Ecology of the Gastrointestinal Parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: Possible Anthropozoonotic Transmission

Julie A. Teichroeb,[1]* Susan J. Kutz,[2] Unaiza Parkar,[3] R.C. Andrew Thompson,[3] and Pascale Sicotte[1]

[1]*Department of Anthropology, University of Calgary, Calgary, Alberta, Canada T2N 1N4*
[2]*Faculty of Veterinary Medicine, Department of Ecosystem and Public Health, University of Calgary, Calgary, Alberta, Canada T2N 4N1*
[3]*WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, School of Veterinary and Biomedical Science, Murdoch University, Murdoch 6150, Australia*

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ABSTRACT Parasite richness and prevalence in wild animals can be used as indicators of population and ecosystem health. In this study, the gastrointestinal parasites of ursine colobus monkeys (*Colobus vellerosus*) at the Boabeng-Fiema Monkey Sanctuary (BFMS), Ghana, were investigated. BFMS is a sacred grove where monkeys and humans have long lived in relatively peaceful proximity. Fecal samples (n = 109) were collected opportunistically from >27 adult and subadult males in six bisexual groups and one all-male band from July 2004 to August 2005. Using fecal floatation, we detected three protozoans (two *Entamoeba* sp., *Isospora* sp.), five nematodes (*Ascaris* sp., *Enterobius* sp., *Trichuris* sp., two strongyle sp.), and one digenean trematode. Using fluorescein labeled antibodies, we detected an additional protozoan (*Giardia* sp.), and with PCR techniques, we characterized this as *G. duodenalis* Assemblage B and also identified a protistan (*Blastocystis* sp., subtype 2). The most prevalent parasite species were *G. duodenalis* and *Trichuris* sp. Parasites were more prevalent in the long wet season than the long dry. Parasite prevalence did not vary by age, and average parasite richness did not differ by rank for males whose status remained unchanged. However, males that changed rank tended to show higher average parasite richness when they were lower ranked. Individuals that spent more time near human settlements had a higher prevalence of *Isospora* sp. that morphologically resembled the human species *I. belli*. The presence of this parasite and *G. duodenalis* Assemblage B indicates possible anthropozoonotic and/or zoonotic transmission between humans and colobus monkeys at this site. Am J Phys Anthropol 140:498–507, 2009.

Parasites can influence host survival, fecundity, and the overall sustainability of wildlife populations (Anderson and May, 1978; Stien et al., 2002; Vandegrift et al., 2008). Stochastic events, combined with parasite-driven reduction in host fitness, can lead to local extinction in isolated populations (Pedersen et al., 2007; Wisely et al., 2008). Anthropogenic disturbance leading to habitat loss, crowding, contact with new reservoirs of parasites (humans and livestock), and nutritional and other stress can result in altered host–parasite dynamics (Eley et al., 1989; Stuart and Strier, 1995; Patz et al., 2000; Gillespie and Chapman, 2006; Trejo-Macías et al., 2007). Baseline measures of parasite richness, prevalence, and intensity in wild populations are thus critical in conservation biology so that the emergence of new parasites or changes in abundance or disease conditions associated with existing parasites can be detected (e.g., Hahn et al., 2003; Brooks and Hoberg, 2006).

Factors such as seasonality, host age, social rank, and changes in steroid levels can also be related to parasite richness, prevalence, and intensity in animal populations. Determining the distribution of parasites across seasons can provide insight into the seasonal patterns of transmission, the quantitative and qualitative role of individuals in transmission, and the physical and behavioral impacts on individuals and group social structure (e.g., Altizer et al., 2008). Some parasite species are common in young animals and remain so throughout life while others show high infection rates for younger individuals but decline as animals develop immunity (Scott, 1988). Nutritional condition can affect immunocompetence, so it follows that social rank may affect parasite richness and prevalence when individuals of higher rank have access to better food resources (Harland, 1965; Suskind, 1977; Bundy and Golden, 1987; Eley et al., 1989). Indeed, some studies have found that parasitized individuals are less likely to be dominant (e.g., mice, Freeland, 1981; red-jungle fowl, Zuk et al., 1998), although this may be due to slower growth and less development of secondary sexual characteristics caused either by parasitism, the lack of access to resources, or both (Hamilton and Zuk, 1982; Møller, 1990; Clayton, 1991; Buchholz, 1995). The contests involved in the loss or

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*Correspondence to: Julie A. Teichroeb, Department of Anthropology, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada T2N 1N4. E-mail: jateichr@ucalgary.ca

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acquisition of rank between individuals can cause a rise in some immunosuppressive hormones (i.e., testosterone and/or cortisol), which may lead to an increase in parasite richness, prevalence, and intensity within hosts (Goymann and Wingfield, 2004; Muehlenbein, 2006). In primates the relationships between seasonality, host age, social rank, and steroids on parasitism have rarely been investigated and when they have, mixed results have been reported (e.g., File et al., 1976; Hausfater and Watson, 1976; Eley et al., 1989; Muller-Graf et al., 1996; Huffman et al., 1997; Stuart et al. 1998; Gotoh, 2000; Gillespie et al., 2004, 2005; Muehlenbein, 2006; Mul et al., 2007).

In this paper, we describe the gastrointestinal parasites of male ursine colobus (*Colobus vellerosus*) based on fecal surveys done at the Boabeng-Fiema Monkey Sanctuary (BFMS) in Ghana, and investigate whether seasonality, host age, social rank, or time spent near human settlements are related to parasite prevalence or richness. Ursine colobus are becoming rare in much of their range due to hunting and habitat loss (Saj and Sicotte, in press) and are in danger of local extinction at several sites, however the population of *C. vellerosus* at BFMS face a somewhat different situation. BFMS is a sacred grove where the monkeys are traditionally protected through local taboos and federally protected by a hunting bylaw (Saj et al., 2005). This has resulted in a relatively high population density (119 ind./km^2, Wong and Sicotte, 2006) at this site and the population is currently increasing (B.O. Kankam, unpublished data). *C. vellerosus* at BFMS live in close proximity to humans, their livestock, and Campbell's mona monkeys (*Cercopithecus campbelli lowei*). There is, therefore, the potential for frequent parasite transmission not only within the colobus population but also among them, people, and the mona monkeys. We thus predicted that male *C. vellerosus* at this site would show high parasite richness and the presence of zoonotic species, especially when they spend more time near human settlements. Parasite species were expected to be more prevalent in younger animals. Rank related differences in parasite richness or prevalence related to nutritional condition were not expected because the folivorous diet of *C. vellerosus* is relatively ubiquitous and scramble competition predominates (Saj and Sicotte, 2007; Teichroeb and Sicotte, 2009). However, stress-induced differences were expected when males changed social rank because these are times of high male-male aggression with associated peaks in testosterone levels (Teichroeb and Sicotte, 2008).

METHODS

Study subjects and site

The ursine colobus (*C. vellerosus*) is a medium sized (male: 8.5 kg, female: 6.9 kg, Oates et al., 1994), arboreal monkey endemic to West Africa. It is one of five species of black-and-white colobus in Africa and is most closely related to *C. polykomos* (Ting, 2008). Research on *C. vellerosus* has been conducted at the Boabeng-Fiema Monkey Sanctuary (BFMS), central Ghana (7° 43′ N and 1° 42′ W) under the direction of P. Sicotte since 2000. This is a dry semi-deciduous forest fragment, 191.6 ha in size, located 350 m above sea level in the Nkoranza district of the Brong-Ahafo Region. BFMS is surrounded by farmland but connects to several smaller forest fragments in the area by a narrow, riparian forest. The vegetation at BFMS is a mosaic of primary forest, regenerating farmland (secondary forest), and woodland (Fargey, 1991; Saj et al., 2005). Nineteen bisexual groups of *C. vellerosus* reside at the site with a growing population of Campbell's mona monkeys (*Cercopithecus campbelli lowei*) (B.O. Kankam, unpublished data). Group composition is uni-male/multi-female, multi-male/multi-female and all-male bands (AMB's) (Wong and Sicotte, 2006). At BFMS, *C. vellerosus* is mainly folivorous, with leaves representing 79–89% of the diet (Saj and Sicotte, 2007; Teichroeb and Sicotte, 2009). Although this species is primarily arboreal, individuals make forays to the ground most days to feed on low vegetation and sometimes soil.

Annually, there are two rainy seasons and two dry seasons at BFMS. The long rains last from approximately March to July and there is a short rainy season in September. There is a short dry season in August and a prolonged one from November to February. The mean annual rainfall from 1985 to 1990 was 1,250 mm (SD: ±21.1; taken in Nkoranza, approx. 20 km from BFMS; Fargey, 1991). During the study, rainfall was monitored daily from a rain gauge <1 km from the range of all the study groups. The annual rainfall at BFMS during this time (July 2004 to June 2005) was 1329 mm (monthly range: 0.4–227.6 mm), with 53% of the precipitation falling in the long rainy season.

Behavioral data and fecal sample collection

Fecal samples were collected opportunistically from 27 individually recognized male *C. vellerosus* in six bisexual groups (RT, B2, DA, WW, OD, and SP) and five samples were taken from unknown males in one AMB during 13-months of observation (July–November 2004, January–August 2005). In all, 109 fecal samples were collected representing at least 20 adult males (>7 years old, n = 74 samples) and 10 subadult males (3–7 years old, n = 30 samples) (the age of AMB males was unknown). Males were classified as subadult when they were smaller or the same size as adult females (range: 3–7 years old) while males were adult (>7 years old) when they achieved full body size and regularly participated in loud call bouts with other adult males. Three males are represented in both the subadult and adult male categories because they matured during the study. Samples from these males were excluded from the comparison of parasite prevalence in adult versus subadults males. We collected a mean of 4 (±3) and a median of 3 (range = 13) fresh fecal samples per male. Immediately after defecation, samples were collected and stored in glass vials in 70% ethanol until they could be transported to the University of Calgary, Faculty of Veterinary Medicine for analysis.

Behavioral data were recorded in four of the groups for which fecal samples were collected (RT, B2, DA, and WW). Group composition varied and each study group was followed for two, two-day periods per month from dawn to dusk (6:00 am to 6:00 pm) (211 days, 2547 contact hours, 433.3 focal-hours) (Table 1). Behavioral observations were done using 10-min focal samples that were alternated among adult and subadult individuals. Ranging scans were taken every 30-min during follows to record all trees occupied by the group relative to 50 × 50 m quadrats on a map of the fieldsite (n = 4950 location scans, RT: 1181 scans; B2: 1166; DA: 1213; WW: 1390). Ad libitum data collection was employed to record rare behaviors (Altmann, 1974). Male dominance rankings

TABLE 1. Study group composition and duration of observations

Name	Group Size	Adults		Subadults		Juveniles/infants	Contact hours[a]	Focal hours
		M	F	M	F			
RT	13	1	5	1	1	5	567.5	106.5
B2	13–17	1–3	4	2–4	0–1	4–5	574.5	102
DA	21–31	3–8	9–10	3–5	1–3	4–5	574	99.8
WW	28–33	6–10	10–11	2–6	2–3	2–5	690	125
AMB	4–10	0–3	0–1	4	0–2	0	101	0
OD	18	1	8	0	4	5	20	0
SP	7	1	4	0	0	2	20	0
Totals							2547	433.3

[a] Including JAT and research assistants.

were determined from the direction of aggressive displacements and submissive and avoidance behaviors during focal samples and ad libitum data collection. Dominance relationships within each group were linear and males could be assigned a number ranking.

Parasitological analyses

Fecal flotation. Floatation in Sheather's solution was done to count helminth eggs, larvae, and protozoans. One to two grams of feces was washed with water and filtered through two layers of cheesecloth, it was then centrifuged with water in a 16 × 100 mm tube for 10 min at 1500 rpm The supernatant was decanted and the sediment was vortexed in 5 ml of Sheather's solution (specific gravity = 1.26). Sheather's solution was then added to the tube until a convex meniscus formed. A coverslip was placed on the meniscus and the tube was centrifuged for 10 min at 1500 rpm. After centrifuging, the coverslip was pulled straight up and transferred to a labeled slide. The slide was scanned under 100× magnification. Helminth eggs and protozoan cysts were identified based on their size and morphology. Photographs and measurements were taken using an ocular micrometer fitted to a compound microscope and Infinity Analyze imaging software (Lumenera Corp., Ottawa, ON).

Fluorescein-labeled antibodies. We tested for the presence of *Giardia* sp. and *Cryptosporidium* sp. cysts and oocysts using an Aqua-Glo G/C direct comprehensive kit (Waterborne, Inc., New Orleans, LA). These kits bind a fluorescein labeled antibody to the cysts/oocysts. Feces were centrifuged for 10 min in 16 × 100 mm tubes at 1500 rpm. A small amount of the sediment was smeared on the slides and dried. Slides were then prepared and mounted according to the manufacturer's directions and observed under 400× magnification using a fluorescence microscope. Samples were compared to positive controls to identify the "apple-green" glow and specific shape of cysts/oocysts. Presence/absence of *Giardia* sp. and *Cryptosporidium* sp. cysts was determined for each sample.

DNA extraction and PCR amplification. Ten of the samples positive for *Giardia* sp. were shipped to the School of Veterinary and Biomedical Science, Murdoch University, Australia, to test for the presence of *Blastocystis* sp. and to type both *Giardia* sp. and *Blastocystis* sp. To type the *Giardia* sp., DNA was extracted from fecal samples using QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol, with the modifications mentioned in Parkar et al. (2007). A fragment of the SSU rDNA for *Giardia* was amplified by a nested PCR using previously described primers. The primary reaction utilized the forward primer, RH11 (5′-CAT CCG GTC GAT CCT GCC-3′) and reverse primer, RH4 (5′-AGT CGA ACC CTG ATT CTC CGC CAG G-3′) (Hopkins et al., 1997). The primers, GiarF (5′-GAC GCT CTC CCC AAG GAC-3′) and GiarR (5′-CTG CGT CAC GCT GCT CG-3′) described by Read et al. (2002) were used in the secondary reaction. Both reactions were performed under conditions described by Santín et al. (2007). For the sequencing analysis, PCR products were also purified from reactions using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI) according to the manufacturer's kit protocol. The PCR products were sequenced in both directions using an ABI 3730 capillary sequencer. Sequences were analyzed using FinchTV and compared with previously published sequences from GenBank using the BLAST 2.2.9 program (http://www.ncbi.nlm.nih.gov/blast).

Three different nested PCRs were used to amplify *Blastocystis* SSU rDNA. In all three nested PCRs, the primary PCR utilized previously published forward and reverse primers (RD3, 5′-GGG ATC CTG ATC CTT CCG CAG GTT CAC CTA C-3′; RD5, 5′-GGA AGC TTA TCT GGT TGA TCC TGC CAG TA-3′) for PCR amplification under the conditions described by Clark (1997). The secondary PCRs utilized one of the previously published forward and reverse primers under the conditions described in Bohm-Gloning et al. (1997), Stensvold et al. (2006), and Wong et al. (2008). To sequence and phylogenetically analyze *Blastocystis*, bands representing amplified PCR products were excised from a gel and purified using the UltraClean GelSpin DNA Purification Kit (MO BIO Laboratories, Inc., Carlsbad, CA). Manufacturer's kit protocols were followed, except that DNA was eluted using 30 μl of ultrapure PCR water and incubated at room temperature for 10 min prior to centrifugation at 10,000 *g* for 30 s. PCR products were also purified from reactions using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI) according to the manufacturer's kit protocol. The PCR products were sequenced in both directions using an ABI 3730 capillary sequencer. Sequences were analyzed using FinchTV and compared with previously published sequences from GenBank using the BLAST 2.2.9 program (http://www.ncbi.nlm.nih.gov/blast).

Data analyses

We report both the prevalence and richness of intestinal parasite species. Prevalence is defined as the number of hosts or samples infected with a particular species

TABLE 2. Prevalence[a] of gastrointestinal parasites for Colobus vellerosus at BFMS

Species	Mean size (μm)	Prevalence (%)	
		Males (n = 26)	All Samples (n = 109)
Protistans			
Blastocystis sp.	NA	37.5[b]	30[b]
Protozoans			
Entamoeba histolytica/dispar	19.6 (±1.6) × 19.1 (±1.8) (n = 9)	23.1	7.3
Entamoeba coli	26 (±2.5) × 25.8 (±2.5) (n = 4)	15.4	4.6
Isospora sp.	26.5 (±3.1) × 12.1 (±1.1) (n = 30)	15.4	6.4
Giardia duodenalis	6.55 (±0.6) × 4.86 (±0.6) (n = 38)	88.5	69.2[c]
Nematodes			
Ascaris sp.	73.4 (±9.4) × 61.6 (±6.6) (n = 2)	11.5	2.8
Enterobius sp.[d]	52.4 (±8.4) × 28.5 (±6.5) (n = 4)	7.7	1.8
Strongyle sp. 1	69.5 (±6.2) × 37.8 (±2.2) (n = 4)	15.4	3.7
Strongyle sp. 2	84.7 (±3.9) × 47.6 (±4.1) (n = 3)	11.5	2.8
Trichuris sp.	55.4 (±3.2) × 25.8 (±2.4) (n = 38)	84.6	80.7
Trematodes			
Digenean trematode[d]	40.4 (±3.6) × 26.5 (±1.7) (n = 7)	26.9	7.3

[a] # positive samples or hosts per # samples or hosts examined.
[b] n = 8 males and 10 samples for *Blastocystis* sp.
[c] n = 107 samples for *G. duodenalis*.
[d] Reported prevalence estimates for *Enterobius* sp. and the digenean trematode are likely underestimates because an invasive tape test and sedimentation, respectively, were not performed to determine the most reliable prevalence values (see Gillespie, 2006).
NA, not available.

divided by the number of hosts or samples examined (Margolis et al., 1982; Muehlenbein, 2005). Richness is the number of individual parasite species present in the host's fecal samples. For each parasite species, the prevalence within all samples was compared to the prevalence within individual males (i.e., the proportion of males that had at least one positive sample) with a Wilcoxon signed rank test.

To determine whether the presence of any parasite species was seasonal we estimated that approximately 1 month would be needed for the parasite species present in fecal samples to react to environmental changes in rainfall (i.e., the number of eggs or cysts in the environment must increase, males must get infected, and the parasites must begin to reproduce within the host). There are two wet and two dry periods at BFMS. If this seasonality has an influence on parasite prevalence, it is expected that it will be particularly obvious during the long seasons (rainfall: long wet = 699 mm; long dry = 122 mm). We therefore compared the prevalence of each parasite species in the long wet season (March 2005 to July 2005) versus the long dry season (November 2004 to February 2005), excluding data from the first month of each season, using a Wilcoxon signed-rank test. Paired comparisons between the seasons within individual males for each parasite species were also done using Wilcoxon signed-rank tests.

Except when otherwise mentioned, only samples from the long wet season (still excluding the first month of rains) were used in the rest of the analyses. To determine if there were age differences in parasite prevalence, adult and subadult males were compared for their proportion of positive samples for each parasite species using Mann-Whitney U tests. The effect of social rank on parasites was investigated in two ways: (1) in males for which rank had not changed during the study, a Pearson correlation was run to see if their average parasite richness (no. of species/no. of samples) was correlated with their rank; (2) for males that changed rank during the study, a Wilcoxon signed-rank test was used to see whether they differed in their average parasite richness at higher versus lower ranks. Since males changed rank in all seasons, this comparison was done using samples from the entire year (Table 3), using only males that changed rank in a single season. To determine whether time spent near human settlements influenced the presence of any parasite species we used Fisher's exact tests. The four focal groups showed a clear division where two groups (B2 and DA) spent <2% of their time near (within 50 m) the village of Boabeng whereas the other two groups (RT and WW) spent >10% of their time there. We thus divided groups for analysis as to whether they spent a small proportion of time near the village of Boabeng (<10%) or a larger proportion of time (≥10%) and the presence or absence of each parasite species for each male was organized into 2 × 2 contingency tables. Tests were two-tailed and alpha levels were set at $P \leq 0.05$. Tests were done using SPSS 15.0, by hand, or using Preacher and Brigg's (2001) interactive tool for Fisher's exact tests.

RESULTS

A minimum of 11 species of intestinal parasites including a protistan, four protozoans, five nematodes, and one trematode species were found in the feces of *C. vellerosus* at BFMS (Table 2). *Blastocystis* sp. (Stramenopiles: Blastocystidae), a protistan parasite, was found in three of 10 samples tested and was identified as subtype two. For the other parasite species, 107–109 samples were tested. Two different *Entamoeba* species (Protista: Entamoebidae) were present (Table 2) (see Fig. 1). The smaller type (mean size: 19.6 × 19.1 μm, 1–4 nuclei, n = 9) is likely *E. histolytica* or *E. dispar* and 23.1% of males were infected with this type. The larger species (mean size: 26 × 25.8 μm, 1–4 nuclei, n = 4) is likely *E. coli* and 15.4% of males had this type. The other two protozoan species found were *Giardia duodenalis* (Protista: Hexamitidae) and *Isospora* sp. with 0–4 sporocysts (Apicomplexa: Eimeriidae). *Giardia duodenalis* was typed as Assemblage B.

The five different nematode species found were *Ascaris* sp. (Ascarididae), *Trichuris* sp. (Trichuridae), *Enterobius* sp. (Oxyuridae) and two strongyle species (Strongylidae). Strongyle eggs were grouped into two different size categories with mean sizes of 69.5 × 37.8 μm (n = 4) and

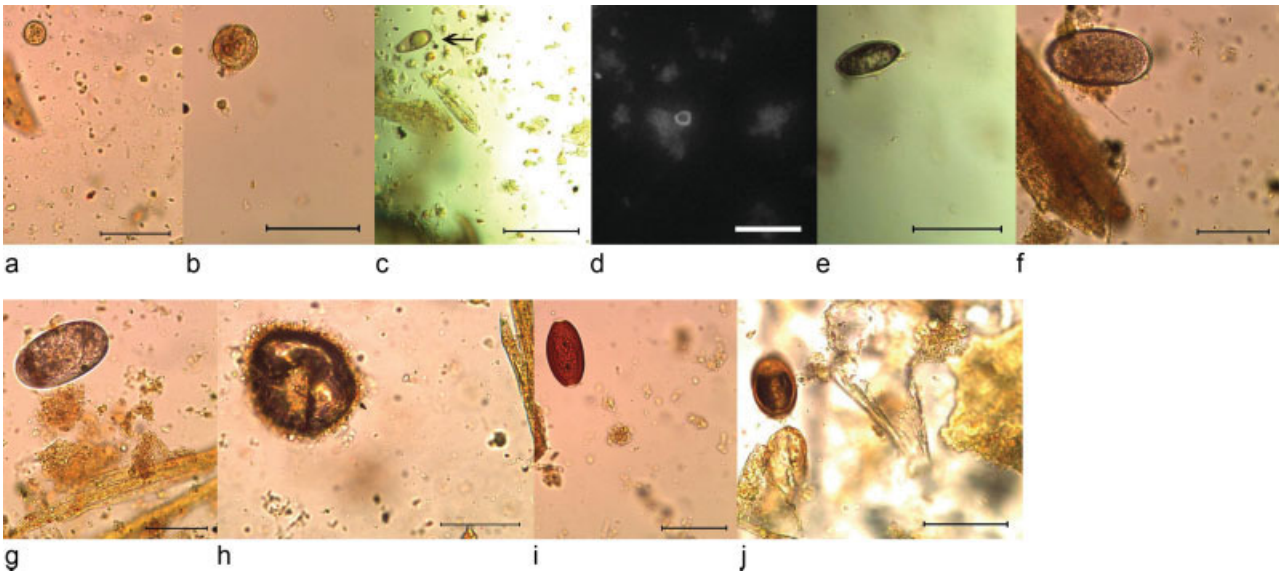

Fig. 1. Photos of the parasite eggs and cysts found in *C. vellerosus* feces, not including *Blastocystis* sp.; (**a**) *Entamoeba histolytica/dispar*; (**b**) *Entamoeba coli*; (**c**) *Isospora* sp. (see arrow); (**d**) *Giardia* sp.; (**e**) *Enterobius* sp.; (**f**) Strongyle 1; (**g**) Strongyle 2; (**h**) *Ascaris* sp.; (**i**) *Trichuris* sp.; (**j**) Digenean trematode egg; all scale bars are 50 μm except for (d) where it is 25 μm; an arrow is used in (c) to point out the *Isospora* sp. oocyst.

84.7 × 47.6 μm (n = 3). It is difficult to identify strongylids to genus and species based solely on their egg morphology (Goldsmid, 1991). However, the smaller of these eggs fell within the size range for an *Oesophagostomum* species reported in other black-and-white colobus (*C. guereza*, 70.2 ± 1.8 × 41.6 ± 1.6 μm, Gillespie et al., 2005), and the larger of the strongylid eggs fell within the size range of *Ternidens deminutus* (70–94 × 47–55 μm; Goldsmid, 1967), which was found in a *C. c. lowei* monkey from BFMS (Schindler et al., 2005). We also found eggs of a digenean trematode (Platyhelminthes) species in the feces of *C. vellerosus* but we were unable to identify it to the genus level.

For every parasite species, prevalence was higher in individual hosts than among samples (n = 11, W = 66, P = 0.004) (Table 2). *Giardia duodenalis* and the whipworm species (*Trichuris* sp.) were the most common parasites, with most males infected with *G. duodenalis* (23/26 or 88.5%) and *Trichuris* (22/26 or 84.6%) at some point during the study.

Seasonality

A total of 56 samples were available from the long wet season and 12 from the long dry. Seasonal prevalence could not be tested for *Blastocystis* sp. but for every other species the proportion of positive samples was greater in the wet season (see Fig. 2). This seasonal difference in prevalence was significant (n = 10, W = 55, P = 0.005). Paired comparisons from eight individual males were available from the long wet and dry seasons; however most males were not infected with many parasites, which lead to a high frequency of zeros and thus many ties in the Wilcoxon signed-rank tests. This lowered the n and made comparisons impossible for nine of 10 parasite species. A comparison was only possible for *G. duodenalis*, which had a significantly higher prevalence in the wet season than in the dry season (n = 6, W = 21, P = 0.05).

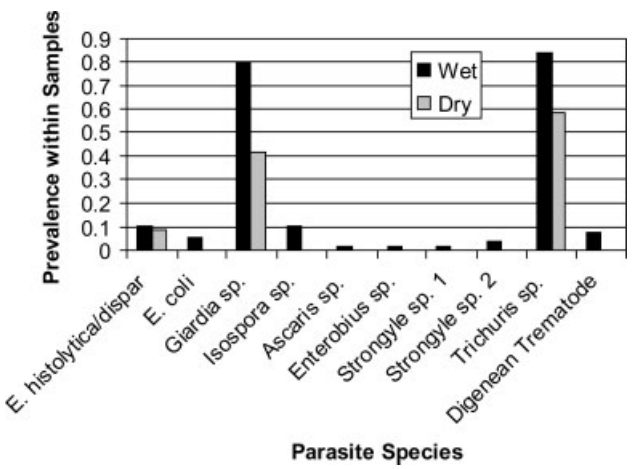

Fig. 2. The prevalence of each parasite species, except *Blastocystis* sp., in samples taken during the long wet and long dry seasons, excluding the first month of each (wet: April 2005 to July 2005, n = 56; dry: January 2004 to February 2005, n = 12). The difference in parasite prevalence between the seasons was significant (n = 10, W = 55, P = 0.005).

Host age

There were no significant differences in prevalence for any parasite species between adult and subadult males in the long wet season (n_{adults} = 13, $n_{subadults}$ = 6, *Entamoeba histolytical dispar* U = 43, P = 0.76; *Entamoeba coli* U = 39.5, P = 1; *G. duodenalis*: U = 33, P = 0.63; *Isospora* sp.: U = 27, P = 0.31; *Ascaris* sp.: U = 33, P = 0.63; *Enterobius* sp.: U = 39, P = 0.97; Strongyle 1: U = 36, P = 0.83; Strongyle 2: U = 43, P = 0.76; *Trichuris* sp.: U = 54, P = 0.2; Digenean trematode: U = 42, P = 0.83; *Blastocystis* was not testable).

TABLE 3. Average parasite richness (no. of parasite species/sample) for males that changed rank during the study

Male	Group	Original → new rank	Rank change	Average parasite richness at high and low rank (*n* in brackets = no. of samples)		Process of rank change	Season
				High	Low		
Fi	B2	2 → 3	Decrease	2 (1)	1.3 (3)	Sudden, new alpha male *Lo* enters group, little increase in aggression to *Fi*	Long dry
Li	B2	6 → 4	Increase	0.5 (8)	1 (2)	Sudden, new alpha male *Wo* enters and evicts 4 males, no increase in aggression to *Li*	Short wet
Wo	B2	2 → 1	Decrease	1 (2)	1 (4)	Sudden, new alpha male *Lo* enters group, increased aggression with *Lo*	Long dry
Do	DA	2 → 1	Increase	1 (1)	2 (1)	Gradual, 1-month period; he increased aggression to the alpha male (*Cy*) before injuring him	Long dry
Cl	WW	2 → 5	Decrease	0.5 (2)	1.5 (2)	Sudden, *Cl* is badly injured after fighting with *Q*	Long wet
Jr	WW	3 → 4	Decrease	1.3 (8)	2 (1)	Gradual, 1-month period where male *Cl* enters the group and pushes *Jr* down in rank, some aggression with *Cl*	Long dry
Q	WW	3 → 2	Increase	1.3 (3)	3 (3)	Sudden, rises in rank after badly injuring *Cl*	Long wet

Host rank

For males whose rank remained unchanged during the study, average parasite richness was not found to correlate with their position in the hierarchy during the long wet season ($n = 14$, $r = -0.33$, $P = 0.25$). However, when males changed rank during the study ($n = 7$), which happened in most seasons (Table 3), they showed a trend for higher average parasite richness when they were lower ranked compared with when they were higher ranked ($n = 6$, $W = -17$, $P = 0.09$). These males varied though in the direction, process, and speed at which rank changes occurred (Table 3).

Time spent near human settlements

The groups varied in the proportion of ranging scans that they spent within 50 m of human settlements, in this case, the village of Boabeng. Individuals of RT and WW spent a greater proportion of time both near and within the village than those in B2 or DA (RT: 13% of scans, WW: 12.9%, B2: 1.6%, DA: 0.6%). Fisher's exact tests revealed that only the presence of *Isospora* sp. was significantly higher for males that spent ≥10% of their time near the village in the long wet season ($n_{<10\%\ \text{time}} = 10$, $n_{\geq 10\%\ \text{time}} = 9$, *Entamoeba histolytical dispar* $P = 0.30$; *Entamoeba coli* $P = 1.0$; *G. duodenalis*: $P = 1.0$.; *Isospora* sp.: $P = 0.03$; *Ascaris* sp.: $P = 1.0$; *Enterobius* sp.: $P = 1.0$; Strongyle 1: $P = 0.47$; Strongyle 2: $P = 0.74$; *Trichuris* sp.: $P = 0.18$; Digenean trematode: $P = 0.09$).

DISCUSSION

This study is the first survey of the gastrointestinal parasites of *C. vellerosus*. Most previous studies on other black-and-white colobus species have found a high prevalence of *Entamoeba* and *Trichuris* spp. *Ascaris* sp., *Enterobius* sp., strongylid worms, and a digenean trematode have also been reported before in black-and-white colobus (Table 4, Bakarr et al., 1991; Gillespie et al., 2005; Okanga et al., 2006). *Blastocystis* sp. has not been reported previously in black-and-white colobus monkeys but is proving to be common in primates and the subtype found here (2) is the same as that characterized for primates in the Perth Zoo (Parkar et al., 2007). Studies of the parasites of black-and-white colobus vary, however, in their methods of preservation and analysis which makes it difficult to compare prevalence among them (Table 4). The use of ethanol as a preservative in this study may also have lead to an underestimation of the prevalence of some parasites.

Increasing human populations and the close phylogenetic relationships between humans and nonhuman primates means that the transfer of pathogens (parasites, bacteria, and viruses) often occurs between species, sometimes with devastating consequences (e.g., HIV/AIDS, Gao et al., 1999; polio, respiratory diseases, Hill et al., 2001; scabies, Kalema-Zikusoka et al., 2002; Ebola, Leroy et al., 2004; bacteria, Goldberg et al., 2008). Many of the parasite species found in colobus in this study have zoonotic potential (e.g., *Blastocystis* sp., Noël, 2005) and others may have originated in people. The finding of *G. duodenalis* Assemblage B and *Isospora* sp. in ursine colobus monkeys suggests that these parasites may circulate among the humans and nonhuman primate populations at and around BFMS. *Giardia* in wild primates has been linked to increased contact with humans and livestock (*Gorilla beringei beringei*, Nizeyi et al., 1999, 2002; *Alouatta pigra*, *G. duodenalis* Assemblages A and B, Vitazkova and Wade, 2006) and may be found more often in primates in disturbed forest fragments than in forest blocks (Salzer et al., 2007). *C. vellerosus* at BFMS reside in a forest fragment where they are in daily contact with humans, sheep, chickens, Campbell's mona monkeys and their waste. This may explain why they are heavily infected with *G. duodenalis* Assemblage B. Assemblages A and B are thought to have evolved in human-canine-livestock cycles and then

TABLE 4. Comparison of the prevalence (within samples) of parasite genera in studies of black-and-white colobus

Parasite genera	*Colobus angolensis*[a] (n = 19)	*Colobus angolensis palliatus*[b] (n = 74)	*Colobus guereza*[a] (n = 476)	*Colobus polykomos*[c] (n = 45)	*Colobus vellerosus*[d] (n = 109)
Protistans					
Blastocystis sp.	*	*	*	*	30[e]
Protozoans					
Entamoeba histolytica/dispar	11	89	8	*	7
Entamoeba coli	16	97	8	*	5
Giardia duodenalis	*	*	*	*	69
Isospora sp.	*	*	*	*	6
Nematodes					
Ascaris sp.			1		3
Enterobius sp.[f]		1	1		2
Necator sp.				2	
Strongyloides sp.	5	9	4	19	
Oesophagostomum sp.			6		
Trichuris sp.	100	9	79	49	81
Unidentified strongyle	11		1		7
Trematodes					
Digenean trematode			1		8
Cestodes					
Bertiella sp.			0.2		

[a] Gillespie et al., 2005, Lake Nabugabo, Uganda, methods: 10% formalin storage, floatation, sedimentation and coproculture.
[b] Okanga et al., 2006, Diani Forest, Kenya, methods: 10% formalin storage, formol–ether sedimentation technique and coproculture.
[c] Bakarr et al., 1991, Tiwai Island, Sierra Leone, methods: 10% formalin storage, fecal smear and coproculture.
[d] This study.
[e] n = 10 for *Blastocystis* sp.
[f] Possibly subgenus *Colobenterobius*.
* Methods may not have allowed detection of these species.

spilled over into wildlife, now transferring freely from humans to wildlife and vice-versa (Thompson, 2004; Appelbee et al., 2005; Kutz et al., 2009). The presence of *G. duodenalis* Assemblage B in *C. vellerosus* at BFMS suggests the circulation of this parasite between humans and monkeys at this site. The relative contribution of, and impact on, each host species requires further exploration.

While common in New World primates, this is the first study to report *Isospora* sp. in an African monkey or a colobine. This may be related to fecal storage techniques; we used ethanol to store fecal material, whereas studies using formalin or PVA (10% buffered formalin and polyvinyl alcohol; Table 4) would not see coccidian oocysts because they are destroyed (Duszynski et al., 1999). The size range (25–30 × 12–15 μm, Wenyon, 1923) and elongated shape of the oocysts found in this study most closely resemble *I. belli*, the human form of *Isospora* rather than the nonhuman primate forms known (listed in: Lindsay et al., 1997 and Duszynski et al., 1999). Gibbons have been successfully infected with *I. belli* (Zamen, 1967) and captive populations of ring-tailed lemurs have also tested positive (Villers et al., 2008), so infection of monkeys may also occur. *Isospora* sp. was the only parasite that was more common in animals that spent a greater proportion of time near human settlements. The colobus at BFMS often come to the ground to forage or move between forest patches. In doing so, they sometimes run through areas that are used by humans as latrines. It is, therefore, possible that that *C. vellerosus* at BFMS are infected with the human form of *Isospora*. Further investigation is needed to determine the identity of this *Isospora* and possible transmission routes between people and colobus.

A number of studies of tropical forest monkeys and apes report higher prevalence for some parasite species during the wet season (Freeland, 1977; Huffman et al., 1997; Setchell et al., 2007; Rothman et al. 2008) while others report no seasonal patterns (Gillespie et al., 2004, 2005). Differences may depend on the level of change from one season to another in different habitats. In this study, parasite species were generally more prevalent in wet season (see Fig. 2). Our finding of a higher prevalence of *G. duodenalis* in individual males in the long wet compared with the long dry season is consistent with the waterborne mode of this parasite (Rendtorff and Holt, 1954). Ursine colobus often drink from natural wells in tree trunks that stay for the duration of the rainy season. Being below the canopy, these wells may contain colobus feces and that of Campbell's mona monkeys, squirrels, and birds. Run-off during the rains is also likely to overflow latrines and spread human (and monkey) feces over the ground increasing the risk of exposure. Eggs and cysts probably survive better in a wet environment as would the intermediate mollusc host required by digenean trematodes (Patz et al., 2000). Seasonality in parasite prevalence, richness, and intensity could have important implications for studies of primate behavior (e.g., Altizer et al., 2008). For example, if individuals are weakened by multiple infections in wet seasons, then rank upheavals, male takeovers, and other social changes may occur more often during these times. The wet season is also the time with the highest intake of mature leaves for *C. vellerosus* at BFMS (Saj and Sicotte, 2007), which is a lower-quality food source (McKey et al., 1981; Baranga, 1983). A diet high in mature leaves may exacerbate infections because it provides less nutrition. In addition, individuals may be more exposed to parasites when feeding on mature leaves in the wet season because defecation high in the canopy leads to feces splattering while falling (J.A.T., personal observation), which could contaminate leaf mat-

ter. Mature leaves are more likely to be contaminated than young leaves because the latter occur at the tips of terminal branches.

No consensus seems to have been reached about the prevalence of infections for younger versus older individuals in primates. Several studies have found no age differences in infections (*Pan troglodytes schweinfurthii*, File et al., 1976; Muehlenbein, 2005; *Pongo abelii*, Mul et al., 2007), while others have found that older individuals are more likely to be infected with certain parasites than younger individuals (*Alouatta palliata* with *Controchis biliofilous*, Stuart et al., 1998; *Gorilla beringei beringei* with *Cryptosporidium* sp., Nizeyi et al., 1999; female *Mandrillus sphinx* with nematodes, Setchell et al., 2007) or that infection rates are higher in juveniles for certain parasites (*Strongyloides fulleborni, Streptopharagus pigmentatus*, and *Trichuris trichiura* for *Macaca fuscata*, Gotoh, 2000). We did not find a difference in the prevalence of parasite species between adult and subadult males in this study. This might reflect an acquired immune response in the subadults (3–6 years of age). Investigation of the parasite prevalence for juveniles or infants is necessary before we can reach a conclusion.

Dominance rank did not correlate with parasite richness for the males in this study whose rank did not vary. However, five of the seven males whose rank changed and who showed a difference in parasite richness between their two ranks had higher parasite richness when they were lower ranked compared to when they were higher ranked, despite variation in the direction, timing, level of aggression, and/or method of the rank change (Table 3). This could be due to the increases in testosterone (fecal testosterone (fT), Teichroeb and Sicotte, 2008) (and perhaps cortisol) that occur during episodes of male-male aggression when the male hierarchy is unstable. Testosterone and cortisol have known immunosuppressive effects (Grossman, 1985; Grossman et al., 1991; Folstad and Karter, 1992; Sapolsky, 1993; Goymann and Wingfield, 2004; Chapman et al., 2006), so stressful, aggressive events could be followed by greater infection/parasite shedding rates for males. Aggression may be more likely to be directed at lower ranking males and could explain why we found greater parasite richness for males when they were lower ranking, regardless of whether the rank change had been an increase or a decrease. However, the direction of causation could also be called into question. It is possible that males that became infected with multiple parasites tended to drop in rank or those with fewer parasites tended to increase in rank because they were in better health. Once male-male aggression ceases in ursine colobus, fT levels decrease to baseline levels and do not correlate with rank (Teichroeb and Sicotte, 2008). Cortisol may follow the same pattern, giving the immune system of males the chance to recover and stabilize in their new position in the male hierarchy. This may explain why we found no correlation between rank and parasite richness when males occupied a stable rank. Further research, with more samples collected around the time of rank changes, is necessary to analyze the causal relationships between steroids and parasites in situations of social change.

Areas of future research

This first survey of the gastrointestinal parasites of *C. vellerosus* found 11 species and high prevalence of *G. duodenalis* and *Trichuris* sp. Both parasite richness and prevalence may have been underestimated though, given that ethanol was used as a fixative. Preservation and analysis techniques are important for understanding and interpreting the results of parasite studies. Currently primatologists are using a variety of methods to analyze parasites within their study animals. In order to optimally compare studies of parasites within primates, primatologists should align their methods (e.g., Gillespie et al., 2008). Most studies sample randomly within a population, but as we were able to show in this study with known individuals, this can lead to a drastic underestimation of the actual prevalence of most if not all parasite species (see also Huffman et al., 1997 for a similar finding).

This study also touches on fascinating areas for future research in primatology. The seasonality of parasite infections and the potential social consequences have not yet received attention. In addition, it is interesting that an effect of changing dominance rank on parasite richness was observed in this study despite the small sample size. The relationships between social interactions, steroids, immune responses, and parasite infections have rarely been studied in the primates (but see Muehlenbein, 2006) and this represents a large gap in our knowledge.

Finally, since two of the parasite species infecting *C. vellerosus* in this study appear to be of human origin (*G. duodenalis* and *Isospora* sp.) and several others have zoonotic potential, a survey of the gastrointestinal parasites of Campbell's mona monkeys and humans at BFMS would prove informative to document the host–parasite interactions at this site. Indeed this would provide a situation to explore the relative contribution of the different hosts to the epidemiology of these parasites in disturbed ecosystems and at the interface of domestic animals, wildlife, and people.

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