Effect of management interventions on helminth levels and body condition of working donkeys in South Africa

by

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ABSTRACT

Twenty-three southern African working donkeys (*Equus asinus*) were allocated to eight experimental groups. The aim of the study was to determine what effect alternative helminth control methods have on the internal parasite burdens and the condition of working donkeys. Three

control methods [monthly removal of faeces from grazing camps (paddocks), pre-winter treatment with the anthelmintic, moxidectin, and a combination of these two methods] and a replicate of each were compared with a set of two control methods. Monthly live weights, body condition scores and certain blood chemistry values differentially improved in those animals receiving the chemical treatment. The eggs and larvae of the cyathostomes represented the largest portion of the helminth species composition in both the faecal egg counts (FEC) and larval cultures. Although both the FEC and pasture larval counts showed seasonal increases during the warm wet summer months, reduced average pasture larval burdens were recorded in the camps from which the faeces were removed monthly, and a 20 % reduction in the average FEC was noted in the donkeys in these camps towards the end of the study. Pre-winter moxidectin treatment resulted in an initial 100 % reduction in FEC, an average egg reappearance period of 42 to 55 days, and a reduced average FEC for up to eight months after deworming in all the donkeys that received this treatment. To obtain total helminth counts, post-mortem examinations were performed on nine of the donkeys at the end of the study. Estimated worm burdens ranged from 3 831 to 29 501 and 38 helminth species were recorded. The latter include a previously unknown cyathostome species, *Cylicocyclus asinus* sp. n. *Cyathostomum montgomeryi* was the most abundant cyathostome and *Triodontophorus hartmannae* the most abundant large strongyle. The large strongyles were less abundant compared to the cyathostomes, and both groups were more prevalent in the ventral colon. Although both the monthly removal of faeces from the camps and pre-winter treatment resulted in a reduction in the total number of lumenal and encysted cyathostome worms, the combination of these two interventions was the most effective. The latter management system had the largest impact on the strongyle parasite burden in the donkeys. The general health and working capacity of donkeys in southern Africa can be significantly improved by implementation of practical and effective disease prevention recommendations, such as those emanating from this study.

Key words: *Equus asinus*, donkeys, helminth parasites, Strongylinae, Cyathostominae, *Cylicocyclus asinus*, helminth control, alternative interventions, pasture larval counts, faecal egg counts, live weight.

Dedicated To My Parents

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CHAPTER 1

GENERAL INTRODUCTION

Equids (donkeys, *Equus asinus*; horses, *Equus caballus*; zebras, *Equus* sp.) can harbour at least 75 nematode species comprising 28 genera, in addition to three cestode and two trematode genera (Lichtenfels, 1975; Lichtenfels, Kharchenko, Krecek and Gibbons, 1998b). Within this diverse group of helminths the small strongyles, also called the cyathostomes (subfamily Cyathostominae within the Nematoda), constitute a large percentage of the internal parasite population (Lichtenfels, 1975; Ogbourne, 1978; Herd, 1990; Herd and Coles, 1995). This group of worms has become increasingly important in horses and their pathogenic effects, such as weight loss and diarrhoea, are well documented in the United Kingdom (Ogbourne, 1978; Love, Mair and Hillyer, 1992; Reilly, Cassidy and Taylor, 1993; Mair, 1994; Murphy and Love, 1970) and the United States of America (Herd, 1990; Uhlinger, 1991; Herd, 1993; Lyons, Swerczek, Tolliver, Drudge, Stamper, Granstrom and Holland, 1994). Unfortunately little is known regarding the clinical symptoms caused by cyathostomes in donkeys or horses in South Africa.

Quantitative nematode faecal egg analysis (with the McMaster technique) is frequently performed (by veterinarians and scientists) to primarily detect nematode parasite burdens and to evaluate the efficacy of an anthelmintic in one individual or a whole herd (Herd, 1992b). However, the information obtained from this technique is limited as a single faecal egg count (FEC) is not an accurate reflection of the host's total worm burden (Rubin, 1967; Michel, 1968; Herd, 1993; Herd and Coles, 1995). Currently, helminth recovery from the gastro-intestinal tract following necropsy is the only reliable method available to give an estimate of the total helminth burden present in the host. Although necropsy techniques have the additional advantage that the parasite species diversity can be identified (Malan, Reinecke and Scialdo, 1981a, b; Duncan, Arundel, Drudge,

Malczewski and Slocombe, 1988), the host animal must be sacrificed and the technique is time consuming.

The value of alternative helminth control methods has been recognised in recent years. These include pasture hygiene (Herd, 1986), strategic anthelmintic treatment (Horak and Snijders, 1968; Herd, Willardson and Gabel, 1985; Pandey and Eysker, 1990) and integrated control strategies (Craig and Suderman, 1985; Reinemeyer, 1986; Herd, 1990; Herd, 1993; Herd and Coles, 1995; Waller, 1999). All these methods share three advantages, namely: 1) reduce the risk of anthelmintic resistance development, 2) reduce the helminth parasite burdens in the host, and 3) reduce the cost of control programmes as a result of less frequent treatments.

Studies on the parasitic burdens of donkeys in Africa and the rest of the world are becoming increasingly important due to: 1) a larger dependence on donkeys to generate some kind of income as a direct result of expanding human populations and unemployment, and 2) an increase in research interest stimulated by the availability of funding for collaborations between Africa and developed countries. Most previous investigations on donkeys, in developing countries, focussed on the helminth species composition in them (Theiler, 1923; Vercruysse, Harris, Kaboret, Pangui and Gibson, 1986; Hilali, Derhalli and Baraka, 1987; Eysker and Pandey, 1989; Pandey and Eysker, 1989; Pandey and Eysker, 1990; Feseha, Mohammed and Yilma, 1991; Mattioli, Zinsstag and Pfister, 1994; Matthee, Krecek and Milne, 2000) while less importance was placed on helminth control (Bliss, Svendsen, Georgoulakis, Grosomanidis, Taylor and Jordan, 1985; Pandey and Eysker, 1990; Khallaayoune, 1991). In addition, recommendations often made regarding the helminth control methods for donkeys are based on the information available for horses. This study was undertaken for the following reasons: 1) the effect of helminth burdens on the health and condition of working donkeys is as yet poorly researched and understood, and 2) there is a need for extensive investigations to determine the value of alternative helminth control methods on the worm burdens and working condition of donkeys in the resource-limited areas of South Africa.

These studies should preferably be extended to different geographical regions, as a single alternative helminth control method will not necessarily be equally effective and practical under all conditions.

Alternative control methods that are cost-effective are of value because they are applicable to developed as well as developing countries. In some of the resource-limited communities in South Africa, where donkeys are used for draught (traction), the removal of faeces from kraals (enclosures) and pastures is a common practice; the "by-product" used for fuel and compost. Moreover, in some instances vegetable farmers exchange vegetables for faeces that is intended for use on the field as compost. It is evident that faeces provide a valuable trading commodity for the local communities (Krecek, Matthee, Milne, Nkungu, Matamotja and van der Meijden, 1998; Krecek and Guthrie, 1999). Empirical data showing the effect of faecal removal on pasture larval counts and the consequence of this on donkey parasite loads is, however, lacking. If this management intervention can be shown to effectively lower helminth levels in the host, such options can be made available to numerous resource-limited donkey and horse owners who are unable to afford more expensive helminth control practices (i.e. regular deworming programmes).

The current recommendation for helminth control to donkey owners is that a single suitable anthelmintic be purchased and administered annually. It is considered to be most effective when given in autumn due to the adverse environmental conditions (cold and dry) generally experienced during winter, in South Africa. As yet, only two studies on horses in South Africa have noted the potential effect of an autumn treatment. The first study reported that a single autumn treatment of horses suppressed their FEC for up to two months following treatment; three months later the counts were still 25 % lower than the pre-treatment counts (Horak and Snijders, 1968). A subsequent selective treatment study on horses noted significant reductions for the first three months after an autumn deworming followed by a clear increase [faecal egg counts > 900 egg per gram (epg) of faeces] in the FEC in the next two months

(Krecek, Guthrie, van Nieuwenhuizen and Booth, 1994b). It is suggested that the cold winter conditions, commonly experienced in the Gauteng province, limited egg and larval survival on the pasture and thus reduced helminth re-infection in the horses in spring. It is evident that more information is needed to confirm the efficacy of such a strategic autumn treatment on equine helminth infections. Additionally, the total helminth burdens of donkeys and the effect of the parasites on the general health and condition of untreated and treated animals warrant further investigation.

The aims of the study were as follows:

1. Establish the influence of helminth parasites on the physical condition of the host by measuring changes in the live weight, body condition score (BCS), haemoglobin concentration (Hb), packed cell volume (PCV), and white cell count (WCC).
2. Determine the value of monthly faecal removal of faeces from pasture and strategic pre-winter treatment in reducing pasture larval burdens as well as the numbers of helminths present in donkeys by means of faecal worm egg counts and necropsy techniques.

CHAPTER 2

LITERATURE REVIEW

1. The role and management of donkeys in South Africa

Since the colonisation of South Africa, in 1652, donkeys have been part of the South African culture (Starkey, 1995). At present, more than 150 000 donkeys are routinely used in South Africa for transport and agricultural practices, especially in the rural and resource-limited areas (Krecek, Starkey and Joubert, 1994a; Starkey, 1995; Wells, 1997; Krecek *et al.*, 1998). Their importance has caused a recent renewed awareness of the socio-economic role that donkeys play, and consequently several recent investigations focussed on the management and health of these animals. For example, Starkey (1995) carried out a rapid rural appraisal over a six-week period and subsequently Wells (1997) intensively studied working donkeys for a 14-month period in three villages in the North-West Province. In the Eastern Cape Province, the socio-economic and health aspects of working donkeys were investigated over a three-month period (Wells, Krecek and Kneale, 1997). From the above it became evident that donkeys are: 1) widely used in resource-limited and rural communities for transport and tillage, 2) easy to handle, 3) inexpensive, and 4) require low "maintenance" compared to modern technology (Wells, 1997). In addition to establishing their socio-economic importance, valuable information was also obtained regarding their helminth burdens and management (Wells, Krecek, Wells, Guthrie and Lourens, 1998). It was found that the working donkeys in the Moretele 1 district in the North-West province, South Africa carried an average nematode faecal egg count of 2 000 epg. Even though this value is almost six times higher than that of the recommended 300 epg cut-off value for anthelmintic treatment in horses (Krecek *et al.*, 1994b) most of the donkeys

recorded acceptable body condition scores (BCS) of between 3.5 to 4, on a scale of 1 to 9 (Wells, 1997). In addition, this study by Wells (1997) was one of the first conclusive research investigations on the management of working donkeys and in it four representative management practices currently used by donkey owners were identified: 1) donkeys are kept in a small yard at all times and fed hay, 2) donkeys roam free and are rounded up and confined to an enclosure when required to work, 3) donkeys are managed in an identical manner to that in system two except that they receive supplemental food in winter, and 4) donkeys are always kept on the owner's land, about 10 ha in extent, and are only rounded up and enclosed only when required for work. More than 50 % of these animals were required to do work (pull carts) on an average of twice a week and travelled between one to five kilometres per day (Wells, 1997; Wells *et al.*, 1998).

2. Helminth parasites of donkeys

Compared to ruminants, such as cattle (*Bos taurus*) and sheep (*Ovis aries*), equids are hosts to the largest diversity of helminth parasites (Theiler, 1923; Lichtenfels, 1975; Craig and Suderman, 1985; Hansen and Perry, 1994). In short, the helminths of the Equidae are represented in more than 75 nematode, five trematode and four cestode species (Lichtenfels, 1975). The largest group of worms (56 of 75 species) present in donkeys, horses and zebras comprise the family Strongylidae within the phylum Nematoda (roundworms). Three subfamilies are recognised: the Cyathostominae (small strongyles), which is also the largest group and comprises more than 50 species, the Strongylinae (large strongyles) and the Oesophagostominae.

In Africa, the availability of limited research funds, and the general perception that donkeys are unimportant, restricted previous research efforts to the economically important production

animals, such as sheep, goats (*Capra hircus*) and cattle. Since 1980 perceptions have, however, changed and helminth studies on donkeys have been conducted in several African countries such as: Burkina Faso (Vercruysse *et al.*, 1986), Egypt (Hilali *et al.*, 1987), Ethiopia (Feseha *et al.*, 1991), Kenya (Lewa, Munyua, Ngatia, Maingi and Weda, 1997), Morocco (Khallaayoune, 1991), South Africa (Malan, Reinecke and Scialdo-Krecek, 1982; Wells *et al.*, 1998; Matthee *et al.*, 2000), The Gambia (Mattioli *et al.*, 1994) and Zimbabwe (Eysker and Pandey, 1989; Pandey and Eysker, 1989; Pandey and Eysker, 1990). Despite the fact that the current information on the helminth species occurring in African donkeys is based on limited geographic coverage, the following has emerged: 1) donkeys in Africa are parasitised by a diverse group of helminths, 2) some helminth species appear to be strictly host specific, and 3) there appears to be a geographical variation in the helminth species composition.

3. The effect of helminth parasitism on donkeys

Parasitism is broadly defined as a negative symbiosis where the relationship between two organisms is beneficial to the one and detrimental to the other (Barnes, 1987). To date, most studies on equids that have attempted to determine the unambiguous effects of helminths have focussed on the horse in developed countries, such as the United States of America and United Kingdom (Round, 1968; Frerichs, Holbrook and Allen, 1976; Smith, 1976; Ogbourne, 1978, Duncan, 1985; Drudge and Lyons, 1989; Uhlinger, 1991; Love *et al.*, 1992; Reilly *et al.*, 1993; Mair, 1994; Murphy and Love, 1997). Results emanating from them indicated that weight loss, colic, diarrhoea and unthriftiness are the typical clinical signs associated with high helminth burdens in this host species. Several of these investigations indicated that diarrhoea and severe weight loss were associated with infections of large numbers of small strongyles (Ogbourne, 1978;

Love *et al.*, 1992; Reilly *et al.*, 1993). These signs appear to coincide with the synchronised emergence of large amounts of fourth-stage larvae (L_4) from the host's gut wall in spring which cause tissue damage, haemorrhage and an intense inflammatory reaction (Herd, 1990; Love *et al.*, 1992; Mair, 1994). Similarly, Murphy and Love (1997) reported that artificially infected ponies, exposed to three million cyathostome third-stage larvae (L_3), showed a significant reduction in weight compared to that of the uninfected control animals (approximately 20 % difference). Based on these findings it seems reasonable to assume that weight loss is probably the most consistent and prominent physiological effect noticed in horses and ponies that are severely infected with helminth parasites. At present, it is unsure if this clinical pattern also holds for donkeys, as previous studies unfortunately restricted their attention only to changes in the BCS of these animals (Bliss *et al.*, 1985; Khallaayoune, 1991; Wells, 1997).

Even though only a few investigations have examined the pathological effect of helminth burdens on the BCS of working donkeys, the results have been very informative. All of them revealed a correlation between the condition score allocated to each animal based on the amount of muscle and fat cover and their helminth burdens (Urch and Allen, 1980; Bliss *et al.*, 1985; Khallaayoune, 1991; Wells, 1997). Bliss *et al.* (1985) noted improved BCS eight months after treating donkeys once with fenbendazole and pyrantel. Along the same lines, Khallaayoune (1991) noted a slight increase in body condition of working donkeys four months following treatment with dichlorvos and a significant ($p < 0.05$) improvement after eight months. It is thus evident that the BCS of donkeys can serve as an indicator of severe helminth burdens. One should, however, be aware of a potential delayed improvement in the BCS subsequent to anthelmintic treatment in donkeys as this might lead to a misinterpretation of the results.

Numerous studies on horses and ponies have reported that strongyle infections might result in alterations in the host's blood composition (Round, 1968; Frerichs *et al.*, 1976; Smith, 1976; Ogbourne, 1978; Drudge and Lyons, 1989; Love *et al.*, 1992; Mair, 1994; Murphy and Love,

1997). These have included changes in the total WCC, eosinophil count, erythrocyte count, Hb and serum protein levels in horses and ponies that harbour large helminth burdens. All these effects appear to be ambiguous indicators of strongyle infection as it is difficult to determine with certainty which group (for example, the large or small strongyles) or which species are responsible for specific alterations in the blood chemistry. In addition, there is variation between studies regarding the diagnostic potential of the blood variables. In contrast to horses and ponies, the field has been mostly unexplored in donkeys (Urch and Allen, 1980; Mattioli *et al.*, 1994). Urch and Allen (1980) concluded that standard haematological variables, such as PCV, Hb and WCC, did not improve in donkeys and ponies following deworming against intestinal and lungworm parasites. However, a recent study by Mattioli *et al.* (1994) recorded a decline in the PCV in donkeys that were infected with strongyles and trypanosomes compared to that of uninfected animals. It is apparent that, as yet, there is no single blood variable whose change in extensive strongyle infections in equids is significantly consistent to be used in their diagnosis.

Extrapolations between closely related species, such as horses and donkeys, are common practice, but it is important to bear in mind that the pharmocokinetics of certain remedies may differ in these animals (Mealey, Matthews, Peck, Ray and Taylor, 1997). Nevertheless, in the current absence of supporting evidence derived from donkeys, most predictions have to be based on the information available for horses.

4. Methods to detect helminth parasites

The recent survey performed in South Africa on working donkeys (Wells *et al.*, 1997) indicated that the most common conditions that could affect the health of donkeys reported by their owners were also the most noticeable, such as tick infestations, harness sores, wounds and mange. Despite an average FEC of 2 000 epg of faeces recorded for donkeys in another survey (Wells *et al.*, 1998), only 5 % of their owners reported worm infections as being present (Wells, 1997). This illustrates that internal parasitic worms are not easily detected with the naked eye, causing the owners of donkeys to perceive the problem as not being present/important.

Some procedures that are used to detect the presence of helminth parasites associated with the host include FEC, post-mortem examination of an animal and herbage larval counts. These methods can be grouped into: the frequently-used non-invasive FEC which is variable, the non-invasive more tedious pasture larval counts and the more informative but invasive, labour intensive and time consuming post-mortem examination. In addition to the usual method used to detect nematode eggs in faeces, procedures have also been described to recover cestode and trematode eggs with ease within a couple of hours. Proudman and Edwards (1992) used a centrifugation-flotation technique to detect the "D-shaped" cestode eggs in faeces, and the washing of faecal material through the Pitchford-Visser apparatus has been proposed to detect trematode eggs (Reinecke, 1983).

Faecal egg counts

Faecal egg determinations are probably the most widely used technique to diagnose helminth infections in animals and it is particularly effective in detecting "strongyle" eggs.

Although the differentiation between large and small strongyles eggs remains problematic with this technique (Reinemeyer, 1986), the ova of *Oxyuris equi*, *Parascaris equorum*, and *Strongyloides westeri* are distinguishable (as illustrated in Thienpont, Rochette and Vanparijs, 1979). Differentiation of the strongyles group is possible by the use of larvál cultures, even though, it is mostly restricted to genus level.

The McMaster technique is a flotation method for quantifying nematode parasite eggs in the faeces (Herd, 1992b). The following information is obtained from FEC: 1) the presence of ova in the faeces is a positive diagnosis of parasitism, 2) the egg count at any given time is a reflection of the eggs produced by sexually matured female worms in the host, and 3) even though there is no indication of the male to female ratio, the presence of sexually matured males is confirmed.

The perception of most veterinarians regarding FEC in the past was, and to a lesser extent still is, that the egg count is a true reflection of the helminth burden in the host (Kingsbury, 1965; Rubin, 1967). Numerous attempts have been made to correlate egg counts and total worm burdens (Kingsbury, 1965; Rubin, 1967; Michel, 1968; McKenna, 1981), but few studies have reported high correlations (Kingsbury, 1965; McKenna, 1981). Consequently, caution should be taken when using FEC in scientific studies because of their inability to record: 1) the nematode, and in particular, the strongyle species composition, 2) the number of encysted and lumenal immature worms, and 3) the number of mature male and female worms in the host (Roberts, O'Sullivan and Rick, 1951; Herd, 1992b). Irrespective of these shortcomings, if planned correctly, FEC is a relatively simple and cheap method to gather information that can be useful for evaluating the drug resistance of worms and the success of different treatments, and for establishing the severity of pasture contamination (Roberts *et al.*, 1951). In addition, the combination of FEC and the larval culture method provides a powerful tool during routine surveys. Use of this combination makes it possible to establish whether the majority of eggs are produced by the cyathostomes or by one or more of the large strongyle species (Poynter, 1954). This information is valuable in determining the

seasonal prevalence of small and large strongyles without the need of sacrificing one or more of the host animals.

Pasture larval counts

Part of the life cycle of most equine nematode species is the free-living larval stages (first-, second- and third-stage larvae). These larvae develop from eggs in the host's faeces that are deposited on the pastures (Ogbourne, 1972). Numerous studies have been performed on the behaviour of, and the environmental conditions required for, parasitic larvae of both equids (Ogbourne, 1972; 1973; Mfitilodze and Hutchinson, 1988) and ruminants (Krecek, Groeneveld and van Wyk, 1991; Krecek, Hartman, Groeneveld and Thorne, 1995) to survive on the pastures. Based on these studies, the two most important abiotic controlling factors that influence the survival of the larvae are moisture content of their immediate surroundings and the ambient temperature (Ogbourne, 1972; 1973). Provided that this moisture content is adequate and the temperatures moderate (9 °C to 29 °C) the L_3 will migrate vertically on the herbage to increase their availability to the grazing host, optimising the latter's infection or re-infection (Ogbourne, 1972; 1973).

The number of L_3 on pasture is valuable for predicting the severity of helminth exposure to the grazing host. The first step that is required to determine the degree of pasture infection is the collection of herbage samples. The "w-shape or zigzag" pattern followed throughout the pasture is one of the earliest methods that were described to collect random herbage samples from pastures (Taylor, 1939). The technique requires that herbage samples should be collected throughout the paddock at regular intervals (determined by a fixed number of paces) without any discrimination being shown towards areas containing faecal material. Taylor (1939) suggested, in fact, that the collector should close his/her eyes when taking the herbage sample. Subsequent pasture studies

have provided new information regarding the grazing patterns of horses and have noted that it takes place in a non-random way and defaecation areas referred to as "roughs" are usually left ungrazed (Herd and Willardson, 1985). As a result of the high concentration of faecal material, the roughs are characterised by the presence of high concentrations of larvae on the herbage which, if sampled, will give an overestimate of the potential risk to the grazing equid (Crofton, 1952; Herd and Willardson, 1985). A more accurate representation would be obtained from multiple herbage samples collected randomly over the entire "lawn" area that is frequently grazed by the herd (Herd and Willardson, 1985).

The methods used for processing soil or herbage samples depend on the size of the samples. The washing of herbage samples in a commercially used washing machine is suggested for larger herbage samples (> 100 g) and also for routine work because it is easy and thorough (Bürger, 1981; RC Krecek, personal communication, 1997). Recovering parasitic larvae from smaller herbage samples (10 – 100 g) can be performed by soaking them in a modified Baermann apparatus (Krecek *et al.*, 1991; Fine, Hartman, Krecek and Groeneveld, 1993). The end product of soaking and/or washing herbage often contains larvae as well as soil residue and other extraneous material. The saturated sugar centrifugation-flotation technique described by Caveness and Jensen (1955) and Dickerson (1977 from Krecek *et al.*, 1991) has been successfully used to clean samples as well as to isolate plant nematode larvae and eggs from soil and plant root samples. Based on the dual advantage of this method, it has been adopted as a cleaning and animal nematode isolation step subsequent to soaking or machine-washing herbage samples (Krecek *et al.*, 1991; Krecek, personal communication, 1997). In addition to the use of a saturated sugar solution, other reports have noted similar advantages with high-density magnesium sulphate, sodium chloride or potassium iodide solutions (Bürger, 1981; Ludwig and Johnstone, 1984; Martin, Beveridge, Pullman and Brown, 1990).

Necropsy techniques that require the euthanasia of animals are probably the most valuable to scientific studies. Even though the procedure is regarded as destructive to the animal, time consuming and costly, it is the only method that provides a complete estimate of: 1) the helminth species composition, 2) the number of immature and mature worms, 3) the total worm burdens in the host, and 4) the sex ratio of the worms. In addition, it provides information regarding the developmental stages, distribution patterns and predilection sites of the different worm species in the host.

The necropsy guidelines of Malan *et al.* (1981a, b) have been used in most worm recovery studies in South Africa. The method involves for worm recovery the sampling of a 1/4 aliquot of the stomach ingesta and a 1/10 aliquot of the contents from each of the small intestine, caecum, ventral colon, dorsal colon and descending colon. In contrast, in a study performed on donkeys in Morocco a combination of the total counts of stomach content and 1/5 aliquots of each of the small and the large intestinal contents were used (Khallaayoune, 1991). Such variations in methodology have prompted the development of guidelines by the World Association for the Advancement of Veterinary Parasitology on the necropsy techniques used for worm recovery from equids in an attempt to standardise and simplify the current methods employed (Duncan *et al.*, 1988). This guideline is, however, incomplete as no information is provided regarding aliquot and sieve sizes.

Another important aspect of worm recovery includes the harvesting of encysted cyathostome larvae or immature worms from the wall of the small intestine, caecum, and ventral-, dorsal- and descending colon. Although two methods (Transmural Illumination [TMI] and Peptic Digestion [DIG]) have been described for the enumeration of the different mucosal larval stages of the cyathostomes, it is suggested that they either be used in combination with one another or DIG on its own (Chapman, Kearney and Klei, 1999; Eysker and Klei, 1999). Transmural Illumination

appears to be easy (illuminate the stretched-out tissue sample and count the number of encysted larvae), faster (if the animals have a low level of helminth infection) and less costly compared to DIG which is time consuming (takes at least two hours for the digestion process), expensive (requires temperature-regulated water baths, chemicals and glass jars) and time consuming (the larvae must be separated from what remains of the digested tissue, and then counted and measured). In addition, there are several disadvantages to using TMI: 1) the sensitivity of TMI is restricted to the counting of the large encysted larval stages, such as the larger late L_3 (LL_3) and developmental L_4 stages (DL_4), and 2) it is not possible to differentiate between the LL_3 and DL_4 while encysted in the mucosa, and thus they are grouped together and identified merely as developing larvae (DL). In contrast, the use of DIG enables differentiation of LL_3 and DL_4 as well as the hypobiotic early L_3 (EL_3). With this technique it is possible to measure the exact length of the larval body and to determine the shape of the buccal capsule in order to differentiate between LL_3 and DL_4 (Klei, Chapman and French, 1997; Chapman *et al.*, 1999). Comparative studies between TMI and DIG obtained contrasting results. In one, the number of DL obtained by TMI has been found to be comparable to the number of DL obtained by DIG when tested on ponies that had not been treated with anthelmintics over a prolonged period (Chapman *et al.*, 1999). Two other studies involving ponies obtained contrasting information. First, Eysker, Boersema, Grinwis, Kooyman and Poot (1997) noted that more DL were recovered with TMI in six untreated ponies, while with DIG larger numbers of DL were recorded in six ponies treated with 0.4 mg/kg moxidectin, which kills both the adult and immature worms in the intestinal lumen as well as the larger encysted larval stages in the gut wall, five weeks before euthanasia. It was suggested that the reason for this difference was that the treated ponies contained higher proportions of very small DL stages that were too small to have been observed with TMI. Second, ponies treated with an anthelmintic effective against the encysted larval stages reported larger numbers of DL with TMI compared to with DIG (Klei *et al.*, 1997). The explanation presented by the authors was that the population of DL in the mucosa

comprised live and some dead encysted larvae, thus the dead larvae were counted by TMI in the treated animals. They maintained that the dead larvae were not seen in DIG as they were completely disintegrated by the digestion process. This is a great danger in using the TIM technique as it will lead to an over estimate of the actual number of encysted larvae that survived the treatment.

Alternative methods have been proposed for the TMI technique. One, described by Reinemeyer and Herd (1986a), consists of 5 x 5 cm in size tissue samples (full thickness) that are collected from the haustral section of the gut wall. The weight of each tissue sample is obtained and the combined weight recorded for each of the intestinal regions (small intestine, caecum, ventral, dorsal, and descending colon) prior to examination. In order to calculate the total number of encysted larvae the combined weight of the tissue samples from each of the regions is used to derive a percentage aliquot of the individual total weight of each region. Each sample is stretched and smoothed out between two petri dishes, one of which contains a grid pattern (to facilitate counting), and is illuminated from below with a strong light source while it is examined under a dissecting microscope. Another TMI method described is that of Chapman *et al.* (1999) in which the tissue samples (full thickness and representing 2.5 % weight of each region's area) are stretched and pinned over a wooden square, 10 x 10 cm in size in which there is a circular open area of 32 cm^2. The tissue in this area is examined under a dissecting microscope.

Although most DIG techniques are consistent in that removal of the mucosa prior to digestion and the use of the pepsin/HCL digestion solution are requirements there is variation in: 1) the duration of the digestion process (reports range from two hours to approximately 12 hours), 2) the incubation temperature (range from 37 °C to 42 °C), and 3) the aliquot sizes (Reinemeyer and Herd, 1986a; Pandey and Eysker, 1990; Xiao, Herd and Majewski, 1994; Eysker *et al.*, 1997; Klei *et al.*, 1997; Murphy and Love, 1997; Chapman *et al.*, 1999; Eysker and Klei, 1999). These differences can potentially affect the results of enumeration (Eysker and Klei, 1999) and it is

therefore not surprising that inconsistencies have been reported regarding which of the two techniques (TMI and DIG) is the most effective in recovering encysted larvae (Eysker *et al.*, 1997; Klei *et al.*, 1997).

5. Helminth control methods for donkeys in Africa

Helminth parasite control in donkeys by their owners is an uncommon practice in South Africa (Starkey, 1995; Wells, 1997; Wells *et al.*, 1997) and several hypotheses can be put forward to explain this phenomenon. First, surveys on health related issues of working donkeys in South Africa that have been done to date mention that a current misconception of donkey owners is that "donkeys never get sick" (Starkey, 1995; Wells, 1997; Wells *et al.*, 1997). Second, there seems to be a general lack of knowledge regarding the existence and effects of internal worm parasites in donkeys (Wells, 1997). Third, in most rural communities there is limited veterinary support and affordable veterinary supplies are not easily available (Starkey, 1995). Fourth, donkeys have a low monetary value, which is approximately equal to the cost of an anthelmintic that is administered orally and can be used for an animal weighing up to 500 kg (Wells *et al.*, 1998). Consequently, in many instances it is more economic to purchase another donkey than to treat a sick animal.

From the above it is evident that future recommendations regarding helminth control for draught animals in South Africa (and other developing countries) should be made with careful consideration to cost and practicability. In recent years, the use of alternative control methods, such as strategic deworming (Horak and Snijders, 1968; Herd *et al.*, 1985; Pandey and Eysker, 1990; Khallayoune, 1991; Krecek *et al.*, 1994b), pasture hygiene (Herd, 1986; 1993; Herd and Gabel, 1990) and the use of integrated control strategies (Craig and Suderman, 1985; Reinemeyer, 1986; Herd, 1990; Herd, 1993; Herd and Coles, 1995; Waller, 1999), have gained increased support for

horses, donkeys and mules in both developed and developing countries. These strategies are laudable because they: 1) reduce the cost of helminth control, 2) reduce helminth burdens, 3) are more environmentally friendly, and 4) reduce or delay the risk of anthelmintic resistance development as a result of fewer treatments with anthelmintics.

Type of anthelmintic

Anthelmintic products that are registered for use in horses are tested and developed on horses prior to registration. Unfortunately, these products are often the only drugs available for use in the other domesticated equids. It is thus not surprising that the dosage and use of these products in donkeys, mules and jennies have been extrapolated from the recommendations made for horses. In the past, the development of anthelmintics has focussed on the most important group of helminths, namely the strongyles, and especially on the control of the adult stages of this group (Duncan, 1985; Herd, 1992a). In recent years attention has shifted from only treating the lumenal adult stages to also include the larval stages of the small strongyles, which are encysted in the gut wall. Three larval stages have been identified that occur in the gut tissue: EL_3, LL_3 and the DL_4. The problem facing pharmaceutical companies is that these larval stages are partially inaccessible to anthelmintics during their encystment in the mucosal and submucosal layers of the gut wall and are unaffected by them. They are thus exposed to small amounts of the drug and therefore able to build up a resistance to them. In addition to the strongyle species, control measures have also included *P. equorum*, which is recognised as an important parasite of foals and young animals (Drudge and Lyons, 1989). Modern day (after 1960) anthelmintics can be grouped into the following classes, namely, benzimidazoles, pyrantel, organophosphates, and avermectins (Duncan, 1985). Most of these drugs are broad spectrum and are more efficient against the adult stages of the strongyles, *P.*

equorum, bots (larval stages of *Gasterophilus* spp.), and *O. equi* (Drudge and Lyons, 1989; Herd, 1992a). Within this group of anthelmintics there are differences in the degree of effectivity against different parasitic species and their developmental stages as well as in the length of the re-treatment interval (Herd, 1992a; Jacobs, Hutchinson, Parker and Gibbons, 1995). Moxidectin is a macrocyclic lactone antiparasitic drug that is related to ivermectin (DiPietro, Hutchens, Lock, Walker, Paul, Shipley and Rulli, 1997). Since its recent release, various scientific studies have been performed on horses and ponies and in addition to its high effectivity against adult strongyles, it was also the first drug that resulted in noticeable reductions, with a single oral treatment, in the encysted larval stages of the cyathostomes (Herd, 1992a; Xiao *et al.*, 1994; DiPietro *et al.*, 1997; Eysker *et al.*, 1997; Vercruysse, Eysker, Demeulenaere, Smets and Dorny, 1998). In addition, moxidectin appears to have a longer residual period of protection (> eight weeks), compared to that of ivermectin or any of the "older" anthelmintics, possibly as a result of its effectivity against the larger encysted larval stages (LL_3 and DL_4) and the fact that it is more lipophilic compared to ivermectin (Jacobs *et al.*, 1995; DiPietro *et al.*, 1997; Boersema, Eysker and van der Aar, 1998; Vercruysse *et al.*, 1998). Its more lipophilic nature reduces the rate at which it is metabolised and excreted, and thus exposes the worms to higher and prolonged levels of it.

Strategic anthelmintic treatment

Strategic deworming of equids is based on the seasonal fluctuations of strongyle egg output (Poynter, 1954). In temperate regions, the egg production of the strongyles increases in spring (Craig, Bowen and Ludwig, 1983; Courtney and Asquith, 1985; Herd *et al.*, 1985; Wells, 1997). This time of the year is also characterised by favourable environmental conditions for the development and survival of the free-living parasitic larvae on pasture (Craig and Courtney, 1986;

Wells, 1997). It is generally recommended that anthelmintic treatments should coincide with this peak in helminth egg production, as this will reduce the pasture larval burdens and, consequently, the risk of re-infection of the host and of first infections in previously unexposed animals such as foals. An additional advantage is that a large percentage of the host's worm burden will be removed with an autumn treatment, as the more susceptible adult stage is the most abundant in the host at this time (Pandey and Eysker, 1990). In South Africa, the value of a strategic autumn treatment in horses has been considered (Horak and Snijders, 1968; Krecek *et al.*, 1994b). Winter in South Africa, is generally characterised by nutrient poor feed and environmentally unfavourable conditions, which in turn cause a reduction in helminth egg production, and consequently, pasture larval counts (Poynter, 1954; Horak and Snijders, 1968). Based on this information, it appears that an autumn treatment will benefit animals by reducing their parasite load and will result in a more gradual re-infection (Horak and Snijders, 1968; Pandey and Eysker, 1990). In support of the autumn deworming, it was noted in Zimbabwe that cyathostomes over-winter as adults in donkeys and thus their hosts would benefit from a strategic pre-winter treatment (Pandey and Eysker, 1990). South African equine advisors recently re-affirmed this practice even to the extent that it is currently incorporated in the curriculum of the veterinary science students at the University of Pretoria (Krecek and Guthrie, personal communication, 1997).

Pasture hygiene

Pasture hygiene has been recommended for many years as a prophylaxis against various helminth parasites in horses (Herd, 1986; 1993). This control method is based on the physical removal of faeces from the pasture, which will result in fewer helminth eggs and larvae that can develop and infect or re-infect the grazing host. To date, very few studies have investigated the

"real" effect of faecal removal on the pasture larval burdens. One of the first to provide empirical data regarding the effect of faecal removal on pasture larval burdens in the camps (paddocks) grazed by horses was by Herd (1986) who determined the value of twice-weekly faecal removal (Herd, 1986). The rationale behind the biweekly faecal removal was that egg-containing faeces were removed before egg dispersal and development of infective larvae from the eggs could take place. From this study, it appeared that this practice had a significant effect on the pasture larval counts two to three months following its commencement and the continued removal of faeces prevented pasture infectivity from exceeding 1000 L_3 per kilogram of the dry herbage (Herd, 1986). The results of a separate study (Fisher, 1997) that also involved the twice-weekly removal of faeces from camps that were grazed by ponies support the finding of Herd (1986). Surprisingly, neither of the studies recorded reductions in the faecal egg counts in the animals grazing the camps.

CHAPTER 3

METHODS USED IN THE STUDY

1. Weekly exercise

Donkeys are generally used for transport of people, goods and water, as well as for agricultural practices in different communities throughout South Africa. The distances and the number of days that these animals are required to trek vary within and between communities. For example, in the North-West Province of South Africa, 40 % of them travel short distances (one to two kilometres in one day), approximately 10 % of their owners use them to travel more than 11 km per day and 50 % of owners use them twice a week (Wells, 1997). Most of the adult donkeys that were purchased for this project were working previously in different communities. An exercise programme was developed for the animals at Onderstepoort so that the results from the study would be applicable to working donkeys in general in South Africa. Therefore, in an attempt to simulate natural working conditions, they were all exercised for four kilometres between one and two days per week in the present study. They were either subjected to a fast trot with no added weight or they were required to carry sandbags that weighed 15 – 20 % of the animal's total weight. The exercise area was frequently shared with horses that were sporadically kept in the paddock to graze.

2. Live weights

As was mentioned previously in section 1.3, on the effect of helminth parasitism on donkeys, a reduction in the live weight of a horse can serve as an indicator of an extensive helminth

burden (Ogbourne, 1978; Love *et al.*, 1992; Reilly *et al.*, 1993; Murphy and Love, 1997) but information on the correlation of helminth parasites with the live weight of donkeys is lacking. In an attempt to gain this much needed information, each animal in the present study was weighed on an Atlas electronic weigh bridge at the end of every week (Fridays) and its weight recorded. The animals were weighed at the same time each day (8:00 – 9:00) to minimise fluctuations due to feeding and drinking. These weekly weight recordings were combined and averaged out to give the monthly figures, which are given in the Results section of Chapter 4 of this study. The monthly live weight recordings therefore represented an average of four to five readings per month to provide a more accurate reflection of the animal's live weight each month. The primary factors for slight weight fluctuations between readings are variations in the amounts of the contents of the large caecum and possibly also water loss during exercise.

3. McMaster technique and pooled larval cultures

Nematode egg counts were recorded bimonthly at the same time of day (7:00 – 9:00) from each animal. As a result of a daily natural variation in the FEC that has been recorded in horses (Warnick, 1992) two readings per month were preferred to one to ensure a more precise representation of the egg counts of each animal during the study period. Faecal samples were collected directly from each animal's rectum and were processed the same day as their prolonged storage (> 1 week) might have lead to artificially low egg counts due to hatching of the eggs (Herd, 1992b). The McMaster technique of Reinecke (1983) was followed using a modification (Krecek, personal communication, 1997). The use of a blender or ball bearings was not required because the faeces were fresh and moist and thus only a wood spatula was required to make a suspension of them in the sugar solution as described by Reinecke (1983). Four grams of faecal material was

weighed off, broken up and mixed in with 56 ml of a saturated sugar solution. The mixture was thoroughly mixed using a wooden spatula. While continuously stirring, an amount of the mixture was transferred to the three counting chambers of the "Eggs-Acto" McMaster slide (Focal Point, South Africa) by means of a wide-mouthed pipette. All three counting chambers were filled and the slide allowed to stand for approximately two to five minutes. This resting period allowed the eggs to rise to the surface, which facilitated examination and counting of the eggs. All the eggs were counted and identified in all three chambers of the McMaster slide. The FEC were expressed as the eggs per gram (epg) of faeces which was calculated using the following equation:

epg = egg count in chambers/number of chambers counted x 100

Faecal cultures are used to determine the larval species composition as well as to differentiation between the large and the small strongyles. This method is based on optimum conditions such as high temperature (28 °C) and high humidity that are both important for the hatching of the eggs and development of larvae into L_3. These conditions were obtained in a room where temperature and humidity were controlled. The pooled faecal samples comprising of equal amounts, based on weight, of fresh faeces from each of the donkeys in each camp were obtained twice a month for the making of pooled larval cultures following the method by Reinecke (1983). The faeces were broken up and mixed with an equal volume of fragmented vermiculite and a small amount of water. The mixture was placed in a one-litre wide-mouth glass jar (9 cm diameter) and tamped down with a flat-bottomed stick while another stick was held in the centre of the jar to produce a hole in the mixture, which reached to the bottom of the jar. The inner surface of the jar was washed down and a screw cap placed on the jar. The cultures were incubated in a humidified room at 28 °C for a period of eight to ten days. One hundred L_3 from each glass jar were harvested

and identified using the guideline of Bürger and Stoye (1968). The larval species and genera identified from each sample were expressed as a percentage of the total count of all L_3.

4. Adhesive tape swab technique for detecting *Oxyuris equi* eggs

The female pinworm, *O. equi*, protrudes from the anus of an equid and deposits her eggs on the skin around the anus. *Oxyuris* eggs in horses are commonly detected by means of an adhesive tape swab technique as they are rarely observed in faecal examinations using methods such as the McMaster technique (Drudge and Lyons, 1989). In contrast, in a recent study on donkeys undertaken by Wells *et al.* (1998) these eggs were regularly recorded in their faeces by use of the McMaster technique. In the present study, faecal samples were examined from all the donkeys for the presence of *Oxyuris* eggs twice a month by means of the McMaster technique. In addition, however, it was decided to include the adhesive tape swab technique (Deplazes and Eckert, 1988) in the study to determine if the infection pattern of this helminth parasite in donkeys differs from that in horses.

The adhesive tape was placed in a loop around one end of a microscope slide, with the adhesive side out. The tail of the donkey was elevated and the elbow of the left arm of the person taking the sample was pushed against its buttock. At the same time, the thumb of the right hand was used to firmly press the adhesive tape to the animal's anal skin fold. The adhesive tape was then attached onto a pre-marked microscope slide, which was examined microscopically for the presence of pinworm eggs (Deplazes and Eckert, 1988; Krecek, personal communication, 1997).

5. Faecal egg count reduction test

Anthelmintic resistance is present when, within a population of helminth parasites, there are a larger number of individuals able to tolerate recommended doses of a specific compound than in a normal population of the same species (Prichard, Hall, Kelly, Martin and Donald, 1980). The frequent use (e.g. every two months) of widely available anthelmintics in economically valuable domestic animal species (cattle, sheep, goats and horses) places increased pressure on these parasites for them to develop resistance. The faecal egg count reduction test (FECRT) is one of the methods frequently used to detect decreased drug efficiency and increased anthelmintic resistance (Johansen, 1989). The test provides an estimate of anthelmintic efficacy based on a comparison of the FEC of animals "before" and "after" treatment (Presidente, 1985).

In the present study the donkeys in four of the eight camps (11 donkeys) received a pre-winter moxidectin anthelmintic treatment. Faecal samples were collected from each of the 11 animals before treatment and again 24, 48 and 72 hours after treatment. Thereafter, faecal samples were collected and examined at seven-day intervals until all the dewormed animals were again positive for parasitic eggs. From the results emanating from previous studies in which the FECRT was used it is recommended that the first FEC after treatment should be performed after 10 - 14 days (Coles, Bauer, Borgsteede, Geerts, Klei, Taylor and Waller, 1992; Presidente, 1985). It is suggested that this delay period is essential in order to reflect the actual worm burden. It is assumed that a shortcoming of FECRT is that anthelmintic treatment may cause temporary suppression of worm egg output without any actual worm loss (i.e. a false positive result) in all hosts. To limit variation, all the faecal samples were collected at the exact same time of day (11:00). The standard McMaster counting method for worm eggs (Reinecke, 1983) was performed on the faeces and individual counts recorded. Larval cultures were set up at the first sign of positive egg counts. The arithmetic mean and percentage reduction were calculated for the treated animals in the four camps

using the formula of Coles *et al.* (1992). The reasons why this method was selected were: First, the sample size in the present study was smaller than 10 animals per group as is required for the method of Presidente (1985). Instead, the sample size of the two groups that received the anthelmintic treatment (four camps) in this study was five and six animals, respectively. Second, the arithmetic mean was calculated instead of the geometric mean. The former is relatively easy to calculate, it also provides a better estimate of the worm egg output and it is a more conservative measure of anthelmintic efficacy (Coles *et al.*, 1992). The percentage reduction was calculated using:

$$\text{FECRT \%} = 100(1 - Xt/Xc)$$

where Xt is the treated group egg count at 10 - 14 days and Xc is the control group at 10 - 14 days.

As was mentioned above, the method of Presidente (1985) is more applicable and practical for larger sample sizes. In addition, the geometric means for the different experimental groups is used rather than the arithmetic. For this technique the percentage efficacy should be corrected for changes that would occur in the control group by the equation:

$$\text{FECRT \%} = (1 - (T_2/T_1 * C_1/C_2)) \times 100$$

where T and C are the geometric means for the treated and control groups and subscripts $_1$ and $_2$ designate the counts before and after treatment, respectively.

6. Standard haematology analysis of blood samples collected from the donkeys

Thus far, in the few studies that have been performed to determine what effect large helminth burdens might have on the blood chemistry of this host there has been no consensus reached (Urch and Allen, 1980; Mattioli *et al.*, 1994). It was therefore decided to assess the diagnostic value of several blood components in working donkeys kept under South African conditions. In the present study blood was collected in EDTA tubes at the same time (8:00 – 9:00) every month from each individual and subjected to standard haematological analyses by the Clinical Pathology Laboratory at the Faculty of Veterinary Science. The Hb, PCV, and WCC in the blood of each donkey were recorded. Only these three blood variables were evaluated for their diagnostic potential as they can be analysed with ease and without the need for expensive laboratory equipment.

7. Filtering of blood samples and staining to detect *Setaria equina*

Setaria equina is a filariid nematode parasite of the abdominal cavity of all equids. The diagnosis of *S. equina* infection is commonly made at necropsy, but microfilariae can also be observed on microscopic examination of the blood, usually after it has been specially prepared. Filtering of a blood sample, collected from the animal in EDTA blood collection tubes, through filter paper (transparent 3 μm aperture polycarbonate filters) is one step in a method described which was used in the present study with a slight modification (Sloss, Kemp and Zajac, 1994). The modification consisted of homogenising the sample without formalin, filtering the homogenised

sample through filter paper and fixing it with methanol before staining. The method is as follows. Clean microscope glass slides were marked with the animal's identification number with a diamond tip pen. The blood sample in the EDTA tube was thoroughly mixed by shaking and 0.5 ml of it was drawn into a two ml syringe. Additional air was drawn into the syringe. The filter apparatus was prepared by placing a filter paper and washer on the filter casing and closing the apparatus. The blood was injected through the apparatus and was collected in the original EDTA tube. Thereafter, 20 ml of distilled water was injected through the apparatus followed by 20 ml of air, which propelled any remaining blood through the apparatus. The filter apparatus was opened and the filter paper removed carefully with forceps and placed on a clean, pre-marked microscope slide. The filter paper was left to air dry before it was fixed for one minute with methanol. The excess methanol was poured off, the slide and filter paper were allowed to air dry and then were saturated with Standard Giemsa Stain (Merck Diagnostics), for one to two hours after which the excess fluid were poured off. The slide was rinsed in running tap water and allowed to air dry before microscopic examination. The washers were alternated and the apparatus rinsed in clean distilled water after each blood sample. Each slide, containing the stained filter paper, was systematically examined under the microscope to record the presence of the purple stained *Setaria* filariids (Sloss *et al.*, 1994).

8. Body condition score of the donkeys

The BCS of an animal is based on its overall muscle development and fat deposition present in different areas on the body and is therefore a more accurate reflection of an animal's physical health than is merely its live weight. In an attempt to improve the methodology and to make more accurate assessments, guidelines to allocate BCS have been developed for most domestic animals

as well as for horses (Henneke, Potter, Kreider and Yeates, 1983). Recently, procedure has been adapted for donkeys after studying 144 animals in Morocco (Pearson and Ouassat, 1996). In short, a nine-point system is used in which a score of one is poor and nine is obese. It takes into account the amount of body fat at the neck, shoulders, back, ribs, pelvis and the rump.

The disadvantage of this guideline, used to assess the condition of an animal, is that the score given is directly dependent on the judgement of the assessor and can be subjective, making it difficult to compare results across studies. It is suggested that the same person should preferably estimate the conditions of all the animals on each occasion that the procedure is performed. This is obviously not feasible, leading Pearson and Ouassat (1996) to perform a repeatability analysis of the guidelines for body condition scoring. They allowed two individuals to assess the same animals and indicated that 74 % of scores allocated were identical and that the largest difference between the two scores was one point. These results suggest that body condition scoring performed by more than one person can be used with a fair amount of objectively and effectivity in scientific studies especially when the suggested guidelines are followed.

In the present study, the BCS of each animal was recorded monthly using the nine-point method described by Pearson and Ouassat (1996). The same person was responsible for assessing the body condition of each animal based on the amount of fat and muscle cover present at the neck, shoulders, back, ribs, pelvis and rump (Table 1).

Table 1. Guide to the body condition scoring of working donkeys.

Score	Description
1 Very thin (emaciated)	Animal markedly emaciated; condemned; bone structure easily seen over body; little muscle present; animal weak; lethargic.
2 Thin	Animal emaciated; individual spinous processes, ribs, tuber coxae, tuber ischii and scapular spine all prominent, sharply defined; some muscle development; neck thin; prominent withers; shoulders sharply angular.
3 Less thin	Vertebral column prominent and individual spinous processes can be felt (palpated); little fat, but superspinous musculature over spinous processes apparent. Ribs, tuber ischii and tuber coxae prominent; loin area and rump concave; little muscle or fat covering over withers and shoulders.
4 Less than moderate	Vertebral column visible; tuber ischii palpable but not visible, tuber coxae rounded but visible; rump flat rather than concave; ribs palpable but not obvious; withers, shoulders, neck with some muscle and fat cover; scapulare less clearly defined.
5 Moderate	Superspinous muscles developed and readily apparent; can palpate vertebral column; tuber coxae rounded; rump rounded, convex; tuber ischii not visible; some fat palpable in pectoral region and at base of neck; can palpate ribs, but not visible.
6 More than moderate	Cannot palpate spinous processes easily; back becoming flat, well covered; rump convex and well muscled; some fat palpable on neck, base of neck and pectoral region; neck filled into shoulder, tuber coxae just visible.
7 Less fat	Back flat, cannot palpate spinous processes; tuber coxae just visible; fat on neck and pectoral region beginning to expand over ribs; flank filling; neck thickening.
8 Fat	Animal appears well covered with body rounded with fat and bones not discernible; flanks filled; broad back.
9 Very fat (obese)	Bones buried in fat; back broad or flat, in some cases crease down back; large accumulations of fat on neck, over pectoral area and ribs; flank filled with fat.

(From: Pearson and Ouassat, 1996)

9. Body measurements of the donkeys

An accurate estimate of the live weight of an animal is advantageous for several reasons: 1) when treating an animal with a drug where the weight of the animal determines the dosage rate of

the drug, 2) to assess the efficacy of a treatment, 3) to assess the animal's general well-being, and nutritional status, and 4) to determine the exact amount of load that a specific animal can carry or pull. Most resourced horse owners are financially capable of transporting their animals to veterinary clinics that are equipped with costly equipment, such as animal scales. In the case of resource-limited donkey owners this is not possible and alternatives have to be found. Therefore, the use of linear body measurements to determine the estimated weight of donkeys has been suggested for use in resource-limited communities (Pearson and Ouassat, 1996; Wells, 1997).

Linear body measurements are based on measurements taken at specific points on the animal's body. These are then used in a mathematical equation to calculate its estimated live weight (Pearson and Ouassat, 1996; Wells, 1997). Pearson and Ouassat (1996) took the following five body measurements from 400 working donkeys in Morocco: heart girth, umbilical girth, height, length, and the circumference of the foreleg cannon bone measured around the narrowest part. The variables that provided the best predictors varied between adult donkeys and young animals (< three years). The best single predictor for the adult donkeys was the heart girth (R^2 = 0.81) and the estimated live weight of the animal can be determined using:

$$\text{Live weight (kg)} = \text{heart girth (cm)}^{2.65}/2188$$

Although the second best single predictor was the umbilical girth (R^2 = 0.59), it was found to be the most difficult measurement in adult animals as well as in pregnant and large animals. This measurement tended to be incorrectly taken over the widest part of the body rather than over the umbilicus. The best overall predictive equation using two variables for the adult donkeys were heart girth and length (R^2 of 0.84), which could then be determined by:

$$\text{Live weight (kg)} = (\text{heart girth [cm]}^{2.12}) \times (\text{length [cm]}^{0.688})/3801$$

In contrast, the best single predictor for the younger animals was the umbilical girth (R^2 = 0.77) and the best overall predictive equation using two variables for the young donkeys were the umbilical girth and length (Pearson and Ouassat, 1996). The suggested combination of heart girth and length for adult donkeys (Pearson and Ouassat, 1996) agrees with another study on working donkeys in South Africa (Wells, 1997). A total of 56 randomly selected working donkeys were measured and their BCS recorded. The aim of the study was to develop an accurate equation that is relatively simple and can be calculated with a readily available calculator or on a piece of paper. The following non-linear equation was developed that includes the BCS of the animal, its heart girth measure as well as its length (R^2 of 0.86):

Live weight (kg) = 4 x condition score + 3 x heart girth + length - 308

In the present study, the following three body measurements were taken from the donkeys and the procedures followed the recommendations made by Pearson and Ouassat (1996) and Wells (1997). The following measurements were taken on each occasion from each animal: 1) heart girth (circumference measured from the caudal edge of the withers around the girth behind the elbow), 2) height (measured with a measuring stick, to the nearest centimetre, from the highest point of the wither with the donkey standing squarely on level ground with its head in a normal position), and 3) length (from the olecranon process of the elbow to the tuber ischii, with the donkey standing in the same position as for the height measurement). The animals stood on an even surface, the heart girth was measured with a measuring tape to the nearest centimetre, and the height and length were measured with a measuring stick.

10. Collection of herbage samples

Herbage samples were collected every month one to two hours after sunrise. It was important that the herbage was collected before the pastures dried out in the sun as the larvae could migrate to the bottom of the grass (Ludwig and Johnstone, 1984). Monthly herbage samples were collected using the method described by Taylor (1939) with a slight modification. They were collected every ten paces following a single "zigzag or W-collection" route. The herbage, which consisted almost entirely of grass, was cut at soil level with a pair of garden scissors at four points, one immediately in front of one foot, and the other three as far as could be conveniently reached to the front, to the left and to the right side of the foot. Care was taken not to include excessive soil on the grass. The method described by Taylor (1939) did not exclude herbage collection next to dung pads/roughs (rarely grazed because of faecal material). Herd and Willardson (1985) noted that counts of infective larvae on herbage samples collected from the whole pasture (grazed and rarely grazed areas) were unlikely to represent the herbage eaten by horses. However, the separation between "roughs" and "lawns" are only distinct in larger sized camps. The donkey camps in the present study were relatively small (473 to 1 242 m^2) and thus not large enough to enable this sort of separation and therefore herbage samples were collected throughout the camps. They were collected, where possible, from each of the four points and, if no herbage was available (for example during winter) or faecal material was present at a specific sampling point, then that point was ignored or a subsequent sample collected a distance away from the point. The average total amount of herbage collected each month from each camp weighed approximately 250 g. The samples from each camp were placed in separate, clearly marked plastic bags and transferred to the laboratory for immediate processing.

11. Processing of herbage samples

It was decided that the washing machine technique as described by Bürger (1981) and Krecek (personal communication, 1997) would be used each month to separate the L_3 from the herbage material due to the weight of the herbage samples (> 100g; see above). Prior to the washing of the material, the wet weight was recorded and the samples were placed in separate gauze bags (1.5 x 1.5 mm hole size). The size of the apertures was small enough to retain the grass, but allowed the soil and larvae to flow out. Each bag was washed separately in a modified heavy duty automatic Speed Queen commercial clothes washing machine (capacity 8 kg) that was set on a regular normal cycle that included two washes (Krecek, personal communication, 1997). The number of washes differed from that used in the method described by Bürger (1981) in which herbage samples are washed three times. The washing machine had been modified by removing its filter so that all washings were drained from it through the machine's pipe (5 cm diameter plastic pipe) and collected after passing through a 25 µm sieve placed outside the machine (Bürger, 1981). Subsequently, the washings were extensively washed over an 850 µm sieve into a 25 µm sieve to finally remove small grass pieces from the larvae. The washed herbage samples were air dried for 30 days at approximately 25 °C and their dry weight recorded.

12. Isolation of parasitic L_3 from suspended soil and extraneous material

The parasitic L_3 were isolated from the soil and finer plant material by using a slightly modified sugar centrifugal-flotation technique originally described by Caveness and Jensen (1955) and Dickerson (1977 from Krecek *et al.*, 1991). The procedure was as follows: the final herbage

washings, containing the larvae and finer material, were concentrated to approximately 30 ml and were poured into centrifuge tubes (50 ml) and water added to the 45 ml level. All the tubes were adjusted to the same weight by the addition of water and were centrifuged simultaneously at 2 500 rpm for five minutes in a Hettich Rotanta/RP after which any material lighter than water was eliminated by carefully decanting the supernatant fluid. The remaining residue in the tubes was suspended in saturated sugar water (484.5 g sugar per litre of water) with a specific gravity of 1.18 by manually shaking and the suspension centrifuged at the same speed and for the same time as before. The supernatant fluid containing the larvae was then washed over a 25 µm sieve with water. The larvae were collected from the sieve, suspended in normal tap water (30 ml) in a clean set of centrifuged tubes and centrifuged again. This procedure differed from that described by Caveness and Jensen (1955) in the final step prior to counting.

A total larval count on a 1/5 aliquot was performed under a microscope on each sample and the first 100 parasitic larvae to be counted were identified, using the guideline of Bürger and Stoye (1968). Non-parasitic nematode larvae were differentiated from the parasitic L_3 by means of the greater development in their internal morphology and lack of a sheath (Caveness, 1964; Krecek, personal communication, 1997). Using the 1/5 count an estimated number of L_3 was calculated for the herbage samples from each camp:

Number of L_3/kg dry herbage = (larval count x 1000/dry weight of herbage [g]) x 5

13. Verification of the efficacy of the combination of machine washing and centrifugation technique used to determine parasitic nematode larvae in herbage samples

For a period of one month three 250 g herbage samples were processed every morning of a working day. The aim was to determine the efficacy of the technique that uses a combination of machine washing and sugar centrifugation, described in Chapter 3 under sections 11 and 12. In order to facilitate comparisons with the "after" larval counts, it was important that the "before" larval counts were known. For this reason, faecal material was collected from donkeys with high faecal egg counts and cultured for eight days at 28 °C. After this time strongyle L_3 were harvested from the culture jars and stored in a refrigerator. For this study, 35 herbage samples, each weighing approximately 250 g, were collected from ungrazed pastures (*Bothriochloa* sp., *Cynodon dactylon*, *Eragrostis curvula*, *Paspalum dilatatum*, *Pennisetum purpurea* and *Sporobolus* sp.). The herbage was cut at soil level and care was taken not to include excessive amounts of soil with the grass. Each sample was placed in a numbered plastic bag, brought to the laboratory and seeded with known numbers of cyathostome L_3 which ranged from 114 to 150 120 per sample prior to washing. Each of the 35 seeded herbage samples was randomly selected for washing. The procedures that were used in the larger herbage sampling trial (monthly processing of herbage samples) described above were also followed in this recovery rate trial: 1) the washing of herbage samples (section 11), 2) drying of herbage material (section 11), 3) isolation of L_3 from suspended soil and extraneous material (section 12), and 4) counting and identification of larvae (section 12).

14. Pasture infectivity as a function of the faecal egg counts and the amount of faecal material produced

The host's faecal egg count as well as the amount of faeces deposited on the pasture influence the degree of pasture contamination. Most faecal deposition in this trial occurred around the feed trough. The presence of the faecal material and constant trampling resulted in the development of an area with a radius of two meters around the troughs in each camp in which there was no pasture growth. In an attempt to determine the amount of faeces that were deposited on the grass in the camps, areas, 20 % the size of each camp, were randomly plotted out once a month, using a piece of string. The faeces in this area were collected, air-dried and weighed.

15. Grazing consumed by the donkeys in each camp

The presence of grazed (shorter grass) and ungrazed (longer grass) in paddocks may be attributed to: 1) the uneven grazing patterns due to non-random defecation patterns of horses and possibly donkeys (Crofton, 1952; Herd and Willardson, 1985), and/or 2) variation in growth rate and palatability of the grass species in a camp. The study area of the present study falls in the summer rainfall region in South Africa. As a result, pasture growth is facilitated during the warm and wet months (the grazing season) and in the dry and cooler months pasture growth is generally limited. Monthly herbage growth measurements were recorded in each camp during the entire experimental period in an attempt to determine the effect of faecal removal on the amount of herbage consumed. It was expected that the grass in the camps subjected to monthly faecal removal would be shorter compared to that in the camps where the faecal material was left to accumulate

during the whole study period. At the beginning of each month, a 0.5 m^2 fenced frame was randomly placed in each camp and left for the month. At the end of the month the grass in this fenced off area was cut at soil level and collected. At the same time a second 0.5 m^2 area was cut and collected at another randomly allocated point in the grazed area of the camp. The cut grass samples were air-dried and their individual dry weight recorded.

16. Necropsy and mucosal larval recovery techniques

The necropsy guidelines of Malan *et al.* (1981a, b) and Duncan *et al.* (1988) for the recovery of helminth parasites were largely followed in the present study. Fasting of the animals prior to necropsy is a common practice as it has been found that it reduces the amount of ingesta in the gastro-intestinal tract, which facilitates parasite recovery and counting. The necropsy guideline of Duncan *et al.* (1988) recommends that feed be removed 24 hours prior to euthanasia. In a separate study on donkeys in Zimbabwe feed was withheld two days before euthanasia (Pandey and Eysker, 1990). In the present study, the recommendation by Duncan *et al.* (1998) was followed and feed was withheld for approximately 24 hours. During the necropsy, faecal material was collected from each individual to determine its faecal egg count (Reinecke, 1983). The age, sex and other features of each animal were also noted. The entire gastro-intestinal tract and abdominal and thoracic organs were removed from the carcass and the latter examined macroscopically for the presence of helminths. The subperitoneal tissues, liver and lungs were examined for the presence of *Strongylus edentatus* larvae and *Dictyocaulus arnfieldi*. The gastro-intestinal tract was divided into the following six sites: stomach, small intestine, caecum, ventral colon, dorsal colon and descending colon, and each handled separately. Aliquots of ingesta of 1/10 by mass from each of these sites were collected for microscopic examination. The 1/10 aliquot used for the stomach ingesta in the

present study deviates from the suggested 1/4 aliquot of Malan *et al.* (1981b) as it was felt that the smaller sample size would be adequate. The walls of the caecum, and ventral, dorsal and descending colons were washed separately and the washings collected for subsequent inspection for worms. The washings, that included helminths, were placed in one-litre wide-mouth glass jar with a screw cap and preserved in 70 % alcohol.

Several gut wall samples (approximate size 25 cm^2) were removed from each of the caecum, and ventral, dorsal and descending colon. The size of the samples concurs with that described in the procedures of Reinemeyer and Herd (1986a) and Xiao *et al.* (1994), but is slightly smaller than that used by Chapman *et al.* (1999). The smaller sample size was used to allow random tissue sampling along the entire length of each of these four gut regions as previous studies have noted a non-uniform distribution of encysted larvae within the caecum and ventral colon of horses (Reinemeyer and Herd, 1986b; Chapman *et al.*, 1999). This was done in the following way: the samples were systematically removed (10 cm apart) along the length of each of the regions, following a "w-shape" pattern. The smaller wall samples could also be easily accommodated in a glass dish (diameter 14 cm) with a grid, comprising squares 2 x 2 cm in size, drawn onto the bottom of the dish with a permanent marker pen. Due to the narrowness of the small intestine, wall samples were collected at regular intervals down its length. The combined weight of all the removed pieces from each compartment was used to provide a 10 % aliquot of the total weight of each compartment.

Transmural illumination (TMI) was performed on each of the gut pieces. The serosa was removed from each sample prior to illumination as it was believed that the larvae would be more visible if there were less tissue layers. This is similar to the TMI method described by Eysker *et al.* (1997), but is in contrast to that used by Reinemeyer and Herd (1986a) and Chapman *et al.* (1999) in which full thickness tissue samples were illuminated. Chapman *et al.* (1999) in fact, recorded no significant differences in the number of DL recovered from stripped mucosa (mean 4.0 ± 3.3)

compared to the full thickness piece (mean 5.2 ± 4.0). The entire piece of mucosa was stretched out on a petri dish containing a grid, illuminated and the encysted larvae if present counted under a dissecting microscope (Reinemeyer and Herd, 1986a; Eysker *et al.*, 1997). After the TMI procedure had been completed the same pieces from the different regions were subjected to DIG. The consensus arising from most studies in which DIG was performed is that the mucosa and submucosa should be removed from the muscle layers of the gut wall prior to digestion (Eysker and Klei, 1999). There is no doubt that the digestion process would be enhanced when less tissue material is present to digest. In contrast to the general consensus, however, this step was not performed in the present study due to difficulties experienced in separating the mucosal and submucosal layers from the rest of underlying muscle layers. The gut pieces were placed in one litre flasks in the following ratio: 30 g of mucosa to 200 ml of standard digestion fluid (10 g pepsin and 15 ml of HCl per litre of water), with a maximum of 90 g or 600 ml per reaction. The digestions were mixed at seven-minute intervals with a total digestion time of three hours at 37 °C (but were monitored after two hours to ensure that the larvae were not disintegrated as this is one of the criticisms against this method). Although the recommended digestion time is two hours at 37 °C (Chapman *et al.*, 1999), it was decided to extend it by one hour in order to compensate for the excess tissue. After three hours of incubation, each digestion solution containing larvae and undigested pieces of gut was poured onto a 63 µm sieve and washed with tap water to stop the digestion process (Chapman *et al.*, 1999). Thereafter all the material that did not pass through the sieve was fixed with 10 % formalin solution, from which two aliquots each comprising 10 % of the volume of the formalin solution was removed. The larvae recovered from the first aliquot were measured (length), their buccal capsule shape noted and counted. If this count was below 50, the second aliquot was processed in a similar manner and the estimated number of encysted larvae was calculated for each of the different gut compartments.

17. Processing of intestinal washings and ingesta for worm recovery and identification

Each of the ingesta and intestinal wall wash content samples was placed into a ten litre bucket which was filled with water to the ten litre level. The water and gut material were mixed uniformly and constantly. Small volumes (50 ml) of the mixture were periodically removed and poured into two separate one-litre wide-mouth glass jars (2 x 1/100 aliquots) until they were 50 % full. The eight litres remaining in the bucket were reduced by pouring the content over a 150 µm sieve and the gut material placed in one or two labelled one-litre wide-mouth glass jars (1 x 8/100 aliquot). Ample volumes of 70 % alcohol were added to each glass jar to preserve the material.

The contents of the two 1/100 aliquot jars were examined microscopically under a stereo microscope. All the worms were counted, removed and placed in a labelled polytop bottle filled with 70 % alcohol. The contents of the 8/100 aliquot were examined under a magnifying glass and all the worms present were counted, removed and placed in a labelled polytop bottle filled with 70 % alcohol.

The identification of helminth species followed the descriptions of Boulenger (1920), Theiler (1923), Zumpt (1965), Lichtenfels (1975), Krecek, Kharchenko, Dvojnos, Malan and Krecek (1997), and Lichtenfels, Pilitt, Dvojnos, Kharchenko and Krecek (1998a). Encysted cyathostome larvae that were recovered from the gut walls of the donkeys necropsied were measured and identified as either EL_3, LL_3 or DL_4, based on the guidelines provided by Popova (1958 from Lichtenfels, 1975) and Chapman *et al.* (1999).

CHAPTER 4

THE EFFECT OF ALTERNATIVE MANAGEMENT INTERVENTIONS ON THE LEVELS OF HELMINTHS IN LIVE DONKEYS AND ON PASTURE

1. Introduction

In most developing countries, donkeys and mules are used extensively for transport and agriculture and South Africa is no exception to it (Krecek *et al.*, 1998). Unfortunately, few attempts have been made to establish the management systems under which these important animals are kept in South Africa. Recent studies have revealed that supplementary feed during winter is restricted and helminth parasite control is virtually non-existent (Krecek *et al.*, 1994a; Krecek *et al.*, 1998; Wells *et al.*, 1998). Several factors, such as limited resources, unavailability of relevant information and of veterinary services, and the perceptions that worms are not important because they can not be seen in an animal, compared to ectoparasites, such as ticks (Starkey, 1995; Wells *et al.*, 1998) all contribute to an apparent "lack" of internal parasite awareness and consequently their control in donkeys and mules.

Most of what is known about the effect of helminth species on equids (Round, 1968; Frerichs *et al.*, 1976; Smith, 1976; Ogbourne, 1978; Drudge and Lyons, 1989; Herd, 1990; Love *et al.*, 1992; Mair, 1994; Murphy and Love, 1997) and the value of alternative control methods (Craig and Suderman, 1985; Reinemeyer, 1986; Herd, 1990; Herd *et al.*, 1985; Duncan and Love, 1991; Herd, 1993; Herd and Coles, 1995; Waller, 1999) is based on studies on horses in developed countries. From these it is apparent that members of the Cyathostominae represent the largest number of nematode worm species (> 50 of the nematode species) in equids and have become

increasingly important following the recognition of a newly recognised disease syndrome, "larval cyathostomiasis". The results of numerous studies noted that this syndrome is most common and pathogenic in young horses but can occur in horses of all ages (Herd, 1990; Mair, 1994). It is normally associated with the start of the warm and wet conditions in most countries when the following clinical signs are often observed: weight loss, colic, lack of vigour, delayed shedding of the winter hair coat, diarrhoea and death (Ogbourne, 1978; Herd, 1990, Love *et al*, 1992; Reilly *et al*., 1993; Mair, 1994; Murphy and Love; 1997).

Results emanating from studies on alternative helminth control methods (commonly characterised by limited anthelmintic use or none at all) indicated that they are indeed effective in reducing helminth burdens on pastures (Herd, 1986; 1993; Herd and Gabel, 1990) and in the host (Herd *et al*., 1985; Duncan and Love, 1991; Herd, 1993; Williams, 1997; Waller, 1999) and are less expensive compared to the traditional exclusive use of anthelmintics. It is therefore hypothesized that such control methods will be viable in developing countries and communities with financial constraints.

Applications of pasture hygiene have been proposed as one of the alternative control methods (Herd, 1986; 1993; Herd and Gabel, 1990). In South Africa, faecal removal from pastures is not an uncommon practice as faeces from donkeys, horses and cattle are valuable sources of fuel and compost and in some communities are often exchanged for vegetables (Krecek *et al*., 1998). However, as yet, there have been no studies in South Africa to determine how frequent the removal of faeces should be practised and what impact it would have on the general health and condition of equids grazing on such pastures.

In addition to pasture hygiene, the strategic use of an effective anthelmintic can also be classified as an alternative helminth control method (Craig *et al*., 1983; Herd *et al*., 1985; Craig and Courtney, 1986). In South Africa, determination of the effectiveness of a single strategic deworming is limited to only one study in horses (Horak and Snijders, 1968). In this study that

took place in the Gauteng province, a summer rainfall area, the animals were administered a single anthelmintic treatment in March (autumn) which reduced the faecal helminth egg counts for up to five months after treatment. It is surmised that the drier, colder winter climate (which is characteristic of the region) interfered with egg maturation and development of infective larvae in the paddocks. The authors considered that an effective dosing programme for horses in this region (and in other regions with similar winter conditions) could thus be based on treatments in autumn, spring and mid-summer. Krecek *et al.*, (1994b) included two strategic treatments (autumn and spring) and demonstrated that a May-June treatment lowers faecal helminth egg counts in horses for at least three months. It is deduced, by extrapolation, that under South African conditions a single annual treatment in the autumn would result in lower helminth burdens in donkeys, with a subsequent improvement in their general health.

2. Materials and methods

2.1. Study area

The Onderstepoort campus of the Faculty of Veterinary Science, University of Pretoria where the study was performed falls in the summer rainfall region in South Africa. The average minimum and maximum temperatures for this area are 12.1 °C and 24.8 °C and it has an average annual rainfall of approximately 710.7 mm (Pretoria Central Weather Bureau). Eight camps were used which had, one month prior to the commencement of the experiment, provided grazing for a few milk cows. The camps differed in: 1) the amount of shade cover, as a few trees were present in two of the eight camps, 2) the pasture species composition, and 3) size (Table 2). The smallest

camp was 473.7 m^2 and the largest 1 242.6 m^2 (Figure 1). To prevent the movement of study animals between and reduce faecal cross infection among camps six-wire-fences were erected to separate the enclosures. In the absence of any flooding it was reasonable to assume that larval migration was restricted. The most dominant grass species that represented more than 80 % of the grass species in each camp was Kikuyu, *Pennesetum purpureum*, followed by quick grass, *Cynodon dactylon* and *Eleusine coracana*. The herb, *Verbena tenuisecta* also grew in some abundance in two of the camps. Apart from the natural grazing, the animals were fed additional grass (*Eragrostis curvula*) hay twice a week and lucerne (*Medicago sativa*) hay three to four times a week. The water in each camp was supplied *ad libitum*. Monthly minimum and maximum temperatures were obtained from the Pretoria Weather Bureau (12 km away) and the daily rainfall was recorded at the camps.

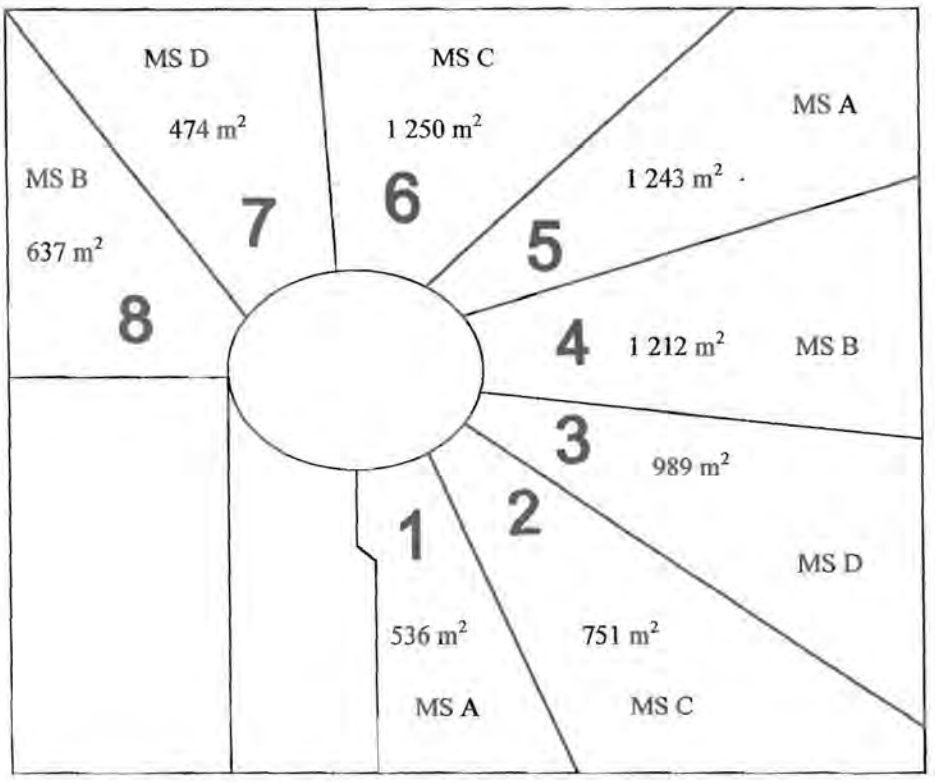


Figure 1. An illustration of the eight camps in which the 24 donkeys were housed during the 16-month alternative helminth control trial at the University of Pretoria, South Africa. Camp size is given in m^2. The management systems (MS) are indicated and correspond to those in Table 4.

Table 2. The seven dominant grass species and one herb species present and their abundance in the eight camps that formed part of the 16-month donkey helminth parasite study.

Species	Camp 1	Camp 2	Camp 3	Camp 4	Camp 5	Camp 6	Camp 7	Camp 8
Grass species								
Andropogon schrensis	-	10 %	-	5 %	-	5 %	10 %	-
Cynodon dactylon	50 %	80 %	15 %	10 %	15 %	5 %	80 %	15 %
Eleusine coracana	10 %	-	-	5 %	5 %	-	-	15 %
Eriochloa mosambisensis	-	-	15 %	-	-	-	-	-
Hermanea tomentosa	-	-	-	-	5 %	-	-	-
Paspalum dilatatum	15 %	-	-	-	-	-	-	10 %
Pennisetum purpureum	25 %	5 %	10 %	80 %	75 %	90 %	10 %	60 %
Herb species								
Verbena tenuisecta	-	5 %	60 %	-	-	-	-	-

2.2. Study animals

Twenty-four adult donkeys were purchased and collected from four different provinces in South Africa: Barkley East (Eastern Cape Province), Hammanskraal and Onderstepoort smallholdings (North-West Province), Marble Hall (Northern Province) and Witbank (Mpumalanga Province) (Figure 2). The group consisted of 15 females and nine males. All animals harboured a natural nematode parasite infection as determined by faecal egg counts (Reinecke, 1983). No information was available on any prior management practices, but to our knowledge none of the animals had been previously dewormed. The age of each animal was estimated based on dental ware and eruption (Miller and Robertson, 1959). Their ages ranged from two to 15 years and they were all in general good health with BCS (Pearson and Ouassat, 1996) ranging from three to four out of nine (Table 3).

Figure 2. Map of South Africa and the provinces from which the 24 donkeys originated include Eastern Cape, North-West, Northern and Mpumalanga.

Table 3. Animal number, place of origin, sex, weight, age and body condition score (BCS) of the 24 donkeys on their arrival July – October 1997 at the University of Pretoria.

Animal number	Origin	Sex	Weight (kg)	Age (years)	BCS (1 – 9)
2	Barkley East	M	158	2.5	4
4	Barkley East	M	156	3.0	4
5	Witbank	F	186	8.0	4
6	Witbank	F	198	4.0	4
7	Witbank	F	216	4.0	4
8	Witbank	M	166	4.0	4
9	Witbank	M	158	2.0	4
11	Witbank	M	110	2.0	4
12	Hammanskraal	F	112	2.0	3
13	Hammanskraal	F	144	4.0	3
14	Hammanskraal	M	172	15.0	3
15	Hammanskraal	F	146	9.0	3
16	Hammanskraal	F	136	2.0	3
17	Onderstepoort	F	170	4.0	3
18	Onderstepoort	F	146	15.0	3
20	Onderstepoort	M	176	3.0	3
23	Marble Hall	F	168	10.0	4
24	Marble Hall	F	164	3.0	4
25	Marble Hall	M	146	3.0	4
26	Marble Hall	F	128	2.5	4
27	Marble Hall	F	140	8.0	4
29	Marble Hall	F	170	5.0	4
31	Marble Hall	M	124	2.0	3
32	Marble Hall	F	174	4.0	4

2.3. Basic outline of the activities and management systems used in the field study

Following the arrival of the initial 24 donkeys, a three-month adjustment period (July 1997 - October 1997) was permitted to facilitate: 1) cross helminth infection between donkeys and between camps in a rotational grazing system of the donkeys in all the camps, 2) the collection of baseline information from the animals and the pasture, and 3) both the animals and the handlers were familiarised with one another, and skills were gained of the different techniques that were to be used during the study. In October 1997, the 24 animals were randomly allocated to eight groups consisting of three animals each, and the study commenced. During the entire 16-month study, the

group of animals within the same management system were rotated every second week between the two camps of that particular management system (e.g. animals in camps 1 and 5 were rotated with each other), thus reducing possible variations in the results obtained due to the differences in the camp sizes. Three management systems, including a replicate of each, and a set of two controls were tested (Table 4). For the first seven months (October 1997 – May 1998) only, monthly faecal removal was performed in camps three, four, seven and eight. Contemporaneously, the faeces in camps one, two, five and six were left untouched. Due to time and financial constraints faecal removal was performed once a month. Moreover, from a management and recommendation perspective, it was hopped that once a month faecal removal will prove adequate in reducing helminth burdens. In December 1997, one animal (number 20) was diagnosed with a terminal lung problem and was removed from the study. The day after the first frost was recorded (18 May 1998) the 11 donkeys in camps two, three, six and seven received a single moxidectin oral gel treatment (0.4 mg/kg). Pre-treatment faecal samples as well as a set of post-treatment samples were collected from these animals at 24, 48 and 72-hour intervals (i.e. six samples from each of the 11 treated individuals). Thereafter, faecal samples were collected at seven-day intervals until positive FEC were once again recorded in all the treated animals. The extent of the individual egg counts was recorded (Reinecke, 1983) and larval cultures were set up at the first sign of positive egg counts. The study continued for eight months following the pre-winter treatment date. The field trial was terminated in the last week of January 1999. At the end of the study period one animal from each of the eight groups was selected, on the basis of its BCS for the final month, for euthanasia and necropsy. An average BCS was calculated for the animals in each camp and the animal with the closest score to the average was selected for necropsy.

Table 4. The four management systems and the associated camp numbers that were tested at the University of Pretoria from 1 October 1997 to 31 January 1999.

Management systems (MS)	ID	Camp numbers
Control	A	1 and 5
Monthly faecal removal	B	4 and 8
Pre-winter moxidectin treatment	C	2 and 6
Monthly faecal removal and pre-winter moxidectin treatment	D	3 and 7

2.4. Variables recorded from the donkeys throughout the study

Each animal was weighed once a week at the same time (8:00 – 9:00) of day on an electronic scale, its weight was recorded and its monthly average weight calculated. Rectal faecal samples were collected bimonthly from each of the animals and processed using the McMaster technique of Reinecke (1983) with a slight modification. Group larval cultures (Reinecke, 1983) for each camp were set up and cultured for eight days and the first 100 larvae to be collected were identified using the guidelines of Bürger and Stoye (1968). The following two procedures were performed monthly: the BCS of each animal was determined according to the nine-point system of Pearson and Ouassat (1996) and recorded, and blood was collected from each donkey and analysed for Hb, PCV and WCC using standard haematological methods. The following three procedures were performed at different intervals during the study. First, the adhesive tape swab, as described in Chapter 3, was used to determine the presence of *O. equi* eggs around the anal opening at three times (February, October and January) during the study (Deplazes and Eckert, 1988; Krecek, personal communication, 1997). Second, blood was collected, filtered and the stained filters examined for *S. equina* at three-month intervals (Sloss *et al.* 1994). Third, the heart girth, height and length of each animal were recorded at three-month intervals during the study (Pearson and Ouassat, 1996; Wells 1997).

2.5. Daily and hourly variation in the donkeys' faecal worm egg counts

In an attempt to establish if strongyle eggs are excreted at a specific time or non-randomly within a day a trial was developed that included the collection of faecal material at three times in an eight hour day. At the start of winter (April to June) faeces were collected directly from the rectums of the donkeys at three different times of the day (7:00, 11:00 and 15:00) for three consecutive days followed by a two-week interval. This was repeated twice. Nematode egg counts were performed on each of the faecal samples using the slightly modified McMaster technique (Reinecke, 1983). Larval cultures were prepared on the last day of the last series (Reinecke, 1983) from the faeces of the individual animals that had provided faecal material on each of the three sampling times that day. The first 100 larvae to be collected were identified using the key of Bürger and Stoye (1968).

2.6. Pasture sampling and determination of its parasitic nematode larval population

The current study modified the herbage collecting method described by Taylor (1939) to some extent in that herbage samples that were close to faecal material were not collected. The methods used for collecting the herbage samples from the eight camps, for the processing of the herbage samples and larval isolations were those that are described in detail in Chapter 3. A total larval count was performed on a 1/5 aliquot of each sample, and the first 100 larvae that were collected were identified, using the guideline of Bürger and Stoye (1968). Based on the 1/5 count, an estimated number of L_3 was calculated for each camp, as described in Chapter 3.

The method used for the isolation of cyathostome L_3 from herbage samples using a technique that combines machine washing and centrifugation in a sugar solution is described in detail in Chapter 3.

2.7. Data analysis

The analysis of Coles *et al.* (1992) was used to determine the percentage effectivity of moxidectin using the FECRT. In this study the arithmetic mean (X) was used and the percentage reduction calculated using:

$$\text{FECRT } \% = 100(1 - Xt/Xc)$$

where Xt is the egg count of the treated group and Xc is the egg count of the control group, both at 14, 30, 42, and 56 days.

Pearson's correlation coefficient was calculated using SAS® to determine the relationship between the bi-monthly FEC, pasture larval counts, and the monthly rainfall. Multiple comparisons were performed to determine the monthly relationship between each of the three experimental animal groups and the control animals for each of the live weight, BCS, linear body measurements, Hb, PCV, WCC, FEC and nematode larval species composition. In addition, comparisons were performed to determine the relationship between the monthly pasture larval burdens (expressed as the number of nematode L_3 per kilogram dry weight of herbage) recorded from the eight camps in the different management systems. Monthly comparisons were also performed to determine the difference in the amount of grazing that was consumed in the different camps. Least square means, using the Fisher's test, were calculated for each of these comparisons. The percentage recovery of

cyathostome L_3 was calculated for each seeded herbage sample and an average recovery rate (± standard deviation) calculated for all 35 samples. Regression analyses were performed on the larval counts before and after washing. The value of the mathematical equation to predict the live weight of the donkeys was established using a linear regression analyses and obtaining the correlation coefficient between the actual live weight and the predicted weights. An analysis of variance (ANOVA) using a general linear model was performed on the daily FEC as well as the egg counts obtained at three different times during a day to determine the effect of time and day on the variation of nematode faecal egg counts. The criteria for the acceptance of a significance probability were set at 90 % ($p < 0.10$) and were adopted throughout the present study.

3. Results

3.1. Egg and larval species composition in the faeces of donkeys

Strongyle eggs represented approximately 95 % of those counted; the remaining 5 % consisted of *S. westeri*, *P. equorum* and *O. equi* eggs. The species composition of the nematode eggs that were obtained with the McMaster technique was similar in all the donkeys throughout the study, with one exception. The animals in the four camps that received the pre-winter moxidectin treatment (referred to as the animals in the MS C and MS D camps) recorded an absence of *S. westeri* eggs in their faecal samples after treatment, which explains the absence of its larval stage in the larval cultures.

Parascaris equorum eggs were sporadically present in low numbers in seven animals during the course of the study. The eggs of this parasite were, however, frequently present in the faeces of two individuals from April 1998 to October 1998 (donkey 29) and October 1997 to

December 1998 and again in March 1998 to May 1998 (donkey 12). No eggs of this parasite were present in the faeces of donkey 12 after it was treated with moxidectin in the middle of May 1998. In February 1998, *O. equi* eggs were detected in two donkeys (numbers 23 and 25) and in October 1998, in three animals (numbers 23, 29 and 31) using the adhesive tapé swab method. The eggs of this parasite were also recorded in the faeces, using the McMaster technique and eight individuals (donkeys 8, 9, 11, 23, 25, 29, 31) were periodically positive in March, May, June and in October. However, *O. equi* eggs were frequently recorded in the faeces of only one animal (donkey 29). The highest egg counts of this parasite were recorded in June, followed by March and May. No eggs of this parasite were present, after treatment, in the faeces of the animals that were treated with moxidectin. Tail rubbing was observed in two individuals and broken tail hair recorded in most donkeys in March (Figure 3). Overall, there was a low prevalence of *S. equina* in the blood of the donkeys. Even though blood was collected on five occasions throughout the study period this parasite was only observed on one of these, in November 1997, in four of the 24 donkeys.

Figure 3. Broken tail hair of a donkey caused by the rubbing of its tail base against a fence in an attempt to ease the irritation caused by the presence of gelatinous substances associated with *Oxyuris equi* eggs around the donkey's anal opening.

The most abundant nematode larvae that were recovered from all the animals were members of the Cyathostominae family. These were followed in numbers by *S. edentatus* and *S. westeri*. The two least abundant species were *Strongylus vulgaris* and *Trichostrongylus axei* (Table 5). *Strongyloides westeri* larvae were present in noticeable numbers in the pooled larval cultures in the animals in one of the control camps (camp one) and in one of the MS B camps (camp eight) (Table 5).

Table 5. The average larval species composition in the 23 donkeys. Each donkey was exposed to one of four different management systems from 1 October 1997 to 31 January 1999. The management systems are indicated and correspond to those in Table 4.

MS	Cyathostomes	*S. edentatus*	*S. westeri*	*S. vulgaris*	*T. axei*
Control	70.30 %	9.48 %	18.73 %	0.84 %	0.22 %
MS B	72.42 %	8.45 %	16.61 %	0.67 %	0.22 %
MS C	78.61 %	9.81 %	2.45 %	1.20 %	0.11 %
MS D	81.91 %	6.39 %	4.41 %	1.06 %	0.14 %

3.2. Fluctuations in faecal worm egg counts

The daily FEC with their average values and standard deviation for 22 of the 23 donkeys are shown in Table 6 (donkey number 12 was left out of this table as only a single egg count was recorded for it throughout the trial). Average daily FEC varied between different days in all the donkeys, but, the variations were not significant ($p > 0.10$). In addition, the FEC varied in samples collected at 7:00, 11:00 and 15:00 but these, too, were not significantly different ($p > 0.10$). Peak egg production was not evident at any specific time of the day. There were no significant differences between the larval species compositions in the faeces collected at the three different sampling times.

Table 6. Average daily faecal egg counts from 22 of the 23 donkeys.

Donkey #	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Average	SD
2	1 099	911	1 011	689	777	666	622	825	184.5
4	944	1 278	1 255	*	*	*	*	1 159	186.5
5	1 044	977	833	533	622	589	866	781	201.0
6	877	911	689	877	689	689	*	789	110.2
7	300	455	466	311	344	*	*	375	80.0
8	577	622	*	*	*	*	*	600	31.6
9	900	889	1 333	966	999	966	*	1 009	164.4
11	1 900	1 200	1 366	1 233	1 344	500	1 111	1 236	414.0
13	255	578	400	355	522	822	*	489	200.1
14	633	799	833	9 11	878	633	989	811	135.0
15	688	700	211	*	*	*	*	533	278.9
16	333	711	678	766	*	*	*	622	195.9
17	1 055	1 389	1 377	1 411	2 033	1 600	1 444	1 473	296.0
18	366	277	578	77	100	133	*	255	193.7
23	600	433	844	589	1 166	*	*	726	286.6
24	1 411	1 177	1 078	866	1 044	1 133	822	1 076	197.9
25	788	977	1 178	1 233	1 533	*	*	1 142	280.5
26	1 200	1 044	1 333	1 166	1 177	*	*	1 184	103.0
27	955	933	944	766	622	733	866	831	127.5
29	1 111	1 111	1 111	978	677	999	1 177	1 023	167.8
31	1 433	711	1 144	1 177	822	1 011	977	1 039	240.0
32	433	500	411	489	244	*	*	415	102.6

* Not sampled; # Number

3.3. Body measurements

The actual live weight (kg) measured in the 23 donkeys at four different times of the year (September 1997, December 1997, March 1998 and October 1998) was compared with the predicted live weight using the body condition score-heart girth-length formula of Wells (1997) for working donkeys in South Africa. Significant correlations (R^2) of 0.66, 0.84, 0.91 and 0.82 were recorded for the individual comparisons between the actual live weights and the predicted live weights for each of the four sampling times. When all the data points (92) were combined a significant correlation of $R^2 = 0.77$ was obtained (Figure 4). However, the correlation coefficient for the combined data set improved to 0.83 with the exclusion of the first 23 data points (the first month's measurements of the 23 donkeys) from the analysis. The average difference between the actual and predicted live weight was 7.8 kg (± 14.1) and the predicted live weight provided an overestimate of 12.8 kg (± 9.7) in 75 % of the 92 data points.

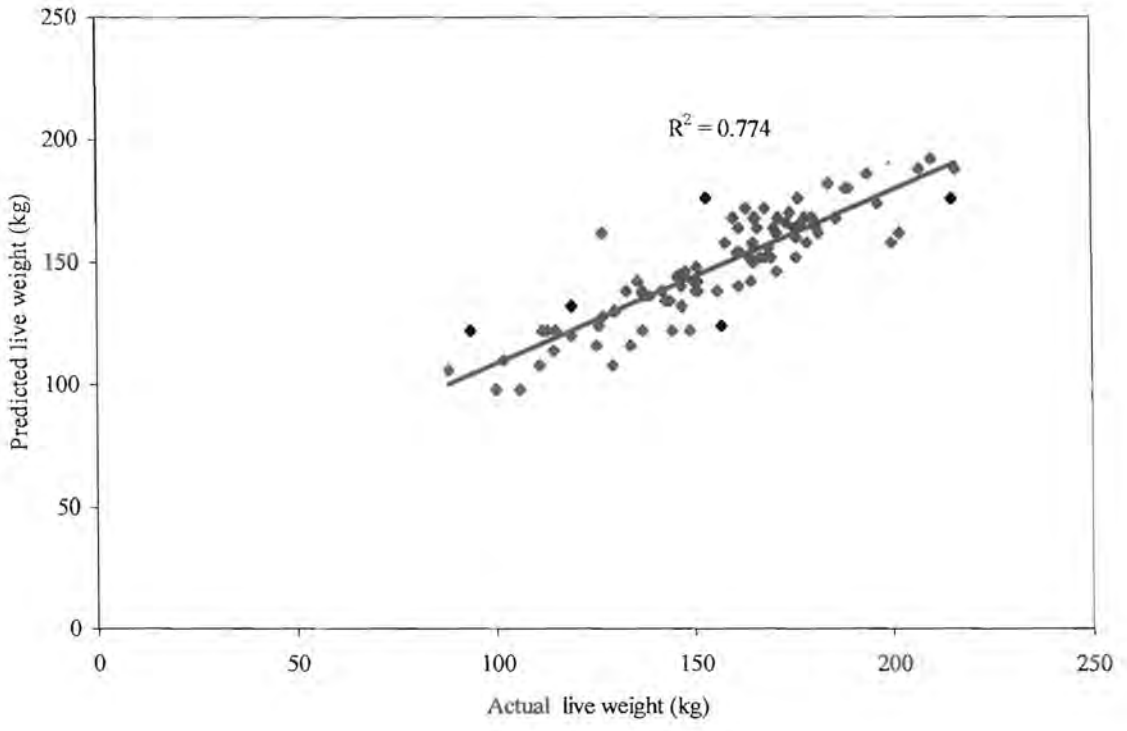


Figure 4. Scatterplot of actual live weight of donkeys compared to the live weight predicted by the body condition score-heart girth-length formula.

3.4. Monthly and seasonal faecal egg counts of the donkeys

The animals in both the control and MS B camps displayed roughly the same seasonal faecal egg output (Figure 5). In the animals in both of these management systems, the lowest counts (680.56 and 633.34, respectively) were observed in April, and the highest counts (1 236.12) were recorded in July for the animals in the camps from which the faeces were removed on a monthly basis, while August recorded the highest counts (1 636.12) for the animals in the control camps. A strong correlation ($p < 0.05$) was recorded between the seasonal FEC of the animals in the MS B camps and the monthly rainfall recorded at the camps (Figure 5).

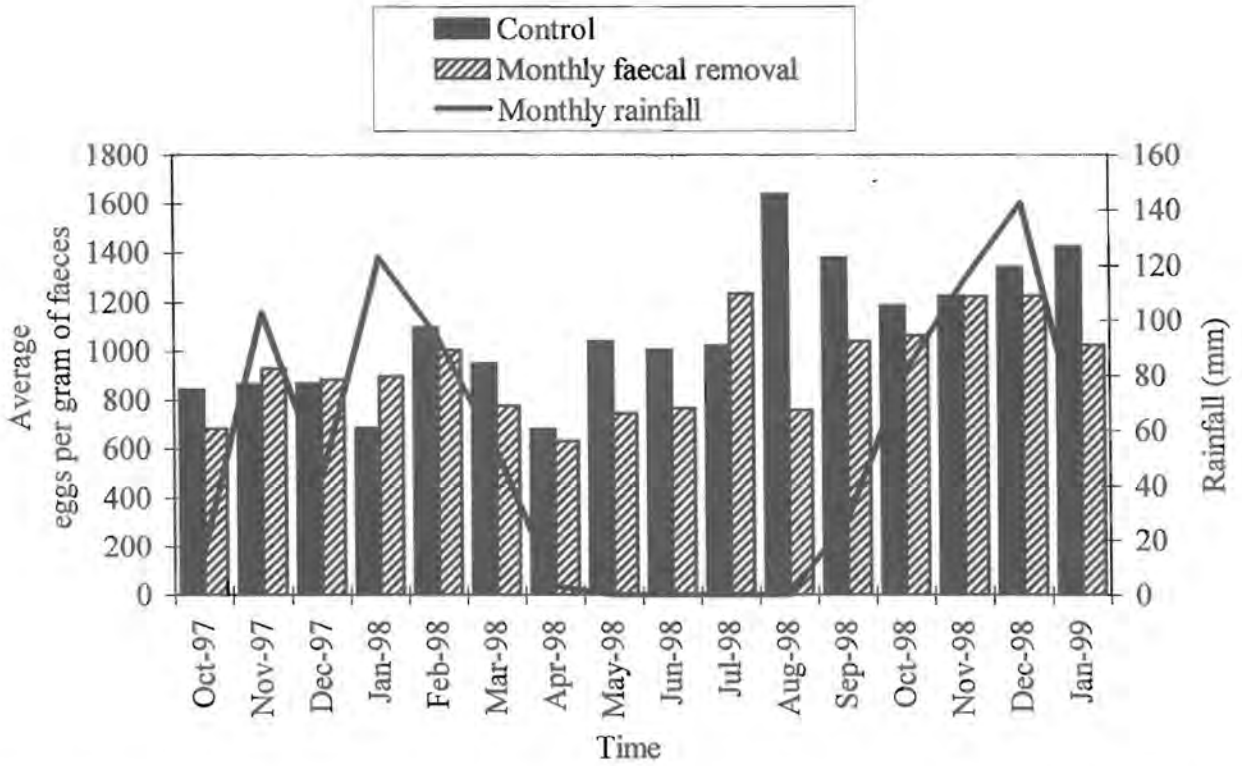


Figure 5. Seasonal average faecal egg counts, based on counts obtained for the animals in the control camps and those in the camps from which faeces were removed from the camps on a monthly basis (MS B), compared to the monthly rainfall that was recorded at the camps.

Although not significant, the monthly removal of faeces from the camps resulted, over time, in a 20 % reduction in the animals' average egg counts ($y = 92x - 499$; $R^2 = 0.77$) when compared to those of the control animals ($y = 112x - 585$; $R^2 = 0.91$). In the first eight months, the average monthly egg counts between all the animals in all the management systems and camps were not significantly different ($p > 0.10$). However, the egg counts decreased to zero within one to two days after the animals in the MS C and MS D camps were treated with moxidectin, and a 100 % reduction was recorded for the first 14 days in the counts (Table 7). Highly significant differences ($p < 0.05$) were noted in the average egg counts between the animals in the MS C and MS D camps and the animals in the control camps from May (deworming) to November at which time the egg counts in the dewormed animals increased to between 300 and 500 epg (Figure 6). For the

remaining two months (December and January) a significance probability of $p < 0.10$ was recorded between the treated animals and the control animals. The donkeys that only received the pre-winter treatment (MS C camps) obtained an average egg reappearance period (ERP) of 55 (± 15) days as opposed to 42 (± 11) days for the animals that were subjected to the combination of pre-winter treatment and the removal of faeces from their camps (MS D camps). At nine to ten weeks after moxidectin treatment patent strongyle infections were detected in all of the donkeys that received the anthelmintic. Egg counts remained reduced for all the treated animals for the subsequent months and at eight months after deworming (January 1999) the average egg counts were still below 500 epg (Figure 6) as compared with 1 425 epg for the control animals (Figure 5). In addition, at the end of the study only 16 % of the animals in the MS D camps recorded egg counts above 1 000 epg compared to 60 % of the animals in the MS C camps (Table 8).

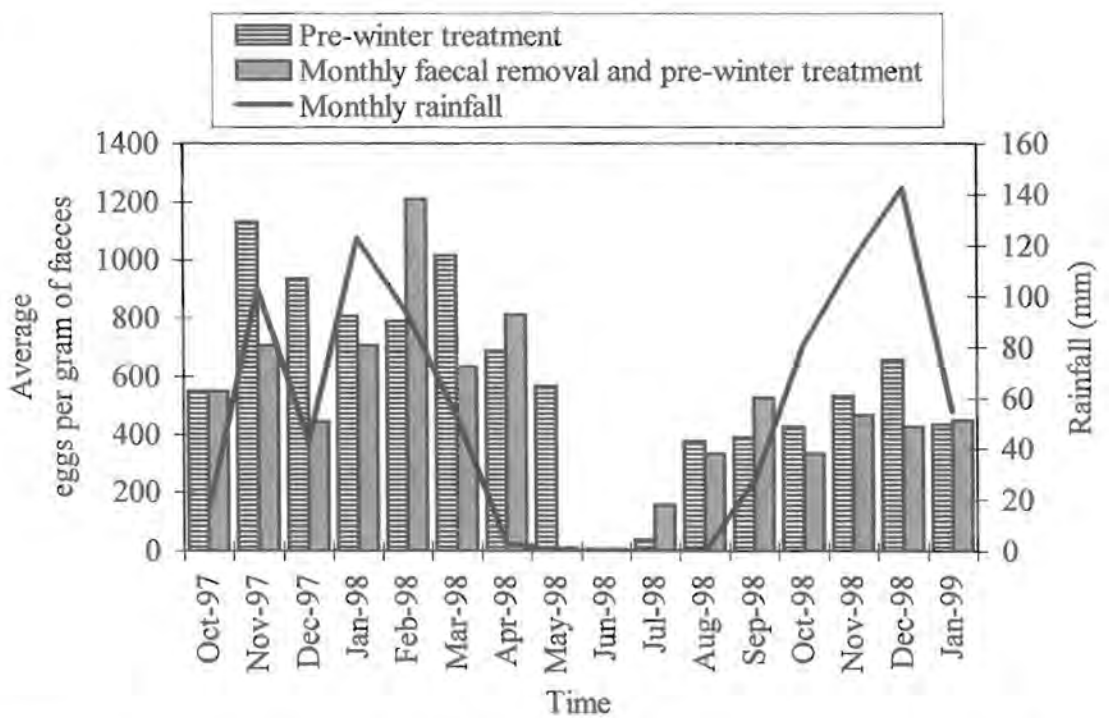


Figure 6. Seasonal average faecal egg counts, based on counts obtained from the donkeys in the pre-winter moxidectin treatment camps (MS C) and from the combination of monthly removal of faeces and pre-winter moxidectin treatment camps (MS D), compared to the monthly rainfall that was recorded at the camps.

Table 7. Average faecal strongyle egg counts from 11 donkeys that received a single treatment of 0.4 mg/kg moxidectin and the control animals.

Group	Eggs per gram				
	Day 0	Day 14	Day 30	Day 42	Day 56
Control	905	955	1 055	878	1 126
MS C (pre-winter treatment MS)	1 106	0	13.3	13.2	66.4
Efficacy	-	**100 %**	**99 %**	**98 %**	**94 %**
MS D (combination MS)	650	0	5.5	83	155
Efficacy	-	**100 %**	**99 %**	**91 %**	**86 %**

Table 8. Average eggs per gram of faeces, range and cumulative percentage above 500 epg for those donkeys treated once with 0.4 mg/kg moxidectin in two of the management systems. MS C = pre-winter moxidectin treatment, MS D = combination of monthly faecal removal and pre-winter moxidectin treatment.

Date	MS C (n = 5)			MS D (n = 6)		
	Average epg	Range	Cumulative % > 500 epg	Average epg	Range	Cumulative % > 500 epg
02/06/98	0		0	0		0
19/06/98	13.33	0 – 66	0	5.56	0 – 33	0
01/07/98	13.33	0 – 66	0	83.33	0 – 200	0
16/07/98	66.67	0 – 233	0	155.56	0 – 333	0
04/08/98	240.00	0 – 966	20	244.44	133 – 333	0
21/08/98	513.33	0 – 1 433	40	333.33	233 – 466	0
01/09/98	440.00	0 – 1 433	40	394.45	100 – 700	33
18/09/98	340.00	0 – 833	40	527.78	166 – 933	50
01/10/98	513.33	0 – 1 400	40	466.67	166 – 866	50
16/10/98	340.00	100 – 966	40	333.33	66 – 733	50
03/11/98	500.00	66 – 1 133	60	544.44	66 – 833	50
20/11/98	566.67	66 – 1 233	60	466.67	200 – 766	66
01/12/98	506.67	66 – 1 200	60	388.89	33 – 600	66
11/12/98	806.67	133 – 1 600	60	427.78	100 – 833	66
12/01/99	433.33	66 – 1 033	60	722.22	100 – 1 500	84
22/01/99	433.33	66 – 1 033	60	450.00	100 – 766	84

3.5. The effect of alternative helminth control methods on the host condition indices

The monthly removal of faeces from those camps in which this procedure was performed had no improved effect on the live weight of the donkeys grazing in those camps. Although not significant, the animals that received an anthelmintic treatment, in the MS C and MS D camps, recorded an improved rate of weight increase in the months following deworming (Figure 7).

The BCS of all the donkeys towards the end of the study ranged from three to five and there were no significant difference in the average rate of increase in body condition for the animals in the MS B and the control camps. During the first six months of the study (before anthelmintic treatment) the average BCS of the animals in the four managements were very similar. Following deworming the BCS of the MS B and control animals reached a plateau while the BCS of the MS C and MS D animals continued to improve, which resulted in a noticeably higher rate of increase during the last two to three months compared to the control and MS B animals (Figure 8).

The average Hb of the animals in the different management systems ranged between 92 and 96 g/dlitre and the average PCV from 0.25 to 0.27 litre/litre (Table 9). The removal of faecal material from the camps on a monthly basis had no significant improved effect on either of the Hb, PCV and WCC of the animals in those camps (MS B camps). In contrast, the animals in the MS C and MS D camps recorded higher averages for Hb and PCV in the period "after " treatment (October 1997 to May 1998) with moxidectin compared to the period "before" treatment (June 1998 to January 1999). The WCC decreased slightly in these animals "after " treatment (Table 9).

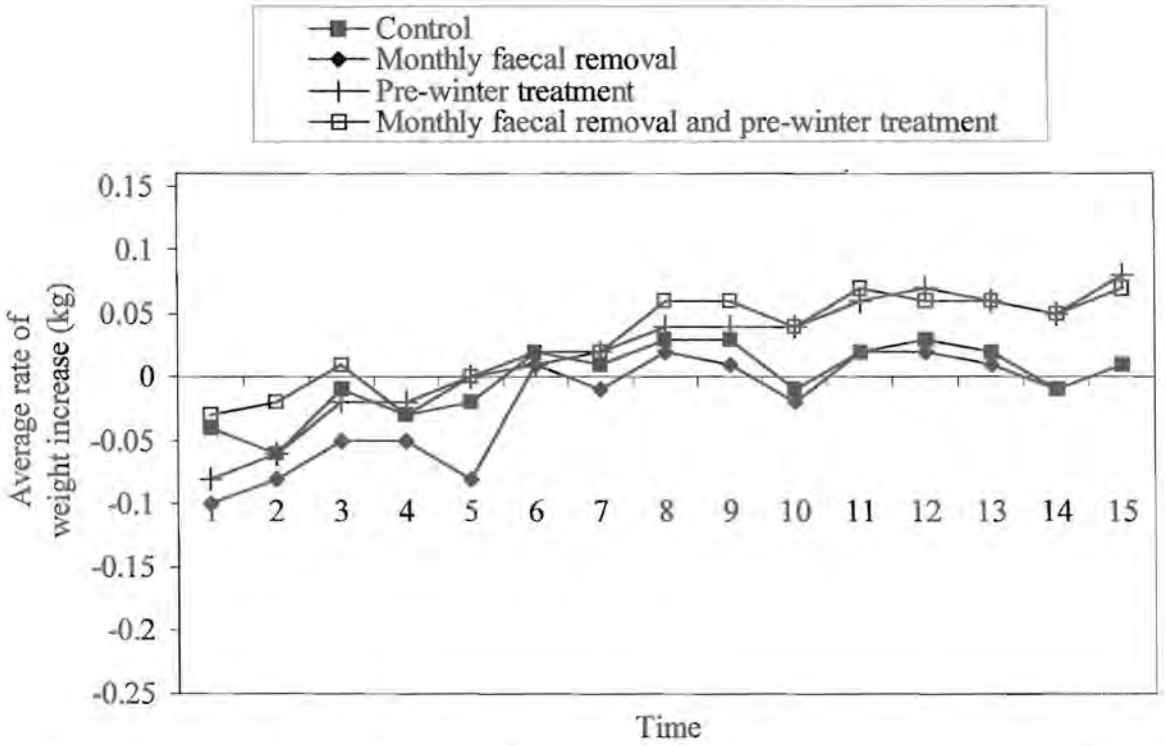


Figure 7. Average rate of weight increase of the animals in the control camps and those in the three different alternative helminth control camps starting at 1 = November 1997; 7 = May 1998; 15 = January 1999.

Table 9. Average ± SD of haemaglobin (Hb), packed cell volume (PCV) and white cell count (WCC) of the 23 donkeys from 1 October 1997 to 31 May 1998 and from 1 June to 31 January 1999.

MS	Group	Hb (g/dlitre)		PCV (litre/litre)		WCC(10^9/litre)	
		Oct. - May	Jun. – Jan.	Oct. - May	Jun. – Jan.	Oct. - May	Jun. – Jan.
MS A	1	102.00 ±9.46	101.13 ±6.49	0.28 ±0.03	0.27 ±0.02	10.42 ±2.76	10.48 ±2.22
	2	85.90 ±20.28	94.13 ±17.42	0.23 ±0.05	0.26 ±0.05	10.97 ±2.82	12.36 ±2.40
MS B	1	88.98 ±15.11	90.63 ±12.18	0.25 ±0.04	0.25 ±0.04	12.84 ±3.92	12.26 ±2.57
	2	98.17 ±15.64	102.33 ±13.84	0.27 ±0.04	0.28 ±0.04	10.96 ±2.62	9.80 ±2.18
MS C	1	76.00 ±22.89	90.13 ±21.42	0.21 ±0.07	0.24 ±0.06	10.56 ±2.06	10.74 ±1.16
	2	93.79 ±13.47	106.17 ±14.06	0.25 ±0.04	0.29 ±0.04	12.02 ±2.89	11.65 ±2.41
MS D	1	83.23 ±12.85	95.29 ±13.14	0.23 ±0.03	0.26 ±0.03	10.47 ±1.91	10.22 ±1.29
	2	94.75 ±13.26	105.00 ±12.03	0.25 ±0.04	0.29 ±0.04	12.22 ±3.24	11.17 ±2.33

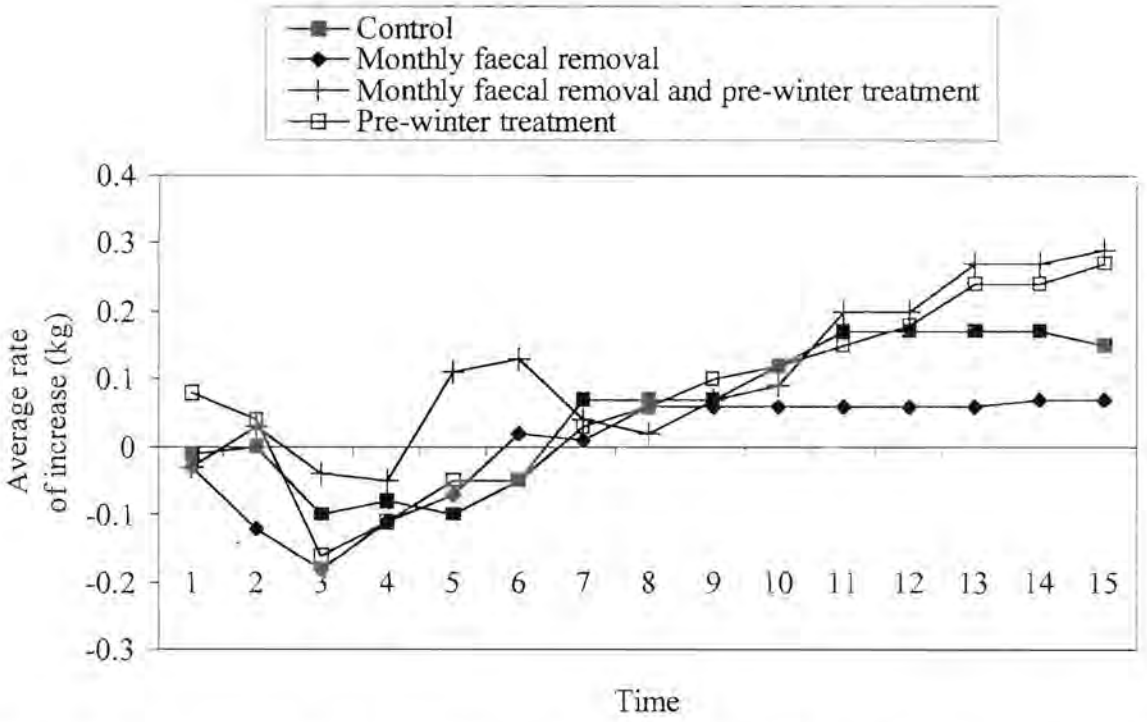


Figure 8. Average rate of body condition increase of the animals in the control camps and those in the three different alternative helminth control camps starting at 1 = November 1997; 7 = May 1998; 15 = January 1999.

3.6. Larval numbers recovered from pasture

The cyathostomes were the most abundant parasites on the pasture (> 90 %) followed by *S. edentatus*. There was no significant difference in the species recorded in the different management systems ($p > 0.10$). The number of L_3 within the eight camps fluctuated monthly. However, in all the management systems there was a noticeable decline in the average number of L_3 in January 1998 (Figures 9 and 10). In addition, an even lower larval burden was recorded in all the camps during the start of the dry winter months (May) followed by a clear increase which coincided with the onset of the spring rains (September and October). A strong correlation ($p < 0.05$; $R^2 = 0.74$) was recorded between the L_3 counts in the control and MS C camps and the monthly rainfall recorded. Higher numbers of L_3/kg dry matter were generally recorded on the pastures where the

faecal material remained (control and MS C camps) in especially the first five to seven months of the study compared to the L_3 counts from the pastures subjected to regular faecal removal (MS B and MS D camps; Figures 9 and 10).

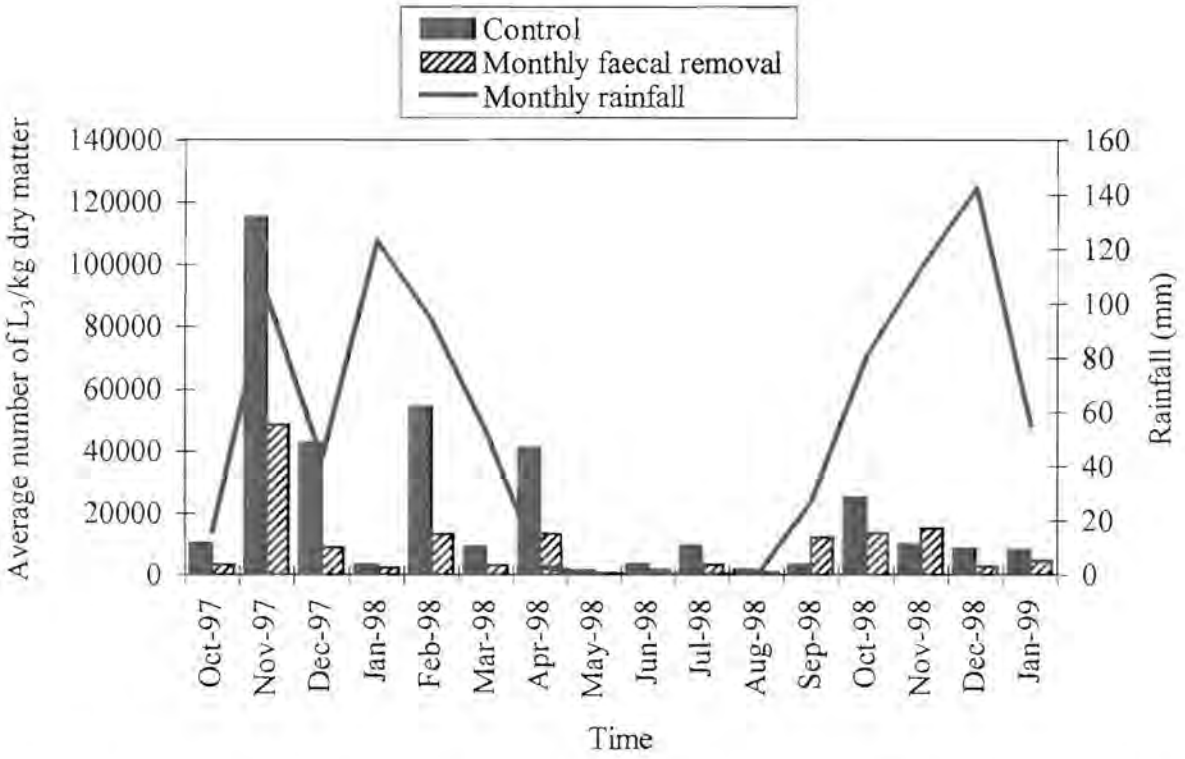


Figure 9. The average number of third-stage larvae (L_3) and the monthly rainfall recorded from the control and MS B (monthly faecal removal) camps from 1 October 1997 to 31 January 1999.

The larval burdens on the pastures varied between camps even within the same management systems. This variation may explain why no significant differences were recorded between those of the control and monthly faecal removal camps. However, there were differences in the amount and extent of the fluctuations in the larval burdens between the control camps and the latter camps. In the control camps, the levels fluctuated extensively (Figure 9). Although fluctuations were also recorded in the MS B camps they were less obvious and the larval burdens were more constant. The same reduced variations were also observed in the MS D camps when compared to those in the MS C camps (Figure 10).

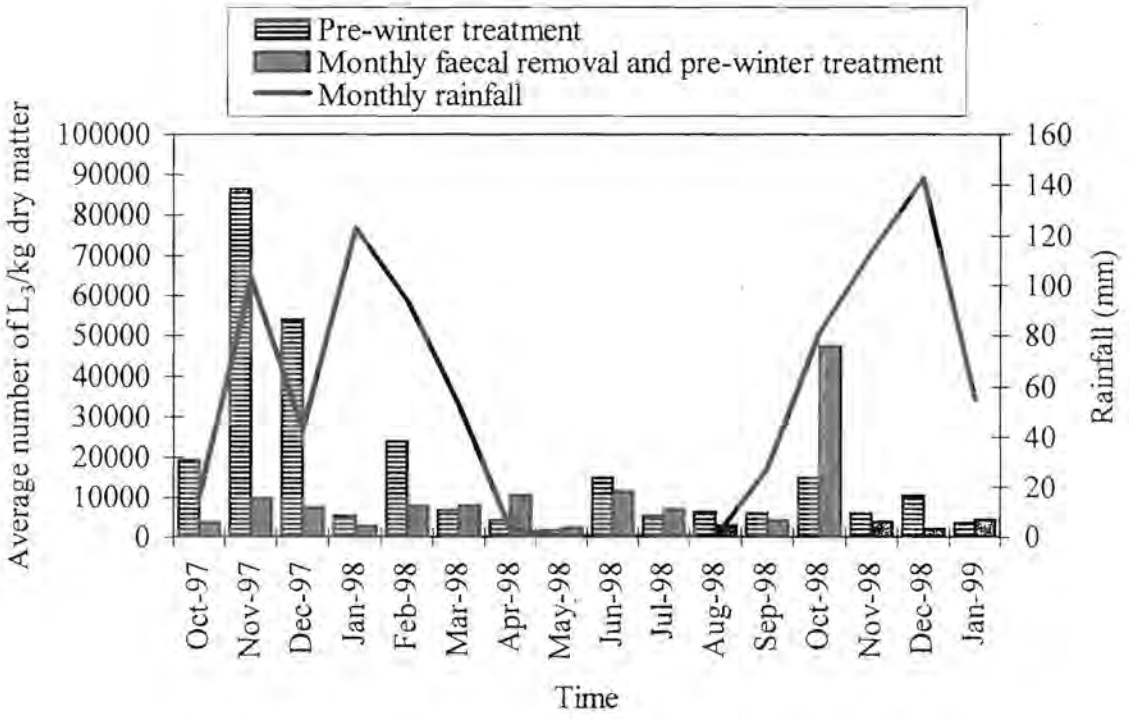


Figure 10. The average number of third-stage larvae (L_3) and the monthly rainfall recorded from the MS C (pre-winter treatment) and MS D (monthly faecal removal and pre-winter treatment) camps from 1 October 1997 to 31 January 1999.

3.7. Results of the technique used to isolate cyathostome third-stage larvae (L_3) from herbage samples

An average recovery rate of 60 % (± 17.2, range 41.7 % to 93.2 %) cyathostome L_3 was obtained using the method, which combined herbage washing and centrifugation in a sugar solution. A significant correlation ($R^2 = 0.91$) was recorded between the "before" and "after" larval

counts (Figure 11). The following equation was obtained with the linear regression analyses to determine the relationship between the predicted L_3 count after washing and centrifugation and the L_3 count before this procedure took place:

$$y = 1.7x + 1854$$

where *y* is an estimations of the number of larvae before washing and isolation and *x* is the number of larvae after washing, isolation and counting.

Use of this L_3 recovery technique and equation will enable pasture larval burdens to be determined in future studies.

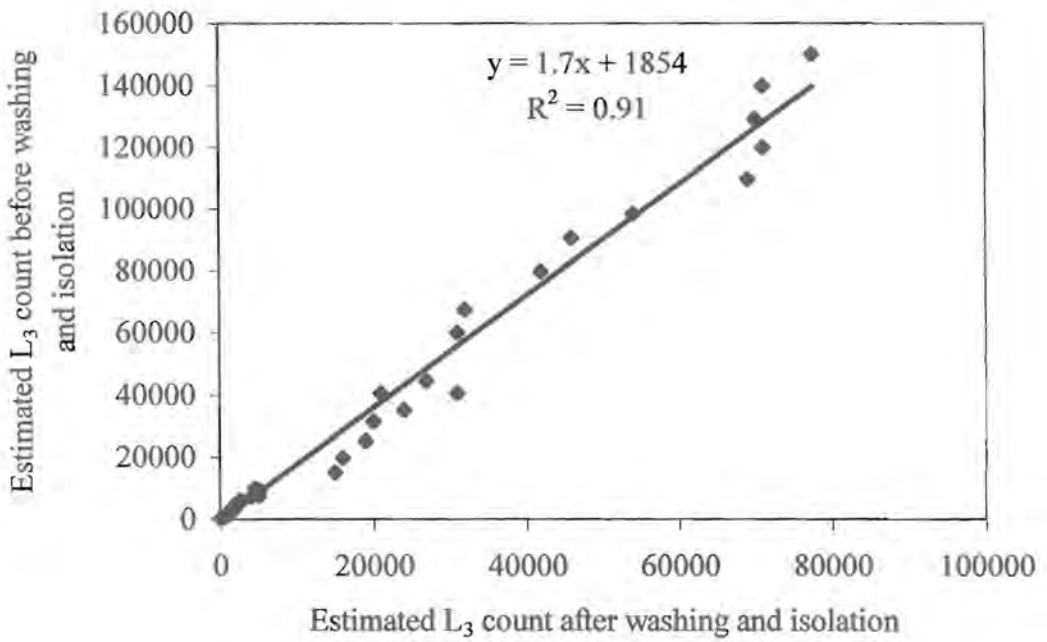


Figure 11. Linear regression analyses of the estimated pasture larval counts before and after using the combination larval recovery technique on 35 seeded herbage samples.

3.8. Pasture grazing and faecal production by the donkeys

There was monthly variation in the length of the grass in the camps between and within the different management systems during the study. This has led to a no significant difference ($p > 0.10$) in the amount of grazing consumed between the donkeys in the four camps from which the faeces were removed monthly and the control and MS C camps, in which the faecal material was left on pasture. In addition, there was no significant difference in the average dry weight of the faeces deposited on the pastures per month in the different camps, the average being 28.3 kg with a range of between 15 and 40 kg (Table 10).

Table 10. The average amount of faeces ± SD (dry weight) recorded per month from the pastures during winter and summer. MS B = monthly faecal removal, and MS D = monthly faecal removal and pre-winter treatment.

MS	Camp no.	Dry weight (kg) of faeces per month			
		Winter	SD	Summer	SD
MS B	Camp 4	37	± 9.3	22	± 8.9
	Camp 8	29	± 17.7	28	±15.4
MS D	Camp 3	20	± 6.3	15	± 5.0
	Camp 7	40	±17.0	36	± 15.4

4. Discussion

4.1. Egg counts, pasture larvae and their seasonal distribution

Strongyle eggs and larvae were the most abundant in the faeces of the donkeys and in the larval cultures, respectively. This is in agreement with previous studies on horses and donkeys (Craig and Suderman, 1985; Wells *et al.*, 1998). Not unexpectedly, within this group, the

cyathostome larvae were the most abundant in both the faecal cultures and on the pasture (Poynter, 1954; Herd and Willardson, 1985; Herd *et al.*, 1985; Herd, 1986; Wells *et al.*, 1998). Wells *et al.* (1998) noted that almost 65 % of the larval cultures of donkey faeces in their study, on 93 donkeys, contained over 90 % small strongyles and concluded that, based on this high proportion of cyathostomes, the eggs in the faeces of the animals were most probably predominantly of this worm group. *Strongyloides westeri* is described as a common parasite of horse and donkey foals (Drudge and Lyons, 1989). In the present study, *S. westeri* was either absent or present in very low numbers in most of the donkeys, which were all adults. However, two individuals were exceptions, as moderate burdens of the eggs of this small nematode were frequently present in their faeces. The estimated age of both these donkeys was two to three years, and a possible reason for the presence of this worm might be that of a delayed immunity. A similar phenomenon has previously been encountered in donkeys in which the prevalence of *S. westeri* ova in the animals younger than six months (12 %) and that in animals between six months and three years (10.2 %), was almost identical (Wells *et al.*, 1998). Both *P. equorum* and *O. equi* displayed peak egg output early in winter as well as in spring, coinciding with the commencement of the spring season. In the present study, *O. equi* eggs were recovered using both the McMaster and adhesive tape swab techniques. This concurs with the findings of Wells *et al.* (1998) who frequently observed *O. equi* eggs, using only the McMaster technique, in the faeces of donkeys in South Africa. The present study's results supports the hypothesis of Wells *et al.* (1998) that the method of egg laying of *O. equi* in donkeys may differ from that in horses, as the eggs of this helminth parasite are not routinely observed during faecal examination of horses (Drudge and Lyons, 1989). However, in contrast to the study of Wells *et al.* (1998) in which the presence of anal pruritis, as manifested by tail rubbing, was not observed, this clinical sign was noted in the present study. It is however uncertain why this condition was not observed in the donkeys that formed part of the study by Wells *et al.* (1998). The eggs of both *P. equorum* and *O. equi* were absent from the faecal material of the treated animals (in

the MS C and MS D camps) after they received the pre-winter moxidectin (0.4 mg/kg oral gel) treatment. This absence was evident for eight months, until the end of the 16-month study. The high effectivity of moxidectin against these parasites has been observed in previous studies on horses and ponies where the percentage reduction, for these two parasites, ranged from 96 % to 100 % for periods ranging from six days to 42 day (Lyons, Tolliver, Drudge, Granstrom, Collins and Stamper, 1992; Xiao *et al.*, 1994; DiPietro *et al.*, 1997; Eysker *et al.*, 1997).

Variations in the L_3 burdens on the pasture of the camps, even between camps within the same management systems, were recorded in the present study. There are several reasons, which may have contributed to these. First, within each of the eight camps there were variations in the grass species present and in the percentage cover provided by each. These variations would have affected the microclimate that was provided to the helminth eggs deposited in the faeces and the survival and development of L_3 on the pasture (Mfitilodze and Hutchinson, 1988; Krecek *et al.*, 1995). Second, the larval burdens at the beginning of the trial may have varied in spite of rotation of the donkeys between camps in the three-month adjustment period. Third, the FEC in the faeces of the donkeys varied during the trial, which resulted in differences in the number of helminth eggs deposited on pasture.

Nevertheless, the larvae in all eight camps did display a seasonal change in their activity. Moisture content and ambient temperature are both very important regulators of the rate of development and survival of strongylid nematode eggs and larvae on the pasture (Poynter, 1954; Ogbourne, 1971; 1972; 1973). Furthermore, they also influence the rate of larval migration from the faeces to the herbage (Ogbourne, 1972; 1973). Monthly faecal egg counts in the donkeys in the control and MS B camps, decreased at the start of the dry winter (April), this might have been due to changes in both the host and in the environmental conditions. First, based on previous studies, it is possible that adult parasite burdens were reduced in the host at this time (Krecek, Reinecke and Horak, 1989; Ogbourne, 1976). Second, the onset of drier and colder (average minimum

temperature 7.6 °C) environmental conditions might have deterred the adult worms from producing large amounts of eggs especially if poor weather conditions would affect the egg and larval survival (Poynter, 1954). This combination of factors was most probably the reason for the low numbers of L_3 observed on the pasture during May (Ogbourne, 1972). Similarly, the significantly high egg counts recorded at the end of winter and during spring may have been related to the presence of large adult burdens in the host as a result of the natural development of larvae that were acquired before and during winter (Poynter, 1954; Ogbourne, 1971; 1976; Craig and Suderman, 1985). This increase in egg production was synchronised with the start of an environmentally favourable time of the year for the development and survival of the free-living stages (Poynter, 1954; Ogbourne, 1971; 1976; Craig and Suderman, 1985; Krecek *et al.*, 1989). A subsequent increase in L_3 numbers was observed on the pastures three to four weeks later as a result of the peak egg production and improved climatic conditions (Herd ***et al.***, 1985). Ogbourne (1972) considered that larval development might be delayed during warm, dry weather; this might explain the unexpected decrease in numbers of L_3 recorded in this study on the pastures during one of the warmest summer months (January). Although rain was recorded during this month it was restricted to the beginning and the end of the month. As a result of an average maximum temperature of 29 °C, it is possible that the faeces and pasture dried out in a very short time, which resulted in decreased larval development and migration during the middle of the month when herbage sampling took place. Another possible explanation is that there might have been a reduction of adult burdens in the host as part of their life cycle and that this resulted in reduced egg production and larval development during January (Krecek *et al.*, 1989).

4.2. Daily fluctuations in the faecal nematode egg counts

As was mentioned previously, strongyle eggs were the most numerous nematode eggs in the faeces of the donkeys, followed in numbers by that of *S. westeri*. The variations in the egg counts recorded at three different times of a day and between consecutive days in 22 donkeys in the current study were not significant. This phenomenon has been previously reported in horses (Warnick, 1992), sheep (Horak, 1967) and cattle (Roberts *et al.*, 1951) and may be related to different rates of egg production of the more than 40 helminth species present in the animals under review. Additional factors to have played a role in this variation might have been an inconsistent distribution of eggs in the donkey's faeces, and/or changes in faecal output (Rubin, 1967; Michel, 1968; McKenna, 1981; Warnick, 1992). It is also suggested that differences in the amount of food consumed during the day and thus in faeces voided at a specific time are likely to concentrate or dilute the number of parasite eggs (Michel, 1968). Although daily variation was recorded in the FEC of the donkeys in the present study and previously in horses (Warnick, 1992), it is considered that this variation is sufficiently low not to influence the identification of animals requiring anthelmintic treatment based on a single egg count (Warnick, 1992).

Based on the results from the FEC obtained at three different times of the day it is hypothesised that the nematode strongyle eggs in the donkeys displayed no increase in egg production in the morning, middle of the day or afternoon. This is in contrast to the results of the study by Horak (1967) who recorded increased egg production of the trematode, *Calicophoron microbothrium* in a sheep, a goat and two cattle at the middle of the day (12:00) followed by a gradual decrease during the afternoon. Similarly, peak egg production was recorded in the middle of the day in cattle infected with the trematode, *Fasciola hepatica* (Dorsman, 1956).

4.3. Isolation of parasitic L_3 from suspended soil and extraneous material using a combination of herbage washing and centrifugation in a sugar solution

Soil, plant roots and herbage serve as a perpetual refuge for both plant and animal nematode larvae (Krecek *et al.*, 1991). Consequently, attempts have been made over the years to develop methods by which nematode eggs and larvae can be effectively recovered and isolated from the different strata (Caveness and Jensen, 1955; Bürger, 1981; Martin *et al.*, 1990; Krecek *et al.*, 1991; Fine *et al.*, 1993). One of these methods for larval recovery uses a modified commercial washing machine that has been recommended for routinely processing larger herbage samples (Bürger, 1981). In the present study, a high cyathostome larval recovery rate was recorded from the seeded herbage samples (250 g) with the combination of machine washing and centrifugation in a sugar solution. The recovery rate of 60 % means that only 40 % of the initial known numbers of larvae on the herbage were lost when the technique was used to determine the number of L_3 on the pasture. A similar method is that in which a heavy-duty washing machine is used to wash herbage contaminated with ruminant nematode larvae followed by the isolation of larvae through centrifugation in a saturated magnesium sulfate solution (Bürger, 1981). With this method Bürger (1981) obtained a comparable recovery rate of 60 %. In South Australia, a recovery rate of 90 % (range: 71 – 100 %) was recorded for sheep nematodes on pasture (Martin *et al.*, 1990). In this study herbage samples (500 – 800 g) were soaked for eight hours in water and larvae isolated with one flotation (centrifugation) in saturated potassium iodide solution. Studies in which smaller (< 100g) but more numerous herbage samples (processed between 50 - 100 samples in 24 hours) were used and employing different methods to recover nematode larvae from pasture have been described (Krecek *et al.*, 1991; Fine *et al.*, 1993). In South Africa, Krecek *et al.* (1991) recorded a ruminant nematode (*Haemonchus contortus* and *Haemonchus placei*) larval recovery rate of 24 – 27 % from 15 – 25 g herbage samples. In this study, a modified Baermann apparatus was used to

recover nematode larvae from the herbage followed by centrifugation in sugar solution (Caveness and Jensen, 1955) for isolating the L_3. As can be seen in the examples given, variation in the recovery rates obtained with different techniques have been recorded. This may be attributed to several reasons, such as: differences in the techniques that were used (soaking of herbage samples with the modified Baermann apparatus compared to the machine washing of herbage samples), differences in the larval species being recovered and isolated (ruminant as opposed to equid larvae), the time interval between seeding of the herbage samples with L_3 and processing of the samples (the same day as opposed to several days after seeding), the age of the larvae, and the size of the herbage samples (< 100 g compared to > 100 g).

In the present study a strong correlation was recorded for all 35 herbage samples between the "before" and "after" larval counts and is supported by the results obtained in the linear regression model. Although the numbers of cyathostome larvae, used to seed the herbage samples with, ranged between 100 and 150 000 there was no distinct increase or decrease in the percentage recovery rate from the samples inoculated with larger numbers of larvae in this study. In contrast, in a previous study by Krecek *et al.* (1991) in which the percentage recovery rates for different ruminant larval treatments were recorded, the results indicated that higher larval recoveries are obtained in lower treatments (32 % for 600 L_3) as compared to those in higher treatments (22 – 23 % for 1 200, 1 800, and 2 400 L_3).

In addition to a high recovery rate, the method used in the present study enabled the processing of 10 herbage samples within an eight-hour working day by a single person. The microscopic examination of each sample requires an additional 30 minutes, depending on the number of larvae per sample. Interestingly, the number of samples processed per day was approximately 50 % less than that recorded by Bürger (1981), the reason for this difference is not clear as both studies share the same processing time. It is possible that Bürger's laboratory was equipped with more than one washing machine and/or more than one person was involved in

processing the herbage material which would explain the higher turn over rate (24 samples in an eight hour day).

An advantage of the centrifugation in sugar solution technique is that cleaner samples are obtained (Caveness and Jensen, 1955). In their study on the recovery of plant nematodes from soil and plant tissue, Caveness and Jensen (1955) compared the sugar centrifugation method to the Baermann funnel and the gravity-screening methods on four different soil types and plant tissue samples. They noted that although both plant nematode eggs and larvae were recovered by the use of the Baermann funnel method the samples were filled with suspended and settled extraneous material. Similarly, Krecek *et al.* (1991) noted that mat samples following Baermannization often contained soil, which complicated microscopic examination. The sugar centrifugation method of Caveness and Jensen (1955) was then applied to these samples and resulted in cleaner samples in which the ruminant larvae were more easily counted and identified.

The method used in this study was performed on freshly cut herbage samples weighing an average of 250 g. However, further studies are required under South African conditions regarding the best method that should be used for recovery of larvae from larger quantities of grass samples. The possibility cannot be excluded that the recovery rate might be higher, or even lower, when larger herbage samples are used.

4.4. Body measurements of the donkeys

The use of body measurements in an equation to predict the live weight of working donkeys is a reliable alternative to the use of expensive, and often, inaccessible manual and electronic scales. Pearson and Ouassat (1996) and Wells (1997) found that the best combination of parameters to predict live weight was heart girth and body length. In addition, in both studies comparably highly

significant correlations (R^2 = 0.84 and R^2 = 0.86, respectively) between the actual live weight and the predicted live weight with their individual predictive equations were reported. The predictive equation developed by Wells (1997), on 55 working donkeys, included the BCS of the animal. The author suggested that the body condition score is a size-independent indicator of the true condition of the animal and can be used as such. It was found that by including the condition score of an animal in the heart girth-length equation the predictive value increased by almost 5 % compared to the original value (R^2 increased from 0.81 to 0.86).

In the present study, linear body measurements were recorded from the donkeys in an attempt to test the predictive value and the repeatability of the body condition score-heart girth-length formula of Wells (1997) on a different group of working donkeys. In the present study the body measurements were recorded four times during the study (September 1997, December 1997, March 1998 and October 1998). The measurements were substituted into the mathematical equation and the predicted live weight calculated for each animal. The significance correlation between the actual live weight (recorded on an electronic scale) and the predicted live weight compared well (0.66, 0.84, 0.91 and 0.82, respectively) with the R^2 recorded in the study of Wells (1997). A poor correlation coefficient (R^2 = 0.66) was recorded for September 1997, which was also the first time that body measurements were recorded from the donkeys and took place the month prior to the start of the study. A possible explanation for the lower R^2 might due to inexperience in taking the different measurements from the donkeys at that time. When the data points from the four collection times were combined in a regression analyses it was found that the correlation coefficient improved from 0.77 to 0.83 if the measurements recorded from the 23 donkeys in September 1997 were excluded from the remaining measurements. An encouraging fact is that the correlation coefficients obtained in the present study and those in the study by Wells (1997) are comparable, although different types of scales were used in each study. In Wells' study the donkeys were weighed on a mobile electronic scale (Ruddweigh G3 cattle scale), but in the

present study the donkeys were weighed on a permanent electronic scale (Atlas electronic weighing bridge) fixed in a crush at the Faculty of Veterinary Science. In the present study it was found that the body condition score-heart girth-length formula is relatively easy to calculate and is a reliable and repeatable alternative in obtaining an estimate of the live weight of working donkeys in South Africa.

4.5. Removal of faeces from the pastures on a monthly basis and its effect on pasture larval burdens, host nematode burdens and the condition of the working donkeys

Several authors have indicated that the removal of faecal material on a twice-weekly basis results in significant reductions in the pasture larval burdens in the United Kingdom (Fisher, 1997) and the United States of America (Herd, 1986). In South Africa, however, empirical data regarding the appropriate interval between faecal removals is lacking. In the present study it was decided to test an interval of one month, which in turn would provide a reference point for future studies. This specific interval was also found to be practical in terms of time and cost.

The rationale behind faecal removal is based upon not only the physical removal of faeces from the pasture to increase the grazing area but also the removal of helminth eggs that are contained within the faeces. This practice should result in fewer nematode eggs that can potentially develop into free-living larval stages on the pasture and thus reduce the risk of pasture contamination and host infection. In the present study, an estimated average of 28 300 g dry faecal material was removed from each of the pastures every month (equal approximately to 56 600 g wet weight/month). If one considers that the average epg content of faeces of the control animals in summer was 1 312 each day, then the pasture contamination would have been 2.228×10^9 eggs per month in summer. However, numerous studies have indicated that larval mortality rates on pasture

are high (Goldberg, 1970; Ogbourne, 1972; Mfitilodze and Hutchinson, 1988) and in one study, in particular, a 99 % mortality rate was recorded (Silangwa and Todd, 1964). With such a high mortality rate the high reproduction rate of nematodes can be viewed as a survival strategy. All the camps in the present study were infested with nematode eggs during the three-month adjustment period at the beginning of the study when the donkeys, which were all relatively heavily infected with worms, were allowed to graze in all eight camps. The monthly removal of faecal material from four of the eight camps limited the monthly variation of L_3 on the pasture and resulted in lower L_3 counts in individual months compared to those in the control and MS C camps, although the results were not statistically significant. This reduced pasture L_3 exposure to the donkeys in the MS B camps resulted in an approximately 20 % reduction in the average faecal egg counts compared to those of the animals in the control camps. This effect was only modest, but, did result in a reduced average FEC of 1 000 compared to 1 400 for the animals in the control camps at the end of the study. This is the first report of the potential effect of faecal removal on the host's parasite load. Interestingly, in two previous studies in which faecal removal on a twice-weekly basis was tested, significant reductions in the number of pasture larvae were recorded, but the ponies' FEC and re-treatment intervals were not affected (Herd, 1986; Fisher, 1997). There may be several reasons for this poor response. First, the paddocks in both the studies had previously been grazed by equids and thus a population of infective larvae was already established on the pastures and re-infection was possible. Second, the ponies carried natural strongyle infections at the start of the faecal removal trials. Third, the prepatent period of naturally infected cyathostomes is approximately three to four months (Reinemeyer, 1986). It is possible that the ponies carried helminth populations that were at different stages in their life cycles and thus if the studies had been extended to 12 months or longer (instead of only five to seven months) an effect might have been noticeable.

Although the effect of the removal of faecal material on a monthly basis on the donkeys themselves in the present study was limited it did result in a 20 % reduction in the donkeys' faecal egg counts. In contrast, there were no noticeable or significant improvements in their live weight, BCS or blood chemistry. It is, however, possible that more frequent faecal removal (i.e. twice monthly or more frequent if practical) would result in improved weight gain as well as improved blood chemistry if practised on a permanent basis.

4.6. Pre-winter moxidectin treatment and its effect on pasture larval burdens, host nematode burdens and the condition of the working donkeys

Based on work done on horses, the cyathostome population of the donkeys in autumn most probably consisted predominantly of adult stages and to a lesser extent encysted L_3 and lumenal L_4 (Krecek *et al.*, 1989). Following deworming, 100 % reduction in nematode egg counts was recorded in the treated donkeys for at least 14 days. This is not unexpected as a positive effect has been noted, in previous studies that used moxidectin, against the larger encysted cyathostome larvae (LL_3 and DL_4), lumenal L_4 and adult stages of strongyle parasites (Lyons *et al.*, 1992; Xiao *et al.*, 1994; DiPietro *et al.*, 1997).

The ERP recorded in this donkey trial was much shorter (six to eight weeks) than that of more than eight weeks that has been recorded in previous studies in horses (Jacobs *et al.*, 1995; DiPietro *et al.*, 1997). There may, however, be several reasons for this. First, the definition of the ERP followed would exert a significant influence on the results obtained. Most studies define the ERP as the time interval after treatment before "substantial numbers" of eggs reappear in the faeces (Herd, 1992a; Jacobs, personal communication, 1999). However, the term "substantial numbers" lacks precision and leads to variation in the results obtained as some researchers regard 50 epg as

substantial and others 100 or even 250 epg (Jacobs, personal communication, 1999). A more robust and stricter cut-off value would be: the first time that eggs are detected in the faeces; this is the cut-off value that was used in the present study. Second, not all the horses in the study by DiPietro *et al.* (1997) were kept on pasture following deworming and it is possible that these animals experienced reduced parasite re-infectivity. Third, even though donkeys and horses are both equids, it cannot be excluded that pharmocokinetic differences between the species may influence the effectivity of anthelmintics developed and registered for horses (Mealey *et al.*, 1997). Fourth, as mentioned earlier, pasture larvae are strongly influenced by environmental conditions (Ogbourne, 1972; 1973). It is therefore possible that abnormally dry conditions can cause reduced pasture challenge resulting in prolonged re-treatment intervals (as suggested by Jacobs *et al.*, 1995). Although all the donkeys in the present study were positive for strongyle eggs much sooner than has been observed in horses, the arithmetic mean egg count still remained reduced (< 500 epg,) for up to eight months following treatment.

The pre-winter moxidectin treatment resulted in reduced FEC and suppression of egg production compared to the untreated animals in the control and MS B camps. This is evident from the higher average nematode egg counts in spring (September) obtained for the control animals and those which grazed in the camps from which the faeces had been removed on a monthly basis (1 400 and 1 000 epg respectively) compared to approximately 340 epg for the animals in both management systems C and D that received the autumn moxidectin treatment. This prolonged and greater suppressive effect of moxidectin on the faecal egg counts was also observed in horses by Jacobs *et al.* (1995) and DiPietro *et al.* (1997) and may be attributed to moxidectin's improved effect on the larger encysted larval stages.

In the present study, all the donkeys received the same type and amount of food, but only those that had received the autumn moxidectin treatment showed noticeable improvements in their live weight, BCS, Hb and PCV values at the start of the grazing season, five months later in

October 1999. This clearly indicates that donkeys with reduced worm burdens are able to optimise the energy and nutrients extracted from poorer quality food during cold and dry winters. The current findings concur with those of previous workers who found that reduced helminth burdens in ponies resulted in improved weight gain (Mair, 1994; Murphy and Love, 1997). Murphy and Love (1997) recorded a reduced percentage weight gain (approximately 50 % lower weight gain) in ponies following artificial infection with more than three million cyathostome L_3. Similarly, Mair (1994) recorded a sudden onset of weight loss in ponies during spring that coincided with a massive emergence of cyathostome L_4 from the gut wall into the lumen. In the present study, the BCS of the animals displayed a delayed response to the pre-winter treatment. It appears that muscle and fat production following anthelmintic treatment is a gradual process especially if the nutrient quality remains unaltered. The same phenomenon was also observed in working donkeys in Greece when the egg counts decreased noticeably following deworming but the improvement in body condition was only evident after eight months (Bliss *et al.*, 1985). In addition, Khallaayoune (1991) recorded reduced egg counts and significantly improved body conditions towards the second half of an 11-month study on donkeys subjected to three strategic deworming treatments. Studies on the effect of helminth burdens on the general blood chemistry of donkeys are sparse and the current results are the first report of a correlation between Hb and PCV values and helminth burdens. This correlation is similar to that obtained in studies on ponies (Round, 1968; Smith, 1976) but, is in contrast to that determined in a previous study in donkeys (Urch and Allen, 1980), in which no improvement in the Hb or PCV values were observed following a single treatment of fenbendazole.

A single pre-winter moxidectin treatment resulted in a consistent reduction in the FEC of the donkeys after treatment. In addition, the strategic administration of moxidectin resulted in an ERP of approximately seven weeks, which lead to improvements in the hosts' general body condition and blood chemistry for up to eight months. It is evident that a strategic autumn

treatment would benefit the health of the donkey and it is therefore suggested that if the owner can afford a dewormer it should be administered pre-winter.

4.7. The combination of monthly faecal removal from camps and pre-winter treatment of donkeys with moxidectin and its effect on pasture larval burdens, host nematode burdens and the condition of the working donkeys

Although an additional helminth control procedure (pasture hygiene) was used in combination to a strategic autumn treatment, the effect on the live weight, body condition and blood chemistry of the donkeys in this management system (MS D) was very similar to that of the animals that only received the pre-winter treatment with moxidectin (MS C). Monthly pasture cleaning did not extend the length of the ERP in the animals in the MS D camps, this may be attributed to the modest effect that the single faecal removal per month had. This integrated helminth control method did, however, prevent the FEC from rising above 1 000 epg following deworming. This is evident from the percentage of animals within the MS C and MS D camps that recorded egg counts above 1 000 at the end of the study (60 % of the donkeys in the MS C camps recorded > 1 000 epg compared to only 16 % for the animals in the MS D camps). Based on this information, it appears that even though monthly faecal removal did not increase the ERP it was responsible for a more gradual re-infection of the donkeys and, consequently, lower FEC. It is possible that if it is practical and practised on a permanent basis, twice-monthly faecal removal in combination with a strategic autumn treatment will result in a longer ERP and lower FEC that will greatly improve the condition of the animal and benefit the owner.

4.8. Conclusion

Knowledge of the pathological effects of helminths on donkeys is mainly based on extrapolation from information generated by studies on horses. The present study on donkeys provides the first empirical evidence describing some of the clinical symptoms observed in donkeys due to helminth infections. Larger nematode FEC resulted in poor body condition (reduced rate of increase in the live weight and BCS) and also had some negative effects on the blood physiology of the animals (lower haemoglobin concentrations and packed cell volumes). The data generated by this study clearly suggest that anthelmintic treatment for donkeys is beneficial and will potentially provide healthier animals with improved working capabilities. Resource-limited donkey owners, however, are faced with several constraints; financing the anthelmintic is merely one of them. An effective helminth control strategy that is practical and inexpensive is therefore of vital importance to the rural communities in developing countries. In the present study the removal of faeces on a once monthly basis resulted in a decrease of 20% in the FEC of the experimental animals. It seems reasonable to argue that frequent faecal removal will ultimately result in a drastic reduction of helminth parasites in donkeys and in rare instances where a single anthelmintic treatment is affordable, it is undoubtedly beneficial to administer the drug. Data gathered from this study suggest that the timing of the anthelmintic treatment is critical. Given that during the cold and dry winter months, in summer rainfall regions, the host is faced with nutrient poor food and the parasites' reproductive cycle is impeded, it is suggested that an anthelmintic treatment would be the most influential if administered in autumn. In this study, a significant reduction in the donkeys' FEC and reduced helminth re-infection rates resulted from a strategic autumn deworming practice, which also led to marked improvements in the animal's general condition. Moreover, the combination of the two above-mentioned control

strategies would be the most beneficial as the greatest residual effect was noted in the FEC of the animals subjected to faecal removal and strategic deworming simultaneously.

CHAPTER 5

THE EFFECT OF THE THREE MANAGEMENT INTERVENTIONS ON THE HELMINTHS AND GASTEROPHILIIDS RECOVERED FROM THE DONKEYS AT NECROPSY

1. Introduction

In recent years, helminth parasite control programmes in domestic animals have shifted their focus from the traditional exclusive use of anthelmintics to include alternative control methods such as selective and strategic deworming, pasture hygiene, and more integrated approaches, such as epidemiology-based methods (Craig and Suderman, 1985; Reinemeyer, '1986; Herd, 1990; Herd, 1993; Herd and Coles, 1995; Waller, 1999). The motivation behind such new approaches for helminth control is: 1) increased reports of anthelmintic resistance as a result of frequent (eight weekly) anthelmintic treatment predominantly with the same drug, 2) reports of shorter ERP, and 3) increased costs (Herd, Miller and Gabel, 1981; Kelly, Webster, Griffin, Whitlock, Martin and Gunawan, 1981).

Although a nematode FEC is an important method with which to obtain a quick and cheap assessment of the status of different helminth control programmes (Herd, 1993; Herd and Coles, 1995), a necropsy is the only method that provides an accurate estimate of the total helminth numbers in an animal. In contrast to the former method, the latter allows for the establishment of the effect of a specific control programme on the adult stages as well as on the different larval helminth stages of individual species in the host (for example, the effect on clinically important mucosal larvae stages of the cyathostomes; Herd, 1990). Therefore, only necropsy techniques that

enable total gastro-intestinal tract worm recoveries would provide an accurate assessment of the total helminth burden of animals managed with alternative helminth control methods.

Cyathostomes constitute a large percentage of the helminth population in equids (Ogbourne, 1978; Herd, 1990; Herd and Coles, 1995), and the encystment and emergence of their larval stages into and from the gut wall have been held responsible for the manifestation of various clinical syndromes in horses (Ogbourne, 1978; Love *et al.*, 1992; Reilly *et al.*, 1993; Mair, 1994; Murphy and Love, 1997). Although not yet standardised, there are currently two accepted methods available for the enumeration of the different mucosal larval stages of cyathostomes (Malan *et al.*, 1981b; Reinemeyer and Herd, 1986a; Eysker *et al.*, 1997; Klei *et al.*, 1997; Eysker and Klei, 1999; Chapman *et al.*, 1999). Transmural illumination of sections of the gut wall is more rapid but its sensitivity is limited to the larger encysted larval stages (LL_3 and DL_4). In contrast, DIG requires at least two hours for the digestion process required in the procedure, but its sensitivity in that all three encysted larval stages (EL_3, LL_3 and DL_4) can be detected is greater (Eysker and Klei, 1999; Chapman *et al.*, 1999). However, inconsistent reports on the sensitivity of these two methods have been recorded and may be attributed to variation in the procedure (Eysker and Klei, 1999).

Irrespective of the drawbacks, recent developments in refinement of TMI and DIG have made it possible to study the anatomic distribution of encysted cyathostome larval stages in the intestine of equids. One of the first studies in which the anatomical distribution of encysted larvae in the gastro-intestinal tract of horses was recorded was that of Reinemeyer and Herd (1986b). They noted that, even though the caecum is smaller than the ventral colon, it harbours the largest number of encysted cyathostome larvae. It is conjectured that because the caecum is the first organ to be encountered by the L_3 after exsheathment in the small intestine, larger numbers of encysted larvae occur at this site. To date, there have only been three studies in South Africa in which the anatomical distribution of encysted cyathostome larvae were investigated in equids (Malan *et al.*, 1981b; Scialdo-Krecek, 1984; Krecek, Reinecke and Malan, 1987a). Large numbers of encysted

cyathostome larvae were noted in the small intestinal wall. Anatomical differences in the digestive tract, such as a possible thinner mucosa and submucosal layer of the small intestine of zebras that can be penetrated more easily might facilitate L_3 encystment at this site. Apart from the possibility that there are anatomical differences in the gut wall between zebras and horses, it is also possible that the small intestine is the area where exsheathment as well as encystment takes place in zebras. It is also possible that sizeable larval burdens might result in a competition for space and therefore altered colonisation patterns (Lyons *et al.*, 1994). As yet, no studies have been performed in donkeys and it is therefore uncertain what the distribution pattern of encysted larvae is in this host.

2. Materials and Methods

2.1. Study animals and experimental design

In January 1998 one of the 24 donkeys (number 20) that was part of the field trial developed a respiratory condition and was euthanased due to a poor prognosis. It and the eight animals euthanased at the end of the 16-month field trial (January 1999) were subjected to detailed necropsy examinations (Malan *et al.*, 1981a, b; Duncan *et al.*, 1988) for the recovery of helminth parasites. The descriptions followed in the identification of the helminth, oestrid fly (Tables 11 and 12) and encysted cyathostome larval identification is described in detail in Chapter 3. The helminth species and their distribution in the gastro-intestinal tract of donkey 20 will be the only information that will be reported for this animal. Representative specimens of adult male and female helminths recovered from the nine donkeys that were necropsied in this study have been deposited in the United States National Parasite Collection in Beltsville, Maryland 20705, USA (Accession Numbers 089130 to 089158).

Table 11. Species of cyathostomes (adult stages) recovered from the nine donkeys (identifications were done according to the descriptions of Boulenger, 1920, Lichtenfels, 1975 and Lichtenfels *et al.*, 1998a and species names follow those of Lichtenfels *et al.*, 1998b).

	Boulenger, 1920	Lichtenfels, 1975	Lichtenfels *et al.*, 1998a
Cyathostominae			
Coronocyclus coronatus		+	
Coronocyclus labiatus		+	
Coronocyclus labratus		+	
Cyathostomum alveatum		+	
Cyathostomum catinatum		+	
Cyathostomum montgomeryi	+		
Cyathostomum pateratum		+	
Cyathostomum tetracanthum		+	
Cylicocyclus auriculatus		+	
Cylicocyclus elongatus		+	
Cylicocyclus leptostomum		+	
Cylicocyclus insigne		+	
Cylicocyclus nassatus		+	
Cylicocyclus radiatus		+	+
Cylicostephanus asymetricus		+	
Cylicostephanus calicatus		+	
Cylicostephanus goldi		+	
Cylicostephanus longibursatus		+	
Cylicostephanus minutus		+	

Table 12. The non-cyathostome helminth and oestrid fly larvae species recovered from the nine donkeys necropsied (identifications were done according to the descriptions of Theiler, 1923; Zumpt, 1965; Lichtenfels, 1975; Reinecke, 1983; Krecek *et al.*, 1997).

	Theiler, 1923	Zumpt, 1965	Lichtenfels, 1975	Reinecke, 1983	Krecek *et al.*, 1997
Anoplocephalidae					
Anoplocephala perfoliata			+		
Ascarididae					
Parascaris equorum			+		
Atractidae					
Probstmayria vivipara	+		+		
Dictyocaulidae					
Dictyocaulus arnfieldi			+		
Habronematidae					
Draschia megastoma			+		
Habronema majus			+		
Habronema muscae			+		
Onchocercidae					
Setaria equina					
Oxyuridae					
Oxyuris equi			+		
Paramphistomatidae					
Gastrodiscus aegyptiacus				+	
Strongylinae					
Strongylus equinus			+		
Strongylus vulgaris			+		
Triodontophorus burchelli					+
Triodontophorus hartmannae					+
Triodontophorus serratus			+		
Trichostrongylidae					
Trichostrongylus axei			+		
Gasterophiliidae					
Gasterophilus intestinalis		+			

3. Results

3.1. Helminth species

The number of helminth species recorded in the donkeys ranged from 10 to 28 with an average of 20 species per animal (Tables 13 and 14). The different management systems appeared to have had no noticeable effect on the number of helminth species present within individual donkeys and therefore the results are discussed for the whole group. Thirty-seven helminth species, including a previously undescribed small strongyle species, *Cylicocyclus* a were recorded. This species was previously recorded in donkeys in South Africa (Matthee *et al.*, 2000) and is described as *Cylicocyclus asinus* sp. n. for the first time in the next chapter. In addition to the already-mentioned helminth species another unknown cyathostome species was recorded in a single donkey (donkey 25) and is referred to as *Cylicocyclus* b throughout. The helminth species recorded in the donkeys include one anoplocephalid, one ascarid, one atractid, one dictyocaulid, three habronematid, one onchocercid, one oxyurid, 26 strongylid taxa (21 small strongyles or cyathostomes and five large strongyles) and one trichostrongylid. In addition, one paramphistomatid and one gasterophiliid species were recovered. Total worm burdens recovered from each animal ranged from 3 831 to 29 501 and are recorded in Tables 13 and 14. *Cyathostomum montgomeryi* was the most abundant small strongyle followed by *Cylicostephanus longibursatus* and *Cylicostephanus minutus* the first two of these species were the only cyathostome species that were present in all nine donkeys. *Triodontophorus hartmannae* was the most abundant large strongyle, followed by *S. vulgaris*, which was present in all the animals (Table 13).

Table 13. Strongyle burdens recovered from the nine donkeys necropsied.

Donkey number	9	12	13	14	17	20	23	25	27	Range
Cyathostominae										
Coronocyclus coronatus	0	0	0	12	780	322	26	615	173	0-780
Coronocyclus labiatus	223	0	0	30	3 491	2 100	21	1 230	4 820	0-4 820
Coronocyclus labratus	0	0	0	10	880	60	0	80	431	0-880
Cyathostomum alveatum	62	0	0	32	140	140	2	54	93	0-140
Cyathostomum catinatum	147	0	11	0	100	0	21	30	0	0-147
Cyathostomum montgomeryi	2 528	1	20	1 928	7 839	452	99	3 062	1023	1-7 839
Cyathostomum pateratum	110	0	0	0	100	0	0	0	0	0-110
Cyathostomum tetracanthum	102	0	0	639	1 510	10	0	0	320	0-1 510
Cylicocyclus auriculatus	928	0	0	110	1 800	0	0	140	1040	0-1 800
Cylicocyclus elongatus	112	0	22	143	1 064	12	0	42	0	0-1 064
Cylicocyclus insigne	0	0	0	0	10	81	0	0	0	0-81
Cylicocyclus leptostomum	5	0	0	1	10	0	0	0	0	0-10
Cylicocyclus nassatus	121	0	0	71	820	1 010	0	20	0	0-1 010
Cylicocyclus radiatus	32	32	0	11	440	472	0	30	11	0-472
Cylicocyclus a	257	2	0	2 146	1 170	630	0	1 564	760	0-2 146
Cylicocyclus b	0	0	0	0	0	0	0	277	0	0-277
Cylicostephanus asymetricus	340	21	2 438	0	701	2	95	794	1 924	0-2 438
Cylicostephanus calicatus	183	0	126	75	140	25	0	73	102	0-183
Cylicostephanus goldi	0	0	0	549	1	0	55	23	1 604	0-1 604
Cylicostephanus longibursatus	174	232	837	6 252	1 237	0	172	498	6 087	0-6 252
Cylicostephanus minutus	707	55	0	2 031	6 015	247	25	790	3 586	0-6 015
Strongylinae										
Strongylus equinus (nodule#)	0	0	0	0	0	0	0	2	0	0-2
Strongylus vulgaris	104	45	288	233	57	20	0	10	545	0-545
In arteries: Adults	5	2	0	4	1	2	4	2	1	0-5
5^{th} stage	44	24	4	13	13	9	5	13	15	4-44
L_4	60	32	8	18	35	18	23	18	42	8-60
In nodules*	0	2	0	0	0	0	0	1	0	0-2
Triodontophorus burchelli	0	484	0	29	52	3	0	30	0	0-484
Triodontophorus hartmannae	0	1 233	0	80	476	71	0	290	10	0-1 233
Triodontophorus serratus	0	1 044	0	0	0	1 322	0	0	0	0-1 322

Dorsal colon, * Ventral colon

Table 14. Non-strongylid burdens recovered from the nine donkeys necropsied.

Donkey number	9	12	13	14	17	20	23	25	27	Range
Anoplocephalidae										
Anoplocephala perfoliata	0	0	0	0	0	155	0	0	0	0-155
Ascarididae										
Parascaris equorum	0	0	0	0	0	4	0	0	0	0-4
Atractidae										
Probstmayria vivipara	0	0	0	1 200	0	0	0	0	0	0-1 200
Dictyocaulidae										
Dictyocaulus arnfieldi	0	0	0	0	0	0	0	0	9	0-9
Habronematidae										
Draschia megastoma										
Lumen	0	0	0	11	0	1	1	0	0	0-11
Nodule	0	0	0	324	2	0	0	0	0	0-324
Habronema majus	1	0	0	22	2	0	2	1	2	0-22
Habronema muscae	66	21	49	53	55	52	136	135	102	21-136
Onchocercidae										
Setaria equina	0	0	0	1	2	0	0	0	4	0-4
Oxyuridae										
Oxyuris equi	0	0	0	0	0	481	1	10	10	0-481
Paramphistomatidae										
Gastrodiscus aegyptiacus	0	16	957	4	0	0	5 598	2 552	10	0-5 598
Trichostrongylidae										
Trichostrongylus axei	0	0	0	0	0	0	20	0	0	0-20
Gasterophiliidae										
Gasterophilus intestinalis										
Third instar	10	10	51	39	24	2	0	0	5	0-51

3.2. The numbers and the distribution sites of the helminths in the donkeys

The total counts recorded for the small strongyle species in the different compartments ranged from 66 550 in the ventral colon; 25 066 in the dorsal colon; 4 117 in the caecum; 536 in the descending colon to 10 in the small intestine (Figure 12). Similarly, the large strongyles were the most abundant in the ventral colon (4 682). The second most preferred site was the caecum (2 917), followed by the dorsal colon (486) and the descending colon (26). Apart from 100 % prevalence in the cranial mesenteric arteries *S. vulgaris* was present in large numbers in the lumen and wall washings of both the caecum and ventral colon. All three *Triodontophorus* species, namely *T. burchelli*, *T. hartmannae* and *T. serratus* demonstrated a preference for the ventral colon. Both *Habronema majus* and *Habronema muscae* were recovered from the stomach, the latter being the

most abundant. Five non-strongylid helminth species were present in different sites respectively: *Anoplocephala perfoliata*, *P. equorum* and *T. axei* in the small intestine, *Probstmayria vivipara* in the ventral colon and *D. arnfieldi* in the lungs. *Gastrodiscus aegyptiacus* was predominantly present in the ventral colon and caecum. *Gasterophilus intestinalis* was recovered from the stomach.

3.3. Helminth numbers recorded from the donkeys

The large strongyles were present in lower numbers, in the donkeys, compared to the number of small strongyles (901 and 10 698/animal, respectively). Each of the three management systems used in the study resulted in reductions in the average helminth counts when compared with the control counts (Table 15). The lowest average helminth count was recorded in the animals that received the pre-winter treatment and grazed in the camps from which the faeces were removed on a monthly basis (6 683). The animals that either received the pre-winter treatment or were kept in the camps from which the faeces were removed monthly obtained average helminth counts of 8 248 and 10 753, respectively. In contrast, the largest average helminth count (26 869) was recorded from the animals in the control camps. The average lumenal L_4 count followed a similar trend as is reflected in Table 15 with the animals in the MS D camps recording the lowest count (163), followed by the animals in the MS B camps (304) and in the MS C camps (376). Not unexpectedly, the largest average larval count was recorded in the animals in the control camps (999).

In the donkeys that had received the pre-winter moxidectin treatment lower average adult *S. vulgaris* burdens in the lumen (39.75) were recorded when compared to the donkeys that had not been dewormed (280.75). Most *S. vulgaris* larvae, present in the cranial mesenteric arteries were in

the fourth-larval stage (L_4) of development (Table 13). The L_4 and fifth-stage *S. vulgaris* larvae, present in the cranial mesenteric arteries, could be distinguished based on the extent of the structural developments. All the anatomical structures in the fifth-stage were well developed.

Table 15. Total helminth count, total number of cyathostomes, number of adult cyathostomes, lumenal L_4 and encysted larvae counts, recovered by TMI and DIG, recorded from eight of the nine donkeys necropsied. MS B = monthly faecal removal, MS C = pre-winter moxidectin treatment, and MS D = combination of monthly faecal removal and pre-winter treatment.

Donkey	MS	Helminth count*	Total cyathostomes	Adult cyathostomes	Lumenal cyathostome L_4	TMI	DIG
17	Control	29 501	28 752	28 248	504	6 310	5 600
27	Control	24 237	23 468	21 974	1 494	21 870	13 750
13	MS B	5 149	3 786	3 454	332	12 000	18 850
14	MS B	16 356	14 323	14 040	283	3 100	5 080
12	MS C	3 831	820	343	477	13 211	10 700
25	MS C	12 665	9 598	9 322	276	5 960	5 350
9	MS D	6 870	6 187	6 029	158	4 270	2 100
23	MS D	6 495	684	516	168	2 130	2 500

*includes *Strongylus vulgaris* larval counts in arteries.

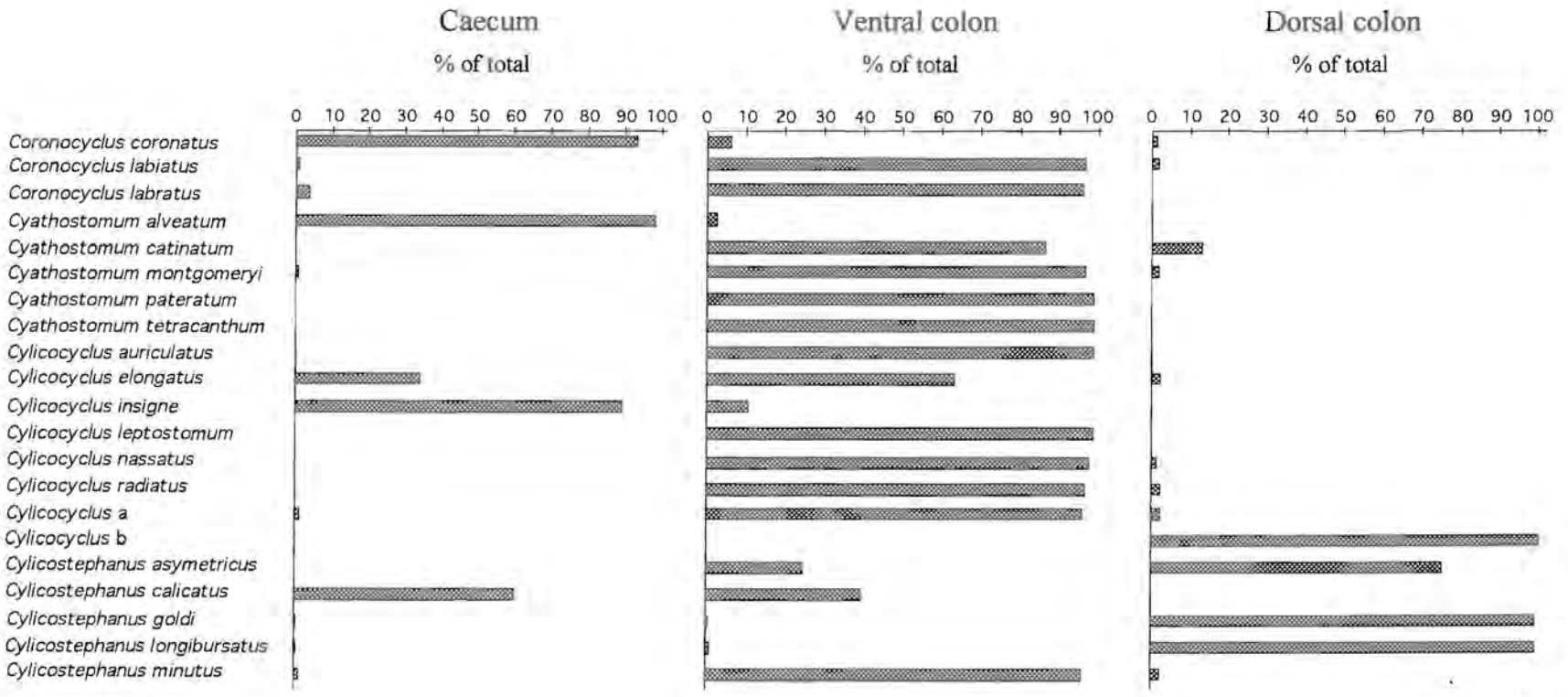


Figure 12. The distrubution patterns of 21 cyathostome species in the large intestine of the nine donkeys necropsied (Percentage of cyathostomes at each site).

3.4. Mucosal larval stages

The lowest average encysted larval counts, recovered by the TMI and DIG methods, (TMI = 3 200; DIG = 2 300) were obtained from the animals that were kept in the MS D camps. The second lowest encysted larval counts (TMI = 7 550; DIG = 3 465) were recorded from the animals that grazed the camps from which the faeces were removed monthly, followed by the counts obtained from the animals that received the pre-winter treatment (TMI = 9 586; DIG = 8 025; Table 15). The highest average encysted larval counts were recorded from the animals in the control camps for both TMI (14 090) and DIG (9 675).

In just over half of the donkeys, the largest numbers of encysted larvae were recorded with TMI (Table 16). This was the situation in both the treated and the untreated animals. No EL_3 larvae were recorded from any site; instead all the larvae represented the larger LL_3 and DL_4. The average estimated total counts indicated that the ventral colon followed by the caecum and dorsal colon contained the largest percentage of encysted larvae per animal (Figure 13). This trend was supported by the counts obtained with both the TMI and DIG methods. Neither the small intestine nor the descending colon walls harboured any encysted larvae. The worms recovered from the scraped stomach walls were identified as belonging to the genus *Habronema*.

Table 16. Comparison of the estimated total encysted larval counts per donkey using the same tissue samples first for counts made by transmural illumination (TMI) and second by peptic digestion (DIG).

Donkey	Technique	Stomach	Caecum	Ventral colon	Dorsal colon	Total
D9	TMI	-	1 570	2 180	520	4 270
	DIG	400	950	1 150	0	2 100
D12	TMI	-	4 780	8 430	1	13 211
	DIG	775	4 900	5 700	100	10 700
D13	TMI	-	2 800	9 060	140	12 000
	DIG	1 375	7 400	11 200	250	18 850
D14	TMI	-	1 830	3 230	20	3 100
	DIG	1 625	900	1 100	1 100	5 080
D17	TMI	-	2 760	3 540	10	6 310
	DIG	1 200	1 700	3 800	100	5 600
D23	TIM	-	970	1 150	10	2 130
	DIG	1 275	900	1 600	0	2 500
D25	TMI	-	2 870	3 090	0	5 960
	DIG	600	2 350	3 000	0	5 350
D27	TMI	-	6 590	15 260	20	21 870
	DIG	1 250	2 350	11 000	400	13 750

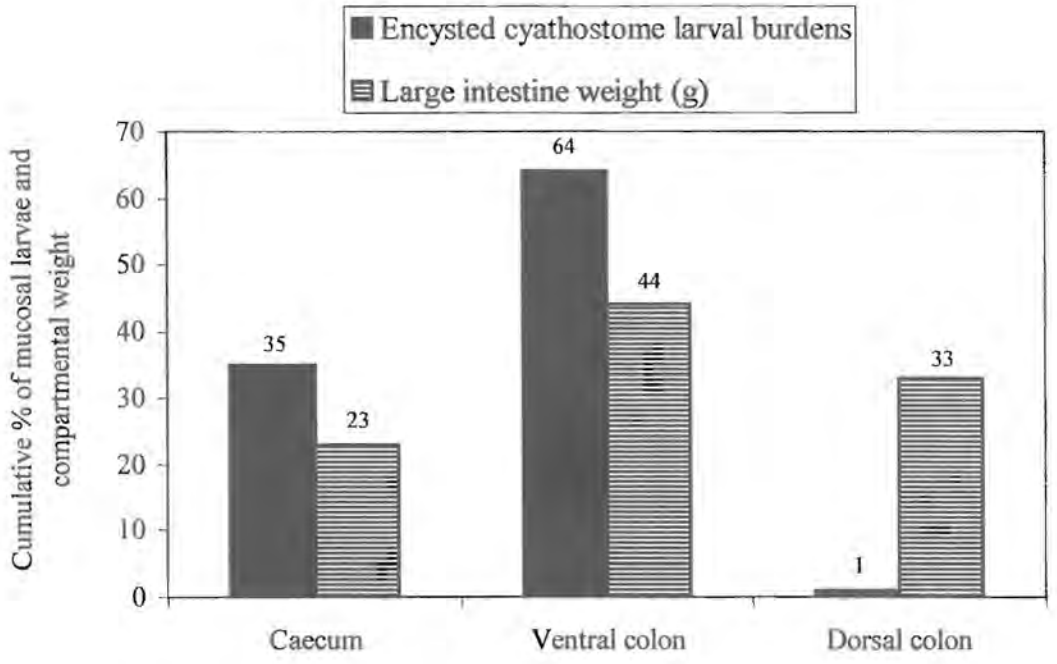


Figure 13. Cumulative percentages of contributions played by the compartments of the large intestine in harbouring the encysted cyathostome larval burdens (LL_3 and DL_4) using TMI and large intestinal wall weight (g) from nine of the donkeys.

4. Discussion

4.1. Prevalence of helminth species in the donkeys

Several hypotheses are proposed to explain the presence or absence of helminth parasites in equids throughout the world. First, from our study it is clear that geography and most probably the association with moisture content and ambient temperature play an important role in the helminth species composition (Ogbourne, 1978; Scialdo-Krecek, 1983a; Scialdo-Krecek, Reinecke and Biggs, 1983; Craig and Courtney, 1986). Overall, there is a large degree of overlap between the species and their abundance recorded in this study and in those of previous studies on donkeys in South Africa (Theiler, 1923; Matthee *et al.*, 2000). The undescribed *Cylicocyclus* species referred to as *Cylicocyclus asinus* sp. n. in the present study was initially noted in this host in the recent study by Matthee *et al.* (2000). Both these studies on donkeys share, amongst others, 12 Cyathostominae species and four Strongylinae species, which displayed similar distribution patterns within the donkeys (Matthee *et al.*, 2000). The African cyathostome *C. montgomeryi* was initially reported in horses and mules in a worm parasite checklist of domesticated animals in South Africa (Monnig, 1928). However, in more recent studies on donkeys in South Africa (Matthee *et al.*, 2000) and Zimbabwe (Eysker and Pandey, 1989; Pandey and Eysker, 1989, 1990) and the present study this worm species was recorded as the most abundant and prevalent small strongyle. In contrast, in a study performed on donkeys in Kentucky, USA this species was not present; *C. longibursatus* being the most abundant worm species found followed by *C. minutus* (Tolliver, Lyons and Drudge, 1985). Similarly, Drudge and Lyons (1989) reported that the lungworm, *D. arnfieldi,* occurs in equids throughout the world and donkeys are regarded as the natural host of this parasite. In South Africa, Reinecke (1983) broadly defines the host of this worm species as the Equidae. The absence of *D. arnfieldi* in horses (Krecek *et al.*, 1989; Krecek, Reinecke, Kriek,

Horak and Malan, 1994c) and zebras (Krecek, Malan, Reinecke and de Vos, 1987b; Krecek *et al.*, 1994) was noted in subsequent studies. Only a single animal in the present study was infected with this species, nine worms being present, possibly reflecting a low occurrence of this parasite in the areas of South Africa from where the study donkeys originated. A higher prevalence for this parasite has been noted in other African countries. In Morocco, Khallaayoune (1991) noted 23 % prevalence in donkeys, and in Kenya Lewa *et al.* (1997) recorded 100 % prevalence of *D. arnfieldi* in the six donkeys that were necropsied. In sharp contrast, larger numbers of *D. arnfieldi* have been reported in studies performed on donkeys in the USA (Lyons, Drudge and Tolliver (1985), 3 – 315 specimens in five donkeys) and the United Kingdom (Urch and Allen, 1980).

Second, farming systems which include donkeys with other domestic stock, such as horses or cattle, can also contribute to the local species composition and abundance (Craig and Suderman, 1985). The second most abundant species in the current study, *C. longibursatus*, was also previously recorded as the most plentiful and prevalent species in the dorsal colon of horses in South Africa (Krecek *et al.*, 1989) and Britain (Ogbourne, 1976). Similarly, moderate to high infection levels for *C. goldi* were also reported in horses (Ogbourne, 1978; Krecek *et al.*, 1989). Both these species were present in noticeable numbers and in most of the donkeys in the present study and in that of Theiler (1923), but they were totally absent in another study on donkeys (Matthee *et al.*, 2000). It is thus reasonable to suspect that cross-contamination between horses and our study animals at Onderstepoort could have occurred during their weekly exercise as the area in which they exercised was frequently shared with horses. Another example is that of *T. axei* which is a common parasite in both equids and ruminants (Drudge and Lyons, 1989). This helminth was present in low numbers (< 100) in the small intestine of a single donkey in the present study. Cattle previously grazed the donkey camps from which they were removed during the winter of June 1998, one month prior to the arrival of the first donkeys at Onderstepoort. This may explain the low prevalence and abundance of the *T. axei* found in the present study (Vercruysse *et al.*, 1986;

Pandey and Eysker, 1990). In contrast, *T. axei* was present in large numbers of donkeys in Morocco (Khallaayoune, 1991), which was ascribed to the traditional farming practice in that country in which equids and ruminants share communal grazing throughout the year.

Third, it appears that the age of the host may influence the species presence and composition. Tolliver *et al.* (1985) recorded low numbers of *P. equorum* in one donkey and attributes this low prevalence in their study to the fact that only animals of an older age group were included in the experiment. In support of the hypothesis that age plays a role Drudge and Lyons (1989) reported that this worm is a common parasite of suckling and weaning foals. In South Africa, however, this parasite has been found in donkeys between the age of six months and three years, but, also in donkeys between the age of three and eight years (Wells, 1997). Both the previous study on donkeys in South Africa (Matthee *et al.*, 2000) and the present study recorded the presence of *P. equorum,* but in low numbers.

There are varying reports concerning the occurrence and prevalence of *O. equi* in equids in Africa. Prevalence rates of 7 % of infected animals in Morocco (Khallaayoune, 1991) to 67 % in Burkina Faso (Vercruysse *et al.*, 1986) have been recorded. In studies on domestic horses (Drudge and Lyons, 1977) and zebras (Krecek *et al.*, 1987b) infections with *O. equi* were mainly in young animals, but, it was suggested that the L_4 may occur in horses and zebras of all ages. The results obtained in the present study support these findings; the parasite being found in four of the nine adult (> 3 years) donkeys. However, no adult worms were found in the animals that were necropsied and the counts of this species included the L_4 stages present in the dorsal colon. The presence of L_4 *O. equi* in the donkeys in January might explain the absence of its eggs in the faecal material, using the McMaster technique, during this month. In support of this hypothesis another study on donkeys noted that during January the faecal egg counts for this parasite were very low (Wells, 1997).

Finally, host-specific preference exhibited by some helminth species may also contribute to their diversity. One example of this in South Africa is *C. leptostomum*, which has been recorded in high numbers in 50 % of the horses (Krecek *et al.*, 1989). It is possible that this species prefers the horse as host as it has not been encountered in zebras (Scialdo-Krecek, 1983a; Scialdo-Krecek *et al.*, 1983; Krecek *et al.*, 1987b; Krecek *et al.*, 1994c) and is either absent or only occurs in low abundance in donkeys (Theiler, 1923; Matthee *et al.*, 2000). Similarly, *C. catinatum* was recorded in moderate to low numbers in donkeys in Burkina Faso (Vercruysse *et al.*, 1986) and in the present study, and was absent in another South African study (Matthee *et al.*, 2000). This small strongyle species may prefer horses, as high numbers of it have been found in this host by Krecek *et al.* (1989) and Ogbourne (1976).

Apart from the nematodes that were recorded, there were also one trematode species and one cestode species present in the donkeys. *Gastrodiscus aegyptiacus* was reported to occur in varying levels of abundance and prevalence in donkeys in Burkina Faso (Vercruysse *et al.*, 1986), South Africa (Wells *et al.*, 1998; Matthee *et al.*, 2000), Chad (Graber, 1970) and Zimbabwe (Pandey and Eysker, 1990) and in horses in Chad (Graber, 1970). Similarly, in the present study, varying numbers of it were recorded in the ventral colon and the caecum. Reports on the pathogenicity of this parasite in horses are conflicting (Azzie, 1975; Soulsby, 1982), but it is possible that significant large numbers in the caecum and ventral colon might limit nutrient uptake by the host from these sites. Information on the presence of tapeworms in horses and zebras in South Africa is limited. In one study on horses (Krecek *et al.*, 1989) and several on zebras (Scialdo-Krecek, 1983a; Scialdo-Krecek *et al.*, 1983; Krecek *et al.*, 1987b; Krecek *et al.*, 1994c) there has only been a single observation of *A. perfoliata* in low to moderate numbers with moderate prevalence in Cape mountain zebras, *Equus zebra zebra* (Krecek *et al.*, 1994c). In addition, there are two recordings of *A. perfoliata* in donkeys in Africa (Khallaayoune, 1991; Matthee *et al.*, 2000). In both of these studies this species was found in the small intestine and in very low abundance and

prevalence. Results emanating from the present study support the probability that this parasite only occurs in low prevalence as it was present in the small intestine in a single animal; it was however, present in moderate numbers. Based on the limited information available, it appears that tapeworms are more prevalent in donkeys in South Africa than in horses or zebras, but further studies on equids in South Africa and other African countries are essential before such a statement can be made with confidence.

Based on previous studies in Egypt (Hilali *et al.*, 1987), Zimbabwe (Pandey and Eysker, 1990) and South Africa (Matthee *et al.*, 2000), it appears that *G. intestinalis* is the most common bot species in donkeys in Africa. Results from the present study are comparable as this species was the only one that was recovered from seven of the nine donkeys that were necropsied.

4.2. Alternative helminth control methods and their effect on the host's helminth burdens

Eight of the animals necropsied formed part of a study to test cost-effective helminth control methods, with attention on pasture hygiene and seasonal regulated treatment. In recent years, studies that focussed on helminth control, in addition to preventing/limiting anthelmintic resistance, have indicated that the combination of pasture management and selective seasonal treatment with an effective anthelmintic is highly successful and sustainable (Herd, 1986; Herd, 1993; Herd and Coles, 1995). Results obtained in the present study support these findings in that the removal of faeces from the camps on a monthly basis in combination with a pre-winter anthelmintic treatment resulted in the lowest average number of adult worms and larvae in the lumen of the gastro-intestinal tract in the donkeys in this management system (MS D). The remaining two management systems, i.e. the removal of faeces monthly from the camps (MS B) and the pre-winter anthelmintic treatment (MS C), also resulted in a decline in the worm burdens, but to a lesser extent.

Although intra-group variation in the lumenal adult and larval worm burdens was recorded between the two groups in each of the three management systems, the individual worm counts for each group were still noticeably lower when compared to the counts of the control animals. Variation between animals, subjected to the same management system, is not unexpected, as there are natural differences in individual animals' susceptibility to helminth infections (Rubin, 1967; Herd, 1992; Duncan and Love, 1991; Lyons *et al.*, 1994; Wells *et al.*, 1998).

Reduced numbers of DL were recorded in the donkeys that were either in the pre-winter anthelmintic treatment camps (MS C) or were grazing in the camps from which the faecal material were removed on a monthly basis (MS B). However, the most significant decrease was obtained in the animals that were subjected to both treatments (MS D) when compared to the numbers in the control animals. Towards the end of the warm and wet season (March – April), the acquired infective L_3 small strongyles entered the mucosal wall where they either encysted and or developed further into lumenal L_4 before they over-wintered in the host (Ogbourne, 1976, 1978; Krecek *et al.*, 1987a). During the months of winter (April – August) very few eggs and larvae were deposited on the pasture that could develop further or survive the cold and dry conditions (Ogbourne, 1976). As a result, there were limited or no additional infective larvae ingested by the host during this time due to reduced pasture larval burdens (< 7500 L_3/kg dry matter) and limited or no grazing by the donkeys due to the poor growth or absence of vegetation. The administration of moxidectin, in May 1998, possibly resulted in the depletion of most encysted LL_3 and L_4 as well as the majority of the lumenal L_4 (98 %) and adult cyathostomes (99 %) (Xiao *et al.*, 1994; Vercruysse *et al.*, 1998). The removal of the adult and larger encysted and lumenal larval stages possibly triggered a portion of the unaffected EL_3 to resume development in the donkeys. This hypothesis has also been put forward in a previous study on ponies to explain why treated ponies, compared to untreated control animals, contained higher proportions of very small DL which could not be detected, using TMI, five weeks after treatment with moxidectin (Eysker *et al.*, 1997). In the present study, however, the

few remaining larvae developed into sexually matured adults during winter that produced eggs in spring. The amount of eggs recorded in the donkeys that received the pre-winter moxidectin treatment was lower compared to those in the animals in the control and MS B camps, which resulted in less infective larvae on the pasture (approximately < 10 000 L_3/kg dry matter, Chapter 4) and therefore a reduced uptake of infective L_3, hence a lower number of mucosal LL_3 and L_4 as well as lumenal L_4 in the hosts in January.

In the studies of horses a significant reduction in the concentrations of infective L_3 on the pasture with twice-weekly faecal removal was reported (Herd, 1986; Fisher, 1997). This study confirms these findings in that a 50 % reduction in the number of infective larvae on the pasture in January 1999 was detected which was probably due to the monthly removal of faeces from the camps and/or pre-winter moxidectin treatment (i.e. less larvae were ingested by the host) and ultimately the presence of less mucosal and lumenal larvae in the host.

The wet and warm climatic conditions in the summer months in Pretoria are ideal for *S. vulgaris* L_3 survival and availability (Pandey and Eysker, 1989). The prepatent period of *S. vulgaris* is more than six months and thus results in a decrease in L_4/L_5 and an increase in adult burdens at the end of the dry and cooler winter months. It is thus suspected that large numbers of adult *S. vulgaris* were present in the donkeys at the time of deworming, May 1998 and that the pre-winter treatment of the donkeys, with moxidectin, decreased the *S. vulgaris* L_4 in the arteries and the adult burdens in the gut (Xiao *et al.*, 1994). The absence of egg producing adults in the host, as well as limited numbers or absence of infective larvae on the pasture, in spring, resulted in a decreased and delayed uptake of infective L_3, hence lower average adult *S. vulgaris* burdens in the dewormed animals in January 1999.

4.3. Mucosal larval stages, their recovery methods and distribution pattern

All the mucosal larvae recorded in the present study were the larger encysted larvae (LL_3 and DL_4) based on their total body length measurements (< 1.2 mm) and buccal capsule shape (Popova, 1958 from Lichtenfels, 1975; Chapman *et al.*, 1999). There are several possible explanations for the poor representation of EL_3 in the donkeys. First, the project commenced in October 1997 and continued for 16 months until the end of January 1999. In both 1998 and 1999, low numbers of L_3 were recorded on all the pastures during January (< 5000 L_3/kg dry matter), which might have been due to a decrease in egg producing adults in the donkeys in December or early January. In previous seasonal studies on horses a decline in the numbers of adult small strongyles as well as lower numbers of L_4 in the lumen, in the host, has been reported during midsummer, December to February (Ogbourne, 1976; Krecek *et al.*, 1989). Thus, low numbers of egg producing adult worms in the host resulted in lower numbers of L_3 on the pasture and probably also in limited ingestion of L_3 by the host and therefore limited EL_3 encysted in the gut wall at the end of January 1999. Another possible explanation for the absence of encysted EL_3 might be that the newly acquired cyathostome L_3 that entered the mucosa might have continued development to the LL_3 and DL_4 stage before they enter an arrested phase in their development. This phenomenon has been previously described in horses by Reinemeyer and Herd (1986a) and Reinemeyer (1986) and may explain the absence of EL_3 in the gut wall of the current study.

In the present study larger numbers of DL were recorded from the gut wall in 75 % of the necropsied donkeys, using the TMI method, in both the treated and untreated animals. The donkeys were euthanased eight months after deworming with moxidectin and it might be possible that dead DL were still visible in the mucosal wall in January 1999. If this was the case they would have been counted during TMI, but, were probably disintegrated by the digestion process of the DIG method (Klei *et al*, 1997). Another possibility might be that the digestion time (three hours) was

too long and resulted in the disintegration of some of the DL even though care was taken to prevent it (a sample of larvae being examined after two hours of digestion). Reinemeyer and Herd (1986a) compared the sensitivity of the two techniques (TMI and DIG) in horses and noted a decrease in the amount of larvae recovered with an increase in time, only 84.1 % of the DL were recovered by digestion after three hours and only 43.5 % were yielded after six hours. This explanation might be more feasible as larger numbers of DL were recorded with TMI in both treated and untreated animals.

Reinemeyer and Herd (1986b) reported the highest cumulative percentage of mucosal larvae from the caecum (57 %), followed by the ventral colon (42 %) and dorsal colon (1 %) in the horse. In a subsequent study on nine ponies, the largest number of DL was present, using TMI, in the caecum of four ponies. However, there were some unexplained exceptions, the largest number of DL were obtained in the dorsal colon of three of the animals and two others recorded the largest numbers in the ventral colon (Murphy and Love, 1997). In sharp contrast, a higher cumulative mucosal larval count was noted in the ventral colon (65 %) in the nine donkeys, followed by the caecum (35 %) and the dorsal colon (1 %). The reason for the larger numbers of larvae in the ventral colon in the donkeys in this study is uncertain. A plausible explanation involves differences in the distribution of the mucosal larval stages of the different cyathostome species in the colon. Studies on zebra have revealed distinct differences in the anatomic distribution of encysted cyathostome larvae in this host (Scialdo-Krecek, 1984; Krecek *et al.*, 1987a). They found that the small intestinal wall harboured very large numbers of L_4 in two zebra species, Burchell's (*Equus burchelli antiquorum*) and Hartmann's mountain zebras (*Equus zebra hartmannae*). In the study by Scialdo-Krecek (1984) a 100 % prevalence of encysted L_4 in 25 Burchell's and three mountain zebra of different ages was recorded. Due to the numerous cyathostome larvae that are known to occur in zebras, in general, Malan *et al.* (1981b) proposed in their guidelines for necropsy techniques that TMI should also be performed on the small intestinal wall. It is possible that large

helminth burdens or anatomical differences between equine species are responsible for the increased rate of colonisation of the small intestine and dorsal colon which would influence the preferred colonisation pattern of the encysted larvae (Lyons *et al.*, 1994). At present, it is uncertain if all the 50 cyathostome species follow the same distribution pattern and thus favour the same predilection site when their larval stages encyst in a horse's or donkey's gut wall. The current predicament is that it is difficult, or even impossible, morphologically to identify the encysted larval stages. It is predicted that the use of DNA based identification procedures may prove to be extremely valuable if not indispensable for the species identification of the different encysted larval stages allowing for the recognition of site-specific preferences by the larval stages in all equine taxa (Nadler, 1990; McManus and Bowles, 1996; Gasser and Newton, 2000).

4.4. Conclusion

Estimated counts of the total helminth burdens in the host, using necropsy techniques, provide the only concrete proof of the extent of an experimental helminth control management system. The information generated in the present study, using gastro-intestinal helminth recovery and identification, provides the first substantial confirmation of the value of alternative helminth control strategies. In this study all three experimental management systems resulted in unambiguous reductions in the hosts' helminth burdens. As expected, the most significant decrease in the internal parasite burdens was consistently observed in the donkeys that were subjected to the combined management system of monthly faecal removal and a pre-winter moxidectin treatment. Based on these findings it is suggested that the donkey, and ultimately the owner, will benefit from the use of alternative helminth control methods. In the present study, both TMI and DIG were used to enumerate the mucosal larval stages and TMI appeared to be superior. It is however suggested

that the lower larval counts, using DIG, might be due to a loss of larvae during the three hours of digestion. In addition, this study reveals a possible variation in the distribution patterns of the encysted larval stages between the gut walls of donkeys, horses and zebras and draws attention to the paucity of information regarding this apparent variation.

CHAPTER 6

Cylicocyclus asinus sp. n. (NEMATODA: STRONGYLOIDAE: CYATHOSTOMINAE) FROM DONKEYS, *Equus asinus*, IN SOUTH AFRICA

1. Introduction

Equids harbour a wide diversity of helminth species that are illustrated in the key of Lichtenfels (1975). The helminths present in equids are grouped into the nematodes, cestodes and trematodes. The nematodes comprise the largest number of genera and the largest number of species (Lichtenfels, 1975). Although more than 50 species of the cyathostomes (subfamily Cyathostominae within the phylum Nematoda) have been described in horses, less than 12 are abundant and prevalent (Uhlinger, 1991; Lichtenfels *et al.*, 1998b).

Studies on donkeys and zebras have contributed further to our knowledge of these equine helminths. Research attention on the helminth flora of zebras has led to the description of six new helminth species, two cyathostome, two large strongyles and two habronematids, in Burchell's, Hartmann's and Cape Mountain zebras, (Scialdo-Krecek, 1983b; Scialdo-Krecek and Malan, 1984; Krecek, 1989; Krecek *et al.*, 1997). Following the array of newly described and/or re-described (Kharchenko, Dvojnos, Krecek and Lichtenfels, 1997; Lichtenfels *et al.*, 1998a) cyathostome species a revised annotated checklist has been constructed for the 51 recognised small strongyle species present in horses, donkeys and zebras (Lichtenfels *et al.*, 1998b).

There are two main reasons why new undescribed species are still found. First, the methods used for helminth recovery and species identification are generally performed on only a portion (1/4, 1/5 or a 1/10) of the intestinal and stomach ingesta, and therefore less abundant species may be

overlooked (Malan *et al.*, 1981a,b). Second, although horses, zebras and donkeys all belong to the Equidae there are differences in the helminth fauna between the taxa (Lichtenfels, 1975).

Two parasitological investigations on domesticated donkeys in South Africa (Matthee *et al.*, 2000; Matthee, current study) have contributed to our current knowledge of equine helminthology. The studies have revealed a previously unknown cyathostome species, which is described and named in this report.

2. Materials and methods

Adult male and female specimens of the undescribed cyathostome species were recovered from the ventral colons of seven donkeys (*E. asinus*) in Pretoria, South Africa (Table 17). Quantitative helminthological studies of the gastro-intestinal tracts were undertaken in January 1998 and 1999 on each donkey after they had been euthanased and thereafter necropsied using the techniques for helminth recovery of Malan *et al.* (1981a, b) and Duncan *et al.* (1988). Nematodes were recovered and stored in 70 % alcohol. The specimens were cleared in lactophenol and examined under a Nikon Optiphot light microscope fitted with disc interference contrast. *En face* cuts of the worm heads were performed to determine the number of elements of the external leaf crown. The heads were cut with a scalpel blade and mounted in lactophenol.

Table 17. Animal number, place of origin, sex and age of the seven donkeys, necropsied during January 1998 and 1999, that harboured the previously unknown cyathostome species.

Animal number	Origin	Sex	Age (years)
9	Witbank	M	3
12	Hammanskraal	F	3
14	Hammanskraal	M	16
17	Onderstepoort	F	5
20	Onderstepoort	M	3
25	Marble Hall	M	4
27	Marble Hall	F	9

Type and paratype specimens were deposited at three museum collection sites: 1) Parasite Worm Division, Department of Zoology, Natural History Museum, Cromwell Road, London, UK [Accession Numbers 1999.11.12.1 - 4 (two male and two female paratype)], 2) The United States National Parasite Collection in Beltsville, Maryland 20705, USA [Accession Numbers 089294 (one male and one female holotypes) and 089295 (one male and one female paratype)], and 3) The National Collection of Animal Helminths based at the Plant Protection Institute, Agricultural Research Council, Rietondale, Pretoria, South Africa [Accession Number T2189 (two male and two female paratype)].

3. Results

3.1. General

Nematoda, Strongylida, Strongyloidae, Strongylidae, Cyathostominae, *Cylicocyclus*. Mouth collar is high (Figures 14a-c, 15a-c). Lateral amphids are broad and the duct extends through the mouth collar (Figure 15d). Submedian cephalic papillae, with candle flame-shaped tips

extend well beyond the mouth collar (Figures 14a-c, 15b, c). Elements of the external leaf-crown (ELC) are inserted deeply and extend beyond the mouth collar (Figures 14c, 15c). Individual elements of the ELC are long, broad and bend slightly towards the centre of the mouth (Figures 14c, 15b, c). Internal leaf-crown (ILC) elements are half the length and almost twice the width of the ELC (Figures 14b, c). Buccal capsule is more than twice as wide as it is deep (Figures 14a-c, 15b, c). Buccal capsule walls are not straight but appear slightly bent anteriorly. The walls are thinner anterior and thicken slightly anterior to large hoop-shaped thickenings at the base of buccal capsule (Figures 14b, c, 15b, c). Dorsal gutter is present (Figures 14a, c, 15b, c). No prominent oesophageal funnel is present. Oesophagus displays a pyriform-shaped swelling and elongated oesophago-intestinal valve (Figures 14a, 15a). Excretory pore is slightly posterior to cervical papillae; both are however posterior to the nerve ring (Figures 14a, 15a).

3.2. Description

Dimensions of the relevant characters are given as ranges in Table 18. In the following two sections, the dimensions are given as mean in micrometers ± standard deviation, unless otherwise indicated.

MALES (N = 10): Total body 6.21 ± 0.6 (mm) long; 332 ± 31.3 wide at oesophago-intestinal junction (O-I). Buccal capsule 33 ± 3.4 long; 70 ± 6.6 wide. The external leaf-crown (ELC) consisted of 40 elements. Dorsal gutter present, extends part way along length of buccal capsule. Distance from the anterior end to the nerve ring, cervical papillae and excretory pore 430 ± 14.9, 500 ± 40.7, 525 ± 42.3 respectively. Oesophagus 0.91 ± 0.04 (mm) long; maximum width 192 ± 16.7. Dorsal ray 666 ± 147.3 long, main division extends to level of externodorsal ray.

Gubernaculum 245 ± 12.8 long, with longitudinal ventral groove and median ventral transverse notch. Spicules 2.5 ± 0.2 (mm) long.

Bursa size average for genus. Dorsal lobe not distinctly set off from the lateral lobes (Figure 15f). One pair of prebursal papillae on the genital cone (Figure 14g). Spicule tips hooked and distally tapered to a rounded point (Figure 14h). Gubernaculum slender, pistol shaped (Figures 15i, j).

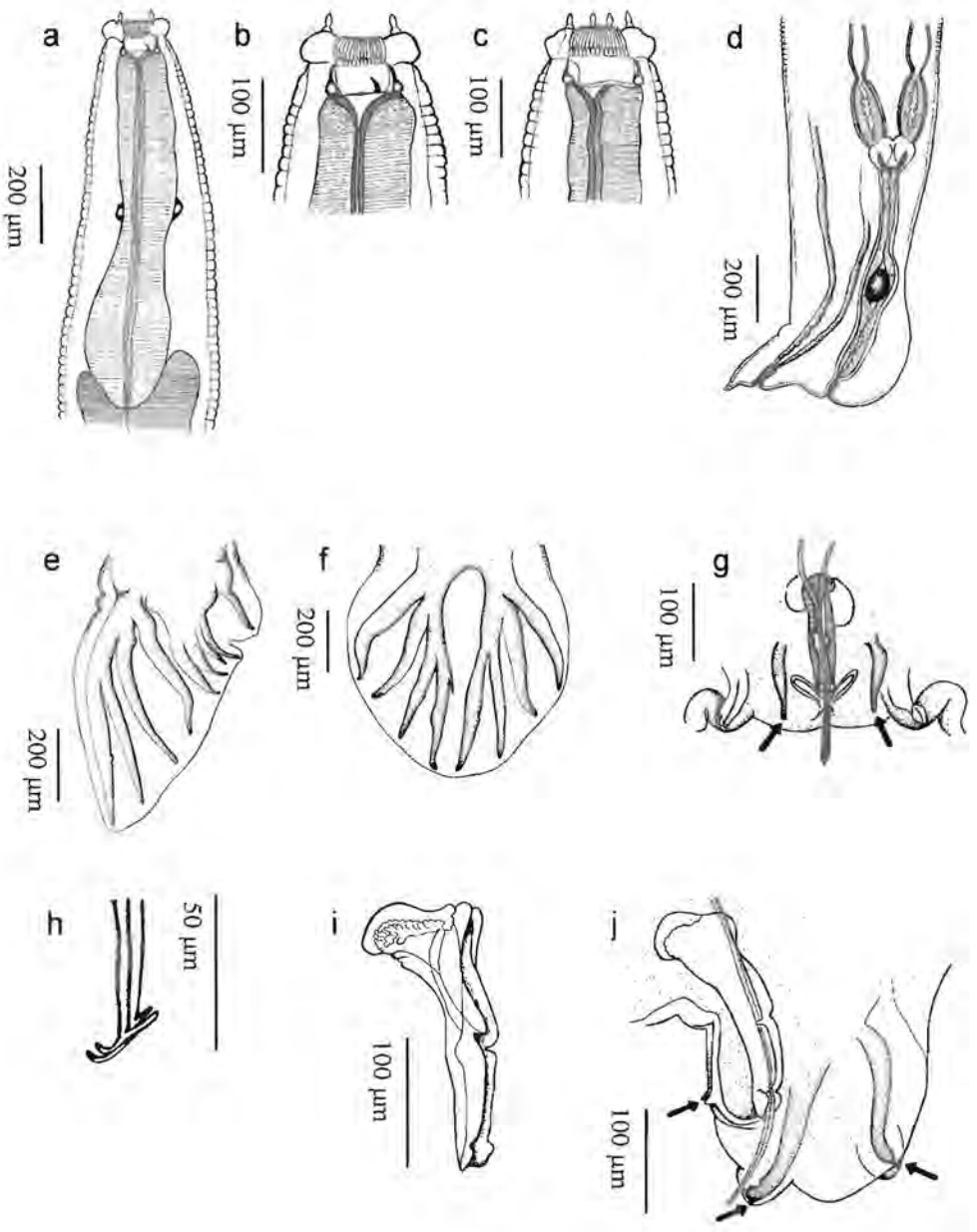
FEMALES (N = 10): Total body 7.94 ± 0.5 (mm) long; 405 ± 30.5 wide at O-I junction. Buccal capsule 34 ± 4.0 long; 74 ± 10.9 wide. The external leaf-crown (ELC) consisted of 46 elements. Distance from the anterior end to the nerve ring, cervical papillae and excretory pore 445 ± 31.0, 546 ± 45.5, 571 ± 30.2 respectively. Oesophagus 1.0 ± 0.1 (mm) long; 221 ± 21.7 wide. Vulva opens 211 ± 19.9 from anus. Tail 116 ± 32.6 long, "club-foot" appearance (Figure 15e). Tail shorter than distance from anus to vulva (Figure 15e). Vagina 667 ± 102.9; vestibule 83 ± 12.4; sphincter 253 ± 39.2; infundibulum 370 ± 75.2 long respectively. Eggs 87 ± 30.5 long; 48 ± 18.2 wide.

HOST RECORD INFORMATION: Total numbers from 2 to 2 146 were recovered from the ventral colons of seven donkeys in Pretoria, South Africa.

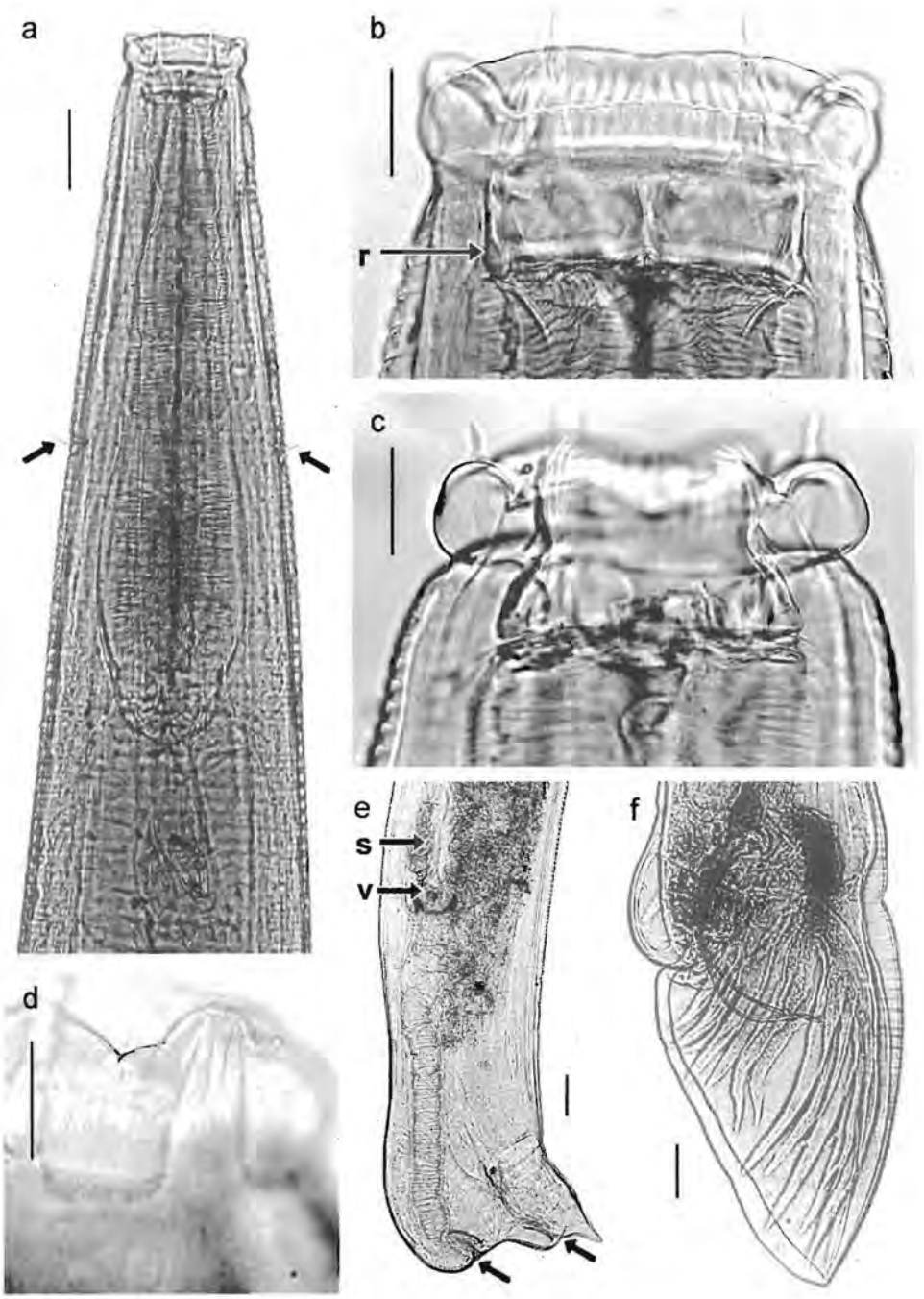
TYPE HOST AND TYPE LOCALITY: *Equus asinus*, Pretoria, South Africa (25°45′S, 28°15′E).

SITE OF INFECTION: Ventral colon.

ETYMOLOGY: This species is named after the donkey, *Equus asinus*.



Figures 14a - j. Drawings of *Cylicocyclus asinus* sp. n. Scale bars = 50 µm (Figure h), 100 µm (Figures b, c, g, i, j) and 200 µm (Figures a, d, e, f). a. Anterior end, lateral view. b. Buccal capsule, lateral view. c. Buccal capsule, dorsal view. d. Female tail, lateral view. e. Male tail, lateral view. f. Male tail, dorsal view. g. Appendages of genital cone, ventral view, showing prebursal papillae (arrows). h. Fused spicule tips of male. i. Gubernaculum of male. j. Genital cone of male with gubernaculum, lateral view, showing paired dorsal papillae (left arrow), ventral papilla (middle arrow) and prebursal papilla (right arrow).



Figures 15a - f. Photomicrographs of *Cylicocyclus asinus* sp. n. Scale bars = 50 µm (Figures b, c, d) and 100 µm (Figures a, e, f). a. Oesophageal region, dorsoventral view, showing the position of the cervical papillae (arrows). b. Buccal capsule, dorsoventral view, showing ring-like thickening at base of capsule (r) and submedian papillae. c. Buccal capsule, lateral view. d. Lateral papilla protruding through mouth collar. e. Female tail, showing anus and vulva (arrows) and ovejectors, including vestibule (v) and sphincters (s). f. Male tail, lateral view.

Table 18. Principal measurements given as ranges and averages of *Cylicocyclus asinus* sp. n. recovered from the seven donkeys (all measurements in micrometers unless otherwise stated).

Character	Males		Females	
	Range	Average	Range	Average
Total length (mm)	5.25 – 6.90	6.21	7.18 – 8.97	7.94
Width	284 – 384	332	368 – 432	405
Buccal capsule width	62 – 78	70	62 – 90	74
Buccal capsule length	28 – 38	33	25 – 40	34
No. of elements in external leaf-crown	40†		46†	
Nerve ring*	410 – 441‡	430	384 – 479‖	445
Cervical papillae*	454 – 536§	500	473 – 611	546
Excretory pore*	428 – 567	525	510 – 611	571
Oesophagus width	158 – 208	192	189 – 252	221
Oesophagus length* (mm)	0.90 – 1.0	0.91	0.88 – 1.12	1.0
Egg (length x width)	-		35 – 115 x 17 – 65‖	84 x 48
Vulva to anus distance	-		176 – 240	211
Vagina length	-		528 – 848	667
Vestibule length	-		63 – 101	83
Sphincter length	-		189 – 302	253
Infundibulum length	-		221 – 428	370
Spicule length (mm)	2.32 – 2.88	2.5	-	
Gubernaculum length	221 – 265	245	-	
Dorsal ray length	441 – 880	666	-	
Tail length	-		64 – 160	116

*Measured from anterior end.
† $N = 1$
‡ $N = 4$
§ $N = 5$
‖ $N = 9$

4. Discussion

According to the annotated checklist provided by Lichtenfels *et al.* (1998b) there are 10 species and one subspecies that comprise the genus *Cylicocyclus* Ihle, 1922. The species are *C. ashworthi*, *C. auriculatus*, *C. brevicapsulatus*, *C. elongatus*, *C. elongatus kotlani*, *C. insigne*, *C. leptostomum*, *C. nassatus*, *C. radiatus*, *C. triramosus* and *C. ultrajectinus*. The worms in this genus are characterised by ringlike, hoop-shaped thickenings at the base of the buccal capsule. In addition, the lateral papillae are usually large, broad and hornlike (Lichtenfels, 1975).

The specimens described are placed in the genus *Cylicocyclus* on the basis of the shape of the buccal capsule and shape of the submedian cephalic papillae. *Cylicocyclus asinus* sp. n. is smaller than some of the other members of this genus (male = 6.21 mm and female = 7.94 mm) and can be grouped with the other smaller species, such as *C. ashworthi* and *C. leptostomus*. The mouth collar is high, similar to *C. nassatus*, yet it does not have a cuticular shelf-like projection midway in depth of the buccal capsule depth (Lichtenfels, Kharchenko, Sommer and Ito, 1997). In addition to its short body length, the buccal capsule is small and more than twice as wide as it is deep which places it in the small buccal capsule group, which comprises the species *C. nassatus*, *C. ashworthi* and *C. leptostomus* with small buccal capsules that are two to three times wider than they are deep (Lichtenfels *et al.*, 1998b). The elements of the ELC are distinguishable and long, yet not as broad and pointed as in the case of *C. ashworthi* and *C. nassatus*. The distinct shape of the oesophagus at the O-I junction is shared with *C. leptostomus*. The oesophago-intestinal valve is elongated and appears to be embedded into the intestine (Lichtenfels, 1975). In the female, the tail length is shorter than the vulva-to-anus distance. This feature is shared by *C. leptostomus, C. radiatus* and *C. triramosus*. It does, however, differ from *C. nassatus* and *C. ashworthi* whose tail lengths are either longer (*C. nassatus*) or similar (*C. ashworthi*) in length to the vulva-to-anus distance. (Lichtenfels *et al.*, 1997). The gubernaculum and spicule lengths are similar to that of *C. triramosus* (Kharchenko *et al.*, 1997) but the spicule shape is similar to that of *C. radiatus* (Lichtenfels *et al.*, 1998a). *Cylicocyclus asinus* sp. n. has distinctive candle flame-shaped lateral papillae that are prominent and extend well beyond the mouth collar. For these reasons the specimens described are considered to constitute a new species *Cylicocyclus asinus*.

GENERAL CONCLUSION

The information generated from this study has made valuable contributions to the existing knowledge available on several aspects of helminths and their control in domestic equids. The cyathostomes were the most abundant helminths in the donkeys based on the FEC, larval cultures and total helminth counts in the gastro-intestinal tract. During the course of the latter an undescribed cyathostome species, *Cylicocyclus asinus* sp. n., was recorded in seven of the nine necropsied donkeys which brings the total number of *Cylicocyclus* species known in equids to twelve. Ambient temperature and moisture was important for the development and survival of both the egg and larval stages on the pastures. It is indeed this environmental dependence, which forms the basis for the seasonal display of helminth egg production in the host and subsequently the availability of parasitic larvae on pasture.

This is the first in-depth study that was performed in South Africa that clearly shows that the general health and working condition of donkeys are adversely affected by helminth burdens. It is suggested that animals with high helminth parasite burdens will show marked improvement in both general body condition and blood physiology following deworming. Moreover, it is the first controlled study that reveals the beneficial effect of practical and cost-effective helminth control strategies for working donkeys in developing countries. Frequent faecal removal (twice monthly) from the pastures grazed by donkeys will reduce the pasture larval burdens and the helminth re-infection rate, which will ultimately result in a reduction of helminth parasites in the donkeys. The strategic deworming of donkeys with moxidectin in autumn will result in significant reductions in their FEC and total helminth burdens. Based on the present study it is suggested that the strategic timing of deworming will probably reduce the helminth re-infection rates during winter and together with the prolonged residual effect of moxidectin will ensure lower worm burdens in the

host for several months. Finally, it is unmistakable that the greatest residual effect on the host's FEC and the most significant reductions in the helminth burdens will be observed in animals subjected to the combined management system of faecal removal and strategic deworming.

SUMMARY

Twenty-three working donkeys (*E. asinus*), allocated to eight experimental groups, formed part of a 16-month study in South Africa to determine the effect of alternative helminth control methods on their helminth levels and general condition. The results of three alternative control methods and a replicate of each (animals in camps from which faeces were removed once a month, those treated before winter with the anthelmintic moxidectin, and those that was subjected to a combination of these two forms of treatment) were compared to those obtained from a set of controls.

The cyathostomes were the most abundant helminths in the FEC and larval cultures. Both the FEC and pasture larval counts displayed increased activity during the warm and wet months (September to March). Towards the end of the study, the positive effect of monthly faecal removal was exemplified by a 20 % reduction in the hosts' average FEC, which could be attributed to the reduced pasture larval burdens that were recorded in these camps. The animals that received a pre-winter moxidectin treatment and those that were subjected to the combined treatment recorded an average faecal ERP of six to seven weeks following deworming. In addition, 100 % reduction in the nematode eggs and a prolonged suppressive effect on the FEC were recorded in all these animals. Monthly live weights, BCS and blood chemistry values concurred that the general condition differentially improved in the animals following deworming. To determine the total helminth parasite loads and biodiversity, post-mortem examinations were performed on nine donkeys at the end of the study period. Thirty-eight helminth species were recorded. In addition, helminths belonging to one trichostrongylid, one paramphistomatid and one gasterophiliid species were recovered. The as yet unknown cyathostome species, *Cylicocyclus asinus* sp. n. was observed in the ventral colons of seven donkeys. *Cyathostomum montgomeryi* was the most abundant

cyathostome, followed by *C. longibursatus*. *Triodontophorus hartmannae* was the most abundant large strongyle, followed by *S. vulgaris*. The large strongyles were less abundant when compared to the numbers of cyathostomes, but the predilection site of both groups was the ventral colon. Worm burdens for each animal ranged from 3 831 to 29 501. All three experimental management systems resulted in reduced total helminth burdens, which included reductions in the mucosal larval stages in the gut wall, however, the most significant decrease was observed in the donkeys that were subjected to the combined management system.

This is the first study to provide empirical data in South Africa on the pathogenic effect of helminths on working donkeys kept on a low quality diet. In addition, it provides information on the value of alternative control methods that can be used to reduce worm burdens. Regular faecal removal from camps and a single pre-winter treatment with moxidectin proved to be cost-effective methods to control helminth parasites. By following these methods the general health and working capacity of donkeys in southern Africa can be greatly improved.

OPSOMMING

In 'n studie oor 16 maande om die effek van alternatiewe wurmbeheermetodes op die algemene kondisie en inwendige parasietlading van werkende donkies te bepaal is 23 volwasse donkies aan agt eksperimentele groepe toegewys. 'n Stel kontroles is met drie alternatiewe wurmbeheermetodes en 'n herhaling van elk vergelyk: maandelikse misverwydering, ontwurming met moksidektien voor die winter en 'n kombinasie van maandelikse misverwydering en ontwurming voor die winter.

Na ontwurming is verbeterings waargeneem in die maandelikse lewende massa, kondisie-indeks en chemiese bloedsamestelling van die vier eksperimentele groepe. Die klein strongiele was die algemeenste groep in die maandelikse mis-eiertellings (ME) en larwekulture. Tydens die warm en nat maande (September tot Maart) is 'n toename in beide die ME en die aantal larwes op die gras waargeneem. Teen die einde van die studie is die positiewe uitwerking van maandelikse misverwydering uitgewys deur 'n verlaging van 20 % in die gemiddelde ME van gashere. Die verlaging word toegeskryf aan die verminderde larwetellings in die vier kampe. By diere wat voor die winter ontwurm is en dié met die kombinasiebehandeling is wurmeiers weer ses tot sewe weke na behandeling herwin. Albei behandelings het 'n 100 % afname in die ME tot gevolg gehad, gevolg deur 'n verlengde onderdrukkende effek op die ME.

Om die totale wurmladings in die donkies te bepaal is nadoodse ondersoeke op nege diere uitgevoer. Die aantal wurms per dier het gewissel van 3 831 tot 29 501. Die onlangs beskrewe cyathostoomsoort, *Cylicocyclus asinus* sp. n., het deel uitgemaak van die 38 wurmsoorte wat gevind is. Een trigostrongilied, een amfistoom en een gastrofilied is ook gevind. *Cyathostomum montgomeryi* was die algemeenste cyathostoomsoort, gevolg deur *Cylicocyclus longibursatus*. *Triodontophorus hartmannae* was die algemeenste groot strongielsoort, gevolg deur *Strongylus*

vulgaris. Groot strongiele was minder algemeen as klein strongiele; beide het veral in die ventrale kolon voorgekom. Alhoewel beide maandelikse misverwydering en behandeling voor die winter 'n afname van wurms in die dermlumen en -wand tot gevolg gehad het, het 'n kombinasie van dié behandelings tot die grootste afname gelei.

Die studie verskaf die eerste eksperimentele gegewens in Suid-Afrika oor die patogeniese uitwerking van wurms op werkende donkies wat 'n lae kwaliteit dieet gevoer word. Daarbenewens word inligting ook verskaf oor die waarde van alternatiewe wurmbeheermetodes vir die verlaging van wurmladings in die gasheer. Gereelde misverwydering en 'n enkele ontwurming met moksidektien voor die winter blyk koste-effektief te wees. Donkies in Suider-Afrika se algemene kondisie en werkvermoë sal noemenswaardig verbeter indien hierdie wurmbeheermetodes gevolg word.

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