A Coprological Survey of Protozoan and Nematode Parasites of Free-Ranging Chacma Baboons (Papio ursinus) in the Southwestern Cape, South Africa

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A coprological survey of protozoan and nematode parasites of free-ranging chacma baboons (*Papio ursinus*) in the southwestern Cape, South Africa

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This study provides data on gastrointestinal parasite infections in the geographically isolated and locally fragmented Cape Peninsula baboon population and two troops from neighbouring populations in the Western Cape Province, South Africa. We obtained data on parasite diversity and prevalence from 616 faecal samples collected from over 350 individuals in eight troops between July 2006 and May 2008. We processed faecal samples using a modified formalin-ether sedimentation technique and identified nematode eggs and protozoan cysts. We recovered seven nematode genera (*Trichuris* sp., *Oesophagostomum* sp., *Trichostrongylus* sp., *Physaloptera* sp., *Ascaris* sp., an unidentified hookworm morphotype, and an unidentified spirurid) and eight protozoan species (*Balantidium coli, Entamoeba coli, E. histolytica/dispar, E. chattoni, E. hartmanni, Iodamoeba bütschlii, Endolimax nana* and *Chilomastix mesnili*). The nematode and protozoan fauna of the Cape Peninsula baboon population was similar to both neighbouring and geographically distinct chacma baboon populations in South Africa. Parasite prevalence was variable across study sites and seasonality did not appear to have an effect on patterns of infection. The finding of the eggs of an ascarid, possibly *Ascaris* sp., in the Cape Peninsula baboon population represents the first report of this nematode genus in wild baboons in South Africa.

Key words: *Papio ursinus,* chacma baboon, gastrointestinal nematodes, gastrointestinal protozoa, South Africa.

INTRODUCTION

The chacma baboon population of the Cape Peninsula in South Africa has become geographically isolated from all other populations through both urbanization and agriculture. Historically baboons occurred throughout the peninsula, but they are currently restricted to two geographical disparate subpopulations that range in part within the Table Mountain National Park. This loss of habitat to suburban development has resulted in a dramatic drop in population numbers, with whole troops having been eliminated in many areas. At the time of this study there were an estimated 380 baboons distributed amongst 13 troops on the peninsula (E.K. Beamish, unpubl. data). Consequently, the Cape Peninsula population is considered to be threatened despite being the only population of this species outside closed national parks and protected by legislation in South Africa.

Parasitic infections have been recognized as an important factor affecting the density and distribution of animal populations in the wild (Anderson 1979; May 1988; Scott 1988). Habitat disturbance by humans can lead to health issues in wildlife that are still poorly understood, such as the alteration of host–parasite dynamics and risk of cross-transmission of pathogens (Daszak *et al.* 2000; Patz *et al.* 2000). As anthropogenic habitat change forces humans and primates into closer and more frequent contact, the collection of baseline data on patterns of parasitic infections becomes very important in infection risk assessment of wild primate populations for management and conservation purposes.

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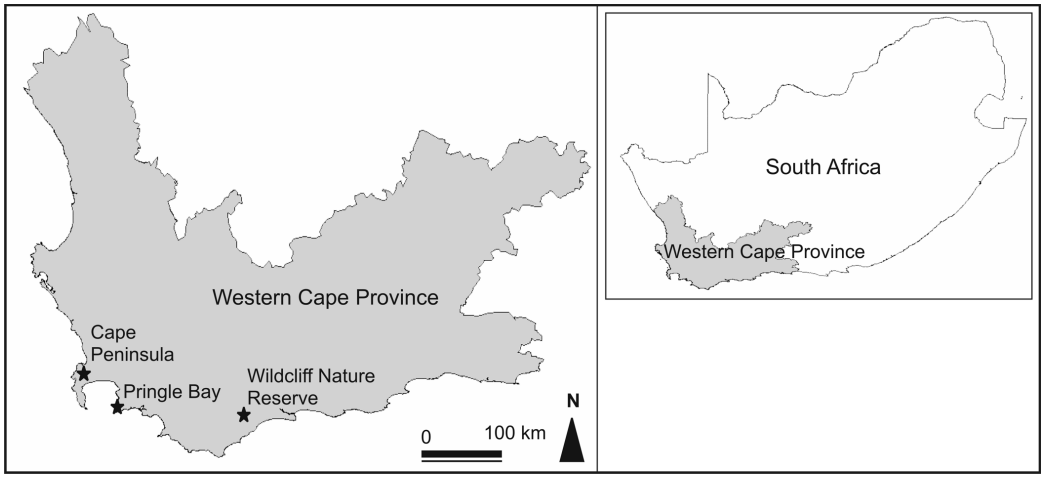

Fig. 1. Location of the three collection sites in the Western Cape Province; (inset) location of the Western Cape Province in South Africa.

Although the parasite fauna of baboons at different localities in Africa, including South Africa, has been well documented (*e.g.* Appleton *et al.* 1991; Appleton & Henzi 1993; Eley *et al.* 1989; Ghandour *et al.* 1995; Goldsmid 1974; Hahn *et al.* 2003; Hope *et al.* 2004; Kuntz & Myers 1966; Legesse & Erko 2004; Meade 1984; Müller-Graf *et al.* 1996; Pettifer 1984), parasitological investigations have never been conducted on the Cape Peninsula baboons. This study aims to quantify the gastrointestinal protozoan and nematode parasites of the Cape Peninsula baboons by coprological examination, and to compare these data with neighbouring populations and other studies in southern Africa. In addition, we examine the effect of season on parasite infections for one troop of the Cape Peninsula baboon population.

METHODS

Study sites

From July 2006 to May 2008, we collected 616 faecal samples from over 350 chacma baboons ranging in three geographically distinct regions of the Western Cape Province of South Africa (Fig. 1). The region of the Cape Peninsula (470 km^2) stretches from the city of Cape Town to Cape Point in the Cape of Good Hope section of the Table Mountain National Park (TMNP) (latitude 33°55′–34°21′S; longitude 18°25′–18°28′E). The Cape Peninsula has, unlike most of sub-Saharan Africa, a Mediterranean-type climate with well-defined seasons and extreme variation in annual rainfall (Davidge 1978; Cowling *et al.* 1996).

Winter months are cool and wet, with rainfall ranging from 503 to 687 mm and an average daily minimum temperature of 10.5°C recorded during the study period. Winters are also characterized by strong northwesterly winds. Summer is warm (average daily maximum temperature of 23.5°C) and dry. In this season the Peninsula is subjected to frequent strong winds from the southeast. We collected 531 faecal samples from six baboon troops of the Cape Peninsula population.

The Cape Overberg region is situated 90 km east of Cape Town and has a climate very similar to that described for the Cape Peninsula. We obtained faecal samples from a single troop (Pringle Bay troop) (Fig. 1), which ranged between the coastal villages of Pringle Bay (latitude 34°21′S, longitude 18°49′E) and Rooiels (latitude 34°18′S, longitude 18°49′E). We collected 35 faecal samples from individuals in this troop. The third study site, Wildcliff Nature Reserve (Fig. 1), is approximately 296 km from the Cape Peninsula, in the Langeberg mountain range. This reserve comprises an area of 9.6 km^2 (latitude 33°58.5′–33°55.9′S; longitude 20°58.9′–21°3.0′ E). We collected 50 faecal samples from the Wildcliff troop.

Data collection

Faecal samples of approximately 1 g were collected immediately after defecation to avoid contamination and stored in 5.0 ml, sterile vials containing 10% neutral formalin solution. They were then transported to the Medical Research Council (MRC) facilities in Tygerberg, South

Africa, where they were processed through a modified formalin-ether sedimentation technique (Allen & Ridley 1970).

We identified nematode eggs and larvae and protozoan cysts using their morphology, shape, size and other visible structures (Ash & Orihel 1991; Ash *et al.* 1994). Egg output was expressed as eggs/gram. A droplet of Lugol's iodine was added in order to facilitate the identification of protozoan cysts. For each faecal sample we measured approximately 10 replicates of each egg type (length and width) to the nearest 0.5 µm using an ocular micrometer fitted to a compound microscope. Due to similarities in their size and appearance, we allowed strongyle eggs and rhabditiform larvae from 10 scat samples from Cape Peninsula troops to develop to the more easily identifiable filariform stage by means of coproculture (Harada & Mori 1955). Larvae and adult nematode pictures were sent to the Division of Parasitic Diseases (Centers for Disease Control and Prevention, Atlanta, U.S.A.) for identification.

Opportunistic necropsies ($n = 5$) were performed whenever we obtained dead baboons (*e.g.* road deaths), allowing the collection of adult and immature nematodes from the gastrointestinal tract. These worms were preserved in 70% ethanol and subsequently identified and matched to the eggs found in faeces.

Prevalence is defined as the number of individuals of a host species infected with a particular parasitic species divided by the number of hosts examined and is usually expressed as a percentage or proportion (Bush *et al.* 1997; Margolis *et al.* 1982). Due to the relatively large number of troops analysed in this study, we could not assign most of the samples (65%) to known individuals. We attempted to collect samples from troops consisting of unknown individuals over brief periods (*e.g.* two hours after sunrise) on a particular day, which greatly reduced the probability of pseudo-replication on a given day. However, repeat samples were likely on repeat days, which while also kept to a minimum were necessary to obtain an adequate minimum sample size. Thus the prevalence estimates reported in this study should be viewed as indices of prevalence (*sensu* Chapman *et al.* 2006).

Data analysis

Statistical tests were conducted using the software SPSS 17.0 (SPSS Inc.) and were two-tailed; significance levels were set to $\alpha = 0.05$. To assess whether parasite prevalence and diversity varied seasonally we used data from a single Peninsula troop (*i.e.* Plateau Road) for which we had a large number of samples evenly distributed across three seasons ($n = 60$, 51, and 83 for winter 2006, summer 2006/2007 and winter 2007, respectively). A Kruskal-Wallis test for several independent samples was used to test for seasonal effects on protozoan and nematode species richness. Mann-Whitney *U*-tests were used for *post hoc* pair-wise comparisons between seasonal parasite species richness. Comparisons of the prevalence between the seasons for each nematode and protozoan taxa were done with Fisher's exact tests. We analysed seasonality of egg output for the nematodes *Trichuris* sp. and *Oesophagostomum* sp., the only taxa for which the sample sizes were large enough. We included only positive samples in this analysis and used a Kruskal-Wallis *H*-test. We performed Mann-Whitney *U*-tests as *post hoc* tests. To avoid any potential confounding bias due to seasonal and year differences in parasite infections, we used only data ($n = 271$) from the 2007 winter season for calculation of parasite species richness and prevalence on the Cape Peninsula. We compared egg emission between parasite taxa, independently from the season, with a Kruskal-Wallis test. We calculated the prevalence estimate for the Cape Peninsula baboon population from the average of the six study troops and compared the prevalence of parasite taxa between localities using chi-square tests of independence. Prevalence of parasite taxa exclusively recovered in the present study (*i.e. Ascaris* sp. and hookworm type) or in only two locations (*i.e.* Spiruridae sp.) were not compared.

RESULTS

Parasite infections

We recovered seven nematode genera and eight protozoan species from the study populations (Table 1). Protozoan taxa included one ciliate, six amoebae and one flagellate. *Balantidium coli* and *Entamoeba histolytica* are potentially pathogenic to baboons, while all the other protozoan species recovered in this study are considered to be non-pathogenic (Cogswell 2007). Cysts of the pathogenic *Entamoeba histolytica* are indistinguishable from those of the non-pathogenic *E. dispar* when using light microscopy. Therefore the two species were regrouped.

We identified the nematode *Trichuris* sp. based

Table 1. Prevalence (%) of gastrointestinal parasites of chacma baboons (*Papio ursinus*) at different localities across South Africa (N/A: comparison not available).

	Location												
	Cape Peninsula (present study)	Pringle Bay (present study)	Wildcliff Nature Reserve (present study)	Mkuzi Game Reserve (Appleton *et al.* 1991)	Giant's Castle Game Reserve (Appleton *et al.* 1986)	Suikerbosrand Nature Reserve (Pettifer 1984)	Loskop Dam Nature Reserve (Pettifer 1984)	Scrutton Private Nature Reserve (Pettifer 1984)	Limpopo Privince (Goldsmid & Rogers 1978)	South Africa (Myers *et al.* 1971)			
Sample size (*n*):	271	35	50	191	122	25	65	18	24	54	χ^2	d.f.	*P*
Nematoda													
Trichuris sp.	66	89	98	6	57	0	2	0	4	22	373.696	9	<0.001
Oesophagostomum sp.	61	91	38	15	49	100	95	100	63	0	284.433	9	<0.001
Ascaris sp.	9	0	0	0	0	0	0	0	0	0	N/A		N/A
Physaloptera sp.	8	54	78	2	44	10	97	78	4	12	414.190	9	<0.001
Trichostrongylus sp.	6	100	48	1	0	92	30	44	8	11	430.281	9	<0.001
Hookworm type	1	0	34	0	0	0	0	0	0	0	N/A		N/A
Spiruridae sp.	1	0	0	5	0	0	0	0	0	0	N/A		N/A
Protozoa													
Balantidium coli	100	77	66	80	26						277.120	6	<0.001
Entamoeba coli	91	94	92	75	70						107.078	6	<0.001
E. histolytica/dispar	12	6	2	0	0						47.507	6	<0.001
E. chattoni	16	6	28	0	0						73.891	6	<0.001
E. hartmanni	38	17	16	0	0						159.000	6	<0.001
Iodamoeba bütschlii	49	40	72	9	2						191.075	6	<0.001
Endolimax nana	2	0	0	13	10						35.377	6	<0.001
Chilomastix mesnili	18	29	10	0	13						57.655	6	<0.001

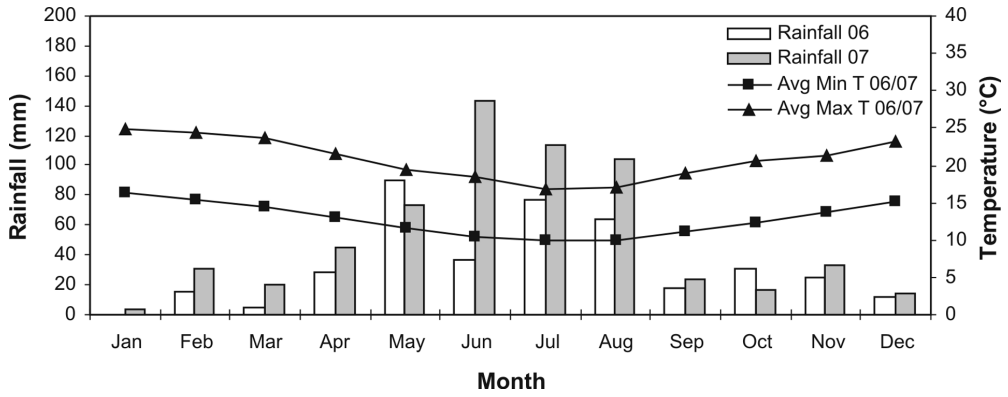

Fig. 2. Monthly rainfall and mean daily minimum and maximum temperature for 2006 and 2007.

on the morphology of its eggs and on adult specimens found during the opportunistic necropsies ($n = 5$). The ascarid roundworm, possibly *Ascaris* sp., was identified based on the egg morphology. We found three morphospecies belonging to the polyphyletic group known as 'strongylids', all from the order Rhabditida: *Oesophagostomum* sp. (identified through the morphology of eggs, cultured larvae, and adult specimens from necropsies), *Trichostrongylus* sp. (identified on the basis of egg morphology), and an unidentified hookworm morphotype. The only species of *Oesophagostomum* reported to date from southern African primates is *O. bifurcum.* Of the two recovered morphotypes belonging to the order Spirurida, we identified one as *Physaloptera* sp. by the eggs and the adult specimens recovered during necropsies. *Physaloptera caucasica* is the only species of *Physaloptera* known from primates in southern Africa. The other spirurid nematode could not be identified further.

The highest nematode prevalence on the Cape Peninsula was found for *Trichuris* sp. (66%; Table 1) followed by *Oesophagostomum* sp. (61%). The highest protozoan prevalence was found for *Balantidium coli* (100%) followed by *Entamoeba coli* (91%). The prevalence of nematodes other than *Trichuris* sp. and *Oesophagostomum* sp. were all below 10% (Table 1), including for the ascarid, possibly *Ascaris* sp. (9%), hookworm morphotype (1%) and unidentified spirurid (1%).

Effects of seasonality

Monthly minimum and maximum temperatures did not differ between the study periods 2006 and 2007 (Wilcoxon for minimum temperatures: $n = 12$, $Z = -0.937$, $P = 0.349$; Wilcoxon for maximum temperatures: $n = 12$, $Z = -0.589$, $P = 0.556$). Therefore, we calculated a monthly average between 2006 and 2007 for both monthly minimum and maximum temperatures (Fig. 2). Monthly rainfall differed significantly between 2006 and 2007 (Wilcoxon: $n = 12$, $Z = -2.158$, $P < 0.05$) and is thus represented separately in Fig. 2. In general, rainfall was reported throughout the year, but peaked between May and August (the austral winter) when ambient temperatures were lowest.

Nematode species richness differed significantly across seasons for the study troop, while protozoa species richness did not (Table 2). *Post hoc* tests showed that nematode diversity did not differ between winter and summer but varied between the two winter seasons, with nematode diversity being significantly higher in winter 2006 compared to winter 2007 (Table 2).

The only significant difference in prevalence between seasons, was found for *Trichostrongylus* sp., which was more prevalent in summer than winter (Fisher's exact test: $P = 0.043$) (Fig. 3). There was no difference in the prevalence of any protozoan taxa across the three sample periods.

Seasonal egg output of *Trichuris* sp. varied significantly for individuals of the Plateau Road troop (Table 2). *Trichuris* sp. egg output was significantly higher in summer than in either winter season. Egg output of *Oesophagostomum* sp. did not vary significantly across seasons (Table 2).

Variation in parasite infections among sites in southern Africa

Parasite prevalence varied significantly among the study sites taken into consideration (Table 1). The prevalence of *Trichuris* sp. was lower in baboons on the Cape Peninsula than in Pringle Bay and Wildcliff, but was higher than in any other

Table 2. Seasonal variation in parasites species richness and nematode egg output (Kruskal-Wallis *H*-test), and *post hoc* comparisons (Mann-Whitney *U*-test); S: summer, W: winter.

Species richness seasonal variation				Egg output seasonal variation			
	χ^2	*n*	*P*		χ^2	*n*	*P*
Nematodes	8.453	194	<0.05	*Trichuris* sp.	7.860	142	<0.05
Protozoa	4.656	194	0.097	*Oesoph.* sp.	5.267	123	0.072
Nematode species richness *post hoc* comparisons				*Trichuris* sp. egg output *post hoc* comparisons			
	U	*n*	*P*		*U*	*n*	*P*
S 2006/2007 *vs* W 2006	1312.500	111	0.175	S 2006/2007 *vs* W 2006	680.000	85	<0.05
S 2006/2007 *vs* W 2007	1901.500	134	0.304	S 2006/2007 *vs* W 2007	656.500	93	<0.05
W 2006 vs *vs* W 2007	1790.000	143	<0.05				

population analysed in South Africa. Apart from Giant's Castle (Appleton *et al.* 1986), this nematode had a low prevalence in the other study sites. *Oesophagostomum* sp. has been found in almost every population studied and its prevalence in the Cape Peninsula population was lower than at five other locations. The prevalence of *Trichostrongylus* sp. and *Physaloptera* sp. on the Cape Peninsula was lower than in the majority of the other baboon populations studied.

The prevalence of protozoa was most similar between the Cape Peninsula and the neighbouring Pringle Bay population, and, to a lesser extent, Wildcliff and Limpopo Province (former Northern Transvaal) (Goldsmid & Rogers 1978). The prevalence of *Balantidium coli* and *Entamoeba coli* was, in general, high in every baboon population studied, and was particularly high in the Cape Peninsula population. The prevalence of *Entamoeba histolytica/dispar* in the Cape Peninsula

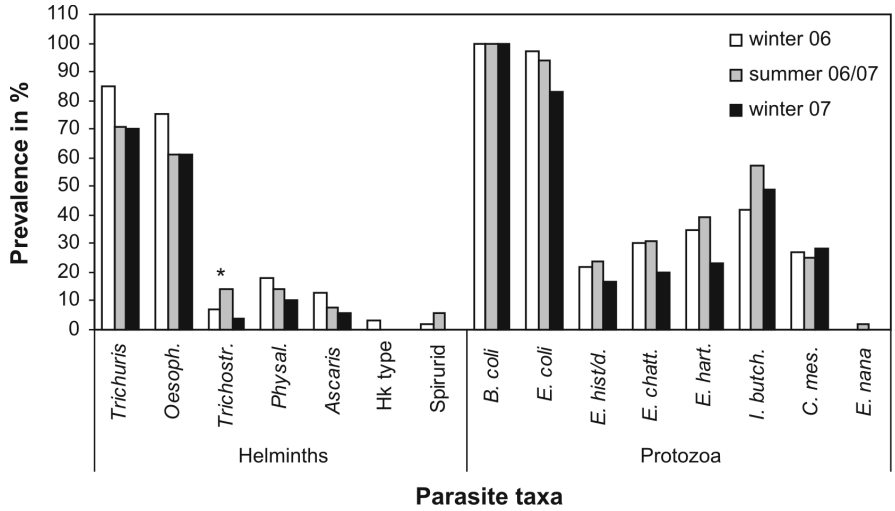

Fig. 3. Parasite prevalence in winter and summer across two years for individuals in the Plateau Road troop (Cape Peninsula). The asterisk indicates statistical differences between summer 06/07 and winter 07. Fisher's exact test: $^*P \leq 0.05$. (*Trichuris* = *Trichuris* sp., Oesoph. = *Oesophagostomum* sp., *Trichostr.* = *Trichostrongylus* sp., *Physal.* = *Physaloptera* sp., *Ascaris* = *Ascaris* sp., Hk = hookworm, *B. coli* = *Balantidium coli, E. coli* = *Entamoeba coli, E. hist/d.* = *E. histolytica/dispar, E. chatt.* = *E. chattoni, E. hart.* = *E. hartmanni, I. butch.* = *Iodamoeba bütschlii, C. mesn.* = *Chilomastix mesnili*; *E. nana* = *Endolimax nana*).

population was higher than in other study areas. However, it must be remembered that, using microscopy, the *Entamoeba histolytica* species cannot be differentiated from the morphologically identical commensal protozoan *E. dispar.* Other protozoan taxa, namely *Entamoeba chattoni, E. hartmanni, Iodamoeba bütschlii, Endolimax nana* and *Chilomastix mesnili,* were found at a low prevalence in all the southern African populations.

DISCUSSION

The nematode diversity found in the Cape Peninsula baboon population was similar to other South African localities studied. Appleton *et al.* (1991) reported higher helminth diversity (12 taxa) in Mkuzi Game Reserve and attributed it to the high humidity associated with the subtropical climate and consequently favourable conditions for the development of parasite infective stages in the soil. Conversely, the low helminth diversity (three taxa) found in chacma baboons in Namibia by Appleton and Brain (1995), was attributed to the hot and dry climatic conditions, which are not suitable for the development and survival of nematode parasites in the soil.

The most common helminth found in chacma baboons in southern Africa appears to be the nematode *Physaloptera* sp., which has been found in all chacma baboon populations studied to date (Appleton *et al.* 1986, 1991; Goldsmid 1974; Goldsmid & Rogers 1978; McConnell *et al.* 1974; Myers *et al.* 1971; Pettifer 1984), except for Namibia (Appleton & Brain 1995). *Physaloptera* is transmitted indirectly to the baboon through intermediate hosts such as beetles, cockroaches and other insects (Brack 1987). Another nematode with an indirect life cycle, *Streptopharagus* sp. is also transmitted through arthropods and has been found in all populations of chacma baboon, including Pringle Bay (this study) but not on the Cape Peninsula or in the Wildcliff Nature Reserve. Other nematodes found frequently across South Africa are *Oesophagostomum* sp. and *Trichostrongylus* sp. (both found at 12 of the 14 localities), and *Trichuris* sp. (present at 10 of 14 localities).

The presence of an ascarid nematode, possibly an *Ascaris* sp., in the Cape Peninsula population is the first record of such a parasite in southern African baboons. *Ascaris* sp. has been reported in only four other studies on baboon parasites, including three studies on olive baboons (*Papio anubis*), one in Kenya (Eley *et al.* 1989) and two in Uganda (Hope *et al.* 2004; Ocaido *et al.* 2003), and one study on hamadryas baboons (*Papio hamadryas*), in Saudi Arabia (Nasher 1988). In all of the above cases a certain degree of interaction with humans was suggested to explain the presence of this parasite in baboons. The high human density on the Cape Peninsula in addition to the medium prevalence (25%) of *Ascaris lumbricoides* eggs in human faeces (Adams *et al.* 2005) from this region could possibly suggest a similar causal link between humans and the presence of *Ascaris* in baboons of the Cape Peninsula. However, confirmation of species identification would require molecular analysis.

In summary, helminth diversity seems to be similar in baboon populations across South Africa. The diversity of intestinal protozoa was also very similar across South Africa with *Balantidium coli, Entamoeba coli* and *Iodamoeba bütschlii* being present at every locality investigated. Interestingly, the flagellate protozoan *Giardia* sp. has only been reported to date in Namibian baboons (Appleton & Brain 1995).

The prevalence calculated for nematodes in the present study was typically similar to or lower than the prevalence recorded at other South African study sites. Thus the prevalence of *Trichuris* sp. and *Oesophagostomum* sp. was similar to the prevalence found at Pringle Bay, Wildcliff and Giant's Castle (Table 1) while the prevalence of *Trichostrongylus* sp. and *Physaloptera* sp. was lower than at the majority of other studies. Nematode prevalence in the Cape Peninsula baboons was most similar (with the exception of *Trichuris* sp.) to that found in Limpopo Province, situated in the northern part of the country. Interestingly, it was significantly different from its two closest locations, namely Pringle Bay and Wildcliff (data not shown). By contrast, prevalence of protozoa was more similar between the Cape Peninsula and Pringle Bay than to Wildcliff and Northern Transvaal baboon populations and showed the highest prevalence for *Balantidium coli* and one of the highest prevalence for *Entamoeba coli.* Climate has been proposed as an important factor explaining the variation of prevalence for one parasite species among various sites (Nunn *et al.* 2005). Climatic conditions vary greatly among the localities in South Africa: from the coastal, subtropical climate of Mkuzi (Appleton & Henzi 1993) to the montane/subalpine climatic conditions found at Giant's Castle (Appleton *et al.* 1986) and the Mediterranean conditions of the Cape Peninsula. It

is possible that much of the variation in prevalence is thus a function of climatic variation and the associated environmental factors that impact on the survival of infective stages and hence on the rate of infection of baboons at the different localities.

Soil moisture is arguably the most important factor for the survival of parasite infective stages (helminth eggs and protozoan cysts) and therefore for infection risk (Rogers & Sommerville 1963). At any particular temperature, survival of infective stages is greatest when conditions are most humid. Furthermore, the survival of eggs at low humidity is greatest when temperatures are low. In the present study, we expected winter conditions, characterized by higher rainfall rates and ground humidity, to correlate positively with parasite infections, by creating better conditions for the survival of infective stages. However, the Plateau Road troop did not present any seasonal patterns in parasite infection except for *Trichostrongylus* sp. prevalence and *Trichuris* sp. egg output, which were significantly higher in the warmer, drier summer months. In the Drakensberg, a region characterized by a montane climate, winter egg output in baboons was significantly higher than summer output for *Oesophagostomum bifurcum* and *Trichuris trichiura* (Appleton & Henzi 1993). The contradictory results across various studies on primates (Appleton & Henzi 1993; Gillespie *et al.* 2004, 2005; Huffman *et al.* 1997; Meade 1984; Stoner 1996; Stuart & Strier 1995; Stuar *et al.* 1993; Stuart *et al.* 1998) suggest that there are no clear and consistent seasonal patterns for standard parasite indices. Further investigations on seasonal output of infective stages could provide more information, as well as an analysis of soil content to detect seasonal differences in nematode soil load and therefore possible seasonal effects on their survival rate.

The baseline data on species diversity and prevalence of gastrointestinal parasites of the Cape Peninsula baboons presented for the first time in this study provides an important step in the infection risk assessment of this population. Despite having lived in close proximity to people for a prolonged period (hundreds of years), the baboons of the Cape Peninsula have a similar gastrointestinal fauna to other populations of baboons in southern Africa. However, the presence of an ascarid, perhaps *Ascaris* sp., raises the question of possible cross-transmission between the baboon and human population and until this has been confirmed by molecular means, it suggests the need to reinforce a management plan of reduced spatial overlap between humans and baboons.

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