

## PREVALENCE OF *CRYPTOSPORIDIUM* AND OTHER ENTERIC PARASITES AMONG WILD NON-HUMAN PRIMATES IN POLONNARUWA, SRI LANKA

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**Abstract.** Cryptosporidiosis is a rapidly emerging disease in the tropics. This is the first report of *Cryptosporidium* and other protozoan infections (*Entamoeba* spp., *Iodamoeba*, *Chilomastix*, and *Balantidium* spp.) in wild primates that inhabit the natural forest of Sri Lanka. It is unclear if non-human primates serve as a reservoir for these parasites under certain conditions. A cross-sectional coprologic survey among 125 monkeys (89 toque macaques, 21 gray langurs, and 15 purple-faced langurs) indicated that *Cryptosporidium* was detected in all three primate species and was most common among monkeys using areas and water that had been heavily soiled by human feces and livestock. Most macaques (96%) shedding *Cryptosporidium* oocysts were co-infected with other protozoans and important anthroponotic gastrointestinal parasites (e.g., *Enterobius* and *Strongyloides*). The transmission of these parasites among primates in the wild may have important implications for public health as well as wildlife conservation management.

### INTRODUCTION

Cryptosporidiosis has emerged as one of the major global public health concern after major outbreaks associated with contaminated municipal water supplies.<sup>1,2</sup> The disease is caused by the apicomplexan protozoan *Cryptosporidium parvum*, which is transmitted by ingestion of environmentally robust oocysts, either directly via the fecal-oral route, or indirectly through contaminated food or water.<sup>3</sup> The infection induces acute and self-limiting diarrhea in immunocompetent hosts, whereas the disease is debilitating and potentially life threatening in immunocompromised hosts, especially those with acquired immunodeficiency syndrome (AIDS).<sup>3,4</sup>

Cryptosporidiosis in humans is the result of either anthroponotic or anthroponotic transmission,<sup>5</sup> and many domestic animals, but only a few wild ones, have been implicated as potential reservoirs for human infections.<sup>6</sup> *Cryptosporidium* is one of the most common protozoan parasites associated with diarrhea in young children in Sri Lanka, and domestic ruminants have been postulated to be the reservoir hosts.<sup>7–10</sup> However, there is no information on the possible role played by wildlife in the epidemiology of cryptosporidiosis in humans, livestock, and wild animals. Monkeys in Sri Lanka may be found near human settlements, including some urban areas, where suitable forest habitat also remains. Monkeys may be conspicuous and cause damage to crops and property; therefore, they often are considered as pests and a threat to public health. Conversely, as humans and their livestock invade progressively more into natural areas, the dynamics of bilateral disease transmission among humans, livestock, and wildlife has important implications for human health and the conservation of biodiversity.<sup>11</sup>

*Cryptosporidium* infection has been recorded from many species of captive non-human primates.<sup>1,6,12,13</sup> However, the data on *Cryptosporidium* infection is scant in free-ranging non-human primates. A recent study of mountain gorillas (*Gorilla gorilla beringei*) in Uganda showed that the preva-

lence of *Cryptosporidium* infection was higher in animals that ranged into areas inhabited by humans than in those living in areas with less human use.<sup>14</sup> *Cryptosporidium parvum* (genotype 2) has been isolated from both gorillas and people that share the same habitat, which suggest a possible anthroponotic mode of transmission for this protozoan.<sup>15</sup> In addition to this protozoan, monkeys, which live in areas with high human presence, are known to harbor many anthroponotic helminths and protozoans that could be potential threat to the health of humans and monkeys.<sup>16–18</sup>

The natural population of non-human primates on the Polonnaruwa Nature Sanctuary in Sri Lanka has been under intense study for more than 35 years, and provides an important refuge for the toque macaque (*Macaca sinica sinica*), gray langur (*Semnopithecus priam thersites*), purple-faced langur (*Trachypithecus vetulus philbricki*), and a variety of other wildlife. Two of these primates, the toque macaque and purple-faced langur, are native to Sri Lanka. The sanctuary also encompasses religious shrines and an archaeological reserve. Therefore, sections of the sanctuary are subject to a continuous flow of local pilgrims and tourists, who along with local residents use areas near water (wells and irrigation channel) as open toilets, for picnicking, and for disposal of food refuse. Local farmers also graze their cattle (cows and water buffalo) in the sanctuary. However, not all areas of the sanctuary are so affected; many groups of monkeys and other wildlife live exclusively in forests that are relatively free from human use and have year-round access to clean water from wells and temporary water sources, such as seasonal ponds and streams. These contrasting environmental conditions, the ecologic differences among the primate species, the phylogenetic closeness between humans and monkeys, and the knowledge base already available for this well-studied population of non-human primates at Polonnaruwa suggest that the site provides an ideal set of conditions for studying the transmission dynamics of emerging anthroponotic pathogens.<sup>19</sup>

The first aim of this cross-sectional study was to determine the presence of *Cryptosporidium* infection based on the Ziehl-Neelsen staining technique and polymerase chain reaction (PCR) amplification of samples from the three diurnal

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monkey species (toque macaque, gray langur, and purple-faced langur) in relation to epidemiologic factors that were predicted to influence this infection in the Polonnaruwa Sanctuary. One risk factor appeared to be frequent fecal contamination of ground and water by humans and livestock in the home ranges of different primate species and groups. A second aim was to determine the prevalence of other gastrointestinal parasites in the toque macaques.

MATERIALS AND METHODS

**Study area.** The study was conducted for a four-month period during the dry season (March to June 2001) in the Polonnaruwa Nature Sanctuary and Archaeological Reserve, which is located in the northeastern dry zone of Sri Lanka (7°56'N, 81°00'E) at an elevation of approximately 150 meters. The natural dry evergreen forest of this site is subject to a mean annual rainfall of approximately 1,671 mm, a temperature ranging from 26°C to 30°C, and 2–5 months of drought (May to September). The sanctuary is bounded by a lake, an irrigation channel, active and abandoned cultivation, scrub forest, and village dwellings (Figure 1).

The geography of the study area had been mapped in detail, and a 100 × 100 meter (hectare) grid was used to help quantify variations in habitat quality as well as home range use patterns for all primate species. Home range and other ecologic data had been charted for more than three decades for individually recognized social groups for the three monkey species.<sup>20</sup> The area varied in the degree of substrate and water contamination involving food scraps as well as feces from humans and livestock (Table 1). Each hectare in the area was scored according to whether any section of it was subjected to promiscuous defecation by humans. Sections within 33 of 273 hectares (12% of the area) were subjected to high visitation rates from religious pilgrims, local tourists, and residents that frequently used them as open toilets. These sections were the shores of the lake, the banks of the irrigation channel, and wooded well areas close to a popular site of worship (Figure 1). Lake water drained into the irrigation channel that served as a source of drinking water for people as well as wildlife. The remainder of the area (240 of 273 hectares) was rarely visited or used for defecation. The entire area was also subject to grazing by cattle and buffalo, the intensity of which varied locally and seasonally (Table 1).

Although streams, water holes, or ponds were common in the area, nearly all dried up for 2–5 months of the year. A scattering of animal feces occurred at all water holes. During the dry season, each group of monkeys had at least one perennial source of water such as the irrigation channel, a small marsh, tube or other wells, and drainage from houses. Only the wells were free of conspicuous fecal contamination by humans and/or livestock (Figure 1 and Table 2).

The density of primates was more or less uniform over the area surveyed, but varied by species (Table 1). These primates were sympatric, home ranges overlapped totally among species, but within the same species, the home ranges of neighboring groups overlapped little or incompletely. Home range sizes of individual groups of toque macaques and gray langurs in the sample area varied from 13 to 48 hectares, and those of purple-faced langurs were 4–6 hectares.

**Animals.** All toque macaques were identified individually by their natural markings and tattoo numbers, whereas individuals of the two langur species were distinguished by their

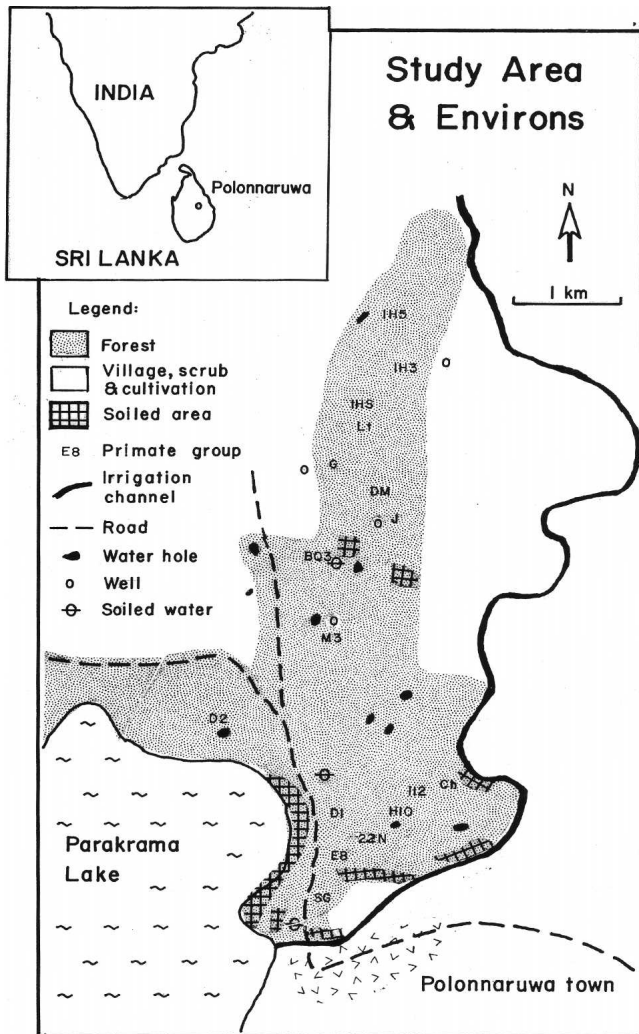


FIGURE 1. Study area in Sri Lanka.

TABLE 1

Comparison of the intensity of use (measured in terms of density per day) by humans, livestock, and different monkey species, with special reference to area variations in human defecation within the home ranges of primate species' social groups in the study sample at the Polonnaruwa Nature Sanctuary

Parameter	Unit of measure	Value
Size of area encompassing home ranges of all primate groups sampled	Hectares (ha)	273
Number of 1-ha plots with soiled sections	N	33
Number of 1-ha plots not soiled (clean area)	N	240
Density of people soiling clean areas	Persons/ha/day	< 1
Density of people soiling soiled areas, mean (range)	Persons/ha/day	14 (2–46)
Animal density in clean or soiled area		
Cattle and buffaloes, seasonal range	Head/ha	0.7–0.9
Toque macaques	Monkeys/ha	2.8
Gray langurs	Monkeys/ha	1.8
Purple-faced langurs	Monkeys/ha	1.1
All diurnal primates	Monkeys/ha	5.7

TABLE 2

Prevalence of *Cryptosporidium* based on microscopy and nested polymerase chain reaction (PCR) assays among three monkey species in relation to differences in the quality of their habitat

Primate species and social group identity	Prevalence among monkeys by habitat No. positive/no. examined				Prevalence by species	
	Soiled substrate and water in home range		Home range not soiled		Microscopy	Nested PCR
	Microscopy	Nested PCR	Microscopy	Nested PCR	Microscopy	Nested PCR
Toque macaque					26/89 (29%)	36/89 (44%)
Groups D1, 22N, Ch1, BQ3	25/39 (64%)	28/39 (72%)				
Groups D2, M3, J, IH3, IH5, IHS, G			1/50 (2%)	11/50 (22%)		
Gray langur					8/21 (38%)	10/21 (48%)
Group SG	8/11 (73%)	8/11 (73%)				
Groups LT, DM			0/10 (0%)	2/10 (20%)		
Purple-faced langur					0/15 (0%)	4/15 (26%)
Group E8	0/8 (0%)	3/8 (37.5%)				
Groups H10, I12			0/7 (0%)	1/7 (14%)		

natural markings and physical characteristics only.<sup>21</sup> The ages of the macaques were known from observed birth, those of the langurs were estimated according to known relations between age and morphologic development.<sup>21</sup> None of these primate species were managed or intentionally provisioned. The study included a sample of 125 monkeys (either sex and variable age) comprising 89 toque macaques from 11 different social groups, 21 gray langurs from 3 social groups and 15 purple-faced langurs from 3 social groups (Table 2). The nature and extent of exposure to soiled ground and/or water differed among species and groups within species. All monkey groups that were exposed to soiled dry or moist ground in their home ranges also drank from soiled water sources. Those monkey groups whose home ranges were more or less free of human feces drank from apparently clean sources of water, such as wells (Figure 1 and Table 2).

We compared the prevalence of parasitic infection among individuals in groups whose home ranges overlapped with areas soiled by humans and who drank from water sources soiled by humans and/or livestock with those that were more or less free from such substrate and water contamination. Of the 125 animals, 58 monkeys (39 toque macaques from 4 groups, 11 gray langurs from one group, and 8 purple-faced langurs from 3 groups) were from ranges that included some soiled ground and/or water, and 67 monkeys (50 toque macaques from 7 groups, 10 gray langurs from 2 groups, and 7 purple-faced langurs from 2 groups) occupied relatively clean areas and water sources in the dry season (Table 2).

The macaques exposed to soiled ground commonly fed on food scraps left in such areas (especially groups D1, 22N, and BQ3), whereas gray langurs in such areas fed mostly only on the vegetation. Although the home range of purple-faced langur group E8 included soiled substrate, being highly arboreal, they came to the ground far less frequently than the other two monkey species. None of the three groups of purple-faced langurs were observed to drink water from ground sources.

**Sample collection.** Individually identified macaques and langurs were followed in their home ranges and the top layers (without soil) of fresh fecal samples (2–8 grams) were collected immediately after defecation, and the scooped samples were stored in sterile plastic containers. Sample collections were done between 7:00 AM and 9:00 AM. Infant macaques (less than one year of age) were individually trapped in the

morning (between 7:00 AM and 9:00 AM) using trap cages and held up to eight hours to collect freshly voided feces.<sup>22</sup> Procedures for the trapping and handling of animals were reviewed and approved by the Department of Wildlife Conservation (Sri Lanka). Each animal selected for this study was sampled only once, and the fecal analysis was done within 4–5 hours of collection.

**Examination of fecal specimens for *Cryptosporidium* oocysts.** The fecal specimens from macaques and langurs were concentrated using Sheather's sucrose solution as described previously,<sup>9</sup> and the concentrated samples were smeared on glass slides and stained using the modified Ziehl-Neelsen procedure, followed by examination under a microscope with an oil-immersion lens (1,000 $\times$ ).<sup>23</sup> Identification of *Cryptosporidium* was based on its acid-fast characteristics and the size (4–6  $\mu$ m) of the oocysts, which was measured by using an eyepiece graticule. In addition, the intensity of oocysts in all positive smears were quantified and expressed as oocysts per gram of feces as described previously.<sup>9</sup>

**Examination of the fecal specimens from macaques for helminths and protozoan parasites.** Identification of trophozoites and cysts of protozoans and helminth eggs were based on light microscopic morphology and morphometry.<sup>24</sup> The fecal samples were analyzed using the salt floatation technique, and the types of helminth eggs were identified on the basis of microscopic morphology.<sup>24</sup> Direct wet fecal mounts with and without staining with Lugol's iodine were checked for the presence of protozoa other than *Cryptosporidium*. For the microscopic detection of protozoa, direct fecal smears were also examined after staining with trichrome stain to confirm the identity of protozoa.<sup>24</sup> Fecal samples from langurs were not tested for other protozoa and helminths because of insufficient amounts of samples.

**Polymerase chain reaction analysis to test the identity of *Cryptosporidium*.** A nested PCR approach based on the 18S ribosomal RNA locus of *Cryptosporidium* spp. was performed on all samples to test the identity and to determine the sensitivity of the staining technique used. Maximum precautions were taken to prevent cross-contaminations between samples during concentration and DNA extraction procedures. Concentrated fecal pellets were used for the DNA extraction after several cycles of freezing-thawing. DNA was extracted from freeze-thawed fecal pellets using a DNA purification kit (Wizard Genomic DNA purification kit; Promega, Madison, WI), and 1  $\mu$ l of genomic DNA was used



as the template for PCR analysis. The nested PCR was performed essentially as described by Jellison and others<sup>25</sup> using the external primers KLJ1 and KLJ2 in the first round of amplification, followed by a PCR using the internal CBP-DIAGE and CBP DIAGR-5 primers. In both PCRs, bovine serum albumin was added to a final concentration of 400 µg/ml and 35 cycles of amplification were performed. One microliter of the first-round PCR sample was used in the second-round PCR. Samples that were negative in the first analysis were tested again using five times as much template to confirm that no trace amounts of *Cryptosporidium* DNA could be detected. Positive controls (10<sup>3</sup>, 10<sup>2</sup>, and 10 *C. parvum* oocysts) and negative controls were included in each set of PCRs. The PCR products were separated by electrophoresis on 1.2% agarose gels and visualized after staining with ethidium bromide.

**Data analyses.** The macaques and langurs were divided into two age categories: ≤ 5 years and > 5 years of age, which corresponded more or less to the juvenile and adult stages, respectively. The chi-square test or Fisher's exact test were used to compare the bivariate prevalence data of *Cryptosporidium*. The influence of species, sex, age, and home range quality on fecal oocyst intensity among *Cryptosporidium*-positive animals were analyzed using a non-parametric Mann-Whitney test.<sup>26</sup> Only the microscopically positive sample data were used for analyses by sex and age.

The potentially independent environmental risk factors of substrate (dry or moist) soiled by human feces and of water sources similarly soiled by humans and/or livestock coincided in the test sample. Therefore, these two factors could not be separated in the analysis. They were considered together as soiled habitat. All statistical analysis was performed using MINITAB release 11.12 (Minitab Inc., State College, PA), except for Fisher's exact test, which was performed using SAS release 8.00 (SAS Inc., Cary, NC). A *P* value less than 0.05 was considered statistically significant.

RESULTS

**Prevalence of *Cryptosporidium* based on microscopy.** Of the 125 single fecal specimens from the three monkey species, 27% (34 of 125) were positive for *Cryptosporidium* oocysts. The oocysts were detected only in the feces of toque macaques and gray langurs; none of the purple-faced langur

samples (n = 15) were positive. The prevalence of infection was slightly higher in the gray langurs (8 of 21, 38%) than in the toque macaques (26 of 89, 29%), but this difference was not statistically significant ( $\chi^2 = 0.62, P > 0.40$ ).

The greatest differences in prevalence of infection among and within species occurred in relation to differences in exposure to soiled ground and/or water. First, both the macaque and gray langur feed extensively on the ground, where they are potentially exposed to such contamination and some members of both species were infected. Conversely, the highly arboreal purple-faced langurs rarely drink or feed on the ground, and none of these animals were infected even though their home ranges overlapped with contaminated ground and water (Table 2). Second, among the ground foraging species, only those gray langurs that were exposed to the contaminated ground and water were infected (*P* < 0.001, by Fisher's exact test) (Table 2). Likewise, the toque macaques whose ranges included soiled ground and water were highly positive ( $\chi^2 = 10.68, P < 0.0001$ ) for the infection compared with those from clean ranges (Table 2).

There was no difference in the prevalence by sex among either toque macaques ( $\chi^2 = 0.326, P > 0.50$ ) or gray langurs (*P* > 0.50, by Fisher's exact test). None of the infants (less than one year of age) toque macaques were positive for fecal oocysts, but all infants sampled were native to one social group (inhabiting clean areas) where older members also were negative. Infant gray langurs and purple-faced langurs were not sampled. The sampled population was divided into two age categories (≤ 5 years of age and > 5 years of age), which corresponded to the reproductively inactive and active classes, respectively. Although, the prevalences were higher in the ≤ 5-year-old age group than in older animals in both infected species (Table 3), the differences were not significant (toque macaques,  $\chi^2 = 0.466, P > 0.50$ ; gray langurs, *P* > 0.50, by Fisher's exact test).

***Cryptosporidium* oocyst intensity in positive animals.** To avoid zero counts, we defined the mean oocyst intensity as total oocyst count divided by total number of animals shedding oocysts in the feces. The mean oocyst count of *Cryptosporidium*-positive samples was 3,633 (range = 166–34,250) for macaques and 2,407 (range = 860–3,500) for gray langurs (Table 3). Except for one juvenile toque monkey that had mild diarrhea with a high oocyst count (34,250), all *Cryptosporidium*-positive animals were asymptomatic.

TABLE 3  
Prevalence and oocyst counts of *Cryptosporidium* among toque macaques and gray langurs

Variable	Toque macaques				Gray langurs			
	Prevalence		Intensity of fecal oocysts*		Prevalence		Intensity of fecal oocysts*	
	No. examined	No. positive (%)	Range	Mean ± SE	No. examined	No. positive (%)	Range	Mean ± SE
Sex								
Male	42	11 (26)	375–34,250	6,673 ± 2,883.95	5	1 (20)	2,616	
Female	47	15 (32)†	166–6,458	3,101 ± 595.92†	16	7 (44)†	860–3,500	2,377.71 ± 342.98‡
Age, years								
≤ 5	46	15 (33)	250–34,250	5,456 ± 2,159	6	4 (67)	2,200–2,616	2,404.50 ± 87.45
> 5	43	11 (26)†	166–6,458	3,462 ± 779†	15	4 (27)†	860–3,500	2,410 ± 638.92†
Home range								
Soiled	39	25 (64)	166–34,250	4,771.6 ± 1,325§	11	8 (73)	860–3,500	2,407 ± 298.52‡
Clean	50	1 (2)‡	624		10	0 (0)§		

† *P* > 0.05 (not significant).  
‡ *P* < 0.001.  
§ *P* < 0.01.

### Prevalence of other enteric parasites in toque macaques.

The result of direct fecal examination showed five different types of nematode eggs and six different species of ciliates, flagellates, and sarcomatigophoran protozoa. The most common nematode eggs were of *Enterobius* spp. (52%), followed by spiruroid type eggs (38%), *Strongyloides* spp. (26%), strongyle type eggs (17%), and *Trichuris* spp. (9%). The prevalences of eggs of *Enterobius* ( $\chi^2 = 4.45$ ,  $P < 0.05$ ), *Strongyloides* spp. ( $\chi^2 = 10.88$ ,  $P < 0.001$ ), strongyle type eggs ( $\chi^2 = 5.76$ ,  $P < 0.01$ ), and *Trichuris* spp. ( $\chi^2 = 6.81$ ,  $P < 0.001$ ) were significantly greater among the macaques exposed to soiled ground and water than among those not exposed. Only *Enterobius* eggs were significantly higher in females ( $\chi^2 = 13.67$ ,  $P < 0.001$ ). There were no significant differences in prevalences between age categories for any of these parasites (Table 4).

Among the protozoa, *Entamoeba coli* showed the highest overall infection rate (52%), followed by *E. histolytica/E. dispar* (34%), *Iodamoeba* sp. (21%), *E. hartmanni* (21%), *Chilomastix* sp. (12%), and *Balantidium coli* (11%). When these rates were compared among macaques exposed and not exposed to a soiled habitat, the prevalences of all protozoan parasites were greater among exposed macaques, and this difference was significant for *E. coli*, and *E. histolytica/E. dispar* (Table 4). There were no significant differences in the rates of infection by these protozoa according to macaque age or sex.

**Co-infection with *Cryptosporidium* and other enteric parasites in toque macaques.** Among the 26 macaques positive for *Cryptosporidium* oocysts, 96% (24 of 26) were co-infected with at least one species of another enteric parasites; among them 58% (15 of 26) were positive for *Enterobius* spp. eggs, 23% for strongyle type eggs, 30% for *Strongyloides* spp. eggs, 42% for spiruroid type eggs, and 11% for *Trichuris* spp. eggs. The same *Cryptosporidium*-infected macaques were also co-infected with the following ciliates, flagellates, and sarcomatigophorans: *E. coli* (53%), *E. histolytica/E. dispar* (76%), *E. hartmanni* (35%), *Iodamoeba* sp. (31%), *Chilomastix* sp. (15%), and *Balantidium* spp. (19%).

**Prevalence of *Cryptosporidium* infection based on a nested PCR.** The nested PCR yielded an expected 434-basepair PCR product, which confirmed that all microscopically positive isolates were *Cryptosporidium* spp. The more sensitive PCR test showed greater prevalence of infection among all three monkey species than was evident by microscopy alone (Table 2)

because of low oocyst counts undetected by microscopy (Figure 2). None of the PCR-negative samples contained *Cryptosporidium*, even when five times more DNA was used. Positive infections were observed among the three monkey species inhabiting both soiled (6 of 39) and clean (13 of 50) areas. Microscopy showed that only 2% (1 of 50) of the macaques from clean areas were positive, whereas the PCR showed that 22% (11 of 50) were positive. The overall pattern of infection shown by microscopy was supported by PCR results: the prevalence of infection was significantly greater among exposed macaques (28 of 39, 72%) than among those from clean habitats (11 of 50, 22%) ( $\chi^2 = 16.12$ ,  $P < 0.001$ ). Likewise, the PCR showed that the prevalence of *Cryptosporidium* infection was significantly greater among exposed gray langurs (8 of 11, 73%) than among those from clean areas (2 of 10, 20%) ( $\chi^2 = 8.41$ ,  $P < 0.01$ ). None of the purple-faced langurs tested positive for *Cryptosporidium* by microscopic analysis. However, the PCR showed that 26% (4 of 15) were positive: 38% (3 of 8) from soiled areas and 14% (1 of 7) from clean habitats (Table 2).

## DISCUSSION

With few exceptions,<sup>14</sup> epidemiologic data on *Cryptosporidium* infection in non-human primates has been based on captive or colony-bred animals.<sup>6</sup> However, these data are not adequate to explain the natural transmission dynamics of pathogens.<sup>27</sup> We investigated a natural population of non-human primates, and this is the first report of *Cryptosporidium* and other protozoan infections among three species of diurnal monkeys found in Sri Lanka.

Earlier work dealt with African species of monkeys. Muriuki and others<sup>13</sup> reported that 51.7% of feral and captive vervet monkeys (*Cercopithecus aethiops*) and olive baboons (*Papio anubis*) in Kenya were infected with *Cryptosporidium*. Nizeyi and others<sup>14</sup> reported a prevalence of 11% in free-ranging mountain gorillas (*G. gorilla beringei*). In our study, the prevalence of the *Cryptosporidium* infection varied between the three species of primates examined (Table 2). Oocysts were detected microscopically in the feces of toque macaques (29%) and gray langurs (38%), but not in the purple-faced langurs (0%). This trend was similar when the same samples were tested using the PCR. Species differences in prevalence of *Cryptosporidium* were related to variations in exposure to the risks of infection from contaminated

TABLE 4  
Prevalence of intestinal parasites by sex, age, and occurrence of soiled substrate in the home ranges of toque macaques

Parasite	Home range includes soiled substrate (n = 39) Positive (%)	Home range is free of soiled substrate (n = 50) Positive (%)	Females (n = 47) Positive (%)	Males (n = 42) Positive (%)	≤ 5 years old (n = 46) Positive (%)	> 5 years old (n = 43) Positive (%)
<i>Enterobius</i> spp.	25 (64)*	21 (42)	33 (70)*	13 (31)	23 (49)	23 (55)
Strongyle type	15 (38)*	8 (16)	12 (25)	11 (26)	10 (21)	13 (31)
<i>Strongyloides</i> spp.	16 (41)*	10 (20)	16 (34)	10 (24)	12 (25)	14 (33)
<i>Trichuris</i> spp.	7 (11)*	1 (4)	4 (8)	4 (9)	5 (11)	3 (7)
Spiruroid type	19 (50)	15 (30)	18 (38)	16 (38)	18 (38)	16 (38)
<i>Iodamoeba</i> spp.	14 (36)	9 (18)	12 (25)	11 (26)	13 (28)	10 (24)
<i>Balantidium coli</i>	8 (20)	4 (8)	7 (15)	5 (12)	4 (8)	8 (19)
<i>Entamoeba coli</i>	25 (64)*	21 (42)	20 (43)	26 (62)	26 (55)	20 (48)
<i>Chilomastix</i> spp.	9 (14)	2 (8)	8 (17)	3 (7)	3 (6)	8 (19)
<i>Entamoeba hartmanni</i>	13 (33)	8 (16)	11 (23)	9 (21)	9 (19)	11 (26)
<i>Entamoeba histolytica/E. dispar</i>	22 (56)*	8 (16)	16 (34)	14 (33)	14 (31)	16 (38)

\*  $P < 0.01$ .

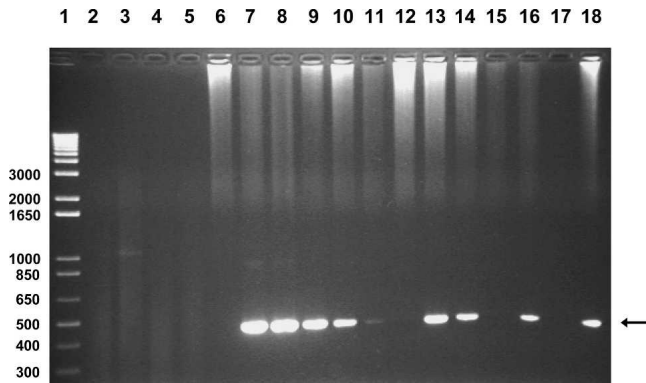


FIGURE 2. Nested polymerase chain reaction (PCR) assay of samples of three non-human primates. Lane 1, 1-kilobase ladder (Invitrogen, Carlsbad, CA); lanes 2–5, primary amplification of negative control and 1,000, 100, and 10 *Cryptosporidium parvum* oocysts per reaction; lanes 6–9, secondary amplification of negative control and 1,000, 100, and 10 *C. parvum* oocysts per reaction; lane 10, secondary amplification of a microscopically and PCR-positive sample from a toque macaque; lane 11, secondary amplification of a microscopically negative, PCR-positive sample from a toque macaque; lane 12, secondary amplification of a microscopically and PCR-negative sample from a toque macaque; lane 13, secondary amplification of a microscopically and PCR-positive sample from a gray langur; lane 14, secondary amplification of a microscopically negative, PCR-positive sample from a gray langur; lane 15, secondary amplification of a microscopically and PCR-negative sample from a gray langur; lanes 16 and 18, secondary amplification of a microscopically negative and PCR-positive sample from a purple-faced langur; lane 17, secondary amplification of a microscopically and PCR-negative sample from a purple-faced langur. Values on the left are in basepairs. The **arrow** indicates the expected 434-basepair PCR product of *Cryptosporidium* isolates.

ground and/or water. Macaques are omnivorous while gray langurs are folivorous, but both species use the ground for travel and foraging at all times of the year, and they drink free-standing water during the dry season. The highly arboreal habits of the purple-faced langurs appeared to offer some protection from acquiring *Cryptosporidium* infection. Munene and others<sup>28</sup> also observed a low prevalence of water-borne or soil-borne parasitic infections in the arboreal Sykes monkeys (*Cercopithecus mitis*) in Africa.

Several studies have shown a high prevalence of *Cryptosporidium* infection in children and animals less than one year of age.<sup>12,29</sup> In mountain gorillas in Uganda, all juveniles up to 2.9 year of age were negative.<sup>14</sup> Infants (less than one year of age) of all species from soiled areas were not sampled in this study, and nine toque macaque infants from unsoiled areas were negative for *Cryptosporidium*. *Cryptosporidium* oocysts were detected in toque macaques more than one year of age, and the prevalence of this infection was slightly, but not significantly, higher in young animals ( $\leq 5$  years of age) than in older ( $> 5$  years of age) ones.

*Cryptosporidium* has generally been associated with intestinal pathology resulting in diarrhea in both humans and animals.<sup>29</sup> In this study, one juvenile macaque was diarrheic with a high oocyst count (34,250 oocysts per gram of feces). This animal was co-infected with *Chilomastix* spp. and *E. coli*, and negative for helminths. Since other bacterial and viral agents that cause diarrhea were not examined, the role of *Cryptosporidium* in this clinical feature is not conclusive.

The prevalence of gastrointestinal helminths in toque macaques in our study was similar to that of the previously reported studies of non-human primates, except for the absence of the cestodes and trematode eggs.<sup>16,28,30,31</sup> Interestingly, an earlier report of the prevalence of helminths in the same population indicated the presence of cestodes (*Hymenolepis* spp.) in post-mortem specimens,<sup>32</sup> and a cestode (*Bertiella studeri*) of monkey origin was reported in humans in Sri Lanka.<sup>33,34</sup> The absence of cestode ova in the present study might be related to the flotation technique used because sedimentation is necessary to isolate cestode eggs. Similar to *Cryptosporidium*, the prevalences of all nematode and protozoan parasites examined in this study were greater among macaques from soiled than clean areas, and this was significant for most parasites (Table 4). Most of the reported parasites have anthroponotic importance; this includes most of the protozoan species (*Entamoeba* and *Balantidium* spp.) detected, as well as the nematodes (*Enterobius* and *Strongyloides*) and possibly some *Trichuris* spp. Other studies have reported many species of *Enterobius* and *Strongyloides* in non-human primates, including the human parasites *Enterobius vermicularis*, *Strongyloides stercoralis*, and zoonotic *S. füllebonii*.<sup>35–37</sup>

We investigated the dynamics and transmission of infection for *Cryptosporidium* and other parasites among the different mammals at the study site. Monkeys, cattle, and buffaloes defecated randomly over large areas, and their densities were approximately the same in soiled and clean habitats. The major difference between infected and non-infected monkey species and individuals (within species), especially for *Cryptosporidium*, was contact with ground that had been soiled by humans through frequent defecation near water sources (Table 1). Since cattle roamed freely, they also may have contributed to the soiling of these water sources. Although further investigations of humans and animals are needed to ascertain the dynamics of infection, it is possible, as demonstrated by other studies, that some of the parasites harbored by the monkey population at Polonnaruwa were of human and/or livestock origin.<sup>17</sup> The common overall occurrence of other protozoa and nematodes in the macaques and the absence of clinical signs of gastrointestinal disease suggest that these parasites might have become established as commensals in the macaque population at Polonnaruwa. Our study suggests that the concentrated use of water distribution channels for toilets by humans increases the risk of transmission of water-borne parasites to monkeys, and probably to humans as well.

Compared with microscopic examination, PCR analysis increased the detection limit for the presence of *Cryptosporidium* spp. In our sample, this was observed in the purple-faced langurs that were infrequently exposed to soiled ground and in other monkeys that inhabited clean areas (Table 2). Thus, low levels of infective oocysts may be present in clean habitats. Further studies are needed on the phylogeny of these *Cryptosporidium* isolates and their modes of transmission in relation to differences in primate species ecologies and environments.

With the ever-increasing expansion of human populations into uninhabited areas, anthroponotic disease assumes an important role in the health of humans as well as wildlife and its conservation.<sup>11,27</sup> This is particularly so for non-human primates that share a close phylogenetic relationship with hu-



mans and where common intestinal metazoan and protozoans parasites have been found.<sup>17</sup> The primates at the Polonnaruwa study site have also been shown to harbor many of these parasites common in humans, including a variety of arboviruses, a strain of dengue virus, as well as a number of helminths.<sup>19,32,38</sup> This study indicates the importance of epidemiologic studies of free-ranging non-human primate populations to gain a greater understanding of the dynamics of disease transmission and the potential emergence of anthrozoanotic infectious agents in Sri Lanka.

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