rates in animals which received the 500,000 larval doses and clinical signs were only seen in this group with diarrhoea of moderate severity being observed in two animals.

The clinical signs presented differently in the two ponies which became diarrhoeic. One developed chronic diarrhoea from Day 47 after infection with a single dose of larvae whereas the other had transient diarrhoea for seven days within two weeks of having been given ten daily doses of larvae. In the case of Pony No. 5 the onset of signs may have coincided with larval emergence

Figure 2.5

Experiment 2: Anatomical Distribution of Individual Cyathostome Species



Species Key to Figure 2.5 and Relative Prevalence of each species

1 *Cylicostephanus longibursatus*	6/6
2 *Cylicostephanus minutus*	5/6
3 *Cylicostephanus goldi*	1/6
4 *Cylicostephanus poculatus*	2/6
5 *Cylicocyclus nassatus*	2/6
6 *Cylicocyclus insigne*	5/6
7 *Cylicocyclus ultrajectinus*	1/6
8 *Cyathostomum catinatum*	6/6
9 *Cyathostomum coronatum*	2/6
10 Lumenal larvae (unspeciated)	4/6

from the intestinal mucosa although this animal had positive faecal worm egg counts from Day 34 post infection. By contrast, patency of infection in Pony No. 7 was not detected until Day 63 but clinical signs occurred between Day 16 and Day 23 which may have been associated with penetration or development of larvae in the mucosa. In previously reported studies of experimental cyathostome infections the times of onset of clinical signs have varied. Tiunov (1951) described severe diarrhoea in foals early after infection but did not specify dose size or frequency. Smith (1976b; 1978) observed mild diarrhoea three to seven days following single reinfections of adult animals with doses from 200,000 to 500,000 larvae and about one month after trickle infections of similar size, with a two to three week duration of signs; Bueno and others (1979) observed transient diarrhoea 39 days post-infection in one of three foals given 300,000 larvae (including 15% large strongyle larvae) which was also the time at which they detected changes in caeco-colic motility patterns. Considering these observations collectively it would appear that changes in the gastrointestinal tract sufficient to result in diarrhoea may occur when larvae either penetrate into the mucosa or emerge from it but the factors which would account for differences between animals in the period of onset of clinical signs cannot yet be determined.

The prepatent periods of the ponies given single infections in Experiment 2 were around 55 days which were comparable to those of the naturally acquired infections of animals of a similar age studied by Round (1969) and also those of ponies in

Experiment 1. Pony No. 5 had positive faecal egg counts in samples taken between Day 34 and Day 41 but then became negative for faecal worm eggs until Day 58 after which it was consistently positive. The early positive counts in this animal may have been true patency since prepatent periods as short as 35 (Reinemeyer and others, 1988) and 42 days (Tiunov, 1951) have been recorded in helminth-naive foals. However this apparently short prepatent period may not be a true result if the faecal worm eggs detected were produced by cyathostome worms of the initial infection which survived the anthelmintic treatments, as occurred in three samples from the control animal. The prepatent periods of the ponies given trickle infections of 50,000 and 500,000 larvae were 55 and 66-80 days respectively, the latter period being similar to the longest individual prepatent period of a yearling animal in Experiment 1 and comparable with times to patency of reinfections of four and five year old animals recorded by Smith (1976b).

The pattern of faecal worm egg output was similar in all ponies which received a single infection; following patency worm egg counts in faeces increased rapidly over two or three weeks and then remained within a range of 500 to 1,200 epg. However, lower values of between 200 and 600 epg were consistently detected in Pony No. 1, which was infected at the lower dose rate and which had a smaller cyathostome worm burden at necropsy. Although patency appeared to be delayed in the pony which received a trickle infection of 500,000 L_3 the subsequent pattern

of faecal egg output was similar to that of single infection animals but in the other trickle infection animal, which received the lower total dose of 50,000 larvae, the faecal worm egg counts were repeatedly negative or low throughout the experiment; this animal had only 2,000 lumenal cyathostome worms at necropsy which was much less than the other animals in Experiment 2 but the low egg output of this animal cannot be solely a result of the size of the worm population since animals in Experiment 1 with comparable worm burdens had much higher levels of faecal egg output. Smith (1978) observed a similar effect on reinfection of his group of adult ponies and it is possible that the low level of faecal egg output in this individual animal was due to a stronger immunological response to previous parasitic infections than that of the other animals.

The cyathostome worm burdens at necropsy of animals in Experiment 2 were smaller in the Group A animals - in which 7,080, 13,350 and 3,880 worms represented 14%, 27% and 11% of the total doses of 50,000 larvae - compared with those of Group B animals in which there were 71,270, 29,680 and 33,880 worms constituting 14%, 6% and 7% of the infective dose of 500,000 larvae. That the percentages of the infective doses present at post mortem were generally slightly less in the animals infected at the higher larval dose rate suggested a possible effect of dose size on parasite survival within the host. Whether this reflects lower establishment rates or higher expulsion rates in the animals which received larger inocula could not be determined. In Group A animals the worm establishsment rate was

lower following trickle infection compared with the single dose but this did not occur in Group B. From this it was concluded that dose frequency alone did not affect parasite survival in the animals studied in Experiment 2. However, in both groups there were markedly higher proportions of the total burdens present as mucosal stages in the trickle-infected animals, at 32% for the 50,000 dose and 13% for the 500,000 dose, compared with 2.5% and 2.9% in those animals given single-doses of 50,000 L_3 and 1.2% and 6.1% in animals infected with a single dose of 500,000 L_3. Following administration of single doses of 20,000 cyathostome larvae to three helminth-naive foals Reinemeyer and others (1988) also observed that low proportions - less than 2% - of the infective dose were within the intestinal mucosa at necropsies performed approximately four months later. In Experiment 1 the ponies were in effect trickle infected by grazing over a five week period and in those animals the proportion of mucosal worms at necropsy was generally similar to the values of the trickle-dosed animals in Experiment 2. These results suggested that repeated exposure to infective larvae had an influence on the occurrence of arrested larval development in both experimental and naturally acquired cyathostome infections.

The anatomical site distributions of both mucosal and lumenal worms was similar in both groups in Experiment 2 and comparable to that found in previous reports (Ogbourne, 1976; Reinemeyer and others, 1984; Mfitilodze and Hutchinson, 1985) and in Experiment 1. Mucosal stages were mainly in the caecum and

ventral colon whereas lumenal stages were generally most numerous in the ventral colon. Significant proportions of the lumenal burden were also present more distally in the dorsal colon and in the animal which received the high dose trickle infection there was 72% of lumenal worms at this site which together with the presence of fairly large numbers of worms within the lumen of the small colon could suggest that expulsion of part of the cyathostome burden was taking place.

In conclusion the results of Experiment 2 suggested that there were effects of both size and frequency of larval dose on subsequent cyathostome development. In the small groups of animals studied there was evidence that clinical signs and parasite survival rate may be related to the size of larval dose since diarrhoea was observed only in ponies receiving the higher cyathostome dose and the same animals had lower parasite survival rates. Although dose size alone did not affect the occurrence of hypobiosis there were indications that repeated, sequential dosing resulted in delayed patency and also a higher level of arrested larval development.

EXPERIMENT 3

Development of Experimental Cyathostome Infection in Helminth-naive Foals

INTRODUCTION

The cumulative evidence of previous studies suggests that arrested cyathostome larval development is multifactorial in origin. Seasonal occurrence of arrested development has been identified (Ogbourne, 1975; Reinemeyer and others, 1986b) but the underlying mechanisms remain unclear although a seasonal progression of various factors including increasing levels of pasture contamination, repeated host reinfection and changing climatic conditions have been considered to be important. Although very high levels of arrested development were not found in Experiments 1 or 2 of the present study, there were sufficient differences between groups in the rate at which it occurred to make the preliminary observations that younger animals and those which received repeated infective doses were predisposed to accumulate burdens of arrested larvae. In studies of naturally acquired cyathostome infections in The Netherlands very high levels of arrested development have been repeatedly observed in ponies in late autumn (Eysker and others, 1984; Eysker and Mirck, 1986; Eysker and others, 1986a, 1986b, 1989b), including in helminth-naive foals exposed to infection at this time, which incriminated climatic conditioning as a contributory factor. Despite the lack of evidence of an effect of climate in the natural infections studied in the West of Scotland in Experiment 1 it seemed worthwhile to investigate the effects of

environmental chilling further since the results of the Dutch work was so striking.

The objectives of Experiment 3 therefore were to examine arrested development of cyathostome infections in helminth-naive foals with special reference to trickle infections and larval chilling.

MATERIALS AND METHODS

Experimental Design

The design of Experiment 3 is summarised in Table 2.6. Three groups of five pony foals each were infected with 450,000 third-stage cyathostome larvae, given as either: a single dose of freshly harvested larvae; or a single dose of larvae which had been cold conditioned for six weeks at $4^{o}C$; or as a trickle infection of 18 doses of 25,000 freshly harvested larvae, administered on week days over one month. Animals given the single doses were killed 16 weeks post infection whereas those given trickle larval doses were killed 16 weeks after the last infective dose was administered. Total worm burdens together with their anatomical site distribution were examined at post mortem. Three uninfected ponies were kept as controls and they were not killed.

Experimental Animals

The animals used in this experiment were Welsh-Shetland crossbred foals which were reared indoors with their dams which received repeated doses of fenbendazole. The foals were considered to be helminth-naive and they were between eight and

Table 2.6

Experiment 3 - Design

	Pony No.	Infective Dose	Necropsy
Group A	55, 63, 64	-	-
Group B	40, 43, 45, 46, 48	1 x 450,000 L_3	Week 16
Group C	41, 42, 47, 49, 51	1 x 450,000 L_3 (chilled)	Week 16
Group D	44, 50, 52, 53, 54	18 x 25,000 L_3	Week 20

ten months old at the time of experimental infection. The control animals were of the same age and they were studied by the same methods as infected animals but at a separate period of time.

Animal Management

The foals received supplementary feeding (Spillers Coarse Mix and Equivite Milk Pellets, Dalgetty Agriculture Ltd., Avonmouth) from one week of age in addition to hay ad libitum. Each foal was consuming approximately 1 kg of concentrate per day at time of weaning when four months old. They were kept in groups on straw bedding which was cleared out daily and they were allowed to exercise in a concrete yard. The milk pellets were discontinued when the foals were seven months old and at this time the quantity of concentrate mix was increased to 1.5 kg. per foal per day. Infective larvae were administered via a nasogastric tube.

Measurements and Sampling

From one month prior to infection bi-weekly faecal samples were analysed for worm egg counts. Blood samples were collected twice weekly for haematological analyses and serum, separated and stored at $-40^{o}C$, was subsequently analysed for serum proteins. Daily clinical records were kept of demeanour, appetite, body temperature, faecal consistency and any other signs of gastrointestinal disease.

RESULTS

Clinical Observations

All animals remained bright, appetant and afebrile following experimental infection but a few animals in each group were observed to pass faeces of soft consistency. Two animals which received trickle doses of infective larvae (Group D) passed soft faeces during the period of infection and then intermittently from about 50 days post-infection. Two animals in Group C, which were infected with chilled larvae, passed soft faeces between 25 and 30 days post-infection and these together with a third animal in this group produced soft faeces for a few days between 62 and 69 days post-infection. A similar biphasic pattern of altered faecal consistency was seen in one pony in Group B which received a single dose of normal larvae although the timing was slightly earlier at Day 5 to 15 and then Days 48 to 50 following infection. Another pony in Group B had soft faeces for 16 days from Day 3 post-infection but it was considered to be clinically normal thereafter.

Prepatent Period

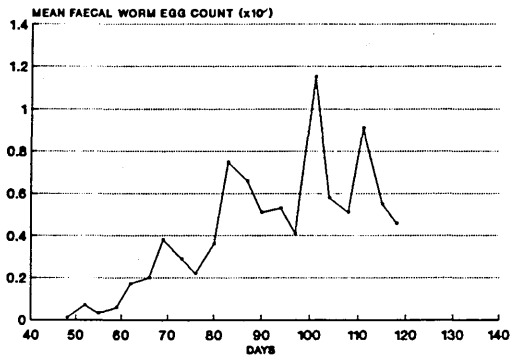
Groups B and C had mean prepatent periods of 53 days with shortest and longest individual values of 48 and 62 days. In Group D, the trickle infection animals, the mean prepatent period was 65 days with a range from 60 to 77 days.

Faecal Worm Egg Counts (Figure 2.6)

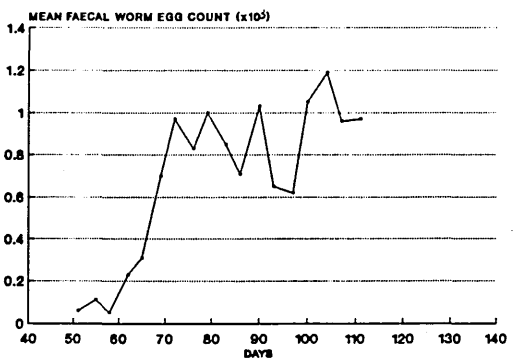
The general patterns of faecal worm egg output were similar in Groups B and C with rises in mean numbers of faecal worm eggs over about a three week period following development of patency

Figure 2.6
Experiment 3: Group Mean Faecal Worm Egg Counts

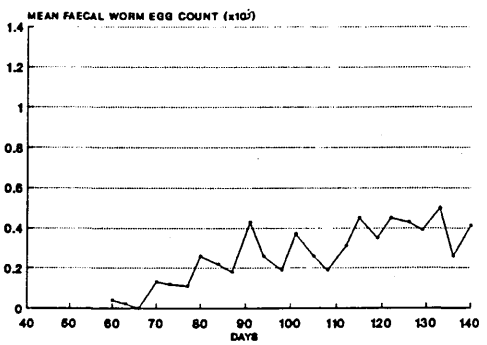
GROUP B



GROUP C



GROUP D



Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

after which the mean worm egg counts in faeces remained between about 400 and 1000 epg. The mean faecal worm egg counts of Group D animals, at approximately 200-300 epg, were lower than the mean values of Groups B and C.

Haematological Profiles

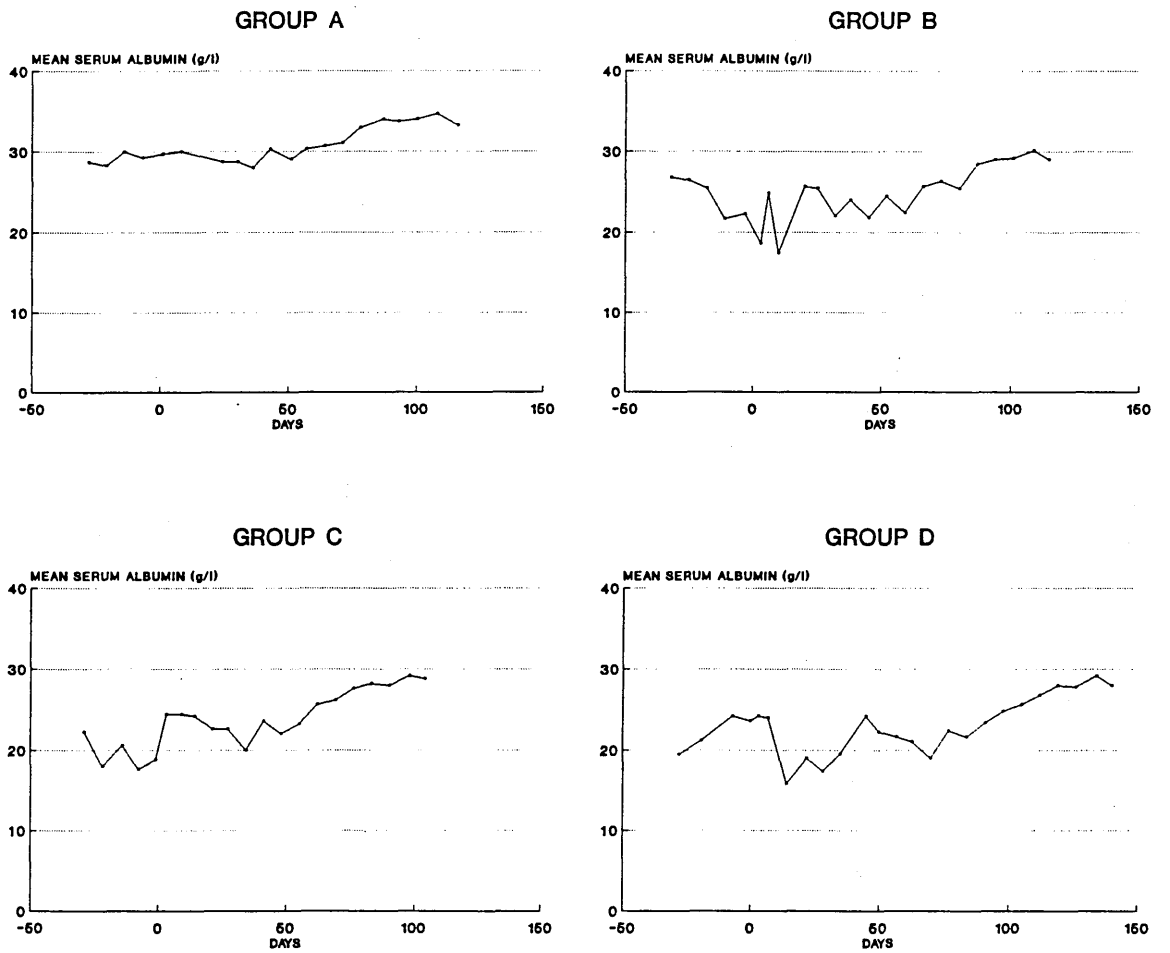
There were no differences observed between the haematological profiles of control and infected groups. Group mean values of total white blood cell, neutrophil, lymphocyte and red blood cell counts together with packed cell volumes are given in Appendices 2.6 -2.10. In the differential analyses eosinophils, monocytes and basophils were found in only very low numbers throughout the study.

Serum Protein Analyses (Figures 2.7 and 2.8)

In the early period of the experiment the mean serum albumin levels of Groups B and C, which received single infections, fluctuated between 17 and 26 g/l without distinct trends until Day 50 at which time the values for both began to gradually rise. In the trickle infected group there were two phases of lowered mean serum albumin levels with minimum values of 17 g/l and 19 g/l at approximately two and ten weeks after first infection followed by a steady increase. The mean values of serum albumin in the control group were fairly constant during the first 50 days after which they steadily rose in a similar manner to the infected groups.

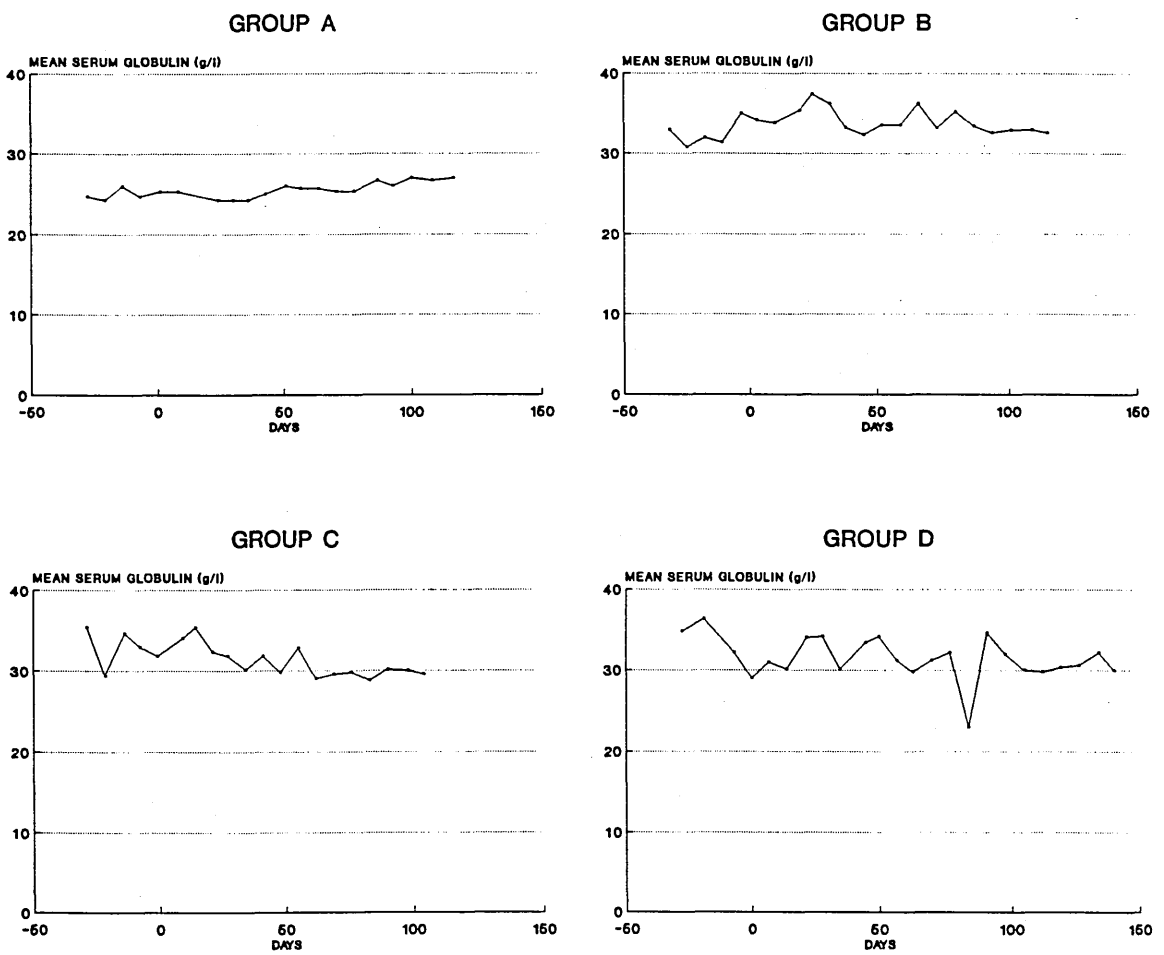
Although there were fluctuations of mean globulin levels of all groups there were no notable changes in response to

Figure 2.7
Experiment 3: Group Mean Values of Serum Albumin



Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

Figure 2.8
Experiment 3: Group Mean Values of Serum Globulin



Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

infection.

Cyathostome Worm Burdens (Table 2.7)

The total cyathostome worm burdens detected post mortem were considerably higher in Group B animals (mean 60,898, range 35,930 - 84,440) than those of ponies in Groups C and D which had mean total counts of 33,592 (range 23,060 - 47,930) and 30,794 (range 23,100 - 39,240) worms respectively. The establishment rates of infective larvae, based on total worm burdens at 16 weeks post-infection as percentages of the infecting doses of 450,000 L_3, were calculated to be 8% to 19% in Group B (single-dose, normal larvae) with a mean of 14% and 5% to 11% in Group C (single-dose, chilled larvae) with a mean of 7%. The comparative values for Group D animals which were killed 20 weeks following first infection were 5% to 9% with a mean of 7%.

The differences in mean total cyathostome burdens and establishment rates were statistically significant ($p < 0.05$) between Group B compared with both Groups C and D but there were no significant differences between values for Group C and Group D.

The numbers of worms present as mucosal stages varied within a similar range of between approximately 6,000 to 26,000 in all groups. Although the group mean mucosal counts were not significantly different, with respective values for Groups B, C and D of 14,806, 13,292 and 14,714 these mucosal burdens constituted varying proportions of the total worm burdens. The highest mean percentage of the total worm count found within the mucosa was found in the trickle infections in Group D, at 49%,

Table 2.7

Experiment 3 - Cyathostome Worm Burdens

	Pony No.	Total Worm Count	% Establishment of Infective Dose	Mucosal Larval Count	% Total Worm Count in Mucosa
Group B	40	84,440	19	11,580	14
	43	35,930	8	5,930	17
	45	58,080	13	16,880	29
	46	54,440	12	14,140	26
	48	71,600	16	25,500	36
Mean		**60,898**	**14**	**14,806**	**24**
Group C	41	32,040	7	14,340	45
	42	47,930	11	15,530	32
	47	23,060	5	7,660	33
	49	40,590	9	19,190	47
	51	24,340	5	9,740	40
Mean		**33,592**	**7**	**13,292**	**39**
Group D	44	37,070	8	21,070	57
	50	23,100	5	16,100	70
	52	39,240	9	11,440	29
	53	29,530	7	16,230	55
	54	25,030	6	8,730	35
Mean		**30,794**	**7**	**14,714**	**49**

Note: i) Necropsy studies - Groups B and C - Week 16 after first infection; Group D - Week 20 after first infection;
ii) % Establishment of infective dose is the total worm count at necropsy expressed as a percentage of the infective dose of larvae;
iii) Group B - 450,000 normal L_3; Group C - 450,000 - chilled L_3; Group D - 450,000 normal L_3 normal L_3 in 18 'trickle' doses.

compared with means of 39% in Group C (single-dose, chilled) and 24% in Group B (single-dose, normal). On statistical analysis of these group mean values there was a significant difference between Group B and Group D ($p < 0.05$) but the value for Group C was not significantly different from either Group B or Group D. All of the mucosal larvae present had a similar gross appearance (Figure 2.9) and were considered to be arrested in development.

Details of the anatomical distribution of cyathostomes detected post mortem in ponies in Experiment 3 are given in Appendix 2.5. In all groups the mucosal worms were found predominantly in the caecum and ventral colon with only negligible numbers in the dorsal colon. Recoveries of lumenal worms were highest from the ventral colon in virtually all animals and generally the dorsal colon contained greater numbers of worms than the caecum. However, in all the animals given the trickle infections (Group D) relatively high proportions of between 17% and 40% of the total counts were within the caecal contents. Also there was a similar distribution of worms within the caeca of some individual animals within both single dose groups.

The cyathostome species present within each region of the large intestine are shown in Figure 2.10. Of the six different species identified, Cylicostephanus longibursatus, Cylicocyclus nassatus and Cyathostomum catinatum were found in every pony and Cylicocylus insigne was present in two thirds of the animals whereas Cylicostephanus minutus and Cylicocyclus brevicapsulatus both only occurred in two individual ponies.

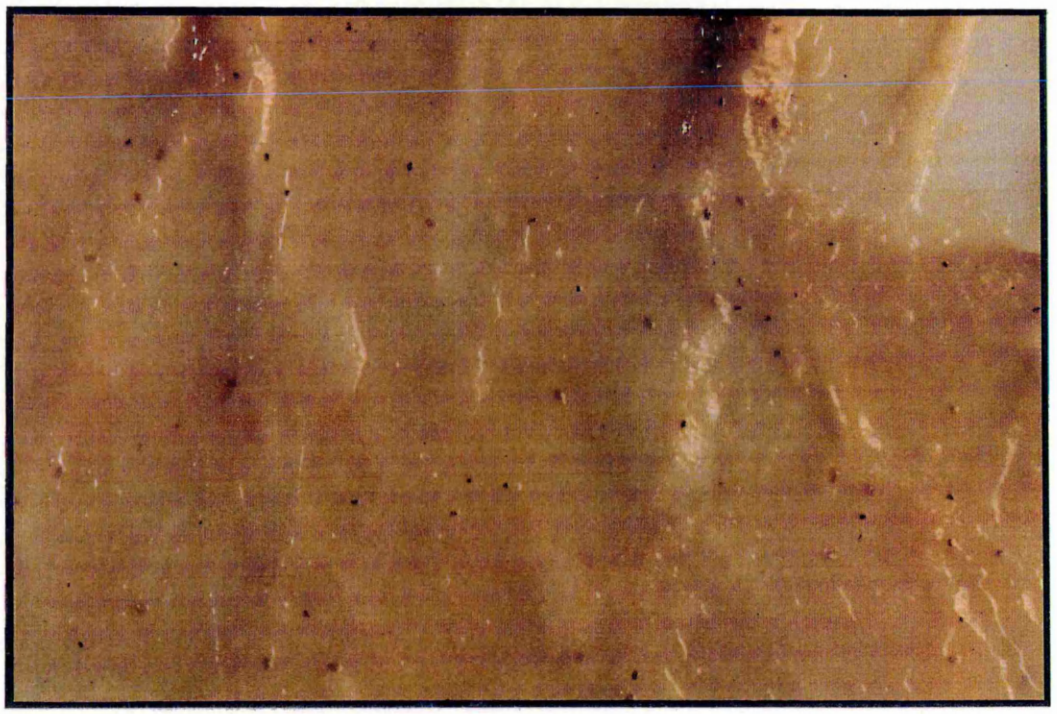


Figure 2.9

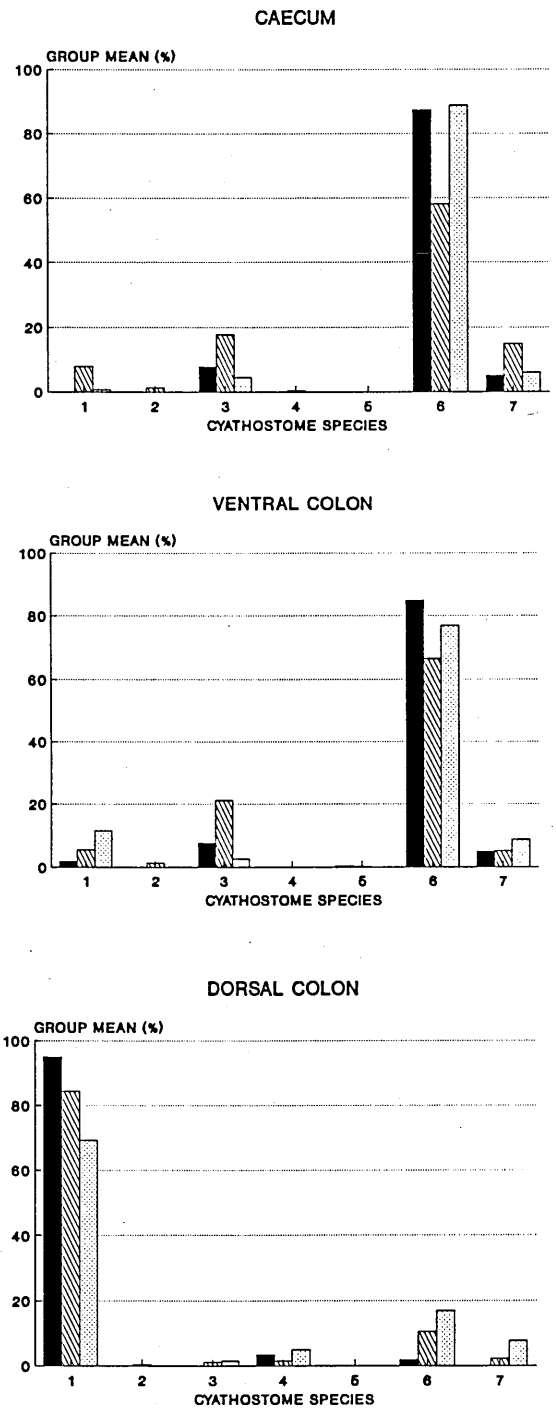
Experiment 3 - Gross appearance of caecal mucosa of Pony No.45 at Week 16 necropsy. All the cyathostome larvae were in arrested development

Species Key to Figure 2.10
and Relative Prevalence of each species

Cyathostome Species	Relative Prevalence		
	Group B	Group C	Group D
1 *Cylicostephanus longibursatus*	5/5	5/5	5/5
2 *Cylicostephanus minutus*	0/5	2/5	0/5
3 *Cylicocyclus nassatus*	5/5	5/5	5/5
4 *Cylicocyclus insigne*	5/5	2/5	3/5
5 *Cylicocyclus brevicapsulatus*	1/5	1/5	0/5
6 *Cyathostomum catinatum*	5/5	5/5	5/5
7 Lumenal larvae (unspeciated)	5/5	5/5	5/5

Figure 2.10

Experiment 3: Anatomical Distribution of Individual Cyathostome Species



Group B: 450,000 normal L3, single dose

Group C: 450,000 chilled L3, single dose

Group D: 450,000 normal L3, trickle dose

DISCUSSION

In Experiment 3 larval chilling and dose frequency appeared to influence faecal worm egg output, parasite establishment rate and the incidence of arrested larval development. Prepatent periods were about two weeks shorter in single-infection ponies compared with those of trickle-infection animals and over the subsequent two months the group mean faecal worm egg counts of the trickle-infected animals were noticeably lower than those of the single infection groups. Larval chilling and trickle-dosing affected establishment rates which were lower in the single-chilled and trickle-infections groups (Groups C and D) at between 5% and 11% compared to 8-19% in Group B which received a single-dose of unconditioned larvae. Although there were clear differences between the group mean percentages of arrested stages with a rank order of: Group B, 24%; Group C, 39%; Group D, 49%, the absolute numbers of mucosal stages were very similar in all groups which suggested that larval chilling and trickle dosing did not directly affect arrested development of cyathostome larvae. During the experiment a few animals from each infected group developed similar clinical signs of mild diarrhoea although none were systemically ill.

The pattern of clinical signs following experimental dosing with 450,000 third-stage larvae were similar in five of the seven affected animals in that two phases of diarrhoea occurred approximately one month apart: in two animals from the trickle-infecion group the second phase was persistent from Day 50 but it was transient between Day 62 and Day 69 in two animals which

received chilled larvae and also from Day 48 to Day 50 in one animal given a single dose of normal larvae. Two animals had only single episodes of diarrhoea: one pony showed signs early after a single infection with normal larvae but was subsequently normal and another passed diarrhoeic faeces for one week from Day 62 following infection with chilled larvae. Although diarrhoea following experimental cyathostome infection has been reported to occur either within a few days of larval dosing or 30-40 days later (Tiunov, 1951; Smith, 1976b; 1978; Bueno and others, 1979) the biphasic pattern of clinical signs observed in several ponies in Experiment 3 has not been described previously. The time course of the clinical phases correlated with the approximate times of larval mucosal penetration and emergence which add to the impression gained in Experiment 2 that either of these events can result in pathophysiological changes within the alimentary tract and lead to diarrhoea.

The mean prepatent period of both single-infection groups was 53 days which was 12 days less than the mean time to patency of the trickle-infected animals. In Experiment 1 yearling and adult ponies had slightly prolonged prepatent periods (approximately 63 days) compared with foals (approximately 55 days) and one trickle-infected animal in Experiment 2 also had delayed patency (80 days). These differences may represent host resistance as a consequence of previous exposure or repeated challenge but they are less pronounced than the protracted patency of 17 to 18 weeks which Smith (1978) recorded in

experimentally reinfected adults.

Following patency the faecal worm egg counts after single infections fluctuated between about 400 and 1000 epg but they were consistently higher in the animals which had received a single dose of chilled larvae. The levels of faecal egg output were lowest in the trickle-infected ponies throughout the experiment. Interestingly, one of the trickle-infected yearlings in Experiment 2 also had markedly lower counts than single-dose animals and it appeared that repeated dosing in these experimental infections moderated the level of faecal egg output in addition to delaying the onset. These findings are in contrast to those of Smith (1976b) who reported that the first animals to develop high faecal worm egg counts were those to which he had administered trickle rather than single infective doses.

The animals given a single dose of freshly harvested larvae had a higher mean total cyathostome burden of 60,898 worms at 16 weeks after infection compared with that of 33,592 found in animals infected with the same number of larvae as a single dose after six weeks storage at $4^{o}C$. A possible explanation of this difference is that the chilled larvae were of reduced viability although equine strongyle larvae have been reported to survive prolonged periods of cold or even freezing temperatures (Ogbourne and Duncan, 1984).

The mean total cyathostome burden at 20 weeks after first infection in animals given freshly harvested larvae by trickle dosing was 30,794 worms which was approximately half of that

found in ponies given the same total dose as a single infection. There are no pre-existing data from equine studies with which to compare this finding but it was taken to reflect a partial resistance to infection in these animals although it cannot be ascertained whether this arises due to decreased establishment rate or increased expulsion rate of the experimental infection.

With regard to the number of larvae arrested in development, both the ranges of individual mucosal burdens and group mean mucosal burdens were similar for all groups but these constituted quite different proportions of the total worm counts. As the establishment rates in this study were apparently affected by both larval conditioning and trickle dosing these variables were therefore having an indirect effect on arrested larval development. Both Gibson (1953) and Smith (1976a) considered that there was evidence of a feedback mechanism from lumenal worms to inhibit the further development of mucosal larvae and in the present study differential worm counts expressed in terms of proportion of arrested stages against total burdens were considered to be a useful index of the population dynamics of cyathostome infections.

Generally the anatomical distribution of cyathostomes in Experiment 3 was similar to that of both Experiments 1 and 2 and also to those previously described (Ogbourne, 1976; Reinemeyer and others, 1984; Mfitilodze and Hutchinson, 1985) with a preponderance of mucosal stages in the anterior regions of the large intestine but with the majority of lumenal stages

established more distally. However, in some animals, including all of those in the trickle-infection group, there were quite high proportions of the lumenal burdens within the caecum. In these animals this was taken to reflect slower rates of larval development since it has been suggested that migration from the caecum to more distal sites occurs as cyathostomes develop to maturity (Reinemeyer, 1986). In Experiment 3 there was evidence of regional variation of the prevalent individual cyathostome species, with both Cyathostomum catinatum and Cylicocyclus nassatus more numerous in the caecum and ventral colon whereas Cylicostephanus longibursatus was found mainly within the dorsal colon which is similar to findings in previous reports (Ogbourne, 1978; Mfitilodze and Hutchinson, 1985).

In the present study, following experimental infections with cyathostome larvae, there was no evidence of cellular haematological responses such as the leucocytosis and anaemia detected in foals by Tiunov (1951) or the eosinophilia recorded in older ponies by Smith (1976b; 1978). However, changes in the serum proteins were observed following infection, although to a certain extent the interpretation of their significance was complicated by the fact that pre-infection levels of serum albumin were relatively low in all groups except the controls. The control animals were studied by the same methods as the infected ponies but at a different period of time which may account for the differences in serum albumin levels. In the trickle-infected group, the mean serum albumin dropped sharply from 24 g/L to 16 g/L within two weeks of first infection and the

hypoalbuminaemia persisted during the time that the animals were continuing to receive trickle doses after which time the mean values increased. There was then a second phase of lowered albumin levels between Day 45 and Day 70. These separate periods of decreased serum albumin levels coincided approximately with times of either larval penetration into or larval emergence from the intestinal mucosa and were also coincident with the episodes of diarrhoea in the two animals in this group which were clinically affected. The trends in serum albumin values observed in the present experiment are similar to those described by Round (1970) although the hypoalbuminaemia in his study was more persistent after primary infection and a biphasic pattern was not detected in the transient hypoalbuminaemia which followed reinfection of the same ponies.

The trends in serum globulin levels reflected increases in the betaglobulin fraction of several, but not all of the infected animals. These variable serum globulin responses between individuals following infection in the present experiment were generally comparable with those reported by Round (1970): in the latter study raised globulins were first detected about five weeks after primary cyathostome infection and then persisted for several months but occurred almost immediately when animals were reinfected. Bailey (1987) has suggested that the globulin responses which have been reported following either experimental or naturally acquired cyathostome infections were probably due to the presence of larvae in the mucosal phase and the trends

observed in the present experiment lend support to the theory that the histotrophic population of larvae may initiate globulinaemia.

In a study of serum protein changes in ponies on different parasite control programmes, Herd and Kent (1986) reported that major serological responses were only detected in young animals and that these apparently bore no relationship to increasing levels of pasture infectivity. These authors observed only minor changes in the serum proteins of mature ponies in the same study and in further considering the wide variations which occurred between individuals, together with the lack of specificity of the responses, they concluded that the study of serum protein responses had limited application in the study of equine intestinal parasitism. In the present study, the interpretation of host responses to cyathostome infection by studying changes in serum proteins was similarly compromised by marked variability between individuals.

From the results of Experiment 3 it was concluded that there were important effects on cyathostome population dynamics consequent to administering repeated, trickle larval doses or single doses of chilled larvae compared with the dynamics following single doses of unconditioned larvae. The apparent effects of trickle dosing were to delay patency, to reduce subsequent level of faecal worm egg output and to reduce parasite establishment/survival rate within the host whereas reduced establishment and/or survivability was the only effect attributed to larval chilling. The main objective of this study was to

investigate trickle larval dosing and larval chilling as possible factors contributing to arrested cyathostome development. Although both were demonstrated to increase the relative proportions of the total burdens which arrested in their development, in both instances this could have resulted from reduced parasite establishment and/or survivability within hosts rather than being direct, primary stimuli for inhibition of larval development.

CHAPTER THREE

STUDIES ON THE PATHOGENIC EFFECTS OF CYATHOSTOME INFECTION IN PONIES

CHAPTER THREE

STUDIES ON THE PATHOGENIC EFFECTS OF CYATHOSTOME INFECTION IN PONIES

INTRODUCTION

The first detailed report of the clinical syndrome of acute larval cyathostomiasis was from the USSR in the early fifties when Velichkin (1952) recorded the severe clinical and pathological consequences of massive cyathostome infections in a large group of horses. Young adult horses (one to three years old), were most severely affected and the clinical signs of marked progressive weight loss, chronic diarrhoea, intermittent pyrexia, limb oedema, weakness, debility with eventual death occurred during late spring and early summer.

More recent accounts from Western Europe describe similar findings in cases admitted to referral centres (Mirck, 1977; Giles and others, 1985) and although there have been other contemporary reports of cases of severe clinical disease associated with cyathostome infection from both UK (Blackwell, 1973; Chiejina and Mason, 1977; Jeggo and Sewell, 1977; Church and others, 1986) and the USA (Jasko and Roth, 1984; Harmon and others, 1986; Reinemeyer and Powell, 1986) the frequent post-mortem finding of very large burdens of adult parasites in apparently healthy horses has led to the general conclusion that cyathostomes are of low or limited pathogenicity.

Uhlingher (1988) has conducted a longitudinal study on the incidence of colic in groups of horses under different regimens

of anthelmintic prophylaxis. The conclusion of this work was that there was a higher incidence of colic in groups where the parasite control programme failed to control cyathostomes. Although this observation provides circumstantial evidence of a pathogenic effect of cyathostomes it is by no means conclusive since the association between the colic cases and cyathostomes is presumptive. There are, however, some grounds for believing that colic may be a consequence of cyathostome infection since in the experimental studies performed by Bueno and others (1979) it was considered that small strongyles contributed to the disruptions of enterocolic motility which were detected in ponies with natural or experimental mixed strongyle infections.

One of the difficulties in settling the issue of the true pathogenic importance of cyathostomes is the lack of suitable criteria on which to base a judgement.

Effects of parasites on 'productivity' are less applicable and less easily appreciated in equine husbandry than in farm animal enterprises. For example production measurements for ruminants such as food conversion ratio, milk yield or carcass quality are inappropriate for the assessment of effects of parasites in the horse. Although poor live weight gains have been recorded in natural strongyle infections (Round, 1968; Frerichs, Holbrook and Allen, 1976) rapid growth and fattening are not usually a planned or desirable goal of equine management systems and so decreased rate of gain may only be noticed if it is grossly retarded.

Alternative indices of the pathogenic effect of animal disease frequently used in clinical medicine are haematological and biochemical analyses of blood and plasma. Such analyses in the clinical disease of cyathostome colitis have shown that affected animals are commonly hypoalbuminaemic (Blackwell, 1973; Mirck, 1977; Giles and others, 1985) which is presumed to be a consequence of increased intestinal plasma protein losses as a result of mucosal damage. Hypoalbuminaemia has also been recorded in experimental cyathostome infection (Round, 1970). Some loss of whole blood may also occur at mucosal sites of adult small strongyle feeding or larval emergence and by incidental ingestion during feeding but anaemia is neither a consistent finding in equine strongylosis (Round, 1968; Ooms, Oyaert, Muylle, Van den Hende and Decraemere, 1976) nor a consistent feature of cyathostomiasis (Giles and others, 1985).

In the assessment of the pathogenic impact of cyathostomes the simple measurement of haematological values or plasma protein profiles on single or sequential samples may be adequate to detect abnormalities if severe enteropathy exists. However, in subclinical disease, increases in catabolism of red blood cells or albumin may be compensated for by increased synthesis such that straightforward measurement of red cell numbers or albumin concentrations will give results within normal ranges. Thus they are relatively unreliable indicators per se of underlying dynamic pathogenic processes.

It is possible to obtain more accurate information on host response to parasitism by studying red blood cell and plasma

protein metabolism using radioisotope tracer techniques. These methods have been fairly widely used for this purpose in studies of the pathophysiological effect of a wide variety of gastrointestinal nematode, trematode and protozoan infections, particularly in sheep but also in cattle and laboratory animals. The principles and application of radioisotope tracer techniques have been reviewed by Dargie (1975) in relation to helminth infections in sheep and Holmes (1987) gives an overview of the knowledge on parasitic infections derived from such studies in various host species.

There are only two reports of the use of radioisotopic methods in experimental studies of equine parasitism: Duncan and Dargie (1975) reported studies of red blood cell and albumin turnover in strongyle infections and later the same group applied these techniques to investigate the pathophysiological changes resulting from infection with Parascaris equorum in foals (Clayton, Duncan and Dargie, 1980). In clinical equine gastroenterology several authors have described the application of radiotracer methods in investigation of cases of chronic enteropathy (Merritt, Cimprich and Beech, 1976; Merritt, Kohn, Rumberg, Cimprich, Reid and Bolton, 1977; Meuten, Butler, Thomson and Lumsden, 1978; Dietz and Nielsen, 1980) and the technique has also been used in drug toxicity studies in horses and ponies (Snow, Douglas, Thompson, Parkins and Holmes, 1981).

The basis of radioisotopic tracer studies is conceptually simple: animals are injected with a known quantity of a substance

which has been labelled with a radioactive isotope and then the concentration of the donor preparation in subsequent samples of blood, urine or faeces is calculated by analysis of their radioactivity in order that the "movement" of the injected material may be traced. The specificity of isotope labels for the study of different pathophysiological changes varies. For example Iodine-labelled albumin is suitable for assessing protein metabolism whereas Chromium-labelled protein is more appropriate for the investigation of loss of plasma protein into the gastrointestinal tract. By using several isotopes concurrently a composite study of a single disease may be made since each label may be analysed separately by counting the activity of samples in specific energy spectra.

Nuclear techniques have been useful in investigating the pathophysiological changes of enteropathies in horses and other domestic species and the objective of the experiments reported in this section was to apply them to the investigation of the pathogenic importance of Cyathostomum spp.

EXPERIMENT 4

Plasma Protein and Red Blood Cell Metabolism in Ponies with Naturally Acquired Cyathostome Infection

INTRODUCTION

The objective of this experiment was to investigate the effects of naturally-acquired cyathostome infection of ponies on plasma protein and red blood cell metabolism for which purpose three radioisotope-labelled preparations were used, namely ^{125}Iodine-labelled albumin (^{125}I-Albumin) ^{51}Chromium chloride ($^{51}CrCl_3$) and ^{59}Ferric citrate (^{59}Fe-citrate).

Albumin labelled with ^{125}Iodine can be used to accurately calculate:-

i) plasma volume by applying the 'dilution principle';

ii) the intravascular pool of albumin (CA) by multiplying the plasma volume by the albumin concentration in the plasma;

iii) the rate of catabolism of albumin which is the proportion of CA broken down daily - usually referred to as the fractional catabolic rate or F(CA).

It has been assumed that the hypoalbuminaemia of cyathostomiasis is due to protein loss into the intestine. Unfortunately this loss cannot be investigated using ^{125}I-albumin because some of the label is reabsorbed from the intestine after enzymatic splitting from the protein (Mulligan, 1973). In order to study enteric protein losses it is necessary to use either a compound which is not degraded or a label which is not reabsorbed, such as ^{51}Chromium. This isotope can be injected

intravenously as $^{51}CrCl_3$ after which it labels, *in vivo*, endogenous plasma proteins - mainly plasma albumin. By dividing the total ^{51}Cr radioactivity counted in 24 hour faecal collections by the activity per ml of plasma at the start of the collection period it is possible to calculate the faecal clearance of plasma and so the rate of plasma protein loss into the intestine.

Although anaemia is not considered a feature of cyathostomiasis (Giles and others, 1985), it has been reported as a non-specific finding in horses with mixed strongyle infection (Round, 1968; Ooms and others, 1976) and the inclusion of ^{59}Fe citrate in this experiment was to study various aspects of red blood cell synthesis and also faecal blood clearance.

MATERIALS AND METHODS

Experimental Design

The design of Experiment 4 is summarised in Table 3.1. Two clean control animals and six naturally infected animals were each injected with ^{125}I-albumin (12 MBq), $^{51}CrCl_3$ (37 MBq) and ^{59}Fe-citrate (9 MBq) via the catheterised left jugular vein on Day 1. On Day 6 the control animals and two of the infected animals were treated orally with ivermectin at a dose rate of 0.2 mg/kg body weight. All the animals were killed and examined post-mortem on Day 14 when measured samples of intestinal mucosa and contents were collected to enable calculation of total larval and adult worm burdens.

Table 3.1

Experiment 4 - Design

	Group A	Group B	Group C
Pony nos.	1, 2	5, 8	4, 6, 7
Day -11 to -6	Fenbendazole 7.5 mg/kg	-	-
Day 1	Isotopes	Isotopes	Isotopes
Day 6	Ivermectin 0.2 mg/kg	Ivermectin 0.2 mg/kg	-
Day 14	Necropsy	Necropsy	Necropsy

Group A - Control

Group B - Infected-treated

Group C - Infected

Experimental Animals

The animals used in this experiment were all mixed, native-breed colt foals which had been reared outdoors with their dams. The animals were from two separate herds and no specific information on the nature of their grazing management was available. Ponies Nos. 2, 6, 7 and 8 were obtained, newly-weaned, from the same source and they were of similar age (approx. five months) and of comparable weight (79 - 97 kg). The remaining ponies were both older (approx. eight months) and heavier (119 - 196 kg).

All of the ponies had a perineal urethrostomy created under general anaesthesia one week prior to the start of the experiment to enable the placement of indwelling Foley balloon catheters.

The clean control animals (Nos. 1 and 2) were treated with fenbendazole at 7.5 mg/kg body weight on five consecutive days (Day -11 to Day -6) before the experiment.

Animal Management

The ponies were tethered in individual stalls on rubber matting for a period of two weeks prior to the experiment where they remained until killed on Day 14. Hay and water was available ad libitum to all animals but consumption rates were not recorded. No other feeding was given.

All ponies were given an oral daily dose of 10 ml of 0.75% potassium iodide solution from Day -4 to Day 14 of the experiment in order to reduce thyroid uptake of ^{125}Iodine and thereby ensure rapid excretion of the radioisotope (Dargie, 1975).

Measurements and Sampling

Urine

All ponies were fitted with a harness constructed from 6 cm rubber webbing. A canvas bag was suspended on the harness at the rear of the pony in which plastic containers for urine collection were placed. The urine containers were three litre Uromatic single dose containers (Travenol Laboratories Ltd., Thetford, Norfolk) which were connected to the urinary Foley catheters (diameter, 7.33 mm/22 Ch; length, 40 cm; balloon volume, 30 cc) by a 10 cm section of 6 mm diameter polythene tubing.

Foley catheters were inserted into the urinary bladder via the perineal urethrostomy observing sterile precautions. The balloon of the end of the catheter was then inflated with 30 ml of sterile water.

The urine collection system was checked frequently for harness movement and catheter leakage (which were uncommon) and adjustment, balloon inflation or catheter replacement were performed as necessary.

The weight of urine collected in each 24 hour period was recorded. After thorough mixing, two samples of 1 ml were taken by syringe into counting vials and weighed accurately.

Faeces

Faeces produced by the ponies were allowed to fall onto the rubber matting on the floor. Four times daily subsamples (handfuls) were taken from the top of the faecal pile, bulked, weighed, thoroughly mixed and five samples of approximately 10 g

were compressed into counting vials. Individual samples were accurately weighed. The remainder of the faecal output was bulked and weighed so that a total daily faecal output could be determined for each animal.

Blood

Blood samples of 10 ml were collected in heparinised, vacuum tubes via 1", 21 g needles.

For the first four days of the experiment all samples were taken from the right jugular vein and thereafter sampling was from alternate jugular veins.

On Day 1 blood samples were taken at 15, 30, 60, 90, 120, 180 minutes after injection and then at 4 p.m. Subsequently, twice daily samples were taken at 10 a.m. and 4 p.m. until Day 5 when only daily (10 a.m.) sampling was carried out.

RESULTS

PCV, Plasma Volumes and Blood Volumes (Table 3.2)

The group mean values for plasma volume were not significantly different. Two of the infected ponies (Nos. 4 and 7) had plasma volumes which were notably higher than the other infected animals and also than the controls.

PCV values ranged between 20% and 31%. Both of the infected-treated animals had low PCVs with correspondingly low mean blood volumes and red blood cell volumes.

Albumin Metabolism (Tables 3.3 and 3.4)

There was some variation in serum albumin levels both between individuals and in sequential samples from the same

Table 3.2

Experiment 4 - Body Fluid Volumes

Pony no.	Plasma Volume ml/kg	PCV %	Blood Volume ml/kg	Red Blood Cell Volume ml/kg
Group A				
1	46.0	31.0	66.6	20.6
2	43.5	21.5	55.4	11.9
Mean	44.8	26.3	61.0	16.3
Group B				
8	43.8	20.0	54.5	10.7
5	46.3	20.0	60.2	13.9
Mean	45.1	21.5	57.4	12.3
Group C				
4	54.1	30.5	77.8	23.7
6	39.5	27.0	54.1	14.6
7	52.3	23.5	68.4	16.1
Mean	48.6	27.0	66.6	18.1
Combined mean for Groups B and C	47.2	24.8	63.6	15.8

Group A - Control

Group B - Infected-treated

Group C - Infected

Table 3.3

Experiment 4 - Albumin Distribution

Pony no.	Plasma Volume ml/kg.bwt.	Serum Albumin* g/l	CA g/kg.bwt	EA^+ g/kg.bwt	TA^+ g/kg.bwt
Group A					
1	46.0	31	1.43	2.50	3.93
2	43.5	23	1.00	1.85	2.85
Mean	44.8	27	1.22	2.18	3.39
Group B					
8	43.8	33	1.45	3.15	4.60
5	46.3	23	1.06	2.07	3.13
Mean	45.1	28	1.26	2.61	3.87
Group C					
4	54.1	40	2.16	3.01	5.17
6	39.5	24	0.95	2.01	2.96
7	52.3	29	1.52	3.43	4.95
Mean	48.6	31	1.54	2.82	4.36
Combined Mean for Groups B and C	47.2	29.8	1.43	2.73	4.16

* Day 0 values

TA^+, EA^+ - extrapolation method

Group A - Control

Group B - Infected-treated

Group C - Infected

Table 3.4

Experiment 4 - Albumin Catabolism of Ponies

Pony no.	Body Wt kg	Serum Total Protein g/l	Serum Albumin g/l	^{125}I half-life hrs	CA g/kg.bwt	Mean F(CA)	Absolute Albumin Catabolism g/kg.bwt/day
Group A							
1	187	82.3	31.7	359	1.43	0.063	0.09
2	82	70.0	29.3	378	1.00	0.074	0.07
Mean	134.5	76.2	30.5	369	1.27	0.069	0.08
Group B							
8	94	66.0	31.7	317	1.45	0.081	0.12
5	116	61.6	27.0	318	1.06	0.079	0.08
Mean	105	63.8	29.4	318	1.26	0.080	0.10
Group C							
4	155	76.6	40.7	341	2.16	0.057	0.12
6	89	58.3	25.0	291	0.95	0.079	0.08
7	76	67.0	33.7	296	1.52	0.057	0.09
Mean	106.6	67.3	33.1	309	1.54	0.064	0.10

Group A - Control
Group B - Infected-treated
Group C - Infected

individuals during the two weeks of the study (Appendix 3.1) although there were no significant differences in the mean values for each group. The serum albumin level of pony no. 6 was consistently low and it also had relatively low intravascular (CA) and total body albumin (TA) values, though these were comparable with those of pony no. 2 (control) and pony no. 5 (infected-treated) which also had similar serum albumin concentrations at the beginning of the experiment. The intravascular albumin pool of Pony no. 4 was considerably larger than the other animals and it also had the highest TA level (calculated by the extrapolation method of Sterling, 1951).

Both groups of parasitised ponies had mean body albumin pools which were larger than those of the uninfected control animals. The values in Table 3.3 were derived from results obtained prior to separate treatments of the infected ponies and so the combined mean values of these five animals were also calculated.

There were no significant differences between groups in either the mean fractional catabolic rates (F{CA}) or the mean absolute amounts of albumin catabolised per day but mean half-life values of labelled albumin were less in both infected groups (Group B - 318 hours, Group C - 309 hours) than that of the control group (Group A - 369 hours). The F(CA) values calculated on each day of the experiment are listed in Appendix 3.2. In all animals the catabolic rates calculated during the first five days of the experiment were high.

Faecal Clearance of Plasma and Blood (Table 3.5)

Group C animals had higher mean values of both plasma and blood clearances than Groups A and B though these differences were not statistically significant. Appendices 3.3, 3.4 and 3.5 contain the faecal plasma and blood clearances on a daily basis. The calculation of faecal plasma loss by ^{125}I activity gave values which were markedly less than those obtained by using ^{51}Cr faecal counts.

Red Blood Cell Metabolism (Table 3.6)

Plasma ^{59}Fe half-life values varied from 96 - 241 hours and the mean value of the control animals was less than that of either of the infected groups and the other variables calculated also indicated more rapid red blood cell metabolism in the control, compared with infected groups but these differences were not significant. Serum iron levels were between 23.9 and 74.5 ug/100 ml.

Worm Burdens

The total worm burdens present in Group C animals were low. All of the parasites examined were cyathostomes and the majority were adult stages within the gut lumen (pony no. 4 - 12,850; pony no. 6 - 11,540; pony no. 7 - 2,190). The Group B ponies were assumed to have similar burdens to Group C initially although since they were treated on day 6 of the experiment they were virtually clean at post mortem. No parasites were found in the intestinal mucosae or contents of the control animals. Details of cyathostome worm burdens are given in Appendix 3.6. With the exception of Pony no. 1 and Pony no. 5, there was evidence of

Table 3.5

Experiment 4 - Mean Daily Plasma and Blood Clearances of Ponies

Pony no.	Mean Faecal Plasma Clearance (^{51}Cr) ml/kg/day	Mean Faecal Blood Clearance (^{59}Fe) whole blood ml/kg/day	Mean Faecal Blood Clearance (^{59}Fe) RBC ml/kg/day
Group A			
1	1.2	0.21	0.08
2	1.6	0.13	0.03
Group Mean	1.4	0.17	0.06
Group B			
8	1.5	0.10	0.03
5	1.8	0.18	0.05
Group Mean	1.65	0.14	0.04
Group C			
4	1.4	0.22	0.07
6	2.0	0.33	0.10
7	2.0	0.19	0.06
Group Mean	1.8	0.25	0.08

Group A - Control

Group B - Infected-treated

Group C - Infected

Table 3.6

Experiment 4 - Red Blood Cell Metabolism of Ponies

Pony no.	PCV %	Serum Iron ug/100 ml	^{59}Fe Plasma half-life mins.	Plasma Iron Turnover Rate mg/kg bwt/day	Percentage Iron Utilisation %	Red Cell Iron Incorporation Rate mg/kg bwt/day
Group A						
1	31.0	42.6	135	0.144	10.5	1.512
2	21.5	45.2	135	0.145	9.8	1.421
Mean	26.3	43.9	135	0.145	10.2	1.467
Group B						
8	20.0	47.8	189	0.110	7.2	0.792
5	23.0	27.9	96	0.134	8.9	1.193
Mean	21.5	37.9	143	0.122	8.1	0.993
Group C						
4	30.5	23.9	127	0.102	11.6	1.183
6	27.0	34.6	233	0.058	7.2	0.418
7	23.5	74.5	241	0.161	8.9	1.433
Mean	27.0	44.3	200	0.107	9.2	1.011

Group A - Control
Group B - Infected-treated
Group C - Infected

infection with Strongylus vulgaris and Strongylus edentatus in the cranial mesenteric arteries and flanks of all of the ponies.

DISCUSSION

Various authors have published values for plasma volume of normal horses calculated following injection of radiolabelled equine albumin (Mattheeuws, Kaneko, Loy, Cornelius and Wheat, 1966; Duncan and Dargie, 1975; Merritt and others, 1977; Clayton and others, 1980; Dietz and Nielsen, 1980). Although the radioisotopes and methods used by each were not identical, the mean values obtained were all very similar, ranging from 39.2 ml/kg to 47.0 ml/kg, with lowest and highest individual animal values of 34.7 ml/kg and 51.5 ml/kg. The plasma volumes calculated in the control ponies in Experiment 4 were of a similar order to these published values ie. 46.0 ml/kg and 43.5 ml/kg and only two of the infected ponies had plasma volumes exceeding these levels (pony no. 4 - 54.1 ml/kg; pony no. 7 - 52.3 ml/kg). Since increased plasma volumes are commonly observed in gastrointestinal parasite infections in ruminants it is possible that the higher values detected in ponies Nos. 4 and No. 7 represent pathophysiological responses to cyathostomes. However, these values did not correlate closely with the size of worm burdens (pony no. 4 - 12,850; pony no. 7 - 2,190).

Values for mean blood volumes of normal horses given by Mattheeuws and others (1966) and by Duncan and Dargie (1975) were 71.3 ml/kg and 62.1 ml/kg respectively which compare acceptably with the control values of 66.6 ml/kg and 55.4 ml/kg obtained in

this study. There is also a range in reported mean values for equine total (TA) and intravascular (CA) albumin pool sizes in normal horses from TA of 2.03 g/kg and CA of 0.81 g/kg (Clayton and others, 1980) to TA of 5.69 g/kg and CA of 1.47 g/kg (Mattheeuws and others, 1966); these values were obtained by extrapolation. The two animals (pony nos. 4 and 7) in Experiment 4 which had values for intravascular albumin pool sizes outwith this range (pony no. 4 - 2.16 g/kg.bwt; pony no. 7 - 1.52 g/kg.bwt) were those with expanded plasma volumes and they also had serum albumin levels which were high (pony no. 4 - 40 g/l) or normal (pony no. 7 - 29 g/l).

The methods by which blood volumes and body albumin pools were calculated in the present experiment are multiplication equations of plasma volumes, using PCV and serum albumin values respectively. There was a wide range of both of these parameters within each group and thus the calculated values of blood volumes and pool sizes were quite diverse. For example, both the highest and the lowest values of blood volume were in ponies in Group C (pony nos. 4 and 6) which in fact had similar worm burdens. These animals also had CA and TA pool sizes at either end of the range of 'normal' values.

Although changes in albumin distribution or hypoalbuminaemia may occur in a disease state this is not invariably the case since it is possible, by increased protein synthesis, for the body to compensate for hypercatabolism of albumin. A better indicator of altered protein metabolism is the rate of albumin turnover which can be assessed by the half-life

or fractional catabolic rate F (CA) of albumin. In the present experiment the results are somewhat paradoxical in that although there were no differences in F(CA) values between groups, the albumin half-life values were shorter in the infected animals. All of the animals in this experiment had albumin half-life values which were less than those given by other authors, who report values of about 20 days. This difference may be due to partial denaturation of the labelled-albumin or to impure albumin due to, for example, contamination by globulins. Some additional support for this explanation exists in that the catabolic rates of albumin in the first five days of the experiment were abnormally high. However, the mean F (CA) values were calculated from the daily values after the sixth day at which time there would be minimal effect of poor quality protein. If there was impurity or denaturation of ^{125}I-albumin then the plasma volumes calculated by the dilution principle will have been overestimates: as already discussed the control ponies had plasma volumes at the upper end of the range of quoted values for normal horses and two infected ponies (pony nos. 4 and 7) had higher levels than the controls.

Accurate daily total excretion of ^{125}I-albumin could not be calculated for two animals (pony no. 2, day 2; pony no. 5, day 10) and so the equilibrium-time method of calculating total body albumin was not used in Experiment 4.

The product of mean F(CA) and CA is the absolute amount of albumin catabolised each day. In this experiment, there was

close similarity in these values for all animals and they also correspond well to control values given by previous workers (Duncan and Dargie, 1975; Clayton and others, 1980; Dietz and Nielsen, 1980).

Faecal clearances of plasma or blood are defined as the amounts of plasma or blood which would have to leak into the gastrointestinal tract in order to account for the faecal radioactivities present. There were two interesting aspects of the faecal plasma clearance values measured in this experiment. First, the infected animals had slightly higher plasma clearances (using ^{51}Cr) than control ponies which might reflect that there was an increase in mucosal permeability, which could be a result of cyathostomes feeding on the gut wall. However, from the preceeding discussion it is evident that the observed differences in plasma loss were not associated with detectable alterations in albumin catabolism.

Secondly, the clearance values which were derived from faecal ^{125}I-activity were lower than those obtained by counting ^{51}Cr-activity. This difference can be explained by enzymatic breakdown of radioiodinated albumin in the intestinal lumen with reabsorption of the label and hence the plasma loss calculated from ^{125}I-activity is an underestimate. The plasma clearances reported by Duncan and Dargie (1975) and Clayton and others (1980) were estimated by measurement of ^{125}I-activity in faeces and although they were not expressed on a body weight basis they appear to have been of similar magnitude to the ^{125}I clearances of albumin in Experiment 4. Merritt and others (1977) calculated

plasma clearances in five normal horses using ^{51}Cr-albumin and obtained a mean value of 0.67 ml/kg.bwt/day, which is substantially less than that of 1.4 ml/kg.bwt/day obtained in control animals in this experiment using $^{51}CrCl_3$. This may well have been due to loss of ^{51}Cr label from the albumin after injection. These differences within and between experiments serve to emphasise that clearance values are indices of plasma loss into the gut rather than absolute quantities.

With regard to faecal blood clearance and red blood cell metabolism there were no significant differences between control and infected animals. The blood clearances obtained here from ^{59}Fe-citrate were comparable with values obtained in the control ponies of Duncan and Dargie (1975) using ^{51}Cr-labelled red blood cells. The serum iron levels (23.9 - 74.5 ug/100 ml) were extremely low compared with normal mean values of 106 ug/100 ml in Shetland ponies given by Osbaldiston and Griffith (1972). Haematological profiles were not obtained in these animals so it was not ascertained whether the animals were anaemic in association with their low serum iron levels. Some indication of anaemia was available from the PCV values which were low normal or low in all animals. There are no published results of studies of plasma iron turnover rate, percentage iron utilisation or red cell iron incorporation rates in horses.

The infected ponies in this experiment were found to have low parasite burdens at necropsy. Therefore, it was not unexpected that there were only minor differences in red blood

cell and plasma protein metabolism between these animals and the controls. Also, since the numbers of animals in each group were small and there were inevitably some individual variations, subtle differences were likely to be masked when group results were averaged. Furthermore, there are only a few papers (also based on small numbers of animals) to which one can refer for 'normal' values and these must be compared with caution due to differences in experimental methods.

In summary, from the results of Experiment 4 it was concluded that even very low burdens of adult cyathostomes may increase plasma losses into the intestine but that this level of parasitism did not affect the metabolism of either albumin or red blood cells. The results confirmed the suitability of radioisotope tracer techniques for the investigation of some pathogenic effects of intestinal parasitism in horses and they provided useful baseline data for further studies.

EXPERIMENT 5

Plamsa Protein and Red Blood Cell Metabolism in Ponies with Experimental Infection

INTRODUCTION

In studies of equine strongyle epidemiology (Ogbourne, 1976; Hass, 1979; Reinemeyer and others, 1984; 1986) it has been observed that animals can commonly harbour cyathostome burdens of several hundred thousand worms without any apparent clinical effect. Although the reports of clinical cyathostomiasis indicate that large numbers of cyathostomes have been identified at post mortem examinations, most authors have not given quantitative counts. In his description of the pathology of the condition in three horses, Velichkin (1952) recorded that 30-50 encysted cyathostome larvae per cm^2 of mucosa were found in the large intestine. Chiejina and Mason (1977) gave details of the worm burden of a single diarrhoeic mare which had a total of 590,000 cyathostomes of which 77% were immature stages.

In Experiment 4 the worm burdens of the ponies were low (2,190 - 12,850) and so, despite the technical success of the radioisotope tracer methods, no major conclusions could be made with regard to the pathogenicity of cyathostomes. The objective of Experiment 5 was to repeat the measurements of red blood cell and protein metabolism in a group of ponies which were parasitised at a level similar to that commonly encountered in the field.

MATERIALS AND METHODS

Experimental Design

The design of Experiment 5 is summarised in Table 3.7. Six animals, three clean controls (Group A) and three experimentally cyathostome-infected (Group B), were injected with ^{125}I-albumin (15 MBq), $^{51}CrCl_3$ (74 MBq) and ^{59}Fe-citrate (6 MBq) via catheterised left jugular veins on Day 1. All the animals were killed and examined post mortem on Day 10 when measured samples of intestinal mucosa and contents were collected to enable calculation of total larval and adult worm burdens.

Experimental Animals

In this experiment all the animals were Dartmoor-cross, yearling fillies. These ponies had been reared naturally in the wild and they were obtained in autumn as part of a consignment of twenty newly-weaned foals. Some of the other animals in the batch were killed in a separate study and they were observed to have heavy burdens of both strongyles and ascarids. The six animals in Experiment 5 had high positive faecal strongyle and ascarid worm egg counts prior to being given ivermectin (0.2 mg/kg body weight). Two further treatments of ivermectin at the same dose rate were given 12 and six weeks prior to cyathostome infection but in subsequent faecal samples from Pony Nos. 57 and 59, low numbers of ascarid eggs were consistently found and Pony Nos. 58 and 62 also occasionally had low counts.

For six months prior to the experiment the ponies were fed a commercially-produced, pelleted diet (Complete Cubes, Spillers). During this time, Group A and Group B were housed separately,

Table 3.7

Experiment 5 - Design

	Group A	Group B
Pony nos.	60, 61, 62	57, 58, 59
Days -104		500,000 L_3
-101	-	500,000 L_3
- 98		500,000 L_3
- 95		500,000 L_3
Day 1	Isotopes	Isotopes
Day 10	Necropsy	Necropsy

Group A - Control

Group B - Infected

bedded on woodshavings with free access to concrete exercise yards. These ponies were also used in Experiment 6 designed to assess intestinal permeability at different stages of infection (vide infra).

Group B animals were infected with a total of two million cyathostome larvae which were given in four doses of 500,000 L_3 via nasogastric tube at intervals of three days from 104 to 94 days prior to the day of radioisotope injection.

Animal Management

The ponies were tethered in individual stalls on rubber matting from one week before the experiment started until they were killed on Day 10. Each pony was fed 6 kg of the commercial ration and water was available ad libitum.

An oral daily dose of 10 ml of 0.75% potassium iodide solution was given to each pony from Day -4 until Day 10 of the experiment to ensure rapid excretion of ^{125} Iodine.

Measurements and Sampling

Urine

Urine collection funnels were constructed from 6 mm rubber strips of aluminium and metal rivets in a modified design of those described by Harris (1988) for urine collection from female horses. The funnels and the methods of fitting them to the animal are illustrated in Figures 3.1. Urine was deflected by the baffle over the vulva, flowed down the funnel and was collected directly into double thickness polythene bags which were held at the neck of the funnel by rubber bands.

Figure 3.1

Experiment 5 - Urine Collection Apparatus

The urine collection system was checked frequently for harness movement or funnel leakage and adjustments made if necessary.

The urine bags were changed four times daily and urine weights were recorded for 24 hours periods. After thorough mixing of the bulked total daily collection two samples of 1 ml of urine were taken into counting vials using a syringe and then weighed accurately.

Faeces

The faeces produced by the ponies were collected directly into polythene bags suspended within canvas bags which were held over the anus of the ponies by attachments to the webbing harnesses. The faecal bags were changed four times daily and the weight collected in each 24 hour period was recorded. Five samples of approximately 10 g of faeces were taken into counting vials after thorough mixing of the bulked total. Individual samples were accurately weighed.

Blood

Blood samples of 10 ml were collected into heparinised, vacuum tubes via 1", 21 g needles.

For the first four days of the experiment all samples were taken from the right jugular vein and thereafter sampling was from alternate jugular veins.

On Day 1 blood samples were taken at 15, 30, 60, 90 and 120 minutes after injection. On Days 2-4, blood samples were collected at 10 a.m. and 10 p.m. and then from Day 5 until Day 10 only daily (10 a.m.) samples were taken.

RESULTS

PCV, Plasma Volumes and Blood Volumes (Table 3.8)

The infected group of ponies had mean values of plasma and blood volumes which were higher, and mean PCV values and red blood cell volumes which were lower than those of the control group. The differences were not statistically significant.

Albumin Metabolism (Table 3.9 and 3.10)

None of the animals were hypoalbuminaemic and although the infected group had a higher mean serum albumin on Day 0 than the control group, this was not a significant difference. In Appendix 3.7 the serum albumin levels measured on Day 0 and Day 5 of the experiment are given - only minimal individual variations were detected. The individual and group mean values of intravascular, extravascular and total body albumin pools (calculated by both extrapolation and equilibrium-time methods) were greater in the infected animals than the control animals. All animals had high F(CA) values on the first four days of the experiment (Appendix 3.8). The individual mean values of F(CA) over the succeeding six days were similar (0.90 - 0.106) except for pony no. 57 which had an F(CA) value of 0.132. The absolute amounts of albumin catabolised per day were greater for those animals with the larger intravascular pools i.e. the infected ponies.

Faecal Clearance of Plasma and Blood (Table 3.11)

One infected animal, No. 57, had a high mean faecal plasma clearance relative to the other infected animals, and also to the controls, and so consequently the mean of Group B is greater than

Table 3.8

Experiment 5 - Body Fluid Volumes

Pony no.	Plasma Volume ml/kg	PCV %	Blood Volume ml/kg	Red Blood Cell Volume ml/kg
Group A				
60	41	32	60.0	19.2
61	37	34	56.3	19.2
62	47	36	73.0	26.3
Mean	41.7	34.0	63.1	21.6
Group B				
57	42	30	60.5	18.1
58	44	32	64.8	20.8
59	49	30	70.2	21.1
Mean	45.0	30.6	65.2	20.0

Group A - Control

Group B - Infected

Table 3.9

Experiment 5 - Albumin Distribution

Pony no.	PV ml/kg	Serum Albumin* g/l	CA g/kg.bwt	EA^{+} g/kg.bwt	TA^{+} g/kg.bwt	TA^{**} g/kg/bw
Group A						
60	41	31	1.27	1.96	3.23	3.27
61	37	27	1.00	1.66	2.66	2.24
62	47	25	1.20	1.74	2.94	2.27
Mean	41.7	27.7	1.16	1.79	2.94	2.59
Group B						
57	42	32	1.34	2.42	3.76	2.93
58	44	33	1.45	2.53	3.98	3.20
59	49	34	1.67	2.78	4.45	3.49
Mean	45.0	33.0	1.49	2.58	4.06	3.21

* Serum Albumin on Day 0

TA^{+}, EA^{+} - extrapolation method

TA^{**} - equilibrium-time method

Group A - Control

Group B - Infected

Table 3.10

Experiment 5 - Albumin Catabolism of Ponies

Pony no.	Body Wt kg	Serum Total Protein g/l	Serum Albumin g/l	^{125}I half-life hrs	CA g/kg.bwt	Mean F(CA)	Absolute Albumin Catabolism g/kg.bwt/day
Group A							
60	146	60.5	29.5	247	1.27	0.093	0.118
61	148	68.5	29.5	268	1.00	0.103	0.103
62	151	59.5	26.0	277	1.20	0.106	0.127
Mean	148	62.8	28.3	264	1.16	0.101	0.116
Group B							
57	138	66.0	31.0	263	1.34	0.132	0.177
58	163	66.5	32.0	272	1.45	0.103	0.149
59	178	67.0	33.5	309	1.67	0.090	0.150
Mean	160	66.5	32.2	281	1.49	0.108	0.159

Group A - Control
Group B - Infected

Table 3.11

Experiment 5 - Mean Daily Plasma and Blood Clearances of Ponies

Pony no.	Mean Faecal Plasma Clearance (^{51}Cr)	Mean Faecal Blood Clearance (^{59}Fe)	
		Whole Blood	RBC
	ml/kg/day	ml/kg/day	
Group A			
60	1.8	0.14	0.05
61	1.5	0.12	0.05
62	1.5	0.11	0.04
Mean	1.6	0.12	0.05
Group B			
57	2.7	0.11	0.04
58	1.6	0.13	0.04
59	1.8	0.15	0.05
Mean	2.0	0.13	0.04

Group A - Control

Group B - Infected

Group A. The clearances of faecal blood were equally low in both groups. The plasma clearances obtained by analysis of faecal ^{125}I-activity were markedly lower than those derived from ^{51}Cr-activity (Appendices 3.9 and 3.10).

Red Blood Cell Metabolism (Table 3.12)

Pony No. 57 had a much shorter ^{59}Fe half-life than all the other animals and the mean iron half-life of Group B was less than that of Group A. The same animal also had a low serum iron level but it had iron utilisation, turnover and red cell incorporation rates which were comparable with those of other parasitised and control animals.

Worm Burdens

In the group of ponies which had been infected with two million cyathostome larvae the burdens found post mortem were 15,790, 37,650 and 46,750 and most of these were immature larval stages within the mucosa but there were 150-200 maturing larvae in each animal. Pony No. 57 was the only animal which had a positive faecal strongyle egg count (50 epg) although lumenal cyathostomes were identified in the intestinal contents of all infected animals. One control and three 'infected' animals had small numbers of Parascaris equorum within the lumen of the small intestine. Details of cyathostome worm burdens are given in Appendix 3.12.

DISCUSSION

The methods used in Experiments 4 and 5 were very similar but in comparing the results one must consider the differences in

Table 3.12

Experiment 5 - Red Blood Cell Metabolism of Ponies

Pony no.	PCV %	Serum Iron ug/100 ml	^{59}Fe Plasma half-life mins.	Plasma Iron Turnover Rate mg/kg bwt/day	Percentage Iron Utilisation %	Red Cell Iron Incorporation Rate mg/kg bwt/day
Group A						
60	32	122.3	180	0.276	15.9	4.39
61	34	200.8	200	0.465	14.8	6.88
62	36	135.6	146	0.434	20.9	9.07
Mean	34.0	152.9	175	0.392	17.2	6.78
Group B						
57	30	83.8	101	0.351	16.8	5.90
58	32	140.9	177	0.380	14.5	5.51
59	30	154.3	171	0.442	15.8	6.98
Mean	30.6	126.3	150	0.391	15.7	6.13

Group A - Control
Group B - Infected

age (foals vs. yearlings), diet (hay vs. complete cubes), and nature of parasite burdens (naturally acquired, adult stages vs. experimentally acquired, mucosal stages).

The body fluid volumes calculated for Experiment 5 were neither significantly different between infected and control animals nor different from the values obtained in Experiment 4 or those recorded by previous authors (vide supra).

Total body albumin (TA) pools were calculated by extrapolation and equilibrium-time methods: these gave different individual values but both procedures gave mean TA values of the infected animals (4.06 and 3.21 g/kg.bwt) which were greater than those of the controls (2.94 and 2.59 g/kg.bwt) (extrapolation and equilibrium-time respectively). The values obtained by the equilibrium-time method are theoretically more accurate but generally the absolute values will be less important than the results relative to controls within the same experiment. The values for TA sizes of normal horses given by previous authors quoted earlier were obtained by the extrapolation method.

In Experiment 5 there were greater absolute rates of albumin catabolism in the infected ponies (0.149 - 0.177 g/kg.bwt/day) than in controls (0.103 - 0.127 g/kg.bwt/day). However this was related to larger mean intravascular pools in the infected animals of 1.49 g/kg.bwt compared with 1.16 g/kg.bwt in controls rather than to differences in the indices of turnover rate. In fact the mean albumin half-life was longer for the infected group (281 hours) than for controls (264 hours) and only pony no. 57 had an F(CA) value (0.132) greater than the control values

(0.093 -0.106). This animal had the highest cyathostome worm burden at post mortem. The extravascular pools of the infected ponies were also greater than those of control animals such that if there was any response in terms of albumin metabolism to cyathostome infection in these ponies then the results would suggest increased synthesis rather than increased breakdown.

Although there were no differences in mean fractional catabolic rates between groups in Experiment 5, interestingly, all of the animals had higher mean F(CA) values than those calculated for animals in Experiment 4. This might have been due to differences in dietary protein intake which have been shown to have an influence on albumin metabolism in sheep (Abbott, Parkins and Holmes, 1986). In that study, individuals on low protein diets had lower fractional and absolute rates of albumin degradation than animals on higher protein rations.

During the first four days of Experiment 5 the calculated F(CA) values were very high in all animals and the albumin half-life values were shorter relative to other reports (Matheeuws and others, 1966; Duncan and Dargie, 1975; Dietz and Nielsen, 1980); this was largely as a result of high urinary excretion of ^{125}Iodine. Similar observations were also made in Experiment 4 and they most probably arise from denaturation or impurities of the ^{125}I-labelled protein preparation (Mulligan, 1973).

The one infected animal, Pony 57, which had a relatively high faecal clearance of plasma (from ^{51}Cr) was found post mortem to have the highest cyathostome worm burden, 99.5% of which were

mucosal stages. As was also the case in Experiment 4, markedly different values for plasma clearance were obtained based on ^{125}I or ^{51}Cr faecal activities. The faecal plasma clearances in Experiments 4 and 5 were of similar magnitude and the faecal blood clearances were also fairly similar in both studies.

Duncan and Dargie (1975) showed increased faecal blood clearance in ponies with experimental infections of Strongylus vulgaris or naturally acquired, mixed-strongyle infections compared with control animals using ^{51}Cr-labelled red blood cells. Although the results are not corrected for body weight their control values were of a similar order to those found in all infected and control ponies in Experiments 4 and 5, which suggested that there was no significant gastrointestinal haemorrhage as a result of the cyathostome burdens present.

Although there were no significant differences in red blood cell metabolism between groups within Experiment 5, the absolute values of serum iron, plasma iron half-life, plasma iron turnover rate, percentage iron utilisation and red cell iron incorporation rate were all greater than those calculated in Experiment 4. These differences in red blood cell metabolism were probably due to the low serum iron values of the animals in Experiment 4.

The objective of Experiment 5 - to study red blood cell and protein metabolism in a group of ponies which were parasitised at a level similar to that commonly encountered in the field - was not achieved due to failure to establish an adequate number of worms following experimental infection.

There is very little information available on establishment and survival rates of experimental cyathostome infections. In helminth-naive foals examined post mortem 10.7 - 32.3% of an inoculating dose of 20,000 larvae were found four months after infection (Reinemeyer and Others, 1985) whereas between 5% and 9% of inocula were present four to five months post-infection in similar animals given 450,000 larvae in Experiment 3. The yearlings used in Experiment 5 were known to have had previous strongyle infections and the low worm burdens might reflect age or acquired immunity. However, it is generally accepted that in the field horses do not develop good immunity to cyathostomes and the 16-week survival rates of either 50,000 or 500,000 larvae given to yearlings in Experiment 2 were between 6 and 27%.

It is possible that the infective cyathostome larvae were of reduced viability but they were cultured and counted by the same methods used in previous experiments and they had normal microscopic appearance when freshly harvested, immediately prior to dosing. Chromium EDTA is not known to have any anthelmintic activity and the other isotope labels have been used widely in gastrointestinal parasitology without apparent effect on worm burdens.

Dose size *per se* has been shown to have an effect on parasite survival in ascarid infections of foals in which there was an apparent expulsion of worms in ponies given either a large single dose or small, trickle doses of *P. equorum* when compared with animals which received a small, single dose (Clayton and Duncan, 1977). The reason for this expulsion is not known and to

date, similar observations have not been made in strongyle infections.

Ross (1963) recorded that the establishment rate in experimental infections of calves with Ostertagia ostertagi was inversely related to the number of larvae given in a single dose. Although this phenomenon has been commonly observed in ruminant gastrointestinal parasitic experimental infections (Bairden, personal communication) there are no reports of dose-dependent establishment rate for equine parasites.

Whatever the reason for the low cyathostome worm burdens in Experiment 5, the level of infection achieved had little significant effect on plasma protein or red blood cell metabolism. The data obtained in Experiment 5 therefore, are effectively 'normal values' for yearling ponies which will be useful for reference in future studies.

EXPERIMENT 6

Intestinal Permeability to ^{51}Cr-EDTA in Ponies with Experimental Cyathostome Infection

INTRODUCTION

Loss of plasma protein into the gastrointestinal tract, which is characteristic of many parasitic diseases, may be the result of two different mechanisms: either directly in whole blood or indirectly by leak of protein molecules through mucosa of increased permeability. In ostertagiasis mucosal hyperpermeability has been shown to be the predominant reason for enteric protein loss (Nielsen, 1968; Murray, 1969; Murray, Jennings and Armour, 1970). The clinical manifestation of larval cyathostomiasis is very similar to ostertagiasis and both conditions are associated with emerging mucosal larvae. It seems probable therefore, that the hypoalbuminaemia of cyathostomiasis could have a similar pathogenesis to that found in ostertagiasis i.e. protein losing enteropathy as a consequence of increased mucosal permeability.

Techniques, of assessing intestinal permeability using ^{51}Chromium-labelled ethylenetetraacetate (^{51}Cr-EDTA), in vitro (Bjarnason and Peters, 1982; 1984) and in vivo (Bjarnason, Peters and Veall, 1983a; Bjarnason, O'Morain, Levi and Peters, 1983b; O'Morain, Abelow, Chervu and Fleischner, 1987; Jenkins, Jones, Goodacre, Collins, Coates, Hunt and Bienenstock, 1987; Jenkins, Ramage, Jones, Collins, Goodacre and Hunt, 1988) have been successfully applied to the study of coeliac disease and

inflammatory bowel disease in human patients. The in vivo test which is based on the demonstration of increased urinary excretion of ^{51}Cr-EDTA following oral administration, reflecting increased intestinal permeability in diseased patients has also been validated in the dog (Hall, Batt and Brown, 1989) and used in the investigation of cases of wheat-sensitive enteropathy, small intestinal bacterial overgrowth and giardiasis in this species (Hall and Batt, 1990).

Intestinal permeability changes have also been demonstrated in experimental studies of trichostrongylosis in gerbils and lambs (Sinski, MacLean and Holmes, 1987; MacLean, Sinski and Holmes, 1989), Nippostrongylus brasiliensis infections in rats (Ramage, Stanisz, Scicchitano, Hunt and Perdue, 1988) and chemically induced enteropathies in rats (Bjarnason, Smethurst, Levi and Peters, 1985) using the ^{51}Cr-EDTA method.

The objectives of this experiment were twofold. Firstly, to investigate the suitability of the absorption of ^{51}Cr-EDTA as a test of intestinal permeability in ponies. Second, to monitor changes in intestinal permeability at various stages of cyathostome infection.

MATERIALS AND METHODS

Experimental Design

The design of Experiment 6 is summarised in Table 3.13 Three animals were studied prior to, and then subsequently at six and ten weeks following, infection with two million cyathostome L_3. The control animals were studied on one occasion only.

Table 3.13

Experiment 6 - Design

Pony Nos.	Group A 60, 61, 62	Group B 57, 58, 59
Week 1	-	^{51}Cr-EDTA
Week 2 - 3	-	Cyathostome Infection 4 x 500,000 L_3
Week 9	-	^{51}Cr-EDTA
Week 13	^{51}Cr-EDTA	^{51}Cr-EDTA

Group A - Control

Group B - Infected

The animals used in this experiment were also used in Experiment 5 and the details of their background and general management are given in the previous section.

Animal Management

The ponies were tethered in individual stalls on rubber matting from one week prior to each period of study. Each pony was fed 6 kg of a commercial diet (Complete Cubes, Spillers) and water was available ad libitum.

Administration of ^{51}Cr-EDTA

Each pony received a weighed dose of ^{51}Cr-EDTA with a total activity of approximately 12 MBq in 10 ml solution which was given via nasogastric intubation using a one metre, foal-size, stomach tube.

The ^{51}Cr-EDTA was then flushed through the tube with 200 ml of water.

Measurements and Sampling

Urine

In the study of Group B animals before infection total urine collection was carried out for seven days using indwelling, urinary, Foley catheters as described for Experiment 4 with the exception that the catheters were introduced through the vulva. On the subsequent occasions, the urine funnel collection system was used and collection was performed for only three days after administration of the ^{51}Cr-EDTA.

Urine was collected and weighed in 12 hour aliquots from which samples of 1 ml were taken into vials for counting (four samples in the first study and thereafter only two samples).

Faeces

For the pre-infection study faeces were allowed to pass onto the rubber floor covering from where they were carefully swept up and bagged. In the succeeding studies, the faeces were collected directly into bags as described for Experiment 5.

Faeces were collected over the same periods of time as urine. The 12 hour collections were mixed very thoroughly and the total weight recorded before samples of approximately 10 g were taken into counting vials. In the first study, six faecal samples were taken but in the subsequent studies just five were counted.

Blood

Daily blood samples of 10 ml were collected into heparinised, vacuum tubes via 1", 21 g needles from the left jugular vein.

RESULTS

The cumulative percentage urinary recoveries of ^{51}Cr-labelled EDTA in the 72-hour periods following administration are summarised in Table 3.14. The excretion levels measured in all of the control animals were between 2.4 and 6.9% and values for the infected animals at both study periods were also within that range. The faecal recoveries of ^{51}Cr-EDTA are given in Table 3.15. In the pre-infection study 49.6 - 55.9% of administered ^{51}Cr-EDTA was detected in faeces which was considerably less than measured in the same animals following infection (76.6 -99.6%) and in the uninfected control ponies

Table 3.14

Experiment 6 - Percentage of Administered ^{51}Cr-EDTA Measured in Urine - 0-72 Hours

Pony No.	Stage of Infection - 1 week	+ 5 weeks	+ 10 weeks
Group A			
60	-	-	3.1
61	-	-	2.4
62	-	-	6.9
Group B			
57	6.8	5.1	5.3
58	5.4	5.1	3.1
59	5.8	5.4	2.9

Group A - Control

Group B - Infected

Table 3.15

Experiment 6 - Percentage of Administered ^{51}Cr-EDTA Measured in Faeces - 0-72 Hours

Pony No.	Stage of Infection - 1 week	+ 5 weeks	+ 10 weeks
Group A			
60	-	-	96.6
61	-	-	93.2
62	-	-	93.6
Group B			
57	49.6	76.6	94.9
58	41.7	94.6	96.4
59	55.9	89.7	99.7

Group A - Control

Group B - Infected

(93.2 - 96.6%).

The details of the proportions of the dose which were excreted in consecutive 12-hour urine and faeces collections are given in Appendices 3.13 and 3.14 respectively. In general the rate of ^{51}Cr-EDTA excretion was fairly constant in the first four collection periods after administration with the exception of Pony no. 62, a control animal, which excreted 4.3% during the first 12 hours. The faecal radioactivity was mainly detected between 12 and 48 hours after dosing.

DISCUSSION

The absence of significant differences in urinary excretion of ^{51}Cr-EDTA between control and infected animals in this experiment may indicate either that there were no differences in intestinal permeability between the groups or that the test was not suitble for assessing this aspect of gut function in ponies.

From the results of the radioisotope tracer studies (vide supra) which were subsequently carried out on the same animals it was concluded that there was no significant intestinal leakage of plasma in the ponies at 16 weeks post infection and so it seems likely that the integrity of the intestinal mucosa was not compromised by the cyathostome infection.

In studies of intestinal permeability in man using ^{51}Cr-EDTA, 24-hour urine collection is usually performed and in control patients the excretion of the oral dose in this time period is between 0.61% and 2.82% (Bjarnason and others, 1983a; 1983b; O'Morain and others, 1986; Jenkins and others, 1987;

1988). In the ponies the urinary excretions of ^{51}Cr-EDTA were comparable with these levels during the first 24 hours.

One control pony, no. 62, had an exceptionally high ^{51}Cr-EDTA excretion of 4.3% in the first 12 hours. During the same period this animal had a low faecal ^{51}Cr-EDTA excretion which may reflect prolonged transit time due to reduced intestinal motility. However, there were variations in the rate of appearance of faecal radioactivity both between individuals and also between studies in the same individual which were not associated with apparent abnormalities in urinary excretion of ^{51}Cr-EDTA. In studies of an experimental enteropathy in rats there was no effect on the urine excretion of ^{51}Cr-EDTA as a result of prolonging intestinal transit time with atropine (Bjarnason and others, 1985). The same workers demonstrated that absorption of ^{51}Cr-EDTA from the gut is affected by osmolarity and composition of the test dose. The ponies were given identical ^{51}Cr-EDTA solutions on each occasion but the protocol did not include strict control of food and water intake around the starting time of the test which may account for the apparently outlying result between 0-12 hours for pony no. 62.

Although this animal showed no clinical signs of intestinal disease and there was no evidence of an enteropathy at post mortem examination seven weeks later it is possible that the apparently high urine excretion of ^{51}Cr-EDTA is a true result and that the animal did have gastrointestinal dysfunction at the time of study.

The apparently low faecal excretion of ^{51}Cr-EDTA in the pre-infection study was most probably a result of experimental technique; collection and sampling was from the floor surface rather than directly into bags. Although the results are not shown, faecal and urine collections in this study were continued for four further days during which time only very low ^{51}Cr-EDTA excretion was detected. It seems that the faecal collection method was very imprecise since the 12-hour faecal weights and the calculated faecal recoveries were much greater in the subsequent studies in which faecal bags were used. Clayton and others (1980) reported ^{51}Cr-EDTA recoveries of a similar order to the pre-infection study in 'from-the-floor' faecal collections in ponies infected with Parascaris equorum although urine was not analysed.

In the initial reports of the clinical application of ^{51}Cr - EDTA to patients with inflammatory bowel disease (Bjarnason and others, 1983a, 1983b) it was considered that urinary excretion of the permeability marker specifically indicated small intestinal disease. However, it has been demonstrated by studies of the temporal excretion pattern of ^{51}Cr EDTA in man that significant amounts are absorbed from the colon (Elia, Behrens, Northop, Wraight and Neale, 1987; Jenkins and others, 1987).

There has been some debate in the medical literature as to whether the measurement of ^{51}Cr-EDTA permeability helps to distinguish between Coeliac Disease, Crohn's Disease and Ulcerative Colitis. Further studies have shown that it is possible to increase the diagnostic specificity of the test by

either simultaneous administration of ^{14}C-labelled-mannitol with ^{51}Cr-EDTA and calculation of the ratio of excretion of the two probes over time (Behrens and others, 1987; Fotherby, Wright and Neale, 1988), or, by separate measurements of excretion following oral and per rectum administration of ^{51}Cr-EDTA to the same patients (Jenkins and others, 1988).

In equine medicine, cases with chronic inflammatory bowel disease are not uncommon but frequently they cannot be definitively diagnosed. The equine caecum and colon have critical functions in digestion and fluid absorption (Argenzio, 1975) but they cannot be readily assessed by physical examination or by aids such as blood chemistry, radiology, endoscopy and biopsy. Although small intestinal function in horses may be evaluated by glucose or xylose absorption (Roberts and Hill, 1973; Roberts and Norman, 1979), to date no function test has been developed for equine large intestine. The findings of altered ^{51}Cr-EDTA absorption in patients with colonic disease in man suggest that there might be a potential role for this test in horses.

In experimental studies of the rate of passage of ingesta through the equine gastrointestinal tract (Argenzio, Lowe, Pickard and Stevens, 1974), it was found that within two hours of intragastric administration, 68% of a soluble marker was within the caecum. The transit time of the caecum was approximately five hours, but thereafter the rate of movement slowed dramatically with a retention of about 50 hours in the colon.

The temporal excretion patterns of ^{51}Cr-EDTA in Experiment 6 lend support to the possible application of this technique to equine studies in that there was fairly consistent amounts of probe in the 0-12 hour aliquots (with the exception of Pony No. 62 which has already been discussed) and from 12 to 60 hours. The first time period should represent absorption from the proximal gut and caecum whereas the latter should relate to colonic absorption assuming that fluid digesta passage rates were similar to those of Argenzio and others (1974).

From the results of Experiment 6 it was concluded that ^{51}Cr-EDTA excretion was not affected by the magnitude of mucosal cyathostome burdens present but the test was considered to be a suitable method for investigating equine intestinal permeability both in the study of the pathogenesis of experimental cyathostome infections and also possibly in parasitic and non-parasitic clinical disease.

GENERAL DISCUSSION

GENERAL DISCUSSION

Despite longstanding veterinary recognition of the fact that intestinal parasites are major factors in the aetiology of gastroenteric diseases of horses there has been only moderate research progress in the field of equine parasitology. The notable exception is the advance in knowledge of the biology of the most potent parasite pathogen of horses, Strongylus vulgaris (reviewed by Ogbourne and Duncan, 1984) whose larval migrations within the intestinal arterial supply have been widely regarded as important in the pathogenesis of colic. Development of anthelmintic regimens which have been effective in treatment and control of this parasite have ensued (reviewed by Drudge and Lyons, 1986) and recently progress towards successful vaccination against S. vulgaris has been described (Klei, French, Chapman, McClure and Dennis, 1990). Comparable advances have not been made for most other equine parasites perhaps with the exception of Parascaris equorum infections (reviewed by Clayton, 1986).

Generally the pathogenic importance of many equine parasites has been considered to be insignificant by veterinarians for several reasons. These include a lack of specific tests for the diagnosis of parasite-induced disease; the absence of suitable production targets which can be used to demonstrate the cost benefit of equine parasite control programmes and the widespread confidence in the efficacy of modern anthelmintics. It has recently become evident that this rather blasé attitude was imprudent in that the small strongyles, or cyathostomes, have been increasingly associated with a distinct clinical syndrome of

severe enteritis known as larval cyathostomiasis. It may not be coincidental that the small strongyles have several mechanisms of surviving anthelmintic onslaught including the fact that many mucosal cyathostomes encysted in the gut are insusceptible to the action of most drugs and that anthelmintic-resistance has become a feature of infections with this group of worms. It is unfortunate that so little is known of the fundamental biology of cyathostomes as both parasitologists and clinicians are therefore in a weak position especially in relation to the development of effective regimens for the treatment and prophylaxis of these parasites. In an attempt to redress this imbalance the work reported in this thesis was undertaken to investigate some aspects of the host-parasite relationship with particular reference to the aetiology of arrested larval development and to study in some detail the pathogenic effects of cyathostome infections.

In Chapter One there is a description of 16 cases referred for investigation of diarrhoea, of which three were diagnosed as larval cyathostomiasis. In each of the cyathostomiasis cases there was a history of sudden onset diarrhoea in late winter which then became chronic and resulted in rapid marked weight loss. The clinical presentations of the small number of previously reported cases were closely similar to those reported here and appear to be typical of the condition (Velichkin, 1952; Blackwell, 1973; Chiejina and Mason, 1977; Mirck, 1977; Jasko and Roth, 1984; Giles and others, 1985; Church and others, 1986).

Further shared features of the cyathostomiasis cases of the present study were the presence of hypoalbuminaemia and neutrophilia. Although these features were not pathognomonic of cyathostomiasis either in this study or in the case series of Mair and others (1990), they were indicative that the condition constitutes a protein losing enteropathy. In subsequent experiments designed to investigate this enteropathy further (Chapter Three) radioisotope tracer techniques - which have been widely applied to the study of gastrointestinal parasitic infections in ruminants (reviewed by Dargie, 1975; Holmes, 1987) - were used successfully to study plasma protein and red blood cell metabolism. The technical success of the experiments however, was not rewarded by convincing verification that cyathostome infection results in a significant protein losing enteropathy in that there was little significant difference between control or infected groups. The two experiments were in one sense parasitological failures in that the animals which were studied were found post mortem to have low cyathostome worm burdens; approximately 2,000 - 13,000 mainly lumenal worms in Experiment 4 and about 16,000 - 47,000 mucosal worms in Experiment 5. Nevertheless these levels of parasitism were representative of the lower end of the range of burdens which have been reported from contemporary post mortem surveys conducted in the United States (Hass, 1979; Reinemeyer and others, 1984) and the United Kingdom (Ogbourne, 1976). In the British study 80% of 86 animals were found to have burdens of less than 200,000 lumenal cyathostomes. The findings of

Experiments 4 and 5 therefore tended to confirm the consensus view that under normal circumstances cyathostomes are of limited pathogenicity. However the clinical evidence that these worms can result in severe, frequently fatal enteritis is strong justification for further studies of protein and red blood cell metabolism in heavily infected animals. Indeed, Experiment 5 of this study was undertaken with this objective in mind and the unexpectedly low 'take' of very large doses of infective larvae not only thwarted the objective but also forcefully emphasised the lack of detailed knowledge of host and parasite interactions in cyathostome infections.

On the positive side, the findings of the studies on natural and experimental cyathostome infections in ponies described in Chapter Two of this thesis, provide some foundation from which to build the detailed parasitological knowledge required in order to elucidate the pathogenesis of larval cyathostomiasis. The main objectives of these studies were to investigate the effects of factors such as host age, previous parasite exposure, size and frequency of larval doses and climatic conditioning of larvae on parasitological parameters such as prepatent periods, patterns of worm egg output, parasite establishment rates and the incidence of arrested larval development.

Smith (1978) considered that the prolonged prepatent periods and smaller worm egg outputs which he observed on reinfection of adult ponies, compared with those obtained when the same animals were younger, reflected an appreciable resistance to

cyathostomes. In the present study previous parasite exposure, in the form of either historical infections earlier in life or accomplished by the administration of divided doses of infected larvae over a period of several weeks, was shown to delay patency by about one or two weeks and to modulate the level of faecal egg output. Further evidence of protracted parasite development in animals with previous exposure to cyathostome infection was obtained from differential worm burdens at necropsy; for example in the two foal groups in Experiment 1 there were higher proportions of the burdens within the mucosa at necropsy 13 weeks post-infection in the previously grazed animals compared with those which were previously helminth-naive.These findings indicated a degree of resistance to reinfection which could be of some importance in the design of parasite control programmes, for example in circumstances where animals of mixed age groups are grazing together.

In the present study a degree of protective immunity to cyathostome infection was manifest from the lower worm burdens in animals which were reinfected following removal of previous infections or in helminth-naive animals subjected to repeated infections. There were some limitations imposed by the design of the experiments on the analyses of the necropsy worm burden data. Specifically, in Experiment 1 the animals acquired infections naturally and in consequence larval intake could not be quantified; also in all of the experiments there was a significant delay between infection and post mortem examination during which time significant losses of parasites might have

occured making it impossible to determine 'real' establishment rates. In Experiment 1 mean total worm burdens were lower in previously exposed animals compared with helminth-naive foals but there was no evidence that multiple infections and/or age increased the level of immunity in that the mean total burdens acquired by previously grazed foals, yearlings and adult animals were of comparable sizes. Trickle doses of larvae appeared to evoke a protective response in that parasite establishment rates in the animals infected in this way in Experiment 3 were lower than in animals given the same size of infective doses as single inocula. In Experiment 2, the size of infective larval dose was also shown to affect parasite establishment rate in that high doses showed lower establishment than low doses. Evidence in support of this observation was obtained in Experiment 5 in that animals infected with very high doses had markedly lower establishment rates than those after other levels of experimental infection. In these animals the inocula of 500,000 L_3 were given in four divided doses over 12 days and so there was also a possible cumulative effect of dose size and trickle dosing.

From the clinical accounts of larval cyathostomiasis it is evident that hypobiosis of cyathostome larvae is important in the pathogenesis of the disease but hitherto the factors which contribute to arrested development have not been elucidated. However comparisons with hypobiosis in trichostrongyles of ruminants have been drawn by various authors (Ogbourne, 1975; Eysker and others, 1984) and in particular to

Ostertagia ostertagia in which host immunity, environmental conditioning and size of the worm burden are considered important (Michel, 1974; Armour and Duncan, 1987). On the basis of finding very high burdens of cyathostomes which were totally arrested in development in two helminth-naive tracer foals, Eysker and others (1986b) suggested that host resistance was not a major factor in inhibition but that it was a seasonal event.

In Experiment 1 of the present study there were low percentages of the burdens arrested in development in helminth-naive foals and although comparable animals with previous parasite exposure had greater proportions of larvae within the mucosa these were considered to be developing more slowly rather than to be inhibited. In two yearling animals given trickle infections in Experiment 2 there was a higher level of arrested larvae compared with single dose animals which gave the impression that the apparent effect of trickle-dosing was to increase the incidence of hypobiosis. However, the results of Experiment 3 suggested that this effect might be an indirect consequence of the decreased establishment of larvae given in trickle doses: significantly the proportions of inocula which inhibited in development were constant in the various groups used in this experiment. Thus, it was concluded that the level of immunity conferred by trickle dosing per se did not increase the incidence of arrested cyathostome larval development.

The only instance in the present studies when the proportions of the total worm burdens which became inhibited in development were comparable to the very high levels reported

following natural infections of yearling ponies in The Netherlands (Eysker and others, 1986b) was following repeated infections with large larval doses in Experiment 5. Although it was not possible to ascertain the larval intakes in the Dutch study it is likely that they were high during a two month period of grazing heavily contaminated pasture. Together, these observations suggest that dose size, possibly in combination with trickle infection, may be an important factor in the occurrence of arrested cyathostome development.

Michel and others (1979) reported that O. ostertagi larvae were not arrested in development in parasite-free calves infected with single doses whereas over half of the established larvae of doses given as trickle infections to similar animals were found present as arrested stages at necropsy. In that study there was also an effect of age on the incidence of arrested development which was higher in 92 week-old helminth-naive animals than in 19 week-old parasite-free calves. Although the effects of age and trickle exposure were shown separately to be significant causes of inhibited larval development it was also demonstrated that they could have a cumulative action in that there was a very high incidence of arrested larvae in older, trickle-dosed animals. In Experiment 1 of the present study the levels of arrested development were higher in grazed foals and yearlings than in adult ponies examined 38 weeks after infection which was suggestive of an age effect on hypobiosis of cyathostomes although this observation was made from data collected from only

small numbers of animals in which details of larval intake, early establishment rates and/or worm expulsion rates were not known.

Environmental conditioning has been considered to be an important stimulus in inhibited larval development in O ostertagi infections (Armour and others, 1969a, 1969b; Armour and Bruce, 1974). However, under the conditions of the studies reported in this thesis there were no apparent direct effects of climate or experimental chilling on the occurrence of arrested development of cyathostome larvae.

It has been suggested that resistance to establishment and inhibition of larval development were separate aspects of the immune reponse to continuous challenge of lambs with either Haemonchus contortus (Barger, Le Jambre, Georgi and Davies, 1985) or Trichostrongylus vitrinus (Seaton, Jackson, Smith and Angus, 1989). However there may be differences in the relative importance of these aspects of immunity in that the initial indication of an immune response to H. contortus was the occurrence of arrested larval development (Barger and others, 1985) whereas the first indication of immunity to T. vitrinus was resistance to establishment with hypobiosis occurring at a later stage (Seaton and others, 1989). In the present study a relatively weak immunity was indicated by modification of patterns of faecal egg output and reduced establishment rates of infective larvae. However, these effects were evident after infections given over a fairly short time course and it is possible that further exposure to infective larvae, as would occur with continuous larval intake in the field, may reinforce

immunity and perhaps result in large populations of mucosal larvae in arrested development. It could be further hypothesised that if large mucosal burdens accumulate as one manifestation of immunity, and that separately there is a sudden loss of lumenal cyathostomes then the feedback mechanism considered by Gibson (1953) and Smith (1976a) to block further development of mucosal larvae may be removed with a resultant emergence of larvae, possibly leading to clinical disease.

With the notable exceptions of Lichtenfel's (1975) taxonomic classification, Ogbourne's (1978) major review of the pathogenesis of cyathostome infections and the recent studies of Reinemeyer and his co-workers (reviewed by Reinemeyer, 1986) there is a paucity of information on the fundamental biology of Cyathostomum spp. The knowledge gained from the experiments reported in this thesis provide some basis for further studies of the biology of cyathostome infections in horses. However, there were two main weakneses in the experiments of the present study which compromised the value of the data obtained. First, the infections described were all of mixed cyathostome species. Secondly, the size of groups of experimental animals were unavoidably small and there was often considerable variation in values or measurements made within a group. Thus, the results and their interpretations should be viewed with this perspective.

In the one and a half centuries since Knox (1836) first associated a diarrhoeic condition in a horse with the presence of parasites within the mucosa of the large intestine veterinary

scientists have made little progress to define the pathogenesis of larval cyathostomiasis. There is now overwhelming clinical, pathological and parasitological evidence that cyathostome infection constitutes a major threat to the health and welfare of horses.

In order to further study the biological behaviour of cyathostomes and the response of their hosts to infection it is desirable to undertake single parasite species infections. Although not reported to date *in vitro* larval culture from eggs recovered from individual adult cyathostome species and *in vivo* monospecific adult parasite transplants could be used to obtain and build up single species cultures. Having obtained these cultures it would then be possible to study, by experimental infection, the behaviour and effects of individual species within the host. Methods of radiolabelling of infective larvae are now available such that it could be possible to compare the host responses to individual species given simultaneously or to primary versus secondary infections. Such studies are required to provide the necessary data from which definitive conclusions on the pathogenesis of cyathostome infections may be drawn and on which future, innovative strategies for treatment and control will be designed.

APPENDIX 1

Appendix 1.1 - Case 1 - Plasma Biochemistry and Haematology Results

	Day 1	Day 6	Day 19	Day 26	Day 37	Day 60	Day 77	Normal Values (range)
Sodium mmol/l	128	128	133	133	128	ND	ND	130 - 151
Potassium mmol/l	4.4	4.1	4.3	4.4	4.1	ND	ND	2.6 - 5.2
Chloride mmol/l	93	98	98	92	98	ND	ND	94 - 113
Calcium mmol/l	2.77	2.52	2.83	2.70	2.52	ND	ND	2.7 - 3.38
Urea mmol/l	3.4	6.3	2.8	2.1	6.4	ND	ND	0.6 - 6.64
Creatinine umol/l	96	97	82	75	82	83	ND	62 - 133
Alkaline Phosphatase iu/l	440	443	515	462	443	497	501	20 - 350
Aspartate Transaminase iu/l	383	391	274	230	391	ND	ND	90 - 240
Gamma Glutamyltransferase iu/l	13	ND	ND	21	10	20	ND	0 - 25
Creatinine Kinase iu/l	387	469	ND	ND	470	ND	ND	40 - 250
Bilirubin umol/l	8	9	9	4	9	ND	ND	0 - 35.6
Total Protein g/l	53	60	60	64	60	73	68	60 - 83
Albumin g/l	9	11	13	13	11	20	21	27 - 40
Globulin g/l	44	49	47	51	49	53	47	30 - 45
White Blood Cell Count x 10^9/l	12.6	6.2	9.6	4.9	ND	ND	7.0	5.4 - 14.3
Neutrophils x 10^9l (%)	9.83(78)	3.72(60)	6.432(67)	1.96(40)	ND	ND	4.13(59)	2.7 - 6.8 (30 - 75)
Lymphocytes x 10^9l (%)	2.27(18)	2.36(38)	2.880(30)	2.01(41)	ND	ND	2.80(40)	1.5 - 5.5 (10 - 70)
Monocytes x 10^9/l (%)	0.13(1)	0.06(1)	0.240(2.5)	0.78(16)	ND	ND	0.07(1)	0 - 0.8 (1 - 7)
Eosinophils x 10^9/l (%)	0.13(1)	0	0.048(0.5)	0	ND	ND	0	0 - 1.0 (0 - 11)
Basophils x 10^9/l (%)	0.25(2)	0.06(1)	0	0.15(3)	ND	ND	0	0 - 0.2 (0 - 3)
Red Blood Cell Count x 10^{12}/l	6.91	5.79	4.72	4.57	ND	ND	5.60	6.8 - 12.9
Haemoglobin g/dl	11.4	9.5	7.9	7.8			8.9	11 - 19
Packed Cell Volume %	31	26	23	22			28	32 - 53

Appendix 1.2 - Case 2 - Plasma Biochemistry and Haematology Results

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 16	Normal Values (Range)
Sodium mmol/l	124	129	125	125	125	129	134	130 - 151
Potassium mmol/l	2.1	3.4	3.0	2.5	2.1	3.0	4.9	2.6 - 5.2
Chloride mmol/l	90	95	95	90	85	91	93	94 - 113
Calcium mmol/l	2.52	2.72	2.71	ND	2.66	3.06	2.87	2.7 - 3.38
Urea mmol/l	6.8	6.3	5.6	3.0	2.6	2.9	3.2	0.6 - 6.64
Creatinine ummol/l	112	ND	99	101	93	87	ND	62 - 133
Alkaline Phosphatase iu/l	1170	1392	1378	1085	1202	934	327	20 - 350
Aspartate Transaminase iu/l	560	594	536	453	360	ND	309	90 - 240
Gamma Glutamyltransferase iu/l	28	ND	33	29	ND	ND	ND	0 - 25
Creatinine Kinase iu/l	515	ND	558	263	ND	ND	ND	40 - 250
Bilirubin umol/l	72	71	56	31	51	26	2	0 - 35.6
Total Protein g/l	55	59	58	56	ND	65	74	60 - 83
Albumin g/l	25	26	27	23	ND	22	23	27 - 40
Globulin g/l	30	33	31	33	ND	43	51	30 - 45
White Blood Cell Count x 10^9/l	6.6	ND	10.9	ND	ND	19.9	13.3	5.4 - 14.3
Neutrophils x $10^9$1 (%)	3.43(52)	ND	7.74(71)	ND	ND	16.32(82)	8.91(67)	2.7 - 6.8 (30 - 75)
Lymphocytes x $10^9$1 (%)	2.90(44)	ND	2.62(24)	ND	ND	2.39(12)	3.59(27)	1.5 - 5.5 (10 - 70)
Monocytes x 10^9/l (%)	0.26(4)	ND	0.44(4)	ND	ND	0.20(1)	0.53(4)	0 - 0.8 (1 - 7)
Eosinophils x 10^9/l (%)	0	ND	0.11(1)	ND	ND	0.99(5)	0.27(2)	0 - 1.0 (0 - 11)
Basophils x 10^9/l (%)	0	ND	0	ND	ND	0	0	0 - 0.2 (0 - 3)
Red Blood Cell Count x 10^{12}/l	10.94	ND	9.89	ND	ND	5.77	6.32	6.8 - 12.9
Haemoglobin g/dl	16.3	ND	16.2	ND	ND	10.0	10.7	11 - 19
Packed Cell Volume %	50	ND	45	ND	ND	27.1	29.7	32 - 53

Note: Figures in brackets are percentages of Total White Blood Cell Count

Appendix 1.3 - Case 3 - Plasma Biochemistry and Haematology Results

	Day 1	Day 14	Day 20	Normal Values (range)
Sodium mmol/l	129	129	131	130 - 151
Potassium mmol/l	4.5	4.5	4.4	2.6 - 5.2
Chloride mmol/l	95	90	95	94 - 113
Calcium mmol/l	2.84	2.69	2.64	2.7 - 3.38
Urea mmol/l	2.1	2.0	3.5	0.6 - 6.64
Creatinine ummol/l	243	162	198	62 - 133
Alkaline Phosphatase iu/l	580	396	442	20 - 350
Aspartate Transaminase iu/l	269	221	291	90 - 240
Gamma Glutamyltransferase iu/l	32	38	28	0 - 25
Creatinine Kinase iu/l	243	162	198	40 - 250
Bilirubin umol/l	16	17	18	0 - 35.6
Total Protein g/l	72	68	65	60 - 83
Albumin g/l	23	16	18	27 - 40
Globulin g/l	49	52	47	30 - 45
White Blood Cell Count x 10^9/l	14.8	ND	ND	5.4 - 14.3
Neutrophils x 10^9l (%)	11.470(77.5)	ND	ND	2.7 - 6.8 (30 - 75)
Lymphocytes x 10^9l (%)	2.20(15)	ND	ND	1.5 - 5.5 (10 - 70)
Monocytes x 10^9/l (%)	0.222(1.5)	ND	ND	0 - 0.8 (1 - 7)
Eosinophils x 10^9/l (%)	0.222(1.5)	ND	ND	0 - 1.0 (0 - 11)
Basophils x 10^9/l (%)	0.666(1.5)	ND	ND	0 - 0.2 (0 - 3)
Red Blood Cell Count x 10^{12}/l	9.18	ND	ND	6.8 - 12.9
Haemoglobin g/dl	12.7			11 - 19
Packed Cell Volume %	45			32 - 53

Note: Figures in brackets are percentages of Total White Blood Cell Count

APPENDIX 2

Appendix 2.1

Experiment 1 - Breed, Age, Sex and History of Animals

	Pony No.	Breed	Age at Turnout	Sex	Previous History
Group A	11	Shetland	20 wks	M	Reared indoors with dams, weaned at 4 months
	13	Shetland	20 wks	M	
	34	Welsh X	40 wks	F	Anthelmintic - fenbendazole, 7.5 mg/kg x 5 days pre turnout
Group B	14	Shetland	4-5 mths	M	Reared in group, at grass, with dams
	15	"	"	"	Weaned 4-5 mths of age
	16	"	"	"	Housed one week prior to Exp. 1
	17	"	"	"	Anthelmintic - fenbendazole
	30	"	"	"	7.5 mg/kg x 5 days pre turnout
	31	"	"	"	
Group C	1	Welsh X	16-17 mths	M	Reared in group (except 35) at grass with dams and grazed for 8 months after weaning i.e. overwinter
	2	"	"	"	
	3	"	"	"	
	4	"	"	"	Housed four months prior to Exp. 1
	5	"	"	"	35 - reared at grass with dam on separate farm and housed for one year (since weaning) prior to Exp. 1
	35	"	18 mths	"	Anthelmintic - Ivermectin, 0.2 mg/kg, at 4 months and 1 month pre-turnout; fenbendazole 7.5 mg/kg x 5 days pre turnout
Group D	21	Shetland	9 yrs	F	Multiple previous grazing seasons (except 28). Housed 5 months prior to Exp. 2 when reared foals (except 28, 24, 26). 28 - reared indoors, one grazing season as yearling, housed one year prior to Exp. 1. Anthelmintic: Ivermectin, 0.2 mg/kg, at 5, 3 and 1 months pre-turnout; fenbendazole 7.5 mg/kg x 5 days pre turnout
	22	"	8 yrs	"	
	23	"	12 yrs	"	
	24	"	13 yrs	"	
	25	"	14 yrs	"	
	26	"	2 1/2 yrs	"	
	27	"	8 yrs	"	
	28	Welsh X	2 1/2 yrs	"	

Appendix 2.2

Experiment 1 - Anatomical Distribution of Cyathostomes at Week 13 Necropsy

	Pony No.	Lumenal Worms					Mucosal Worms			
		Caecum	Ventral Colon	Dorsal Colon	Small Colon	Total	Caecum	Ventral Colon	Dorsal Colon	Total
Group A	11	3,600	17,800	21,800	0	43,200	1,220	120	0	1,340
	13	0	27,200	20,200	0	47,400	1,910	2,420	10	4,340
	34	8,200	41,200	30,000	0	79,400	2,950	1,840	30	4,820
Group B	16	700	3,800	2,800	0	7,300	1,770	470	0	2,240
	15	1,000	9,000	900	0	10,900	900	590	0	1,490
	31	800	9,400	4,000	0	14,200	680	180	20	880
Group C	4	800	2,800	1,000	0	4,600	580	380	0	960
	35	1,100	7,600	11,500	25	20,225	4,470	3,410	80	7,960
	2	7,500	61,000	28,400	600	97,500	2,880	2,190	20	5,090
Group D	28	1,650	26,100	24,600	200	52,550	5,930	6,760	10	12,700
	22	300	8,080	2,100	0	10,480	120	390	30	540
	23	1,900	10,600	5,600	400	18,500	120	1,370	30	1,520

Group A - helminth-naive foals; Group B - foals; Group C - yearlings; Group D - Adults

Appendix 2.3

Experiment 1 - Anatomical Distribution of Cyathostomes at Week 38 Necropsy

	Pony No.	Lumenal Worms					Mucosal Worms			
		Caecum	Ventral Colon	Dorsal Colon	Small Colon	Total	Caecum	Ventral Colon	Dorsal Colon	Total
Group B	17	40	1,000	1,000	0	2,040	250	140	50	440
	30	100	5,200	4,000	0	9,300	1,400	990	0	2,390
	14	100	1,600	1,200	0	2,900	560	390	1,280	2,230
Group C	5	6,900	13,200	8,200	100	28,400	11,020	8,810	0	19,830
	3	900	49,400	92,600	0	142,900	3,220	26,860	80	30,160
	1	80	8,400	4,200	0	12,680	1,300	1,230	320	2,850
Group D	27	120	16,600	5,600	400	22,720	170	1,400	140	1,710
	25	10	40	1,680	0	1,730	30	130	0	160
	21	0	40	240	0	280	120	100	10	230

Group B - foals; Group C - yearlings; Group D - adults

Appendix 2.4

Experiment 2 - Anatomical Distribution of Cyathostomes at Necropsy

	Pony No.	Lumenal Worms					Mucosal Worms			
		Caecum	Ventral Colon	Dorsal Colon	Small Colon	Total	Caecum	Ventral Colon	Dorsal Colon	Total
Group A	1	700	4,800	1,400	0	6,900	90	90	0	180
	6	960	7,000	5,000	0	12,960	70	320	0	390
	8	80	3,400	400	0	3,880	750	920	160	1,830
Group B	3	1,840	64,200	4,200	200	70,440	300	450	80	830
	5	1,400	23,600	2,800	60	27,860	750	1,070	0	1,820
	7	3,900	2,800	21,200	1,600	29,500	2,590	1,790	0	4,380
Control	4	0	0	400	0	400	40	0	0	40

Group A - 50,000 L_3; Group B - 500,000 L_3

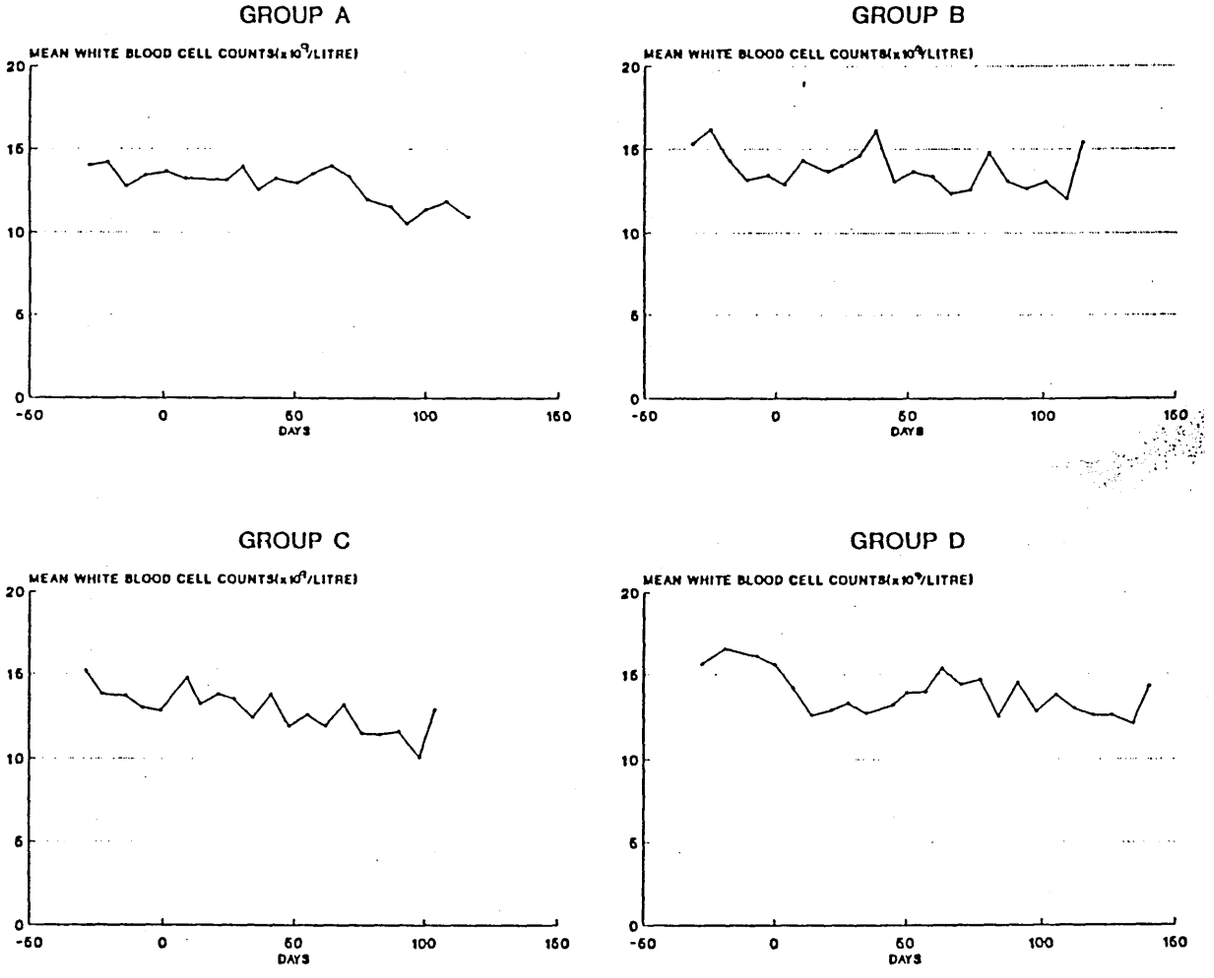
Appendix 2.5

Experiment 3 - Anatomical Distribution of Cyathostomes at Necropsy

	Pony No.	Lumenal Worms					Mucosal Worms			
		Caecum	Ventral Colon	Dorsal Colon	Small Colon	Total	Caecum	Ventral Colon	Dorsal Colon	Total
Group B	40	18,700	36,800	17,200	160	72,860	6,090	5,490	0	11,580
	43	1,200	19,600	9,000	200	30,000	3,780	2,150	0	5,930
	45	5,400	28,200	7,600	0	41,200	13,170	3,660	50	16,880
	46	6,500	16,600	15,200	2,000	40,300	9,700	4,290	150	14,140
	48	2,700	26,800	16,200	400	46,100	13,400	12,050	50	25,500
Group C	41	2,300	11,000	4,200	200	17,700	11,900	2,280	160	14,340
	42	10,600	9,200	12,600	0	32,400	9,750	5,770	10	15,530
	47	1,400	7,600	6,400	0	15,400	3,610	3,990	60	7,660
	49	2,600	9,200	9,600	400	21,400	16,720	2,400	70	19,190
	51	2,400	9,000	3,000	200	14,600	3,860	5,870	10	9,740
Group D	44	3,200	5,000	7,200	600	16,000	12,940	8,090	40	21,070
	50	2,800	3,800	400	0	7,000	8,720	7,290	90	16,100
	52	9,600	10,000	7,200	1,000	27,800	5,950	5,400	90	11,440
	53	3,500	7,200	2,200	400	13,300	6,260	8,930	1,040	16,230
	54	2,800	5,800	5,700	2,000	16,300	4,430	4,290	10	8,730

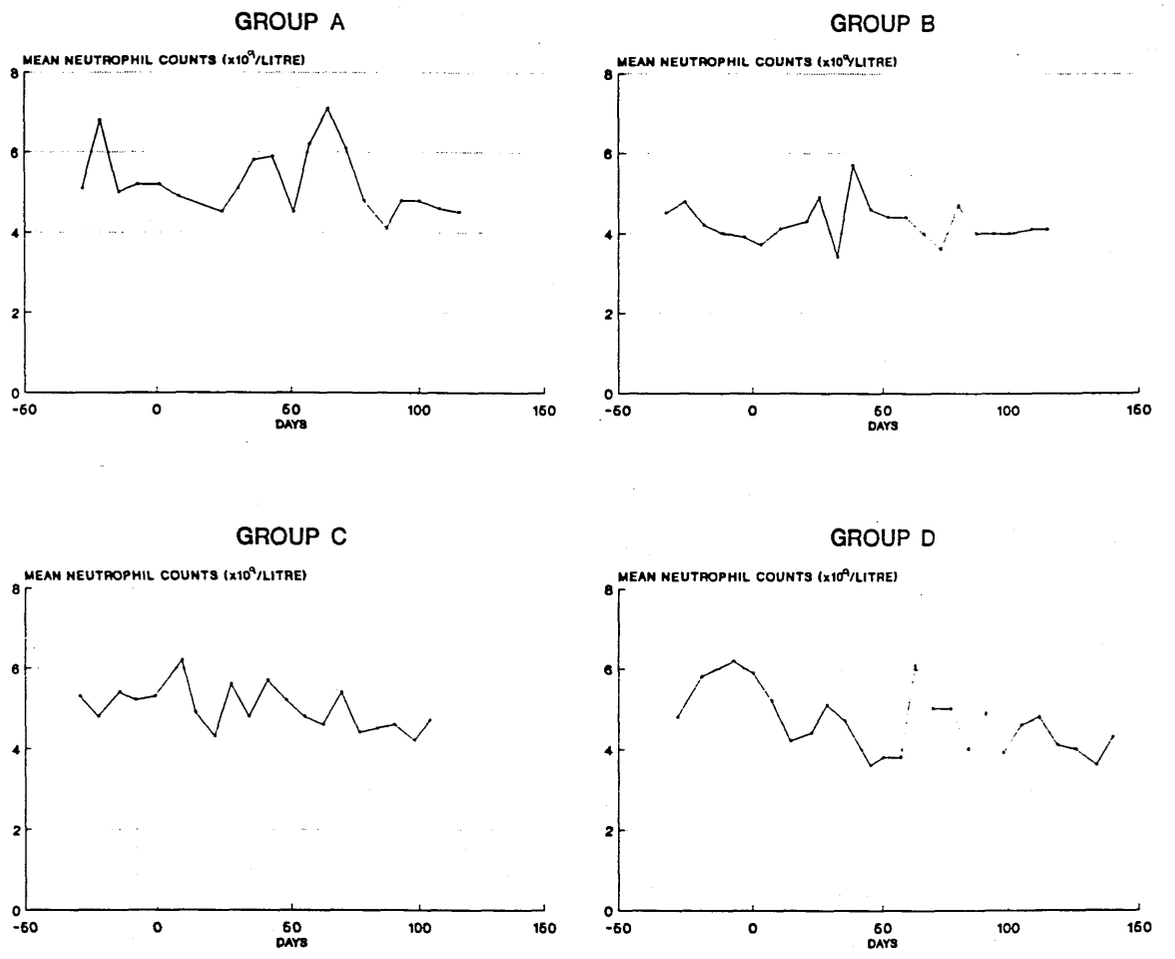
Group B - normal L_3, single dose: Group C - chilled L_3 single dose; Group D - normal L_3, trickle dose

Appendix 2.8
Experiment 3: Group Mean Total White Blood Cell Counts



Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

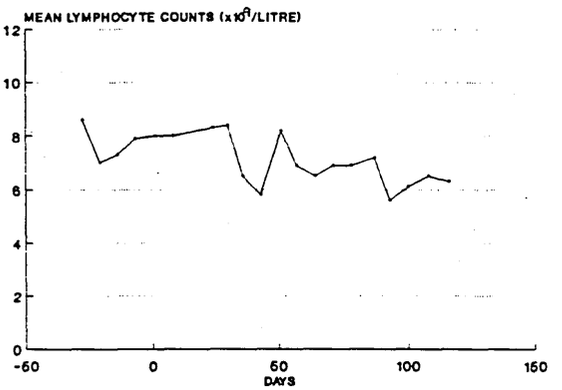
Appendix 2.7
Experiment 3: Group Mean Neutrophil Counts



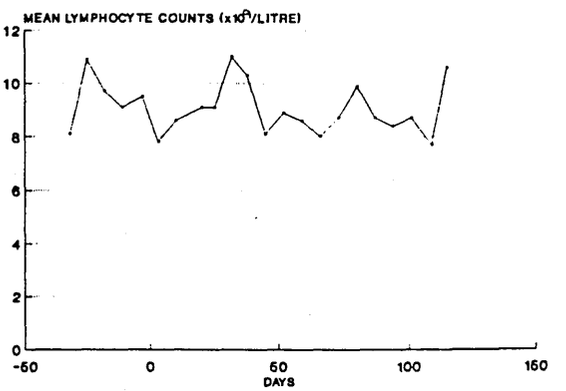
Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

Appendix 2.8
Experiment 3: Group Mean Lymphocyte Counts

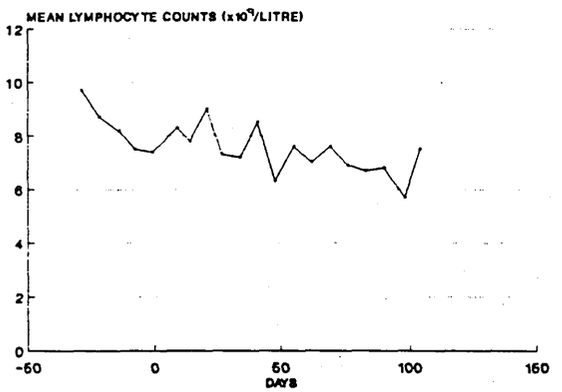
GROUP A



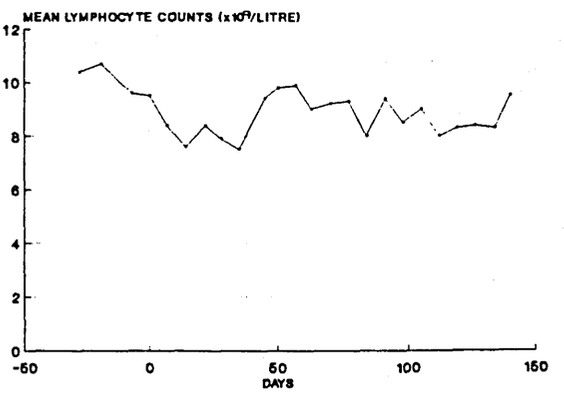
GROUP B



GROUP C

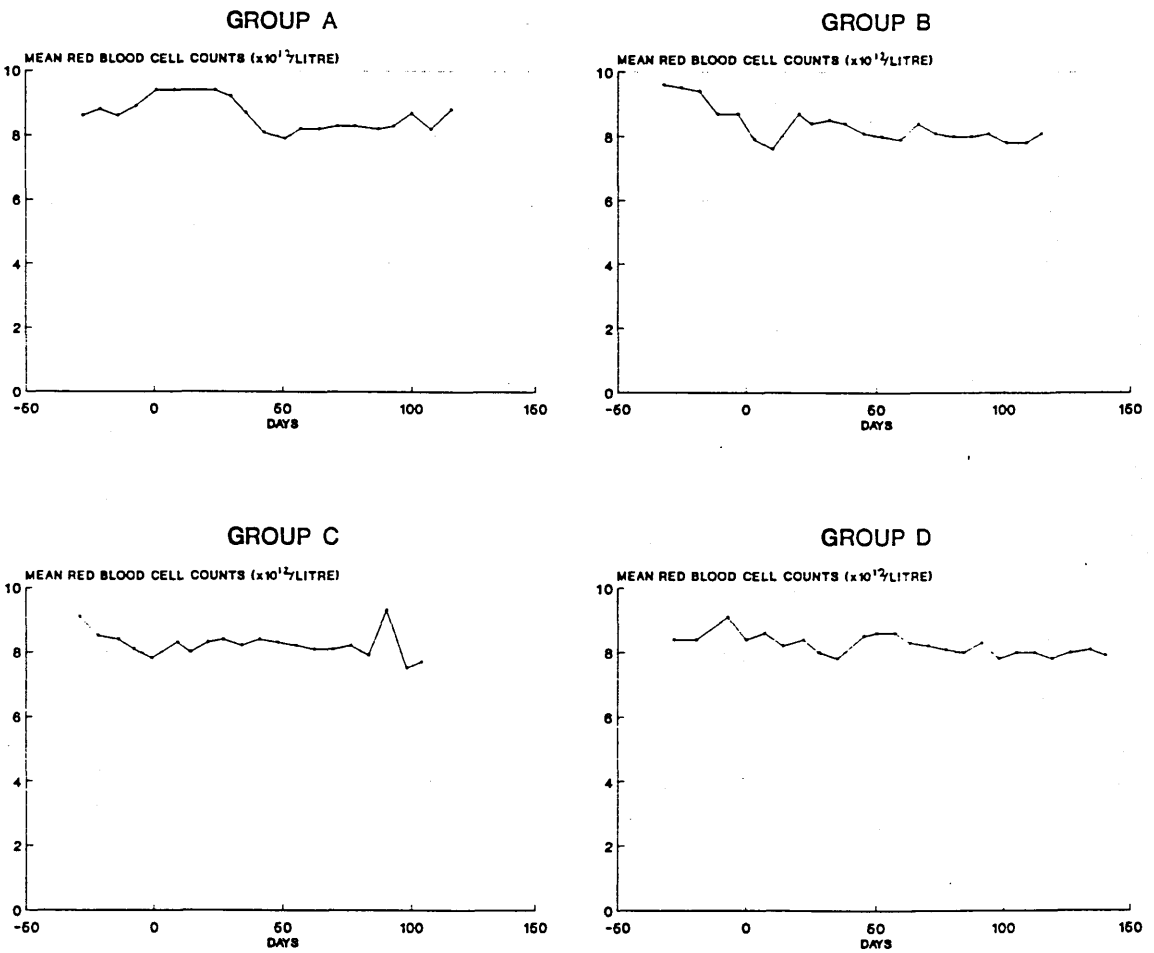


GROUP D



Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

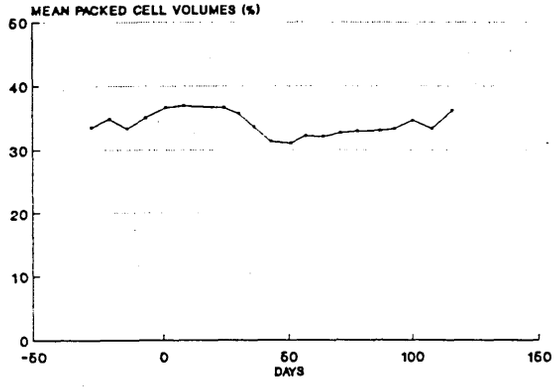
Appendix 2.9
Experiment 3: Group Mean Red Blood Cell Counts



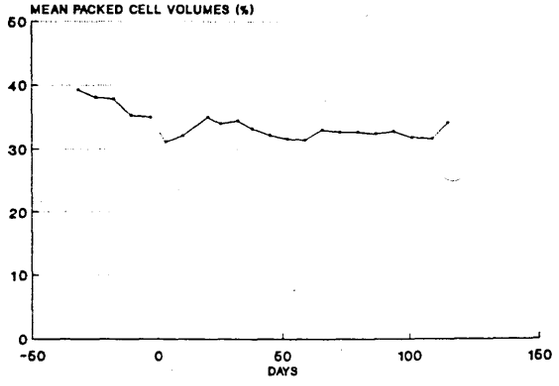
Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

Appendix 2.10
Experiment 3: Group Mean Packed Cell Volumes

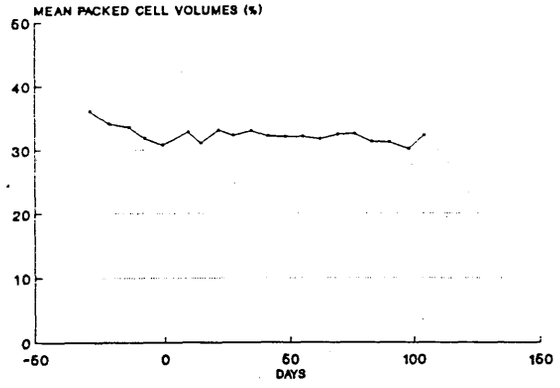
GROUP A



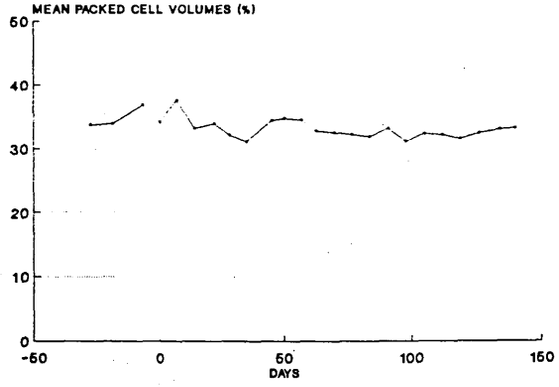
GROUP B



GROUP C



GROUP D



Group A: Control
Group B: 450,000 normal L3, single dose
Group C: 450,000 chilled L3, single dose
Group D: 450,000 normal L3, trickle dose

APPENDIX 3

Appendix 3.1

Experiment 4 - Serum Total Protein and Albumin Values

Pony no.	Total Protein (g/l)				Albumin (g/l)			
	Day				Day			
	0	6	14	Mean	0	6	14	Mean
Group A								
1	78	84	85	82.3	31	34	30	31.7
2	66	70	74	70.0	23	31	34	29.3
Group B								
8	61	68	69	66.0	33	25	37	31.7
5	50	63	72	61.6	23	30	28	27.0
Group C								
4	68	78	84	76.6	40	39	43	40.7
6	47	64	64	58.3	24	24	27	25.0
7	59	70	72	67.0	29	28	44	33.7

Group A - Control

Group B - Infected-treated

Group C - Infected

Appendix 3.2

Experiment 4 - Daily F(CA) Values

Day	Group A		Group B		Group C		
	Pony no.						
	1	2	8	5	4	6	7
1	.568	.471	.480	.376	.447	.616	.415
2	.354	-	.390	.360	.361	.437	.362
3	.246	.200	.270	.313	.292	.287	.227
4	.190	.181	.170	.201	.188	.203	.154
5	.126	.126	.157	.151	.118	.120	.134
6	.085	.104	.122	.119	.081	.107	.065
7	.091	.084	.104	.106	.067	.094	.053
8	.071	.072	.085	.075	.056	.072	.054
9	.048	.070	.057	.068	.058	.077	.061
10	.064	.067	.082	-	.041	.071	.057
11	.053	.061	.075	.059	.047	.072	.053
12	.078	.081	.069	.117	.058	.075	.057
13	.040	.073	.075	.048	.058	.077	.058
14	.040	.057	.057	.041	.051	.070	.052
Days 6-14 Mean	.063	.074	.081	.079	.057	.079	.057

Group A - Control

Group B - Infected-treated

Group C - Infected

Appendix 3.3

Experiment 4 - Daily Faecal Clearances of Plasma (ml/kg) Calculated from ^{125}I-Activity

Day	Group A		Group B		Group C		
	Pony no.						
	1	2	8	5	4	6	7
4	2.5	1.9	2.1	5.3	1.8	2.3	3.2
5	2.0	2.5	2.1	3.1	1.3	1.4	1.8
6	1.3	1.3	1.8	1.6	1.2	1.3	1.4
7	0.8	1.0	1.1	1.5	0.8	1.0	0.8
8	0.8	0.9	1.0	1.1	0.6	1.0	0.8
9	0.8	0.7	0.8	0.8	0.5	0.4	1.0
10	0.8	0.6	0.7	0.6	0.6	0.5	0.8
11	0.6	0.5	0.6	-	0.6	-	0.6
12	-	0.5	0.7	0.6	0.4	0.5	0.7
13	0.7	0.7	0.6	0.7	0.6	0.5	0.6
14	0.4	0.4	0.5	0.6	0.5	0.5	0.5
Days 6-14 Mean	0.8	0.7	0.8	0.9	0.6	0.7	0.8

Group A - Control

Group B - Infected-treated

Group C - Infected

Appendix 3.4

Experiment 4 - Daily Faecal Clearances of Plasma (ml/kg) Calculated from ^{51}Cr-Activity

Day	Group A		Group B			Group C	
	Pony no.						
	1	2	8	5	4	6	7
4	1.1	1.4	1.6	1.6	1.5	3.0	1.8
5	1.2	2.0	2.1	1.7	1.3	2.0	1.9
6	1.3	2.0	2.2	1.1	1.5	2.2	2.0
7	1.0	1.4	1.4	1.9	1.3	2.0	1.5
8	1.0	1.6	1.4	2.0	1.2	2.4	1.7
9	1.1	1.5	1.2	1.7	1.2	1.2	2.5
10	1.2	1.3	1.2	1.9	1.6	1.6	2.4
11	1.0	1.4	1.1	-	1.6	-	2.0
12	-	1.2	1.5	2.1	1.1	1.7	1.9
13	1.7	2.0	1.4	2.3	1.8	1.9	1.9
14	1.4	1.8	1.2	1.9	1.7	2.3	2.1
Days 4-14 Mean	1.2	1.6	1.5	1.8	1.4	2.0	2.0

Group A - Control

Group B - Infected-treated

Group C - Infected

Appendix 3.5

Experiment 4 - Daily Faecal Clearances of Blood (ml/kg) Calculated from ^{59}Fe-Activity

	Group A		Group B			Group C	
	Pony no.						
Day	1	2	8	5	4	6	7
6	0.22	0.17	0.16	0.19	0.30	0.49	0.27
7	0.20	0.13	0.12	0.22	0.26	0.47	0.21
8	0.20	0.15	0.13	0.22	0.21	0.37	0.24
9	0.22	0.12	0.12	0.18	0.19	0.28	0.20
10	0.25	0.13	0.10	0.16	0.23	0.30	0.18
11	0.17	0.13	0.07	-	0.21	0.30	0.18
12	0.19	0.10	0.09	0.16	0.14	0.22	0.14
13	-	-	0.07	0.16	0.21	0.26	0.12
14	0.19	0.13	0.06	0.16	0.20	0.26	0.17
Days 6-14 Mean	0.21	0.13	0.10	0.18	0.22	0.33	0.19

Group A - Control

Group B - Infected-treated

Group C - Infected

Appendix 3.6

Experiment 4 - Cyathostome Worm Burdens

	Caecum		Ventral Colon		Dorsal Colon		Large Intestine		
Pony no.	Mucosal	Lumenal	Mucosal	Lumenal	Mucosal	Lumenal	Mucosal	Lumenal	Total
Group A									
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
Group B									
8	0	0	0	0	0	0	0	0	0
5	80	0	40	100	0	10	120	110	230
Group C									
4	320	0	0	4,600	30	7,900	350	12,500	12,850
6	600	1,600	500	4,040	0	4,800	1,100	10,440	11,540
7	0	100	20	1,920	0	150	20	2,170	2,190

Group A - Control; Group B - Infected-treated; Group C - Infected

Appendix 3.7

Experiment 5 - Serum Total Protein and Albumin Values

Pony no.	Total Protein (g/l) Day 0	Day 5	Mean	Albumin (g/l) Day 0	Day 5	Mean
Group A						
60	61	60	60.5	31	28	29.5
61	68	69	68.5	27	32	29.5
62	57	62	59.5	25	27	26.0
Group B						
57	68	64	66.0	32	30	31.0
58	67	66	66.5	33	31	32.0
59	67	67	67.0	34	33	33.5

Group A - Control

Group B - Infected

Appendix 3.8

Experiment 5 - Daily F(CA) Values

	Group A			Group B		
	Pony no.					
Day	60	61	62	57	58	59
1	.601	.515	.559	.657	.608	.614
2	.265	.252	.216	.495	.220	.241
3	.159	.149	.145	.215	.142	.123
4	.105	.133	.170	.213	.132	.131
5	.125	.122	.166	.138	.111	.083
6	.122	.098	.101	.134	.105	.081
7	.100	.083	.080	.140	.105	(.143)
8	(.193)	.101	.102	.105	.081	.102
9	.108	.110	.093	.139	.118	.092
10	.008	.105	.095	.136	.095	.092
Days 5-10 Mean	.093	.103	.106	.132	.103	.090

Group A - Control

Group B - Infected

(Figures in brackets omitted)

Appendix 3.9

Experiment 5 - Daily Faecal Plasma Clearances (ml/kg) Calculated from ^{125}I-Activity

	Group A			Group B		
	Pony no.					
Day	60	61	62	57	58	59
2	0.3	0.5	0.4	0.3	0.5	0.6
3	0.2	0.3	0.2	0.3	0.2	0.3
4	0.1	0.2	0.1	0.2	0.1	0.2
5	0.1	0.1	0.1	0.2	0.1	0.1
6	0.1	0.1	0.2	0.2	0.2	0.2
7	0.1	0.1	0.1	0.2	0.1	0.2
8	0.1	0.1	0.1	0.1	0.1	0.1
9	0.1	0.1	0.1	0.2	0.1	0.1
10	0.1	0.1	0.1	0.1	0.1	0.1
Days 4-10 Mean	0.1	0.1	0.1	0.2	0.1	0.1

Group A - Control

Group B - Infected

Appendix 3.10

Experiment 5 - Daily Faecal Plasma Clearances (ml/kg) Calculated from ^{51}Cr-Activity

Day	Group A			Group B		
	Pony no.					
	60	61	62	57	58	59
2	0.8	1.2	1.1	1.3	1.0	1.3
3	1.3	1.2	1.2	3.0	1.1	1.7
4	1.6	1.2	1.1	3.0	1.2	1.6
5	1.4	1.4	1.5	2.5	1.7	1.3
6	1.8	1.5	2.1	2.7	1.8	2.6
7	1.7	1.7	0.9	2.5	1.6	1.7
8	1.6	1.4	1.3	2.3	1.5	1.7
9	2.4	1.6	2.0	3.0	2.0	1.8
10	2.4	1.7	1.9	2.4	1.7	2.1
Days 4-10 Mean	1.8	1.5	1.5	2.6	1.6	1.8

Group A - Control

Group B - Infected

Appendix 3.11

Experiment 5 - Daily Faecal Blood Clearances (ml/kg) Calculated from ^{59}Fe-Activity

Day	Group A			Group B		
	Pony no.					
	60	61	62	57	58	59
7	0.16	-	0.09	0.12	0.17	0.17
8	0.12	0.12	0.10	0.10	0.11	0.16
9	0.16	0.13	0.13	0.12	0.13	0.20
10	0.16	0.11	0.11	0.08	0.10	0.20
Days 7-10 Mean	0.14	0.12	0.11	0.11	0.13	0.15

Group A - Control

Group B - Infected

Appendix 3.12

Experiment 5 - Cyathostome Worm Burdens

Pony No.	Caecum Mucosal	Caecum Lumenal	Ventral Colon Mucosal	Ventral Colon Lumenal	Dorsal Colon Mucosal	Dorsal Colon Lumenal	Large Intestine Mucosal	Large Intestine Lumenal	Total
Group A									
60	0	0	0	40	0	40	0	80	80
61	30	0	0	40	0	0	30	40	70
62	0	0	20	0	0	40	20	40	60
Group B									
57	16,960	20	21,580	120	7,990	80	46,530	220	46,750
58	5,000	460	18,570	4,120	8,180	1,320	31,750	5,900	37,650
59	7,750	320	3,170	120	4,350	80	15,270	520	15,790

Group A - Control
Group B - Infected

(Note: P. equorum - 57 three adults; 58 three adults and two immature stages; 59 five adults and three immature stages; 62 three immature stages)

Appendix 3.13

Experiment 6 - Percentage of Administered ^{51}Cr-EDTA Measured in Urine

Pony no.	Stage of Infection	0-12 hrs	12-24 hrs	Day 1 Sub-total	24-36 hrs	36-48 hrs	Day 2 Sub-total	48-60 hrs	60-72 hrs	Day 3 Sub-total	Total 0-72 hrs
57	- 1 wk	1.2	1.7	2.9	2.3	0.8	3.1	0.5	0.3	0.8	6.8
	+ 5 wks	1.2	1.8	3.0	1.2	0.6	1.8	0.2	0.1	0.3	5.1
	+ 10 wks	1.2	1.4	2.6	1.0	1.2	2.2	0.3	0.2	0.5	5.3
58	- 1 wk	1.3	1.9	3.2	1.4	0.5	1.9	0.3	<0.1	0.3	5.4
	+ 5 wks	1.0	1.5	2.5	1.6	0.7	2.3	0.2	0.1	0.3	5.1
	+ 10 wks	1.0	1.0	2.0	0.6	0.3	0.9	0.1	0.1	0.2	3.1
59	- 1 wk	0.8	1.5	2.3	1.7	1.3	3.0	0.3	0.2	0.5	5.8
	+ 5 wks	0.8	1.9	2.7	1.1	0.5	1.6	0.7	0.4	1.1	5.4
	+ 10 wks	1.6	1.2	2.8	0.7	0.3	1.0	0.1	<0.1	0.1	3.9
60	Control	0.9	1.0	1.9	0.7	0.2	0.9	0.2	0.1	0.3	3.1
61	Control	0.5	1.1	1.6	0.5	0.2	0.7	0.1	<0.1	0.1	2.4
62	Control	4.3	1.1	5.4	0.8	0.4	1.2	0.2	0.1	0.3	6.9

Appendix 3.14

Experiment 6 - Percentage of Administered ^{51}Cr-EDTA Measured in Faeces

Pony no.	Stage of Infection	0-12 hrs	12-24 hrs	Day 1 Sub-total	24-36 hrs	36-48 hrs	Day 2 Sub-total	48-60 hrs	60-72 hrs	Day 3 Sub-total	Total 0-72 hrs
57	- 1 wk	0.1	7.7	7.8	20.9	7.9	28.8	8.9	4.1	13.0	49.6
	+ 5 wks	0.4	23.8	24.2	25.2	17.0	42.2	6.0	4.2	10.2	76.6
	+ 10 wks	10.0	33.8	43.8	25.4	13.6	39.0	8.2	3.9	12.1	94.9
58	- 1 wk	<0.1	15.2	15.2	13.4	4.1	17.5	8.1	0.9	9.0	41.7
	+ 5 wks	<0.1	29.2	29.2	30.8	19.5	50.3	11.4	3.7	15.1	94.6
	+ 10 wks	5.9	34.2	40.1	32.4	16.1	48.5	5.8	2.0	7.8	96.4
59	- 1 wk	0.2	4.8	5.0	23.9	16.1	40.0	8.1	2.8	10.9	55.9
	+ 5 wks	<0.1	5.0	5.0	26.9	24.7	51.6	12.8	14.3	27.1	83.7
	+ 10 wks	8.0	40.0	48.0	31.9	10.8	42.7	6.8	1.5	8.3	99.0
60	Control	12.0	37.7	49.7	26.1	13.7	39.8	4.0	2.5	6.5	96.0
61	Control	11.8	46.9	58.7	24.3	8.7	33.0	1.3	0.2	1.5	93.2
62	Control	<0.1	33.9	33.9	32.8	18.0	50.8	5.5	1.9	7.4	93.6

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