CHAPTER 1

GENERAL INTRODUCTION

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

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

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

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

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

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

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

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

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

The aims of the study were as follows:

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

CHAPTER 2

LITERATURE REVIEW

1. The role and management of donkeys in South Africa

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

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

2. Helminth parasites of donkeys

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

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

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

3. The effect of helminth parasitism on donkeys

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

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

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

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

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

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

4. Methods to detect helminth parasites

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

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

Faecal egg counts

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

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

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

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

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

Pasture larval counts

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

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

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

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

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

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

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

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

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

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

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

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

5. Helminth control methods for donkeys in Africa

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

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

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

Type of anthelmintic

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

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

Strategic anthelmintic treatment

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

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

Pasture hygiene

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

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

CHAPTER 3

METHODS USED IN THE STUDY

1. Weekly exercise

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

2. Live weights

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

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

3. McMaster technique and pooled larval cultures

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

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

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

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

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

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

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

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

5. Faecal egg count reduction test

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

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

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

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

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

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

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

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

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

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

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

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

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

8. Body condition score of the donkeys

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

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

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

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

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

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

(From: Pearson and Ouassat, 1996)

9. Body measurements of the donkeys

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

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

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

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

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

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

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

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

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

10. Collection of herbage samples

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

11. Processing of herbage samples

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

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

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

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

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

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

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

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

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

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

15. Grazing consumed by the donkeys in each camp

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

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

16. Necropsy and mucosal larval recovery techniques

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

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

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

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

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

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

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

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

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