

# Chapter 7

## PARASITES

### Introduction

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This was a study on factors influencing productivity of two sympatric antelope species of South Africa, the mountain reedbuck (*Redunca fulvorufula*) and grey rhebok (*Pelea capreolus*). The mountain reedbuck has the potential to be cropped for meat production because it is fecund (Irby, 1979; Skinner, 1980), has the potential to breed aseasonally (Irby, 1979), and produces good quality, edible meat that is generally free from parasites (Irby, 1975; Skinner, 1980). Additionally, it does not compete with other grazers because it utilises steep rocky habitat that is marginal for animal husbandry. Reproduction and body condition are the subjects of other chapters, but a potentially important aspect of antelope ecology that can play a significant role in productivity is parasitology. Parasites can negatively affect numerous fitness traits of their hosts, potentially causing a loss of condition, reduced productivity, disease and even death. The degree to which parasite effects translate into changes to host populations will likely depend on many factors including dispersion of parasites among hosts, magnitude of parasite effects, and degree to which ages and sexes differ in parasitism (Schalk & Forbes, 1997).

The parasites of mountain reedbuck are not well known despite these antelopes' relative abundance in South Africa (Boomker *et al.*, 2000). Baker & Boomker (1973) found 17 helminth species (14 nematodes, two cestodes and one trematode) in mountain reedbuck at Loskop Dam Nature Reserve and four species at Mountain Zebra National Park. Genera found included *Cooperia*, *Gongylonema*, *Haemonchus*, *Impalaila*, *Moniezia*, *Nematodirus*, *Oesophagostomum*, *Paramphistomum*, *Setaria*, *Skrjabinema* and *Taenia*. Boomker *et al.* (2000) additionally found *Ostertagia* type females and a *Trichostrongylus* sp. in mountain reedbuck at Mountain Zebra National Park. Mean adult nematode burdens were found to be negligible (283 worms).

The parasites of grey rhebok are even less well known (Boomker *et al.*, 2000) and have only been studied at Bontebok National Park. Work by Boomker, Horak & de Vos (1981), Horak, de Vos & De Klerk (1982), Boomker (1990) and Boomker & Horak (1992) found 12 nematode and one trematode species. Genera included *Fasciola*, *Haemonchus*, *Longistrongylus*, *Nematodirus*, *Ostertagia*, *Paracooperioides*, and *Trichostrongylus*. As with mountain reedbuck, nematode burdens were found to be negligible.

The aim of this component of the study was to identify and quantify helminths of mountain reedbuck and grey rhebok and to investigate effects of host gender and season on the occurrence and numbers of parasites. Mountain reedbuck occur at both Sterkfontein and Tussen die Riviere, while grey rhebok occur at the former but not the latter. As helminths from both of these antelopes were poorly known, and neither of the two areas had been surveyed quantitatively previously, this study provides new helminthological data for both the antelope species and the geographic localities.

Mountain reedbuck were regularly culled in the Free State, including Sterkfontein and TdR. Considerable parasite material was made available from animals culled during the study period from both reserves, and management assistance permitted seasonal and gender comparisons between host-parasite populations. Grey rhebok were not culled for meat production because they are less common and not favoured for consumption. Although they are occasionally utilised for trophy hunting, no systematic removal of grey rhebok occurred during the study. Parasite collection from the gastro-intestinal tracts (GIT) of these antelope could, therefore, only be carried out from animals that died naturally or accidentally, and as a result was only useful for limited helminth species identification. In an attempt to set a baseline of intestinal parasites in grey rhebok for comparison with mountain reedbuck at Sterkfontein, a limited amount of nematode egg quantification as well as faecal culture of nematode larvae was carried out. However, the relationship between these indirect measures and actual worm burden is very complex (Shaw & Dobson, 1995) and egg counts are not considered a reliable method for estimating the numbers of parasites within the GIT (Reinecke, 1983).

To accomplish the aims, the following questions were considered:

1. What are the helminth species of mountain reedbuck at Sterkfontein and TdR?
2. What are the prevalence's and abundance's, and how do these vary seasonally?
3. Are there any differences between the sexes, between animals in different reproductive condition, or animals of different ages?
4. Do animals with higher parasite burdens have poorer body condition?
5. Can faecal egg counts from mountain reedbuck be used as a means of estimating true worm counts from the GIT?
6. What are the helminth species of grey rhebok at Sterkfontein?

## **Methods**

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### ***Study sites and animals***

Alimentary helminths were recovered from mountain reedbuck culled at two Free State Provincial Nature Reserves, Sterkfontein and TdR. Culling schedules differed between the two sites as a result of differing management programmes. At Sterkfontein a total of 41 animals were culled over a two-year period. Eight separate culls were carried out at three-month intervals, and during each cull five or six animals were shot within a week. The culling schedule was as follows: five animals were shot in March 2000, five in June 2000, five in September 2000, five in December 2000, six in May 2001, six in August 2001, six in November 2001, and four in February 2002. Numbers culled in the final two periods were reduced because a large number of mountain reedbuck died in heavy snowfalls in September 2001. In a typical culling period, two adult males, two adult females, and one juvenile of either sex were selected. The age and condition of adult mountain reedbuck were not known before they were shot, and because animals were located randomly, the selection was not considered biased.

The spacing of the culls, spanning eight different months over a two year period, meant that parasitic loads could be compared in both warm and cold periods as well as wet and dry periods. They hence covered times when animals had abundant food

supplies available and times when food resources were limited. For some analytical purposes these months were divided into four seasons: autumn = February/March, winter = May/June, spring = August/September, and summer = November/December.

At TdR mountain reedbuck were culled on three separate occasions, covering one summer and two winters. Seven animals were culled in December 1999 (summer), 10 in June 2000 (winter), and eight in June 2001 (winter). Most of the animals were culled at night using spotlights to locate them and were, therefore, selected randomly.

Grey rhebok were not culled at Sterkfontein during the study period, so no systematic investigation of their endoparasites could be made. However, three animals died naturally and one was shot for necropsy because it was visibly sick, and these animals were sampled for helminths.

### ***Recovery of alimentary helminths***

Alimentary tracts were removed as soon as possible after animals were shot, and the abomasal/duodenal and small-intestine/large-intestine junctions ligated to prevent transfer of parasites from one site to the other (Urquhart *et al.*, 1994). They were then stored in a cool sheltered place until they could be processed, usually within three hours.

The rumen and reticulum were opened and their walls and contents examined for paramphistomes. Any found were collected and preserved in 10 % formalin. The abomasum was then opened along the side of greater curvature and the ingesta poured into a large plastic bowl (50 cm diameter x 20 cm depth). The inside of the abomasum wall was thoroughly rinsed with approximately 1 L of water to remove the rest of the contents, and this was added to the rest of the ingesta. Once the inside lining appeared clean of any remaining contents, the abomasum was discarded. The ingesta and water were then thoroughly mixed in the bowl using a small beaker, and a one-fifth aliquot removed using a measuring jug. This one-fifth aliquot was then rinsed using a 15 µm sieve and stored in 10% formalin or 70% ethanol.

Similarly, the ingesta of the small intestine were emptied into a clean plastic bowl (see above). To achieve this, the intestine was cut into manageable segments and the contents of all segments squeezed out into the bowl. Each segment was then cut along its entire length and rinsed thoroughly in 1 L of water. Again, the water plus ingesta was thoroughly mixed in the bowl, a one-fifth aliquot removed and rinsed using a 15 µm sieve before being stored in 10% formalin or 70% ethanol. Finally, the contents of the large intestine and caecum were collected in the same way as the small intestine and stored in 10% formalin or 70% ethanol.

### ***Helminths of the heart, lungs and liver***

In addition to the alimentary canal, the heart, lungs and liver were examined for the presence of helminths at both Sterkfontein and TdR. The heart chambers were opened and examined macroscopically for visible parasites, then the walls of the heart muscle were cut into 10 mm slices and placed into a jar with normal saline, and incubated in a warm water bath at approximately 40° C for 2 h. The trachea and bronchi were opened, examined macroscopically for parasites and then rinsed with water over a sieve with 15 µm apertures. The right lung was also rinsed over the sieve then cut into 20 mm cubes and put into a separate jar of normal saline for incubation in a warm water bath for 2 h. Finally, the liver was cut into 10 mm strips, palpated to express any visible parasites and again placed into a separate jar of normal saline and incubated in a warm water bath for 2 h. The solutions were then sieved separately (15 µm apertures) and the residues stored in 10 % formalin. At a later stage, these solutions were examined under a dissection microscope for helminths. Nothing was found in any of the mountain reedbuck from either Sterkfontein or TdR, so no results are presented.

### ***Worm identification and quantification***

To extract the worms from storage, the samples were again rinsed over a 15 µm sieve to remove the formalin or ethanol preservative, and the ingesta then remixed with water to make a volume of about 200 ml. Approximately 2 ml red dye (Eosin) was then mixed with the sample and left for an hour to stain the worms. A small amount of

the mixture was poured into a Perspex counting dish and all the helminths, including the fourth larval stages (L<sub>4</sub> larvae), extracted using a dissection microscope. Once completed, the remaining material was discarded, another small amount of mixture poured into the dish and the procedure repeated. The aliquot was thus worked through piecemeal until all the worms were extracted. All worms were stored in 70 % ethanol.

At a later stage nematodes were cleared in a drop of lactophenol on a microscope slide and examined under a compound microscope using magnifications between x 10 and x 40. Species were identified using species descriptions (Boomker, 1977, 1991; Boomker, Horak & de Vos, 1981; Gibbons, 1981; Boomker & Reinecke, 1989). Male nematodes were identified to the species level, and in most cases females could also be identified to species level by extrapolation. In cases where male nematodes were not present, or when two or more species of the same genus were found in the same animal, females were identified to the level of genus only. Trematodes and cestodes were identified to the generic level only.

### ***Faecal egg counts***

Faeces were only collected from animals at Sterkfontein. From mountain reedbuck they were collected from 18 culled animals with the aim of making comparisons between egg counts, larval counts (see below) and counts of adult worms from the GIT (as above). This was done to test whether egg or larval counts could be reliably used to estimate numbers of adult nematodes in grey rhebok. In grey rhebok, faeces were collected fresh off the ground while following live animals. Samples were taken twice a month from five animals within one habituated herd, between September 2001 and April 2002. Faeces were kept cool until microscopic analysis could be carried out, usually within 12 h.

Two grams of faeces were weighed and added to 58 ml saturated sugar solution (800 g sugar to 1 000 ml tap water). Nematode eggs float in this solution because they have a relatively lower specific gravity. A food blender was then used to break up the faeces and thoroughly mix it with the solution. One drop of amyl alcohol was added to the mixture to reduce surface tension and remove bubbles. The solution was then stirred well, a small amount extracted with a pipette and this placed into a McMaster

slide (“Eggs-Acto” McMaster egg counting chamber, Focal Point, South Africa, [www.mcmaster.co.za](http://www.mcmaster.co.za)). This was left to stand for two minutes to allow any eggs to float to the top, placed under a compound microscope under low magnification (x 10) and the eggs counted in all three chambers. No attempt was made to identify the nematode species from the eggs.

### ***Coproculture***

After carrying out egg counts, the remaining faeces were used to culture larval nematodes. Faeces were weighed, crushed and thoroughly mixed with vermiculite at a ratio of 1:1 to aerate the faeces and allow movement of larval worms. A small amount of water was added if necessary so that mixture held together like clay. The faeces/vermiculite mix was then lightly compressed in the bottom of a 1 l fruit jar, leaving a circular space of about 2 cm diameter at the bottom. Using a pipette, a few millilitres of water were added to the mix and to the inside surface of the jars to increase humidity. Lids were then loosely screwed onto the jars to allow a small amount of air circulation, and the jars placed near a small heater to maintain warmth (approximately 20° C). After 10 days, cultured larvae were extracted from the inside walls of the console jars where they migrated to after hatching. This was achieved by splashing water onto the walls using a pipette and tipping the water into a petri dish without letting it come into contact with the faeces. The walls were rinsed twice using the same amount of water each time to make sure that all possible larvae were taken out. The jars were then placed back near the heater for another four days to collect a second set of larvae. After day 14, the faeces were discarded. Larval numbers were estimated in a Perspex counting dish using a dissection microscope. If very large numbers were present a sample was counted using a McMaster slide under a compound microscope and the numbers multiplied according to the total volume used. Species identification was not attempted.

### ***Statistical methods***

Two-way ANOVA were used to test for differences between genders and months. As with most parasite population data, the nematodes were strongly aggregated, so the data had to be Log<sub>10</sub> transformed. Because the occurrence of one species of nematode

in the GIT was not dependent on or affected by the occurrence of another species, it was not of interest to test for differences between nematode species. Therefore, three separate two-way ANOVA's were carried out for the three main nematode species. One-way ANOVA's and Kruskal Wallis ANOVA on ranks were used to test for differences in parasitic loads of animals of different ages and females in varying degrees of pregnancy. Spearman Rank Correlation Coefficients were used to compare faecal egg counts with cultured larvae counts and adult worm counts. They were also used to determine whether there was a correlation between the number of nematodes extracted and the kidney fat index of each animal (Chapter 6).

## Results

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### *Helminth species prevalence and abundance*

Seventeen species of helminths, including fifteen nematodes, one trematode, and one cestode were recovered from mountain reedbeek at Sterkfontein and TdR (Tables 25 & 26). The most prevalent species at Sterkfontein were *Cooperia yoshidai*, *Longistrongylus schrenki* and *Haemonchus contortus*, found in 98 %, 80 % and 66 % of animals respectively (Table 25). *C. yoshidai* followed by *H. contortus* and *L. schrenki* were the most abundant species. The other ten species demonstrated low prevalence and abundance. There was one new species of *Cooperia*, as well as six new host records for mountain reedbeek at Sterkfontein. These were *L. schrenki*, *L. namaquensis*, *Ostertagia* sp., *Trichostrongylus deflexus*, *Impalaia nudicollis* and *Paracooperioides peleae*.

Eleven species were recorded at TdR (Table 26), including seven that were also found at Sterkfontein. The most prevalent was *Nematodirus spathiger*, found in 58 % of animals, while the most abundant species was *T. falculatus*. Overall, nematodes were considerably less prevalent and abundant in mountain reedbeek at TdR than at Sterkfontein. New parasite records were *C. rotundispiculum* and *I. nudicollis*.



**Table 25.** Prevalence and abundance of helminths recovered from 41 mountain reedbuck culled at Sterkfontein between March 2000 and February 2002. (Prev. = prevalence, Std. Dev. = standard deviation, T = trematode, N = nematode, C = cestode, L<sub>4</sub> = fourth larval stage, Rum = rumen, Abo = abomasum, SI = small intestine, LI = large intestine, \* = new species).

Nematode genera and some species	Prev. %	Site in host	Mean	Std. Dev.	Range
<i>Calicophoron</i> sp. (T)	5	Rum	6.1	27.8	0 – 150
<i>Haemonchus</i> sp. L <sub>4</sub> (N)	32	Abo	21.4	61.9	0 – 350
<i>Haemonchus contortus</i> (N)	66	Abo	115.8	218.2	0 – 1050
<i>Longistrongylus</i> sp. L <sub>4</sub> (N)	44	Abo	72.9	135.0	0 – 470
<i>Longistrongylus schrenki</i> (N)	80	Abo	36.5	56.8	0 – 232
<i>Longistrongylus namaquensis</i> (N)	2	Abo	0.2	1.6	0 – 10
<i>Ostertagia</i> sp. (N)	2	Abo	0.2	1.6	0 – 10
<i>Cooperia</i> sp. L <sub>4</sub> (N)	66	SI	312.3	682.3	0 – 4050
<i>Cooperia yoshidai</i> (N)	98	SI	2037.5	2594.2	0 – 14880
<i>Cooperia</i> sp. (N) *	2	SI	3.7	23.4	0 – 150
<i>Trichostrongylus falculatus</i> (N)	12	SI	5.4	26.7	0 – 170
<i>Trichostrongylus deflexus</i> (N)	2	SI	0.2	1.6	0 – 10
<i>Impalaia nudicollis</i> (N)	2	SI	0.5	3.1	0 – 20
<i>Paracooperioides peleae</i> (N)	2	SI	0.2	1.6	0 – 10
<i>Skrjabinema</i> sp. (N)	39	LI	187.9	1140.4	0 – 7310
<i>Moniezia</i> sp. (C)	5	SI	0.1	0.2	0 – 1

**Table 26.** Prevalence and abundance of helminths recovered from mountain reedbuck at TdR between December 1999 and June 2001. (Prev = prevalence, Std. Dev. = standard deviation, T = trematode, N = nematode, C = cestode, Rum = rumen, Abo = abomasum, SI = small intestine, LI = large intestine, Vis = visceral cavity).

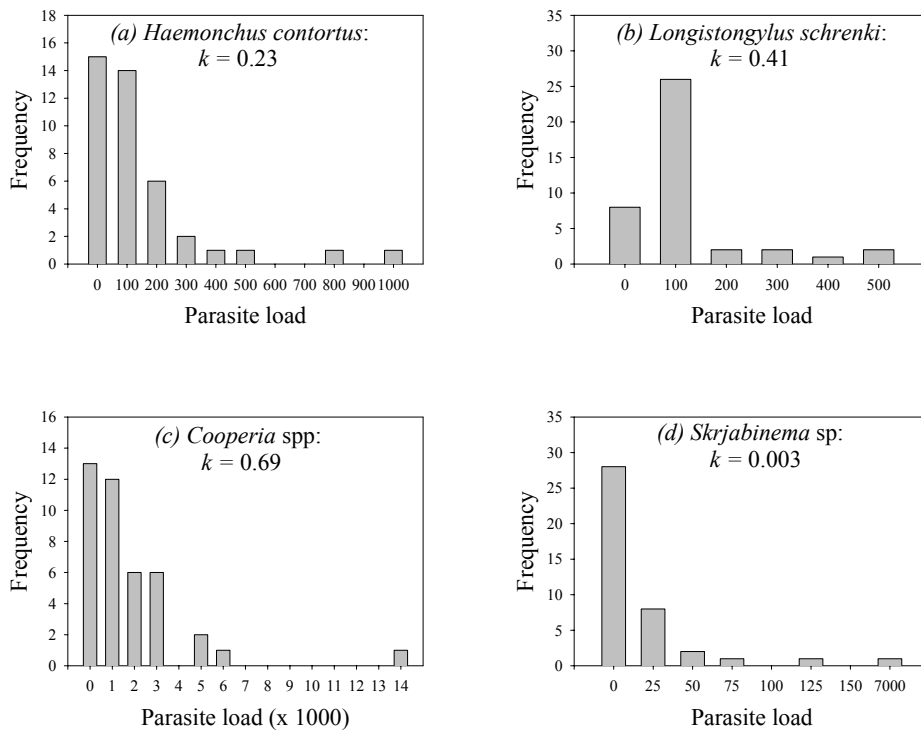
Nematode genera and some species	Prev %	Site in Host	Mean	Std. Dev.	Range
<i>Calicophoron</i> sp. (T)	4	Rum	2.0	12.5	0 – 80
<i>Haemonchus contortus</i> (N)	28	Abo	4.0	10.2	0 – 50
<i>Longistrongylus albifrontis</i> (N)	8	Abo	0.3	1.1	0 – 4
<i>Nematodirus spathiger</i> (N)	58	SI	15.1	22.5	0 – 100
<i>Trichostrongylus falculatus</i> (N)	31	SI	29.6	70.0	0 – 240
<i>Cooperia rotundispiculum</i> (N)	31	SI	19.3	64.2	0 – 320
<i>Cooperia yoshidai</i> (N)	4	SI	0.4	2.0	0 – 10
<i>Impalaia nudicollis</i> (N)	8	SI	1.7	6.0	0 – 24
<i>Skrjabinema</i> sp. (N)	4	LI	0.4	2.0	0 – 10
<i>Setaria</i> sp. (N)	4	Vis	0.1	0.2	0 – 1
<i>Moniezia</i> sp. (C)	4	SI	0.1	0.2	0 – 1

### ***Frequency distributions of nematodes at Sterkfontein***

Figure 33 shows the frequency distributions of the four most common nematode species found in mountain reedbuck at Sterkfontein. The degree of aggregation was tested using the corrected moment estimate of  $k$  (Wilson *et al.*, 2002):

$$k = (m^2 - s^2/n)/(s^2 - m)$$

where  $m$  = mean,  $s^2$  = variance,  $n$  = sample size.



**Figure 33.** Observed frequency distributions of (a) *H. contortus*, (b) *L. schrenki*, (c) *Cooperia* spp. and (d) *Skrjabinema* sp. found in 41 mountain reedbucks culled at Sterkfontein between March 2000 and February 2002.  $k$  = the corrected moment estimate for aggregation.

All four species were highly aggregated and their distributions could best be described by the negative binomial distribution. Out of a total of 4 499 adult *H. contortus* found, 78 % occurred in only 20 % of the animals. For *L. schrenki* (total 1 518 adult worms), 67 % occurred in 20 % of the animals, while in *Cooperia* spp. (total 89 759 adult worms), 52 % occurred in 20 % of the animals.

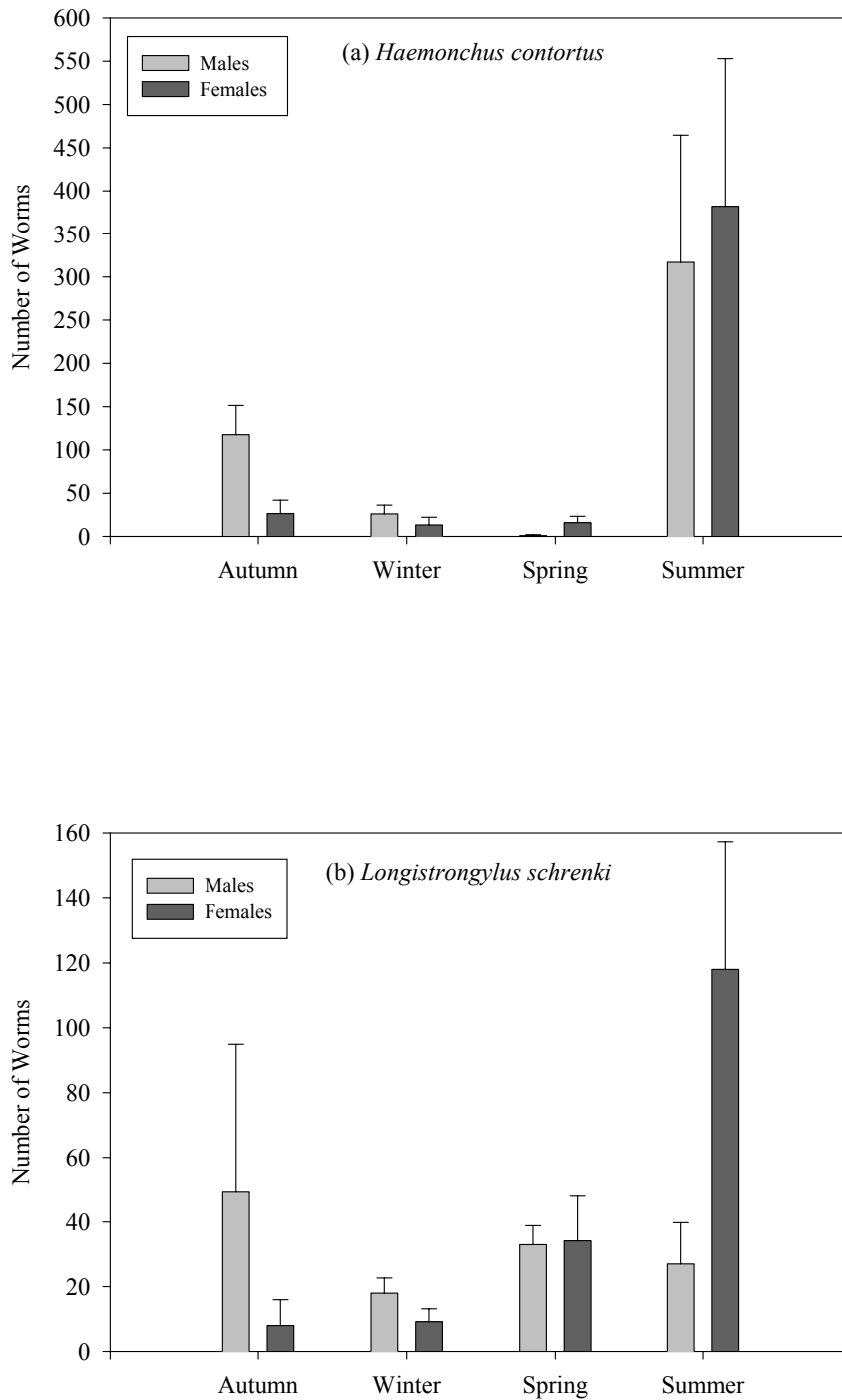
### ***Abomasum nematodes* at Sterkfontein**

Numbers of *H. contortus* were highest in summer (November/December) for both males and females, and lowest in spring (August/September) for males and winter (May/June) for females (Figure 34a). Male mountain reedbucks had more worms than

females between February and June, while females had more worms than males between August and December.

Similarly in *L. schrenki*, male mountain reedbuck had more worms than females between February and June, while females had more worms than males between August and December (Figure 34b). Males had most worms in February/March and least in May/June, while females had most worms in November/December and least in February/March.

Differences between genders and between months (not seasons) in the numbers of *H. contortus* were tested for using a two-way ANOVA (Table 27). The data were Log<sub>10</sub> transformed. There was strong evidence of a difference in the number of parasites between months but no evidence of a difference between males and females. Although there was no evidence of an interaction at the 5 % level, the P value was quite close to being significant. This meant that the effect of different levels of gender was marginally dependent on the level of month present. Males had more *H. contortus* than females in autumn and winter, while females had more *H. contortus* than males in spring and summer (Figure 34). Multiple pairwise comparisons using the Tukey test indicated that numbers of *H. contortus* were higher in December than May, June, August and September.



**Figure 34.** Seasonal variation in (a) *Haemonchus contortus* and (b) *Longistrongylus schrenki* in the abomasums of 20 male and 21 female mountain reedbuck at Sterkfontein. Numbers of animals per gender and per season varied between 4 and 6 (mean = 5). Autumn = February/March, winter = May/June, spring = August/September, summer = November/December. Error bars represent standard error.

**Table 27.** Two-way ANOVA comparing the differences between genders and between months in the numbers of *H. contortus* in 41 mountain reedback at Sterkfontein. Data were Log<sub>10</sub> transformed.

Source of Variation	df	SS	MS	F	P
Gender	1	0.190	0.190	0.365	0.551
Month	7	19.505	2.786	5.352	< 0.001
Gender x month	7	7.448	1.064	2.044	0.089
Residual	25	13.016	0.521		
Total	40	42.334	1.058		

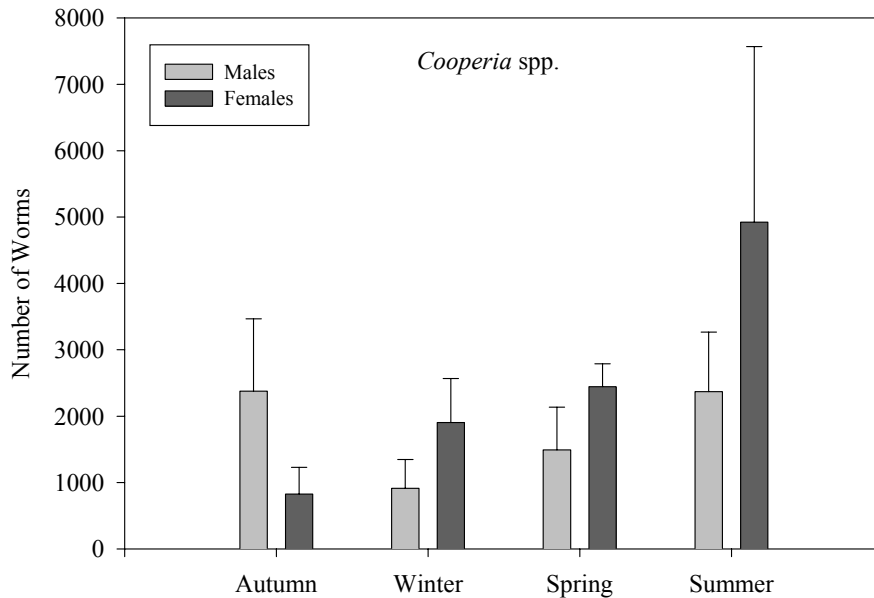
Differences between genders and between months were also tested for *L. schrenki* using a two-way ANOVA (Table 28). There was some evidence of a difference between months, but no evidence of a difference between genders and no interaction. Multiple pairwise comparisons using the Tukey test indicated that numbers of *L. schrenki* were higher in females in December than February.

**Table 28.** Two-way ANOVA comparing the differences between genders and between months in the numbers of *L. schrenki* in 41 mountain reedback at Sterkfontein. Data were Log<sub>10</sub> transformed.

Source of variation	df	SS	MS	F	P
Gender	1	0.009	0.009	0.025	0.875
Month	7	6.464	0.923	2.464	0.045
Gender x month	7	3.686	0.527	1.405	0.247
Residual	25	9.367	0.375		
Total	40	19.603	0.490		

***Small intestine nematodes at Sterkfontein***

Female mountain reedbuck harboured most *Cooperia* spp. in November/December (twice as many as the other seasons) and least in February/March (Figure 35). In both February/March and November/December males had a similarly high numbers of worms, while they had the least amount in May/June. A two-way ANOVA comparing variation in numbers of *Cooperia* spp. found no evidence of any differences between genders or between months (Table 29).



**Figure 35.** Seasonal variation in *Cooperia* spp. in the small intestines of 20 male and 21 female mountain reedbuck at Sterkfontein. Numbers of animals per gender per season varied between 4 and 6 (mean = 5). Autumn = February/March, winter = May/June, spring = August/September, summer = November/December. Error bars represent standard error.

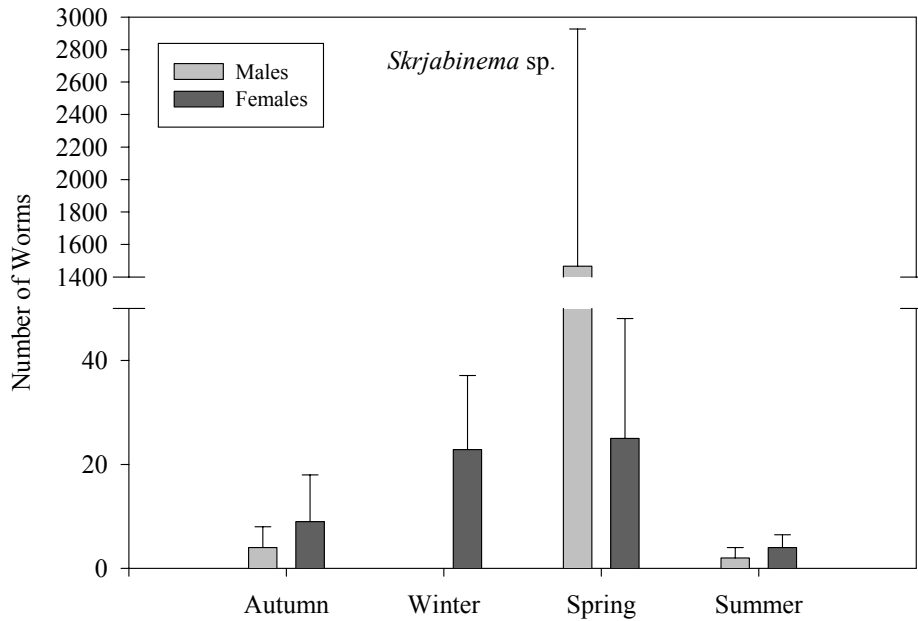
**Table 29.** Two-way ANOVA comparing differences between genders and between months in the numbers of *Cooperia* spp. in 41 mountain reedbuck at Sterkfontein. Data were Log<sub>10</sub> transformed.

Source of Variation	df	SS	MS	F	P
Gender	1	0.032	0.032	0.050	0.825
Month	7	1.946	0.278	0.435	0.871
Gender x month	7	4.835	0.691	1.081	0.404
Residual	25	15.972	0.639		
Total	40	22.753	0.569		

### ***Large intestine nematodes at Sterkfontein***

*Skryabinema* sp. occurred in very low numbers in mountain reedbuck at Sterkfontein (Figure 36). The relatively very large numbers of worms found in males in spring compared to the other seasons, resulted from the occurrence of a large number of worms in only one animal. Due to low prevalence and very low abundance (except one animal) of this species, statistical analysis of differences between genders and between months was not carried out.





**Figure 36.** Seasonal variation in *Skrjabinema* sp. in the large intestines of 20 male and 21 female mountain reedbucks at Sterkfontein. Numbers of animals per gender per season varied between 4 and 6 (mean = 5). Autumn = February/March, winter = May/June, spring = August/September, summer = November/December. Error bars represent standard error.

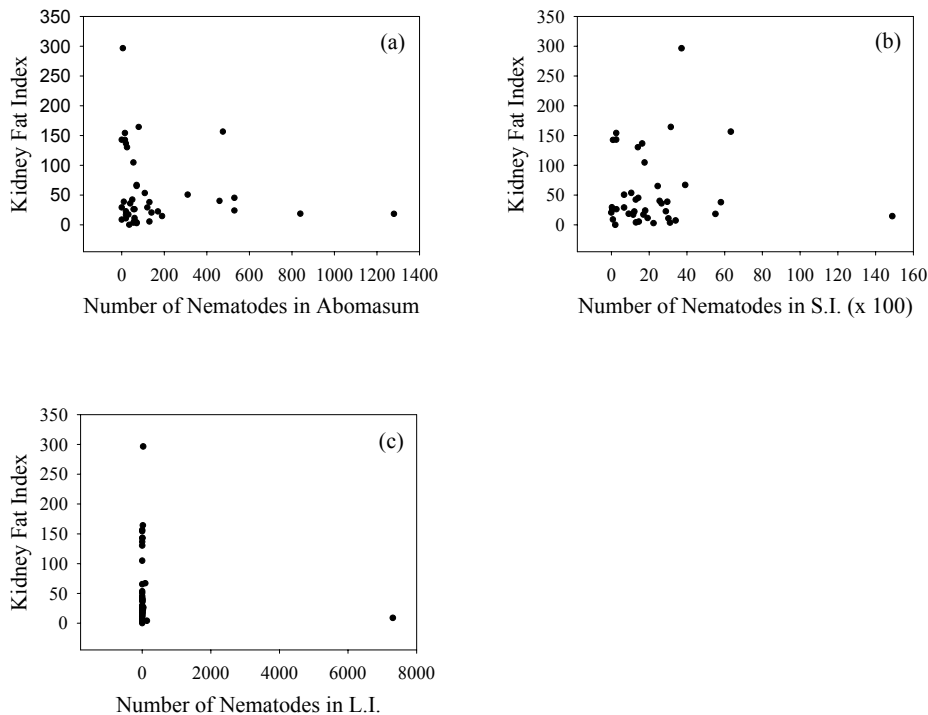
### *Age differences*

Analysis of the effect of age on parasite distributions in mountain reedbucks at Sterkfontein was limited because only a small number of young and old animals were sampled. The small sample size of the former was by design, where only one young animal was collected per cull. For old animals, sample sizes often decline with age due to mortality (Wilson *et al.*, 2002), and because sampling was random for adults, less old individuals were collected. Variation in abundance of *H. contortus* and *C. yoshidai* were tested for mountain reedbucks in three different age classes. These included animals < 25 kg (juveniles), animals between 25 and 30 kg (young adults), and animals > 30 kg (adults over 2.5 years age). Old animals were included in the last group. Data for males and females were pooled because no statistical differences were found between them and because the sample size was too small to keep them separate.

Because the nematodes were considered independent, two separate Kruskal-Wallis tests were carried out. There was no evidence of any differences between the age groups for either *H. contortus* ( $H = 1.695$ ,  $df = 2$ ,  $p = 0.429$ ) or *C. yoshidai* ( $H = 2.426$ ,  $df = 2$ ,  $p = 0.297$ ).

### ***Host body condition (kidney fat index)***

Part of the present study involved the determination of body condition by means of a kidney fat index (KFI) (see Chapter 6). To investigate whether there was any correlation between the numbers of nematodes harboured and body condition of animals, numbers of parasites from the abomasums, small intestines and large intestines were plotted separately against KFI (Figure 37). A Spearman Rank Correlation Coefficient found no evidence of a correlation between the numbers of parasites in either the abomasum or small intestine with KFI (Abomasum:  $r = -0.13$ ,  $p = 0.435$ ; S.I.:  $r = 0.03$ ,  $p = 0.843$ ). The large intestine was not tested.



**Figure 37.** Scatter plots of kidney fat index against (a) number of nematodes in the abomasum, (b) number of nematodes in the small intestine, (c) number of nematodes in the small intestine.

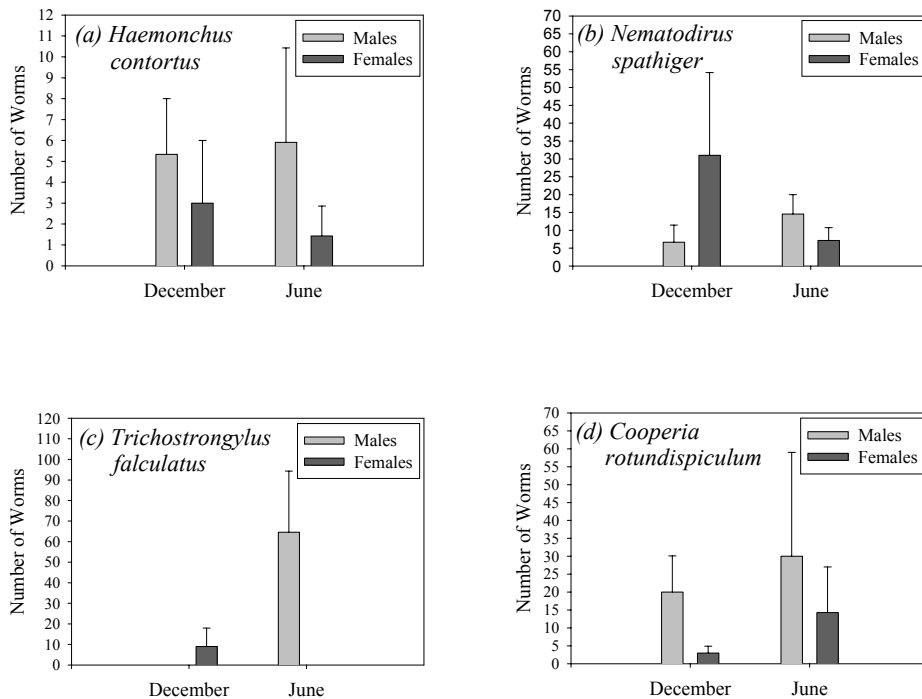
### ***Nematodes and pregnancy***

Variation in the number of nematodes found in females at varying stages of pregnancy were tested for because of the possibility of an effect of hormones on the susceptibility of females to infection. Comparisons were made between non-pregnant females, pregnant females within the first half of gestation, pregnant females within the second half of gestation, and females that had recently given birth. Stage of pregnancy was determined by calculating foetal age using the Hugget & Widdas (1951) formula, adapted for mountain reedbuck by Norton (1989).

Although sample sizes were small using four groups, comparing all pregnant females with all non-pregnant females, regardless of the stage of pregnancy or whether they had recently given birth, was considered to have little biological meaning. Results should, however, be treated with caution. There was evidence of a difference in the number of *H. contortus* between females at different times of pregnancy and non-pregnancy (ANOVA:  $F = 5.11$ ,  $df = 3$ ,  $p = 0.011$ ), but no evidence of a difference in the number of *C. yoshidai* (ANOVA:  $F = 0.780$ ,  $df = 3$ ,  $p = 0.522$ ). For *H. contortus*, pairwise comparisons using the Tukey test indicated that females that had recently given birth had more worms than pregnant females within both the first and second halves of pregnancy.

### ***Nematodes of Tussen die Riviere***

Figure 38 shows the seasonal variation in numbers of the four most common species of nematode in mountain reedbuck at TdR between December 1999 and June 2001. Because of the low prevalence and abundance of the nematodes in the mountain reedbuck of TdR, and because of the lack of any apparent pattern in variation between genders and seasons, there was no reason to test the data statistically.



**Figure 38.** Seasonal variation in (a) *H. contortus*, (b) *T. falculatus*, (c) *N. spathiger*, and (d) *C. rotundispiculum* in 14 male and 11 female mountain reedbuck at TdR in one summer (December 1999) and two winter (June 2000, 2001) periods. Error bars represent standard error.

### *Nematodes of grey rhebok at Sterkfontein*

Five nematode species were extracted from four grey rhebok that died at Sterkfontein during 2001 (Table 30). Four of these were also found in mountain reedbuck, while one, *Ostertagia* sp., was only found in grey rhebok. Due to the small sample size, not too much should be read into the details of the table. No gender or seasonal comparisons were attempted. *C. yoshidai* was a new parasite record.

**Table 30.** Prevalence and abundance of nematodes recovered from four grey rhebok at Sterkfontein in 2001 (Prev = prevalence, Std. Dev. = standard deviation, Abo = abomasum, SI = small intestine).

Nematode species	Prev %	Site in Host	Mean	Std. Dev.	Range
<i>Haemonchus contortus</i>	50	Abo	32.5	42.7	0 – 90
<i>Longistrongylus schrenki</i>	25	Abo	15.0	30.0	0 – 60
<i>Ostertagia</i> sp.	75	Abo	168.8	243.3	0 – 520
<i>Cooperia yoshidai</i>	100	SI	145.0	97.5	10 – 230
<i>Paracooperioides peleae</i>	50	SI	213.2	282.2	0 – 595

### ***Faecal egg counts and coproculture in mountain reedback***

Faecal egg counts, larval counts from coproculture and counts of adult worms in the GIT were carried out in 18 culled mountain reedback from Sterkfontein and compared using a Spearman Rank Correlation Coefficient (Table 31). Egg counts were very highly positively correlated with larval counts, but there was no correlation between egg counts and adult worms or between larval counts and adult worms. This means that neither egg counts nor larval counts were reliable methods for estimating numbers of adult nematodes in the GIT of mountain reedback.

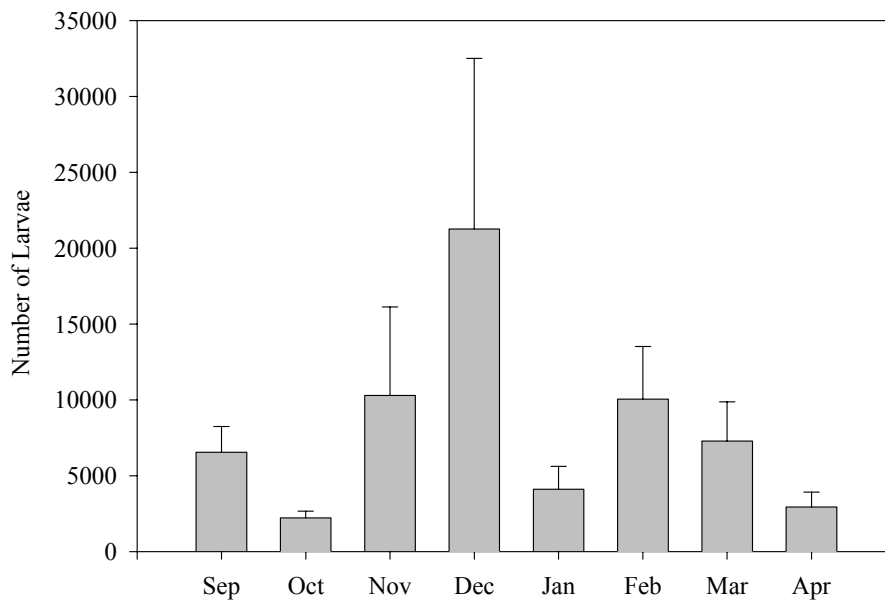
**Table 31.** Spearman Rank Correlation Coefficient comparing faecal egg counts, number of larvae in faeces from coproculture, and the actual number of nematodes found in the GIT of 18 mountain reedback at Sterkfontein.

	Larvae from coproculture	Adult worms from GIT
Egg counts	$r_s = 0.952$ $P < 0.001$	$r_s = -0.173$ $P = 0.565$
Larvae from coproculture		$r_s = -0.211$ $P = 0.409$

### *Faecal egg counts and coproculture in grey rhebok*

Faecal egg counts and larval counts from coproculture were carried out every two weeks from faeces of five live grey rhebok over a period of eight months between September 2001 and April 2002. A Spearman Rank Correlation Coefficient was carried out to test correlation between the two techniques. As occurred in mountain reedbeek, there was a strong positive correlation between the two sampling techniques ( $r_s = 0.747$ ,  $n = 57$ ,  $p < 0.001$ ).

Figure 39 shows the monthly variation in nematode larval counts from the five grey rhebok between September 2001 and April 2002. Although larval counts were not found to correlate with the actual number of adult nematodes in the GIT of animals, there was a peak in average larval counts in December. The average number of larvae cultured from 100g of mountain reedbeek faeces was 223 187, compared to 7 702 in grey rhebok.



**Figure 39.** Monthly variation in nematode larval counts from coproculture of faeces from five grey rhebok at Sterkfontein between September 2001 and April 2002. Numbers of larvae were adjusted for 100 g faeces.

## Discussion

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### *Species prevalence and abundance*

The 17 species of helminths found in mountain reedbuck at Sterkfontein and TdR compares favourably with the 17 species found at Loskop and Mountain Zebra National Park (hereafter MZNP) (Baker & Boomker, 1973). There were six new host records for nematodes at Sterkfontein (*L. schrenki*, *L. namaquensis*, *Ostertagia* sp., *T. deflexus*, *I. Nudicollis* and *P. peleae*) and one new host record at TdR (*C. rotundispiculum*). Half (7/14) the nematode species found were, therefore, new host records, emphasizing the fact that nematodes of mountain reedbuck in South Africa are poorly known. Genera of helminths found at Loskop and MZNP but not found in the present study were the nematodes *Gongylonema* and *Oesophagostomum*.

Four studies of grey rhebok at Bontebok National Park (Horak & De Vos, 1981; Horak, De Vos & De Klerk, 1982; Boomker, 1990; Boomker & Horak, 1992) recorded 12 nematode species, compared to five found at Sterkfontein. The species of the present study were, however, extracted from only four animals. Four of the species found at Sterkfontein were also found at Bontebok NP, while one (*C. yoshidai*) was a new host record. *P. peleae* has only ever been found in grey rhebok and, until the present study, only at Bontebok NP. At Sterkfontein it was again found in grey rhebok in two out of the four animals sampled. It was also found in one mountain reedbuck, but in very low numbers, so was probably an accidental parasite record.

### *Frequency distributions of nematodes*

The nematode populations in mountain reedbuck at both Sterkfontein and TdR were highly aggregated. All of the animals at Sterkfontein had some worms, while at TdR there were four animals without any. In terms of overall numbers, the majority of the parasite population were concentrated into a minority of the host population. A relatively small number of individuals in the “tail” of the parasite distribution were then responsible for most parasite transmission and would have played an important role in the persistence of the parasite (Wilson *et al.*, 2002).

Heterogeneities in parasitic loads occur in most natural populations (Shaw & Dobson, 1995; Wilson *et al.*, 2002), and these could have a differential effect on host species fitness and ultimately population dynamics. However, it is difficult to tell whether parasite loads, as occurred in the most heavily infected individuals at Sterkfontein, were large enough to have a negative impact. Little information on the numbers of nematodes necessary to produce clinical disease in antelope is available (Boomker, 1990).

Pathogenesis caused by *H. contortus* is essentially an acute haemorrhagic anaemia due to the blood-sucking habits of the worms. Each worm removes about 0.05 ml of blood per day by ingestion and seepage from the lesions so that sheep with 5000 worms may lose about 250 ml daily (Georgi & Georgi, 1990). At peak infection, naturally acquired populations of *H. contortus* may remove one fifth of the circulating erythrocyte volume per day from lambs. The pathogenic effects of *H. contortus* result from the inability of the host to compensate for blood loss. Infections of up to 500 worms, however, have been found to have little effect on growth or wool production of sheep under conditions of satisfactory nutrition.

In mountain reedbuck the numbers of *H. contortus* needed for clinical signs of disease to be shown is unknown, but is probably in excess of 2000 worms (Boomker, pers. comm.). The highest count of *H. contortus* at Sterkfontein was 1050 worms. This number might have had some effect on the individual, especially if the animal was compromised in some way, but the mean of 116 worms per animal should certainly be considered negligible.

*Cooperia* spp. usually play a secondary role in the pathogenesis of parasitic gastroenteritis of ruminants although they may be the most numerous trichostrongyle present (Georgi & Georgi, 1990). However, in some tropical and subtropical areas, some species are responsible for severe enteritis in domestic calves. They may penetrate the epithelial surface of the small intestine and cause disruption, leading to villous atrophy and a reduction in the area available for absorption. In heavy infections diarrhoea has been reported. Approximately 300 000 larvae are needed to infect a cow within ten days for there to be any clinical effect (Boomker, pers. comm.). Although mountain reedbuck are about ten times smaller than domestic



cattle, the average burden of 2 040 worms per animal would not have been enough to cause clinical signs. It is possible, however, that the 15 000 worms found in one animal might, in conjunction with other extenuating factors, result in some detrimental effects. This is speculative because such effects have never been tested in wild antelope. No information is available about the seasonal patterns in *L. schrenki* for comparison.

It is possible that the sample size of mountain reedbuck at Sterkfontein was not large enough to detect the animals with the largest parasitic loads (Wilson *et al.*, 2002), so there may have been individuals with loads large enough to cause clinical signs. However, Shaw & Dobson (1995) in their quantitative review of parasite abundance and aggregation used a minimal host sample size of 30 animals to reduce the effects of sampling error. By that standard, the 41 animals sampled at Sterkfontein should have been adequate.

### ***Possible causes of aggregation***

Aggregation, or variations in parasitism rates, may be associated with heterogeneities in the host population, including host age, gender, body condition, behaviour and genetics (Wilson *et al.*, 2002). It may also be associated with heterogeneities in the parasite population genetics or extrinsic factors such as the spatial distribution of the parasites.

Host age could affect parasite distributions by a number of mechanisms. These include parasite-induced host mortality, acquired immunity, age related changes in predisposition to infection, and age dependent changes in exposure to parasites (e.g. changes in behaviour) (Wilson *et al.*, 2002). Although there was no evidence of a difference in parasitic loads between animals of different ages, a thorough evaluation of age-associated heterogeneities was not possible in this study because of the relatively small numbers of young and old animals sampled. Wilson *et al.*, (2002) stated that sample sizes often decline with host age due to mortality and, if sampling effort is not directed at obtaining equal numbers of hosts in all age classes, then it might appear that average parasite loads decline in old animals and that parasite aggregation declines with age, purely due to sampling biases. Collecting equal

numbers of animals in all age classes was not an aim of this study, while investigating parasite-induced mortality, acquired immunity and predisposition to infection were not within its scope.

Schalk & Forbes (1997) found that in 12 out of 136 field studies on mammals, males exhibited higher rates of parasitism than females. In all 12, however, male biases were small (<5%). When meta-analyses were carried out using pooled data from these studies, including those that did not find male biases, males were still found to exhibit higher rates of parasitism. Poulin (1996) found that prevalence and intensity of nematode infection tended to be higher in male mammals, while Schalk & Forbes (1997) found that such male bias only occurred when arthropod data were also included. Moore & Wilson (2002) found that the mean prevalence of infection was male biased for helminths in mammals in general, but not for Artiodactyla alone. Even if sex biases exist, determining the relative importance of the different mechanisms capable of generating them may prove extremely difficult, due to the fact that many of the ecological and physiological factors covary (Wilson *et al.*, 2002). It has even been suggested that the small differences found between males and females will have little impact on parasite epidemiology, although Poulin (1996) argued that an increase in even a few parasites could be biologically meaningful.

Intrinsic biological differences between host sexes could lead to one sex being more prone to parasite infections than the other. Physiological, morphological and behavioural differences between sexes could operate to create a slight but consistent sexual bias in infection levels. The present study, however, found no evidence of differences between males and females in parasite abundance.

Moore & Wilson (2002) found that increases in sex-biased parasitism (SBP) were associated with increases in sexual size dimorphism (SSD), and that this resulted in an increase in adult male mortality when males were larger than females. Moreover, they also found that there was a significant positive relation between SBP and sex-biased mortality (SBM), even when SSD was controlled for. Thus sexual selection leads to an enhanced risk of parasitism and elevated mortality in males, and this is due in part to the negative impact of parasites. In mountain reedbuck, however, males are only

slightly larger than females (approximately 6 % heavier) (Irby, 1975; Skinner, 1980; Anderson & Koen, 1993; see Chapter 6), so SBP and SBM should be less prominent.

Significant male biases may be found more often than expected by chance, but this does not mean that statistically significant male biases are a general rule. Sex biases in experimental studies, where hosts were artificially infected, were much stronger than those detected in field studies where hosts were naturally infected. This suggests that the main differences may lie in the host immune responses rather than the infection processes. Quantitative support for sex biases in parasite infection rates remains inconclusive (Wilson *et al.*, 2002).

Although no statistical differences were found between males and females in the present study, patterns of parasitic loads were slightly different. Males had more worms than females between February and June, while females had more worms than males between August and December. One physiological aspect implicated in male biased parasitism is that high testosterone levels can cause immuno-suppression (Grossman, 1985). The main breeding season for mountain reedbuck was April/May, but if testosterone levels were higher at this time, and males were immuno-suppressed, they should have had larger parasitic loads. This was not the case (see Figures 34 & 35). The only nematode species that showed significant seasonal variation was *H. contortus*, and at Sterkfontein males had their highest loads in December. Moreover, mountain reedbuck were considered aseasonal, so there were unlikely to be significant peaks in testosterone secretion.

In females, there is evidence suggesting that oestrogens stimulate humoral and cell-mediated immunity (Schuurs & Verheul, 1990). In contrast, energetic costs of pregnancy and maternal care, plus the immuno-suppressive effects of some hormones produced during parturition and lactation, may increase the susceptibility of females to parasites. Measuring immuno-competence is, however, fraught with difficulties (Wilson *et al.*, 2002) because it is not clear whether there is a simple relationship between immune function and disease susceptibility. Females at Sterkfontein had significantly more *H. contortus* in December than May, June, August and September, which is more consistent with the immuno-suppression theory during late pregnancy and parturition. Moreover, females that had recently given birth, and were therefore

lactating, had significantly higher worm burdens than females that were still pregnant. Boomker (1990) found that the mean worm burden of lactating female kudu was more than double that of pregnant or quiescent females. The difference was ascribed to the stress associated with terminal pregnancy, parturition, lactation and anxiety during the first few weeks of the newborn calf's life.

High parasitic loads might decrease body condition and this will in turn reduce resistance to parasitic infection. Body condition is also likely to affect the hosts' ability to compensate for damage inflicted by parasites, such as repairing tissues or replacing critical nutrients. At Sterkfontein, however, there was no correlation between numbers of parasites and body condition.

Behavioural differences in feeding have been suggested as a possible cause of heterogeneities in parasitism, with host age or sex being potential factors. Feeding behaviour of mountain reedbuck was the subject of Chapter 5. The only change that is likely to occur with age would result from an older male being forced off a territory by a stronger one. Males so affected might be forced into peripheral areas where grazing is not so good, but such areas would not necessarily have greater densities of infective nematode larvae. In fact, they might have lower densities because there are less animals defecating in the area. Males defend resources (Irby, 1976; Dunbar & Roberts, 1992; pers. obs.) and territory selection is based on availability of females, which in turn is based on the availability of cover and feeding resources (Dunbar & Roberts, 1992; pers. obs.). Parasite avoidance is not a factor. Females don't hold territories so would not undergo changes in behaviour that might alter infection rate. Males of some antelope species feed less during their breeding periods because they spend more time guarding territories or females. In this case they should be less exposed to parasitic infection. In the case of mountain reedbuck, males do not have a defined rut, and do not feed any less during the breeding period than at other times (see Chapter 5).

There are very few good examples of genetic variation in disease resistance in natural host populations, particularly in vertebrates. There has been even less research conducted on the importance of parasite heterogeneities. The effect of host or parasite genetics on parasitic infection rates was not within the scope of the present study.

The most likely cause of heterogeneity in parasite loads, specifically in *H. contortus*, was temporal variation in distributions of the parasite populations. Horak (1978a, b, c, d, 1981) found that burdens of *H. contortus* in sheep, cattle, blesbok and impala peaked between October and March at a number of areas in South Africa, including the Hennops River (Bankenveld), Tonteldoos (North-Eastern Sandy Highveld), Lunsklip (Sour Bushveld) and Boekenhout (Mixed Bushveld). Reinecke (1964, 1983) found that the abundance of *H. contortus* was positively correlated with ambient temperatures and rainfall. In summer rainfall areas, infective larvae on pasture increased after rains in excess of 15 ml per month and temperatures of over 17° C. Sheep acquired infection in November and adult worms were dominant until February. At Sterkfontein monthly rainfall only exceeded 15 ml after August, and at the time of the September 2000 and August 2001 culls, the rains had barely started (Figure 3). Temperatures had also not yet exceeded 17° C. Under this scenario, peak infections would only have been expected in the next culling periods, i.e. December 2000 and November 2001 respectively, and this was indeed the case. *H. contortus* was more abundant in both male and female mountain reedbuck during December, after good rainfall, than during May, June, August and September.

### ***Cross-transmission with domestic livestock***

As this chapter was part of a larger study examining factors influencing productivity, it is worth adding a note here on the cross-transmission of nematode parasites between wild and domestic ruminants. Although it was not covered in the present research because these groups of animals did not mix in the areas surveyed, cross-transmission could be relevant in areas where they do mix because of the possible effect on productivity. There are conservation areas within South Africa where wild and domestic ruminants coexist, such as Qwaqwa National Park in the Free State, and there are many places where they mix on private land. In the case of the latter, wild ruminants such as mountain reedbuck can provide extra income from meat production and other by-products on top of that obtained from cattle and sheep husbandry. Cross-transmission would be of importance if either group was responsible for causing or even aggravating (by acting as a reservoir) increased parasitic burdens that might adversely affect productivity.

Boomker (1990) found that the majority of worms of domestic ruminants were found in wild browsers, but less of the worms found in wild browsers occurred in domestic ruminants. Both groups can act as reservoir hosts of each other's worms, but browsers may be better hosts for the worms of domestic stock than vice versa. As mountain reedbuck are grazers, they are more likely to have contact with nematode species of cattle and sheep. Horak (1979) stated that many of the helminths recovered from antelope (e.g. impala and blesbok) are those usually encountered in sheep and cattle (Horak 1978a, b, c, d).

Sheep were successfully infected with *H. contortus* larvae from blesbok (Horak, 1979), indicating that this nematode is well adapted to both host species and that cross-transmission can readily take place. Other nematode species, however, were not so easily cross-transmitted, e.g. *Cooperia hungi* from impala to sheep (Horak, 1979). In most nematode species, cross-transmission generally did not take place when the adult worm burden of a particular species in the donor antelope was less than 200 worms. This can probably be ascribed to the fecundity of the female worms and hence their contribution to the larval pool. *Haemonchus* spp. are particularly fecund and thus a few adult females would make a considerable contribution to the larval pool. Other species that are less fecund would contribute fewer larvae. Consequently, the chances of cross-transmission would be minimal from those donor animals in which only a few adult worms of the latter species were present. In impala and cattle utilising the same pasture in the Boekenhout area there was very little natural cross-transmission of nematodes (Horak, 1978c, d; 1979).

Horak (1978b) found that the main difference between the *H. contortus* burdens of sheep and blesbok was numerical. Whereas sheep generally harboured considerable burdens in the summer (Horak & Louw, 1977; Horak, 1978a), the worm counts in blesbok in the same season were relatively low. This may be one of the reasons why nematodes appear to be less pathogenic in wild antelope than in domestic animals (Boomker, 1990). However, the pathogenicity of nematodes in antelope is unknown.

Both Sterkfontein and TdR adjoin farmland where animal husbandry is practiced. The main study site at Sterkfontein was adjacent to an area of cattle farming, and cattle were seen almost every day. The only thing separating the wild and domestic

ruminants in this case was a game fence so cross-transmission might have been possible, but only on the peripheries. At TdR the surrounding farmland was used primarily for sheep farming, but the mountain reedbuck culled on the reserve were separated from the farmland by the Gariiep River (formerly the Orange River) and had no contact with it. They were generally not within sight of the domestic animals. Both reserves were previously used for domestic animal husbandry prior to their designation as Provincial Nature Reserves. Sterkfontein was used in this capacity as recently as 1980, while TdR was set aside for game farming in 1967. It may be that some of the helminth species found in the mountain reedbuck were there initially as a result of agriculture.

### ***Nematodes of Tussen die Riviere***

No statistical tests were conducted on the parasite data from TdR because prevalence and abundance of species were very low, so any patterns would have had no biological meaning. Nematode burdens at TdR were much lower than at Sterkfontein. This lower abundance may have resulted from lower densities of mountain reedbuck at TdR. Arneberg *et al.* (1998) showed that for strongylid nematodes of mammals, abundance may depend on host population density because as host densities increase, each parasite egg or larva enjoys an increased probability of contacting a host. Differences in habitat may also have played a role. At TdR grasses were clumped and numerous bare patches of earth occurred between tufts. In contrast, at Sterkfontein, percentage grass canopy cover was very high, allowing infective larval nematodes greater opportunity to attach themselves to grass clumps to be eaten by grazers such as mountain reedbuck.

### ***Nematodes of grey rhebok at Sterkfontein***

Only four species of helminth were found in grey rhebok at Sterkfontein, but this low number no doubt resulted from the very small sample size of four animals. Because the sample size was so small, no meaningful analyses were carried out. Faecal egg counts and larval counts from coproculture provided a more systematic data set for seasonal variation in grey rhebok nematodes. There was a peak in average larval counts in December, which was similar to the GIT results for mountain reedbuck, but

because adult worm numbers did not correlate with egg counts, these faecal results should be treated with caution. One thing that does seem apparent, however, from both the limited GIT worm counts and faecal egg and larval counts, was that grey rhebok harboured considerably less helminths (on average) than mountain reedbuck. This might be explained in terms of feeding habits because, in contrast to mountain reedbuck which are grazers, grey rhebok are browsers. Wild ruminants that browse generally have lower parasitic burdens than those that graze (Boomker, 1990), and this probably results from differences in feeding habits. Grass cover is generally much higher than herbaceous cover, so more infective larvae should occur on grass and grazers, therefore, have a greater chance of acquiring worms. Grey rhebok at Sterkfontein do, however, feed on forbs that grow very close to the ground (pers. obs.), and to get to these plants the animals have to push through grass tufts. They hence feed from a very similar “microhabitat” to mountain reedbuck so, although forb cover is much lower than grass cover, grey rhebok should be exposed to the same nematode species as mountain reedbuck.