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GASTROINTESTINAL PARASITES OF CRITICALLY ENDANGERED PRIMATES ENDEMIC TO TANA RIVER, KENYA: TANA RIVER RED COLOBUS (*PROCOLOBUS RUFOMITRATUS*) AND CRESTED MANGABEY (*CERCOCEBUS GALERITUS*)

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ABSTRACT: We conducted fecal egg counts of gastrointestinal parasites of 2 critically endangered primates endemic to the forest of Tana River, Kenya. We aimed to use the fecal egg counts as proxies to quantify the prevalence of gastrointestinal parasites between the 2 primates. The Tana River red colobus (*Procolobus rufomitratus*) and crested mangabey (*Cercocebus galeritus*) are of similar body size, but their behavioral ecology is very different. We predicted that mangabeys would have a higher prevalence of parasites because they are mostly terrestrial omnivores, live in larger social groups, and therefore range widely. We detected 10 nematodes and 3 protozoans in mangabeys and 7 nematodes and 2 protozoans in colobus. We detected a higher number of different parasite species in individual mangabeys, and 4 of the 5 nematodes requiring intermediate hosts were found in mangabeys. The overall prevalence of parasites was higher for mangabeys, but this difference was not statistically significant. For colobus, we found a trend whereby the number of different parasite species in individual monkeys was higher in males and in lactating females. However, there was no difference in the prevalence of parasites between the sexes or between lactating and nonlactating females.

Parasites and infectious diseases of wildlife are a major threat to conservation of endangered species (Lyles and Dobson, 1993). Thus, there is a great need for studies documenting the prevalence of parasites among endangered species in the wild (Daszak et al., 2000). One ecosystem where there is great need for information on the prevalence of parasites among endangered species includes the Tana River forests in eastern Kenya (Mbora and Meikle, 2004). The forests are of great conservation importance because they provide the only known habitat of 2 endemic primates, i.e., the Tana River red colobus (*Procolobus rufomitratus*) and crested mangabey (*Cercocebus galeritus*). Nevertheless, habitat loss and fragmentation in these forests have been so severe that the populations of both primates have declined precipitously in the past 30 yr, and extinctions have occurred in several fragments (Mbora and Meikle, 2004). Consequently, both species are now critically endangered and ranked among the top 25 most endangered primates in the world (Grubb et al., 2003). No studies have ever been done to document the prevalence of parasites in these primates.

The Tana colobus and mangabey are of similar body size, but their behavioral ecology is very different. The red colobus is a putative habitat specialist that lives in relatively small social groups that exhibit high site fidelity and limited vagility (Marsh, 1981). A canopy dweller, it depends on a diet of leaves obtained from a limited number of canopy tree species (Marsh, 1981; Mbora and Meikle, 2004). In contrast, the mangabey is a putative habitat generalist that lives in much larger social groups (Wieczkowski, 2004). It is mostly terrestrial (80% of the time), highly vagile, and its diet comprises seeds and ripe fruit from a variety of tree species and substantial amounts of animal prey (Wieczkowski, 2004). Thus, because these 2 species use the habitat in fundamentally different ways, we expected them to exhibit clear differences in the prevalence of their parasite fauna.

Here, we report a detailed assessment of the gastrointestinal parasite fauna of the 2 Tana primates by using parasite fecal egg counts as a proxy to quantify the prevalence of parasites. We focused on gastrointestinal parasites because they are common in primates, relatively easy to sample from feces (Munene et al., 1998; Gillespie et al., 2005a), and invasive methods of sampling parasites are inappropriate for critically endangered species. We expected mangabeys to exhibit a higher prevalence of parasites and to harbor a higher diversity of parasites requiring intermediate hosts because, unlike colobus, they are omnivorous and live in larger social groups. Thus, mangabeys use a larger home range than do colobus, and therefore they are likely to encounter more parasite infective stages in the environment than colobus.

MATERIALS AND METHODS

The study area consisted of 11 gallery forest patches of various sizes on both sides of the lower floodplain of the Tana River in eastern Kenya (see Fig. 1 in Mbora and Meikle, 2004). The study forests were located within 20 km of each other and were systematically selected so that approximately equal areas were sampled east and west of the river. The general topography of the area is flat, with a maximum elevation of 40 m above sea level and a mean annual rainfall of 400 mm (Hughes, 1990). The forest is created and maintained by groundwater, and by periodic flooding, and its lateral extent is limited to about 1 km on either side of the river (Hughes, 1990). Thus, there is no climatic variability among study forests, and the main gradient in forest community composition is decreasing density and basal area of trees with increasing distance from the main river channel (Mbora and Meikle, 2004). The intervening matrix comprises cultivation, riparian grassland, and dry shrubs.

As part of a larger demographic study of primates in the area, we surveyed each forest for all primate species, and systematically selected mangabey and colobus social groups that were easy to locate and to identify by using ''marker'' animals on subsequent surveys, as focal study groups. We have surveyed the forests periodically for primates and have monitored the study groups since 2001 (Mbora and Meikle, 2004; D. Mbora, unpubl. obs.).

Within a period of 23 days in July and August 2005, we collected fecal samples from both habituated and semihabituated free ranging study groups, by following them from 0600 to 1130 hr and then from 1500 hr until nightfall. Upon observing an animal defecate, a sample of the feces was collected and stored in a 20-ml vial containing 10% formalin as a preservative. The vial was labeled with the date, species identity, age, and sex of the monkey and a code identifying the troop. We made every effort to sample individual animals only once and to sample the populations as widely as possible. Preserved samples were subsequently transported to the Institute of Primate Research in Nairobi

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TABLE I. Density of individual monkeys and the number of different primate species found in study forests in Tana River, Kenya.

	Colobus forests		Mangabey forests	
Study forest	Density (monkeys/ha)	Total species	Density (monkeys/ha)	Total species
Congolani	1.4	3		
GuruN	4.4	4		
GuruS	3.3	4	3.3	4
MakereE	5.9	4	5.9	4
MakereW	7.3	4		
McheleloE	7.3	4		
McheleloW	2.1	4	2.1	4
MnaziniN	4.9	4	4.9	4
SifaE	2.6	4		
WenjeE			2.5	3
WenjeE2			3.1	3

where they were diagnosed for gastrointestinal parasites in August–September 2005.

We used a modified formalin-ether-sedimentation method to diagnose the presence of ova, cysts, and larval parasite stages in stool samples (Long et al., 1985). One gram of stool, fixed in 5 ml of 10% formalin, was strained through a brush wire sieve to remove course debris. Three μl of ether was then added to the filtrate, and the mixture was centrifuged at 1,610 rpm, equivalent to 500 *g* for 10 min. This process formed a floating layer of fat and fine debris, which was then removed by an applicator stick and the supernatant discarded. The remaining sediment was then thoroughly mixed using a Pasteur pipette. To stain ova and cysts, a drop of the mixture was transferred onto a glass microscope slide to which was added a drop of iodine containing 1 part Lugol's solution in 5 parts distilled water (Ash and Orihel, 1991). The mixture was then covered with a coverslip, and the total area of the coverslip was scanned with the microscope by using the 10 and ×40 lenses. We used standard gastrointestinal parasite examination and identification criteria, based on morphology and size characteristics (Ash and Orihel, 1991), to identify the different parasites. We then used the McMaster fecal floatation method to quantify the number of eggs per gram of feces for the samples determined to have various helminths (Kahn, 2005). Three grams of stool was mixed with 42 ml of supersaturated sodium salt solution, the sample was strained through a tea strainer, pipetted into the 2 chambers of the McMaster slide, and the eggs were counted after letting the suspension stand for 5 min. We calculated the number of eggs per gram of feces by using the procedure described by Kahn (2005).

We were able to identify and classify several of the parasites we detected to the species level. However, we report most of the parasites we found at the genus level, because, based on host fecal examination alone, classification of these parasites to the species level was deemed unreliable. In addition, we report differences in parasite prevalence between the sexes and between lactating and nonlactating females only for the red colobus because the mangabey sample for unambiguously sexed individuals was too small. We classified any parasites requiring intermediate hosts as exhibiting an indirect life cycle; otherwise, we classified parasites as having direct life cycles.

We used data from the larger survey of primates in the study forests to compute the density of primates and the number of primate species found in each forest. We then used a 2-sample *t*-test to determine whether the density of monkeys and the number of primate species differed between the mangabey and colobus study forests. We used a contingency table analysis and a chi-square (likelihood ratio) test to determine whether the prevalence (percentage of infected hosts; Margolis et al., 1982) of gastrointestinal parasites was a function of the host species, and among colobus, a function of the sex and lactating status among females. We further used a 2-sample *t*-test to determine whether (1) the number of parasite species in individual monkeys and the abundance of helminth eggs per gram of feces differed between the 2 species; and (2) the number of parasites in individual monkeys and the abundance of helminth eggs per gram of feces differed between colobus males and females and between lactating and nonlactating females.

RESULTS

We obtained 150 fecal samples from 15 groups of colobus in 10 forests and 82 samples from 6 groups of mangabeys in 5 forests. The density of monkeys and number of different primate species was higher in forests where colobus were studied (Table I, $t = -2.3$, 230 df, $P = 0.02$). We detected a total of 15 different parasites, 12 nematodes, and 3 protozoans in the 2 primate species (Table II). Nine parasites were identified in colobus compared with 13 in mangabeys (Table II). The number

TABLE II. Prevalence (% of individuals infected) of gastrointestinal parasites in primates of Tana River forest, Kenya.

Parasite species	*Procolobus rufomitratus*	*Cercocebus galeritus*	Life cycle
Nematoda			
Abbreviata sp.	1.1	0.0	Indirect
Ascaridia galli	1.1	0.9	Direct
Capillaria sp.	0.0	1.8	Indirect
Heterakis sp.	0.0	10.0	Direct
Oesophagostomum sp.	1.1	0.0	Direct or indirect
Physaloptera sp.	0.0	1.8	Indirect
Streptopharagus sp.	0.0	0.9	Indirect
Strongyloides fuelleborni	4.0	1.8	Direct or indirect
Toxascaris sp.	13.1	0.9	Direct
Toxocara sp.	0.0	0.9	Indirect
Trichostrongylus sp.	5.1	6.3	Direct
Trichuris trichura	6.3	15.5	Direct
Protozoa			
Entamoeba coli	16.6	20.0	Common in primates, benign
Entamoeba hystolytica	0.0	0.9	Pathogenic in humans
Entamoeba hartmani	6.9	7.3	Nonpathogenic
Overall prevalence*	48.0	57.3	

* Not the sum of the column because some individuals had multiple parasite species.

TABLE III. The number of nematode eggs per g of feces of gastrointestinal parasites in primates of Tana River forests, Kenya.

Parasite	Colobus			Mangabey		
	Mean	SD	n	Mean	SD	n
Abbreviata sp.	150.0	0.0	2			
Ascaridia galli	225.0	35.4	2	200.0		1
Capillaria sp.				100.0		1
Heterakis sp.				1045.5	358.1	11
Oesophagostomum sp.	400.0	0.0	2			
Physalaptera sp.				400.0	353.6	2
Strepropharagus sp.				150.0		1
Strongyloides fuelleborni	600.0	132.3	3	225.0	106.1	2
Toxascaris sp.	521.8	206.1	23	50.0		1
Trichostrongylus sp.	244.0	123.6	9	242.9	185.8	7
Trichuris trichura	350.0	212.1	11	425.0	232.7	14

TABLE IV. Prevalence (% of individuals infected) of gastrointestinal parasites in the Tana River red colobus, Kenya.

Parasite species	All females	All males	Lactating females	Other females
Nematoda				
Abbreviata sp.	0.9	2.1	0.0	1.1
Ascaridia galli	0.9	2.1	3.7	0.0
Oesophagostomum sp.	0.9	2.1	0.0	1.1
Strongyloides fuelleborni	3.4	6.3	0.0	4.4
Toxascaris sp.	14.4	12.5	14.8	14.3
Trichostrongylus sp.	7.6	0.0	7.4	7.7
Trichuris trichura	5.9	6.3	3.7	6.6
Protozoa				
Entamoeba coli	19.5	10.4	25.9	17.6
Entamoeba hartmani	8.5	4.2	22.2	4.4
Overall prevalence*	55.1	40.9	70.0	50.0

* Not the sum of the column because some individuals had multiple parasite species.

of different species of parasites in individual monkeys was significantly higher in mangabeys ($t = -2.29$, 230 df, $P = 0.023$), and 4 of the 5 nematodes unambiguously determined to require intermediate hosts were only found in mangabeys. In addition, the overall prevalence of parasites was higher among mangabeys, although this difference was not statistically significant ($\chi^2 = 1.85$, 1 df, $P = 0.17$; Table II). Seven parasites were found in common between the 2 species (Table II), but only 2 significantly differed in prevalence. The prevalence of *Trichuris trichiura* was significantly higher in the mangabey ($\chi^2 = 6.212$, 1 df, $P = 0.013$) and that of *Toxascaris* sp. was higher in the colobus ($\chi^2 = 17.12$, 1 df, $P < 0.0001$). Generally, the number of nematode eggs per gram of feces was very high for both species (Table III; Kahn, 2005). However, of the 3 nematodes whose prevalence was high enough to allow a statistical comparison of the number of eggs per gram of feces, only *Toxascaris* sp. was higher in colobus (Table III; $t = 4.89$, 230 df, $P < 0.00$).

Of the 9 parasite species identified in colobus, 7 were nematodes and 2 were protozoans (Table IV). There was a trend for the number of parasites to be higher in females ($t = 1.80$, 140 df, $P = 0.07$), but there was no difference in the prevalence of parasites between the sexes ($\chi^2 = 2.46$, 1 df, $P = 0.12$). Except for *Trichostrongylus* sp., which was only found in females, ($\chi^2 = 6.35$, 1 df, $P = 0.011$), there was no difference in the prevalence of individual parasites between the sexes. Four nematodes and 2 protozoans were found in lactating females (n = 20) compared with 6 nematodes and 2 protozoans found in nonlactating females (n = 78; Table IV). There was a trend for the number of parasites to be higher in lactating females ($t = -1.81$, 96 df, $P = 0.07$), but there was no difference in the prevalence of parasites between lactating and nonlactating females (Table IV; $\chi^2 = 2.64$, 1 df, $P = 0.1$). Nevertheless, *Strongyloides fuelleborni* was only found in nonlactating females, and the prevalence of *Entamoeba hartmani* was significantly higher in lactating females ($\chi^2 = 7.07$, 1 df, $P = 0.001$). The number of eggs per gram of feces did not differ for any of the parasites between males and females or between lactating and nonlactating females, but the trend was for a higher abundance of eggs per gram of feces of *Toxascaris* sp. in males compared with females ($t = -2.02$, 21 df, $P = 0.057$).

DISCUSSION

Consistent with our expectations, individual mangabeys had a higher number of parasite species, and 4 of the 5 parasites unambiguously determined to require intermediate hosts were found in mangabeys. Additionally, absolute levels of parasite prevalence were higher in mangabeys (Table II). Many factors may influence the abundance of parasites in these primates. However, the most important factor in driving the abundance of helminth parasites, which dominate the parasite fauna of these primates, is probably levels of environmental contamination. This relationship is so because these macroparasites need to develop in the environment before becoming infective; therefore, a high density of hosts as well as sympatry with related species can lead to high parasite prevalence. In addition, sociality may play an important, albeit indirect, role in determining parasite prevalence in primate hosts. Omnivorous hosts that live in large social groups must typically use a large home range to satisfy their food requirements. Such hosts, therefore, encounter more parasites' infective stages through their contact with the environment. In our 2 study species, the density of all primates and the number of sympatric species were higher in forests where colobus were sampled, yet colobus individuals had fewer parasite species, and all but 1 of the parasites requiring intermediate hosts were found in mangabeys. Although this finding suggests that differences in the prevalence of macroparasites between the 2 species may be due to the manner in which they use the forest habitat, other factors may contribute to these differences. In particular, interactions with sympatric species of primates may be an important source of infection risk (Ezenwa, 2003), whereas differences in immune responses may contribute to differential susceptibility to infection.

Evidence for the role of individual differences in determining parasite prevalence was equivocal. Just like studies of other primate species (e.g., Gillespie et al., 2005a), we found no difference in the prevalence of parasites and in the number of nematode eggs per gram of feces between the sexes, but the number of parasite species in individual monkeys tended to be higher among the females. Contrary to our expectation, we

found more parasite species in nonlactating females than in lactating females (Table IV). However, we attribute this difference to the larger number of nonlactating females sampled because lactating females had a higher prevalence of parasites.

The gastrointestinal parasite fauna of the 2 endemic Tana primates is very similar to that of forest primates found elsewhere (e.g., Munene et al., 1998; Gillespie et al., 2005a). However, our results suggest that there may be important differences between the Tana River and the other sites. For example; we examined 232 fecal samples and detected 15 species of gastrointestinal parasites from the 2 host species (Table II). In contrast, Gillespie et al. (2005a) examined 2,396 fecal samples and detected 14 species of gastrointestinal parasites in 7 primate host species. Thus, using the same sampling methods as we did and applying a sampling effort 10 times larger than ours on twice the number of species we studied, Gillespie et al. (2005a) detected a similar number of parasites species as we did. In addition, Gillespie et al. (2005b) found that the infective stages of generalist primate parasites were at much higher densities in logged forests than in unlogged forests. This finding suggests that primates living in disturbed habitats such as the Tana River forests may have a much higher risk of acquiring infections. More research is needed to determine whether the highly disturbed Tana River forest contains a higher diversity of parasites compared with less disturbed sites.

Given that primates that live in disturbed habitats may have a much higher risk of acquiring infections than those living in undisturbed habitats (Gillespie et al., 2005b), it would be useful to know whether primates living in disturbed habitats also exhibit higher levels of parasite intensity. However, parasite intensity data are almost impossible to obtain for wild endangered primates. This is so because fecal egg counts are probably the only conscientious approach to study parasite prevalence in endangered primates, yet this approach cannot give us a reliable measure of parasite intensity because many factors influence the occurrence, recognition, and numbers of parasite eggs found in a fecal sample. In particular, the number of eggs counted in a sample is not necessarily indicative of the number of worms present (Mes, 2003; Stear et al., 1996).

In conclusion, we speculate on the possible effects of habitat change on parasite prevalence among primates endangered by habitat change. In engaging in this conjecture, we are aware that the data we have presented here cannot speak to the issue of habitat change. However, this speculation is warranted as a means of identifying testable hypotheses on the effects of habitat change on the prevalence of parasites among endangered primates in future studies. Primate species endangered by habitat changes are vulnerable to increased parasite prevalence because primate social groups facilitate parasite transmission among individuals (Freeland, 1976) and because habitat change may increase parasite prevalence among primates for the following reasons. Increasing human population size, the main driver of habitat change, results in human encroachment into primate habitat, with at least 3 consequences. First, habitat loss leads to crowding of animals in habitat fragments, which increases rates of environmental contamination, creates more habitats for parasites to thrive, and thus may increase rates of parasite transfer among residents (Arneberg et al., 1998). Second, encroachment by humans into native primate habitats leads to loss of food resources for primates, which leads to deterioration of their body condition and therefore a heightened risk of acquiring infections (Eley et al., 1989). Finally, intrusion into native habitats increases the frequency of contact between humans, domestic animals, and primates, which may cause the spread of infections from humans and domestic species to primates in the wild. Thus, given the habitat changes apparent at the Tana River site (Mbora and Meikle, 2004), it is not surprising that many of the gastrointestinal parasites we detected are potentially zoonotic, epizoonotic, or anthropozootic (Table II). Thus, we strongly recommend for further studies to examine the effects of habitat change on the prevalence of parasites, and the role that parasites might play in influencing the population status of primate species threatened by habitat change.

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