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.

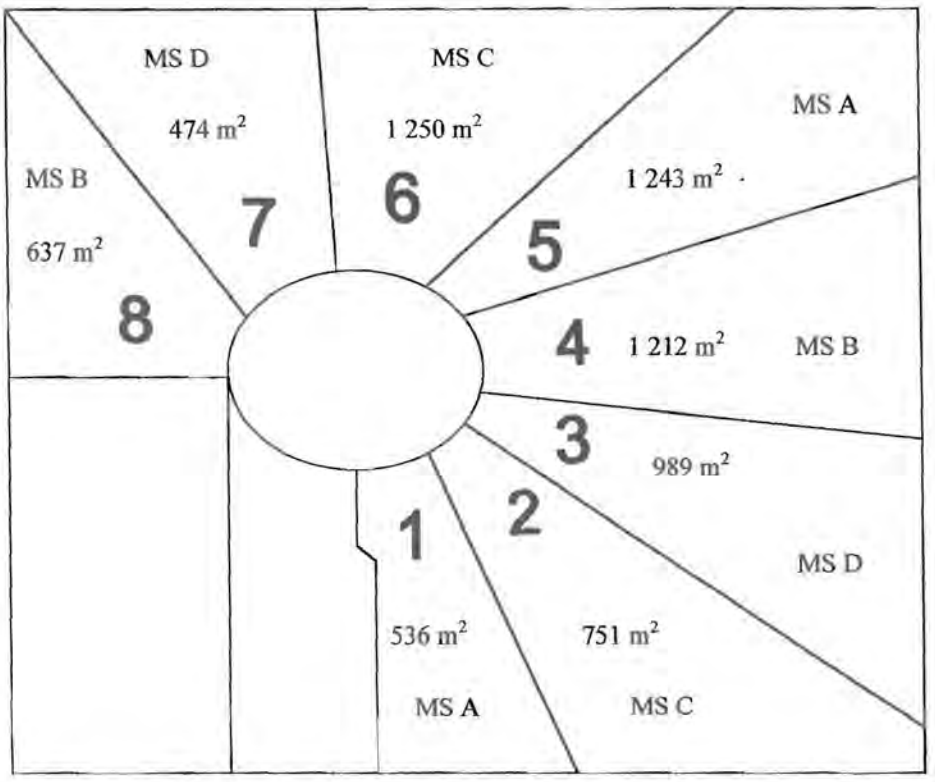


Figure 1. An illustration of the eight camps in which the 24 donkeys were housed during the 16-month alternative helminth control trial at the University of Pretoria, South Africa. Camp size is given in m^2. The management systems (MS) are indicated and correspond to those in Table 4.

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.

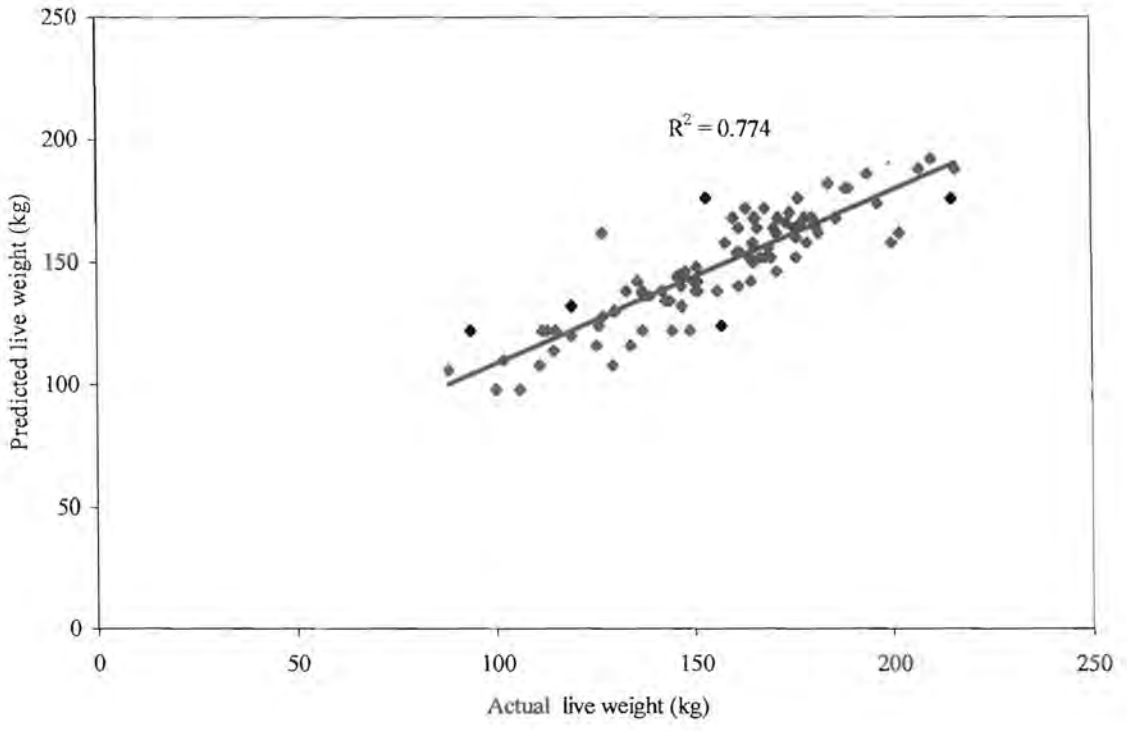


Figure 4. Scatterplot of actual live weight of donkeys compared to the live weight predicted by the body condition score-heart girth-length formula.

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).

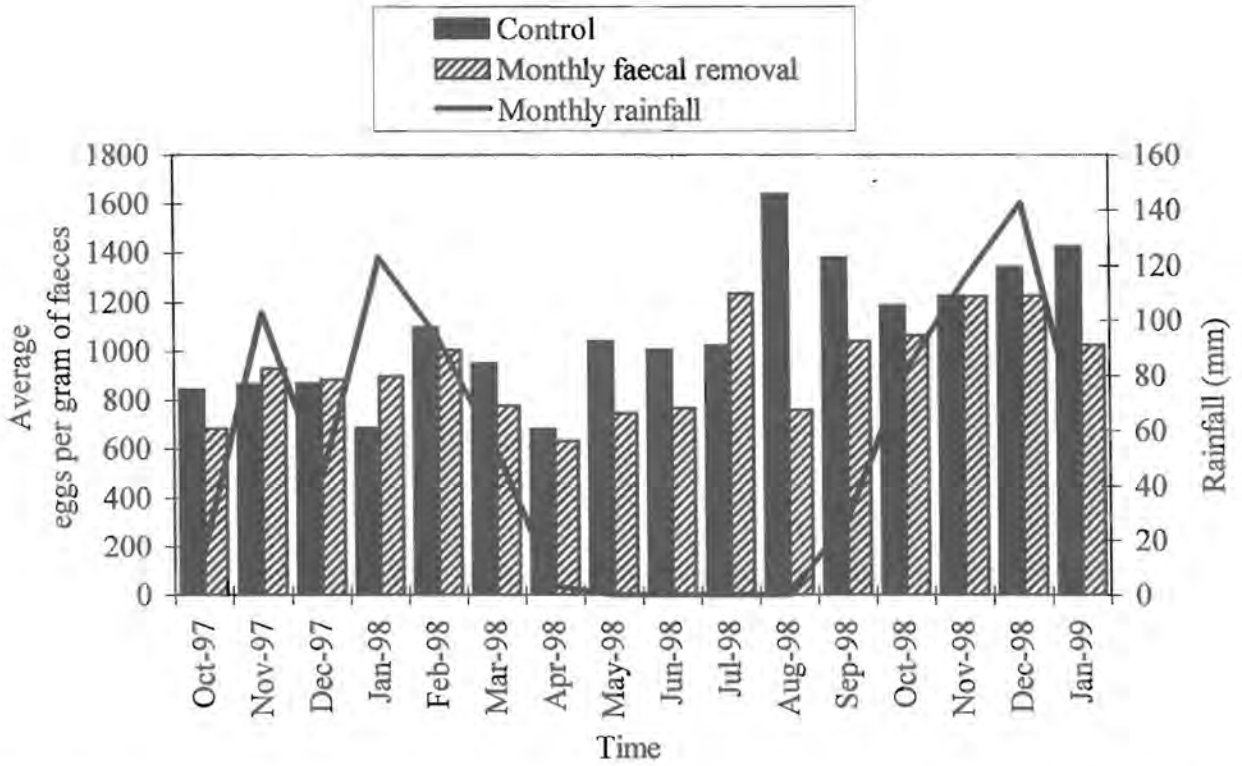


Figure 5. Seasonal average faecal egg counts, based on counts obtained for the animals in the control camps and those in the camps from which faeces were removed from the camps on a monthly basis (MS B), compared to the monthly rainfall that was recorded at the camps.

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).

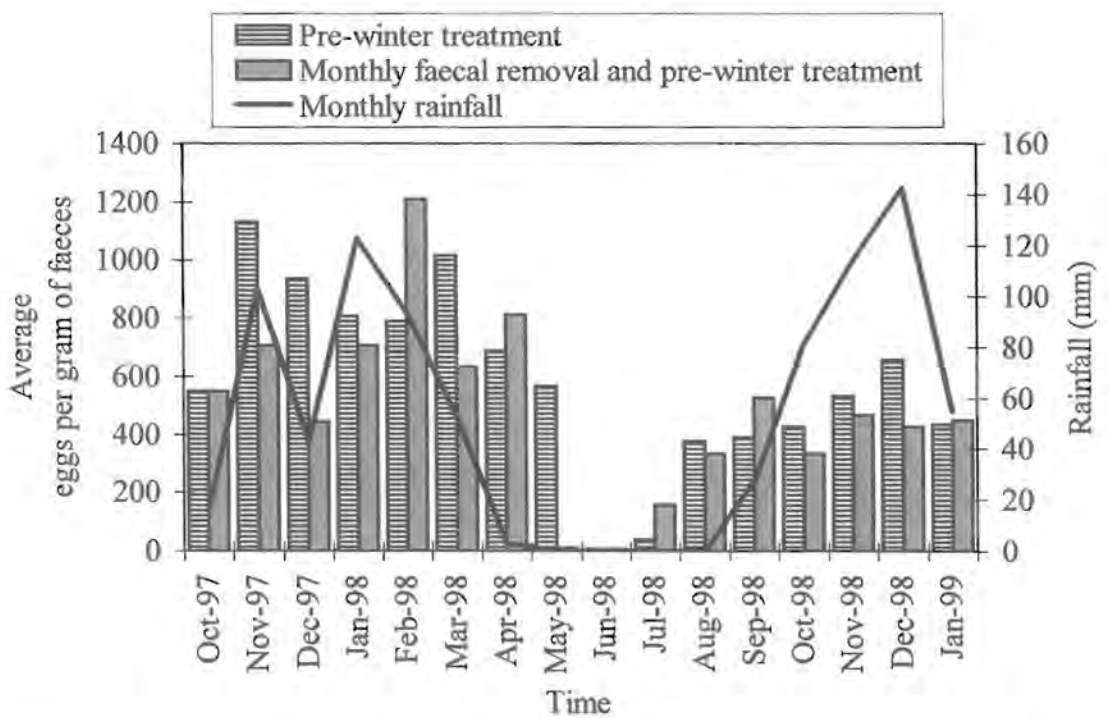


Figure 6. Seasonal average faecal egg counts, based on counts obtained from the donkeys in the pre-winter moxidectin treatment camps (MS C) and from the combination of monthly removal of faeces and pre-winter moxidectin treatment camps (MS D), compared to the monthly rainfall that was recorded at the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

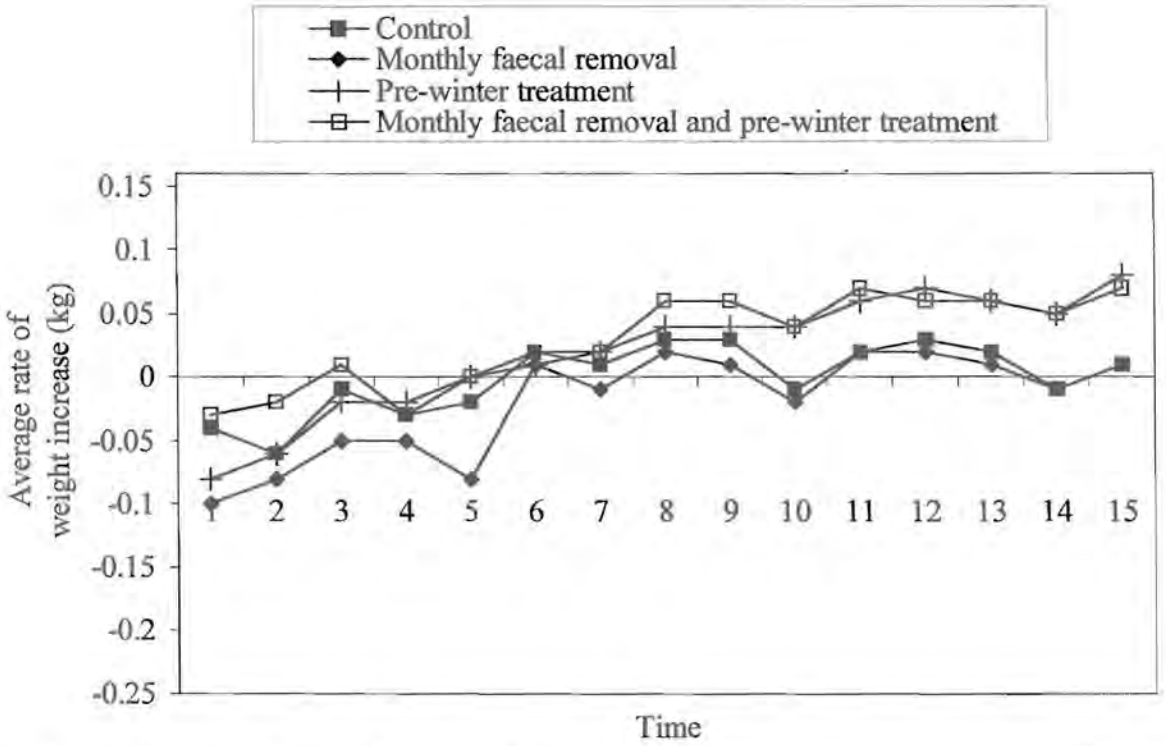


Figure 7. Average rate of weight increase of the animals in the control camps and those in the three different alternative helminth control camps starting at 1 = November 1997; 7 = May 1998; 15 = January 1999.

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

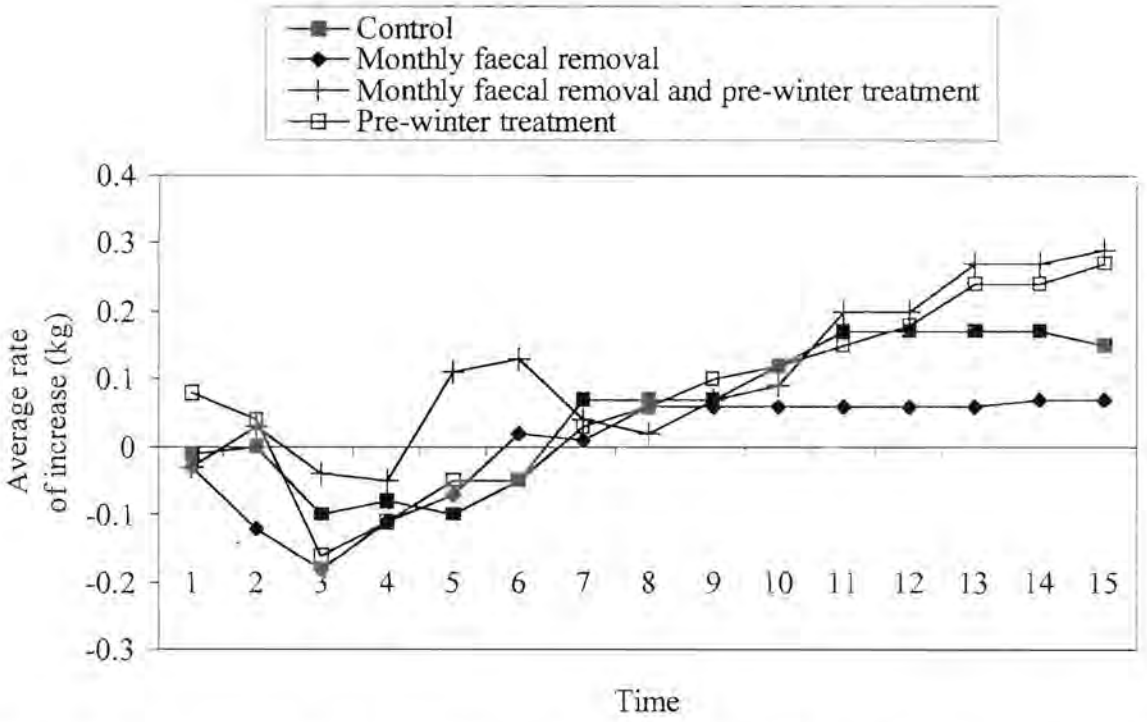


Figure 8. Average rate of body condition increase of the animals in the control camps and those in the three different alternative helminth control camps starting at 1 = November 1997; 7 = May 1998; 15 = January 1999.

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).

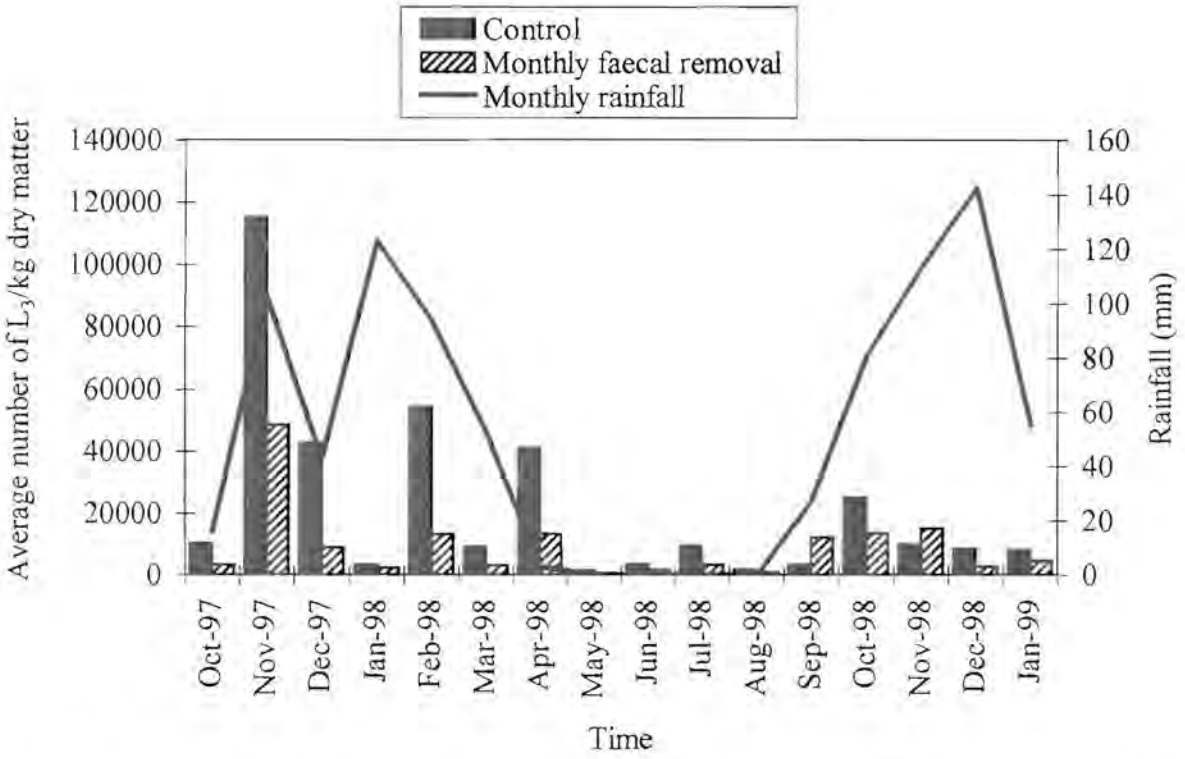


Figure 9. The average number of third-stage larvae (L_3) and the monthly rainfall recorded from the control and MS B (monthly faecal removal) camps from 1 October 1997 to 31 January 1999.

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).

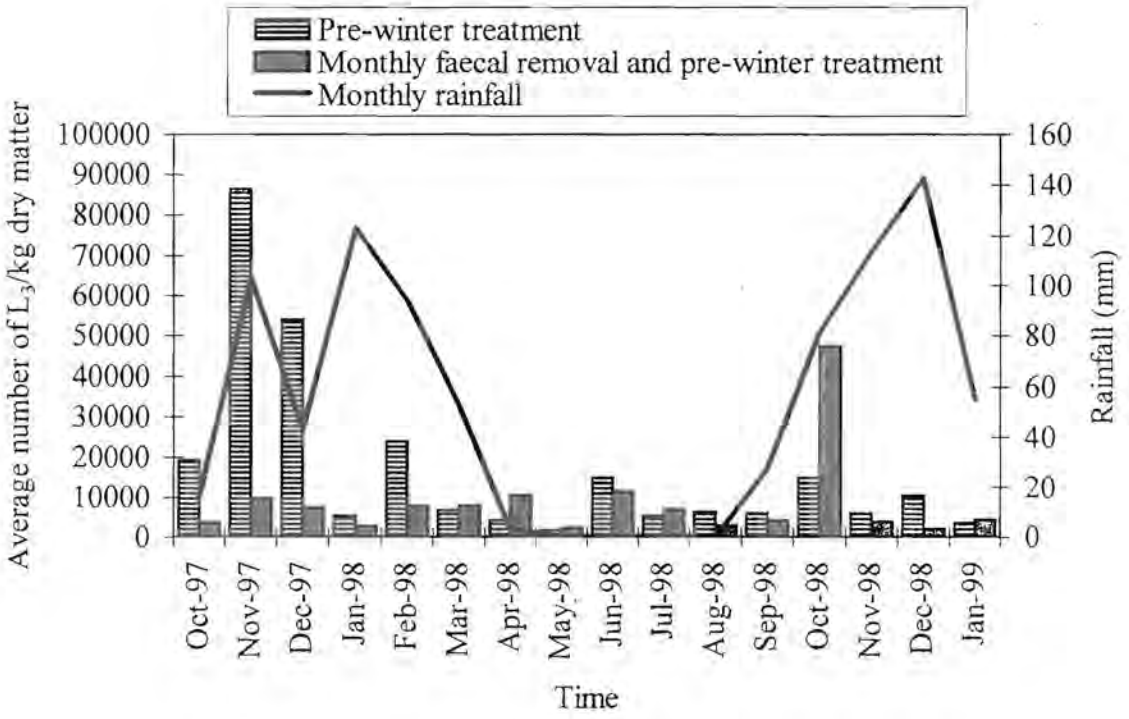


Figure 10. The average number of third-stage larvae (L_3) and the monthly rainfall recorded from the MS C (pre-winter treatment) and MS D (monthly faecal removal and pre-winter treatment) camps from 1 October 1997 to 31 January 1999.

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.

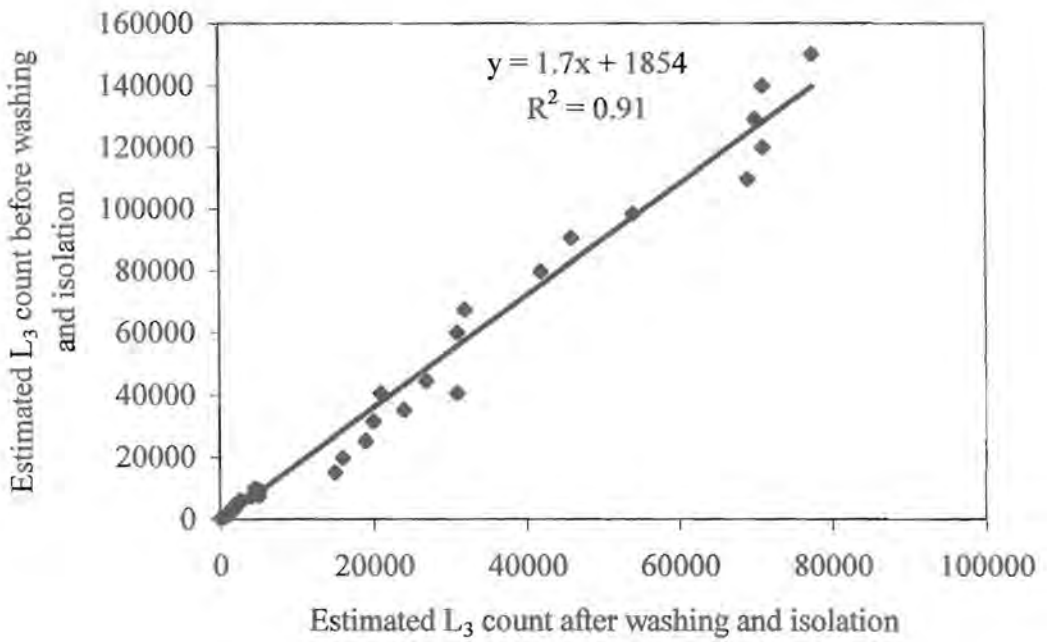


Figure 11. Linear regression analyses of the estimated pasture larval counts before and after using the combination larval recovery technique on 35 seeded herbage samples.

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.

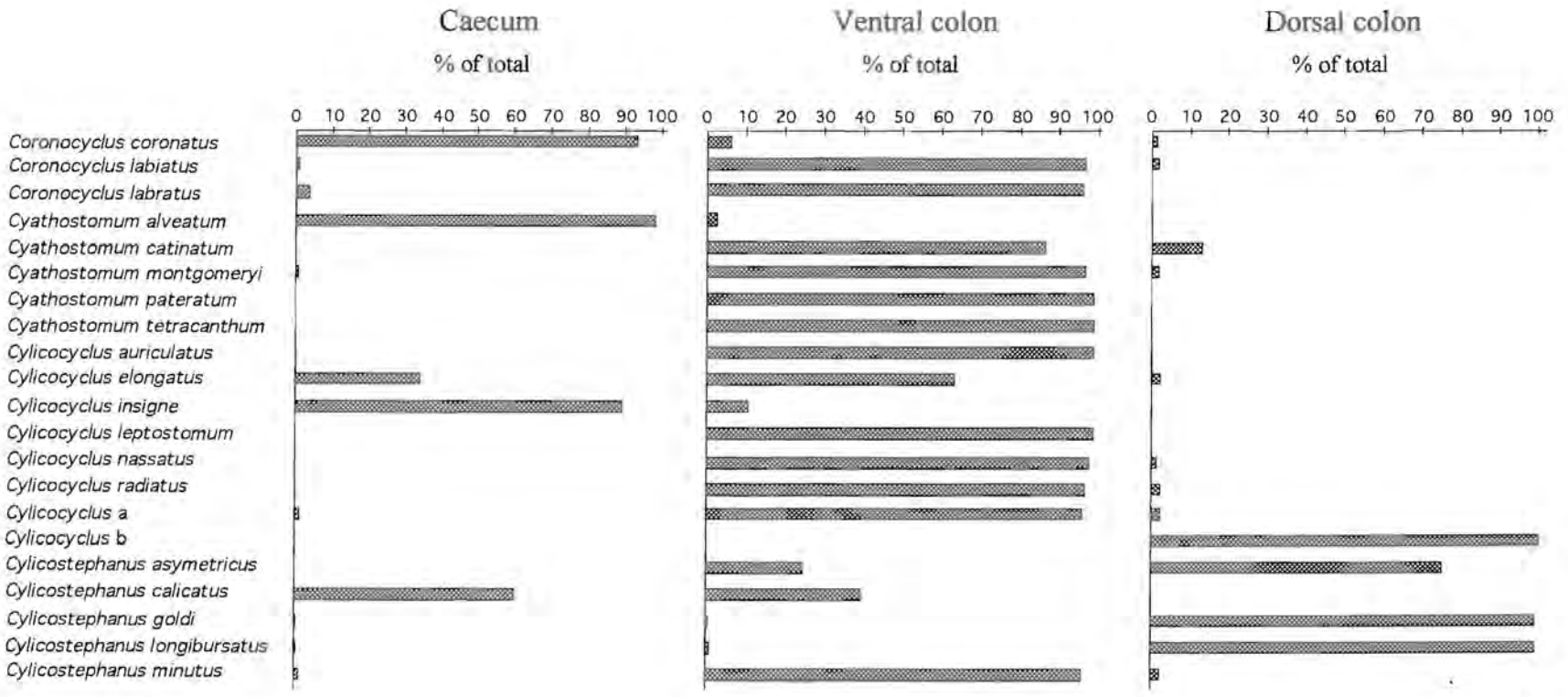


Figure 12. The distrubution patterns of 21 cyathostome species in the large intestine of the nine donkeys necropsied (Percentage of cyathostomes at each site).

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

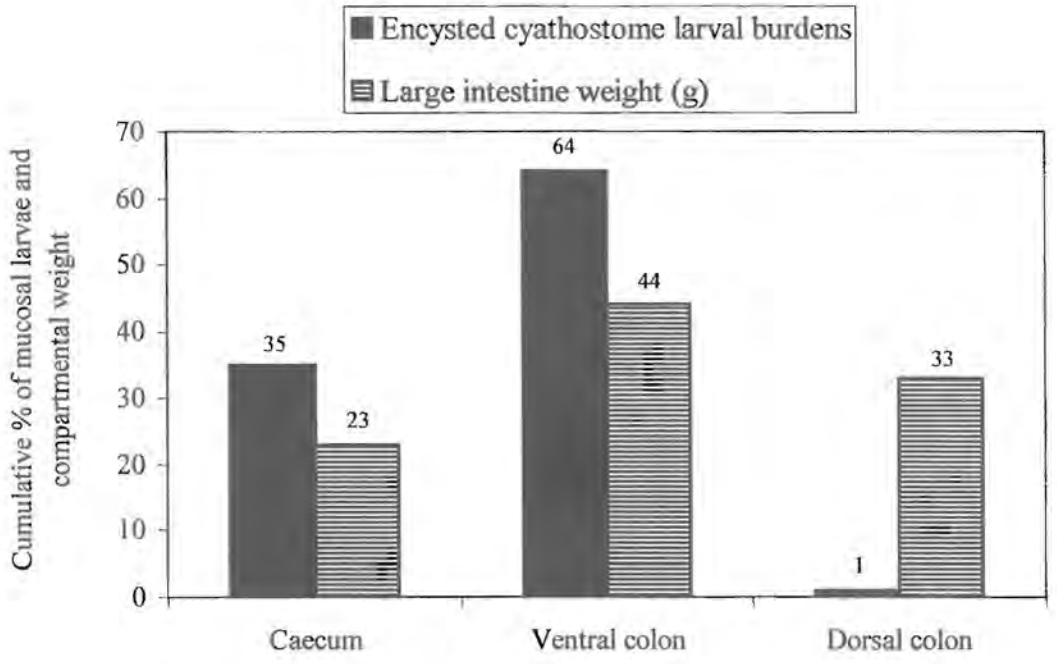


Figure 13. Cumulative percentages of contributions played by the compartments of the large intestine in harbouring the encysted cyathostome larval burdens (LL_3 and DL_4) using TMI and large intestinal wall weight (g) from nine of the donkeys.

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).

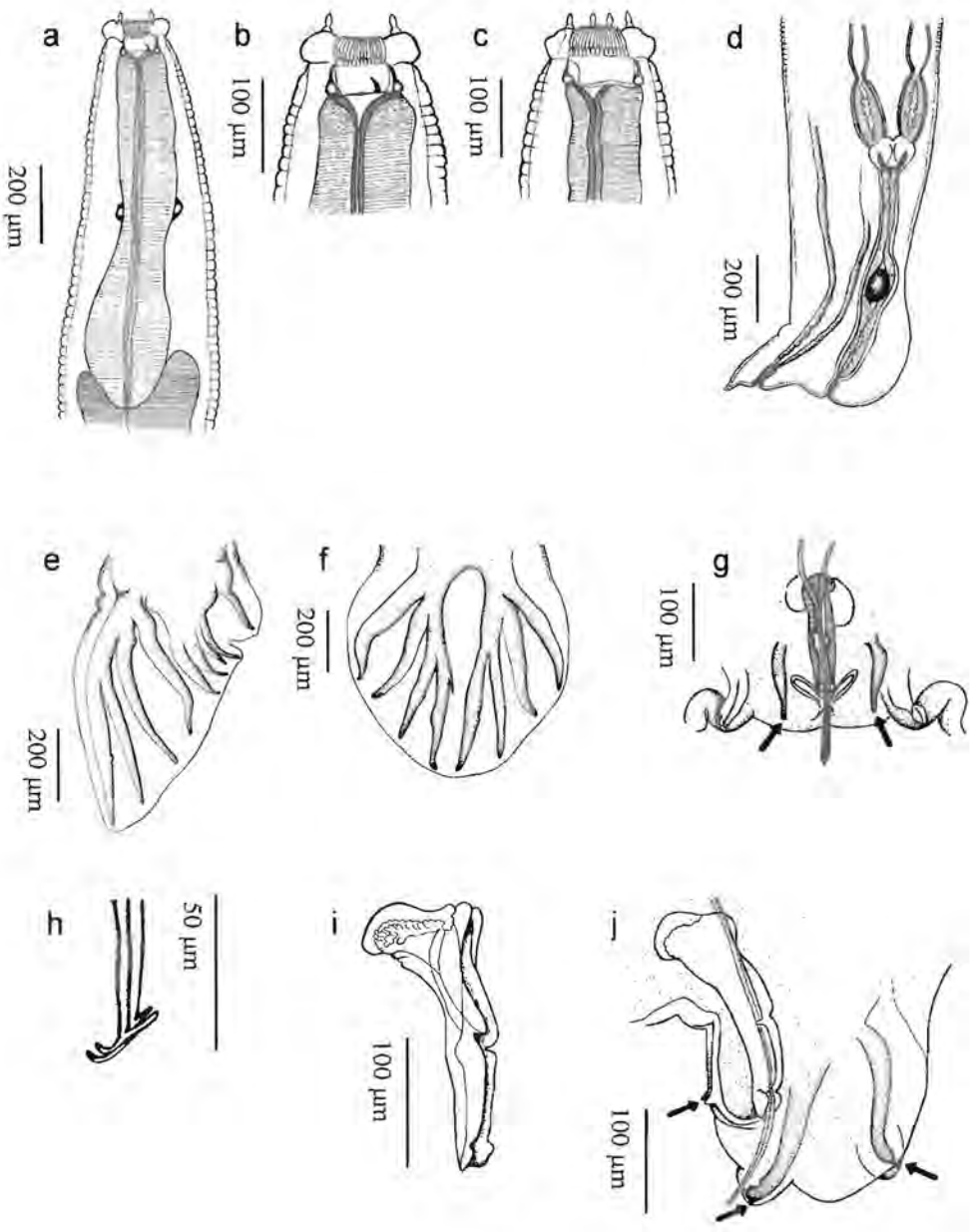
FEMALES (N = 10): Total body 7.94 ± 0.5 (mm) long; 405 ± 30.5 wide at O-I junction. Buccal capsule 34 ± 4.0 long; 74 ± 10.9 wide. The external leaf-crown (ELC) consisted of 46 elements. Distance from the anterior end to the nerve ring, cervical papillae and excretory pore 445 ± 31.0, 546 ± 45.5, 571 ± 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.

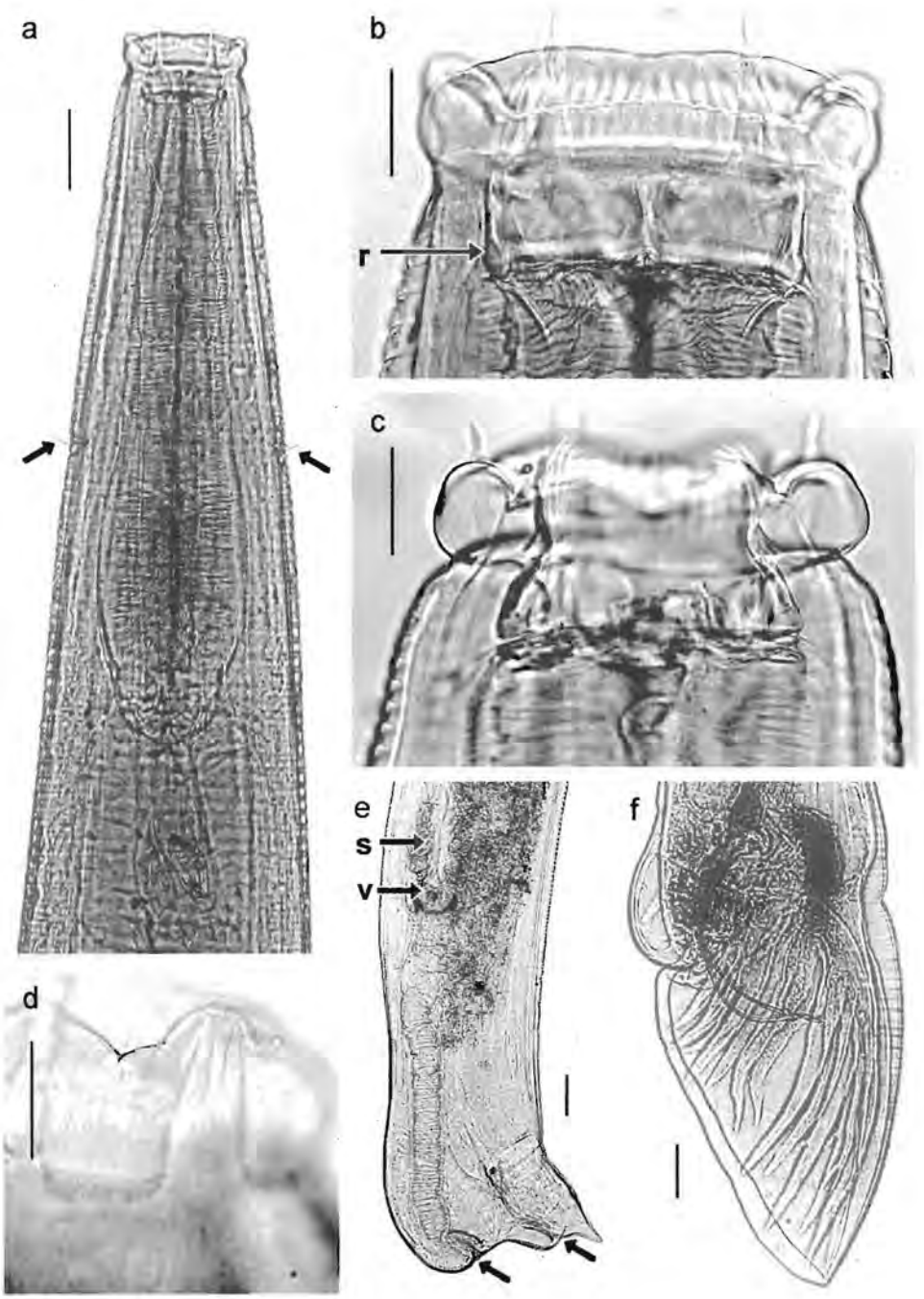
TYPE HOST AND TYPE LOCALITY: *Equus asinus*, Pretoria, South Africa (25°45′S, 28°15′E).

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*.