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RESEARCH ARTICLE

Preliminary survey of the distribution of four potentially zoonotic parasite species among primates in Sri Lanka

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Abstract : The occurrence of four parasitic species of zoonotic potential, *Entamoeba coli*, *Entamoeba histolytica / dispar*, *Trichuris* sp. and hookworm was investigated in the toque macaque, grey langur and the purple-faced langur at 32 sites across Sri Lanka. The study was carried out during the rainy season months of February - March in both 2007 and in 2009 and in December of 2010. 93 faecal samples were collected from 49 monkey troops at representative locations in altitudinal /climatic zones across the country where toque macaques (58 samples), grey langurs (21 samples) and purple-faced langurs (14 samples) naturally occur. Overall, the most common parasitic species found in all three primates were *Trichuris* sp. (28 %) and *E. coli* (25 %). Notably, hookworms were present in 23 % of the grey langur samples and 33 % of the toque macaque samples but absent in the purple-faced langur samples collected. Statistically significant variability in the prevalence levels across altitudinal/climatic zones was noted for toque macaques. Overall, group prevalence values in toque macaques decreased with increasing altitude; the highest values were found in the intermediate to arid lowland zones, and were lowest in the upland wet zone. Only *Trichuris* sp. and hookworm were found (13 %, 7 %, respectively) in the highland/ wet zone. Molecular analysis will be necessary to genetically type the parasite species before drawing firm conclusions about the status of zoonotic transmission between humans and non-human primates in the country. However this study highlights the need to systematically survey the human parasite population in areas where primates are commonly found to harbour these parasite species.

Keywords: Country-wide survey, *E. coli, E. histolytica/dispar,* hookworm, *Trichuris* sp., zoonoses.

INTRODUCTION

It is known that 27.5 % of all parasites found in wild primates, have also been reported in humans (Pedersen *et al*., 2005). Given the genetic relatedness of non-human primates with humans, the potential for zoonotic transmission between them is expected to be high in areas of close contact (Wolfe *et al*., 1998). Five species of non-human primates occur in Sri Lanka; the toque macaque (*Macaca sinica*), the grey langur (*Semnopithecus priam thersites*), purple-faced langur (*Semnopithecus vetulus*), and two slender loris species (*Loris tardigradus, Loris lydekkarianus*) (Molur *et al*., 2003). With the exception of *S. priam* and *L. lydekkerianus*, all are endemic species classified as endangered or critically endangered (Dela, 2007; Rudran, 2007; Nahallage *et al*., 2008).

Periodic reviews of the parasitic zoonoses in Sri Lanka have been made over the years, and the list of such species is extensive (Dissanaike, 1993 a, b; 2002). According to Dissanaike (1993a, b), of the 33 species of parasitic zoonoses detected in humans in Sri Lanka, 5 species (*Balantidium coli*, *Plasmodium* spp., *Sarcocystis* sp., *Bertiella studeri* and *Echinococcus granulosus*) were reported for monkeys presumed to be animal host reservoirs. Gunawardena *et al*. (2011) have also reported moderate to heavy intensity infections with

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potentially zoonotic nematodes, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm in children living in five districts of the plantation sector, namely, Nuwara Eliya, Ratnapura, Kandy, Badulla and Kegalle. In a hospital in Colombo, *Cryptosporidium* sp., *A. lumbricoides*, *T. trichiura*, *Giardia lamblia*, *Entamoeba histolytica* and hookworms were detected and significantly associated with diarrhoea in young children (Perera *et al*., 1999). The first case of human infection due to *B. studeri* in Sri Lanka was reported from the Central Province (Edirisinghe & Kumaranjan, 1976). Thereafter, several cases were reported from the Southern Province (Weerasooriya *et al*., 1988; Karunaweera *et al*., 2001) and Sabaragamuwa Province (Morawakkorale *et al*., 2006). According to Karunaweera *et al*. (2001), *B. studeri* infection of children in the Dikwella area along the southern coast was 'presumably transmitted from the toque macaque or grey langur'. However, grey langurs are only found in the lowland dry zone, so the toque macaque is more likely to be the reservoir host for these transmissions.

Investigations into the naturally occurring parasites of monkeys in Sri Lanka is limited mainly to the archaeological site of Polonnaruwa (Dewit *et al*., 1991; Ekanayake *et al*., 2006, 2007), located in the lowland arid zone in the North Central Province. Of the parasite species reported at Polonnaruwa, *E. coli* was found only in toque macaques and *T. trichiura* was found in toque macaques, grey langurs and purple-faced langurs. Other primate parasite species of potential anthropozoonotic importance that were not listed by Dissanaike were reported at Polonnaruwa, including *Cryptosporidium parvum*, *E. histolytica* /*dispar* and *Entamoeba* sp., (Dewit *et al*., 1991; Ekanayake *et al*., 2006).

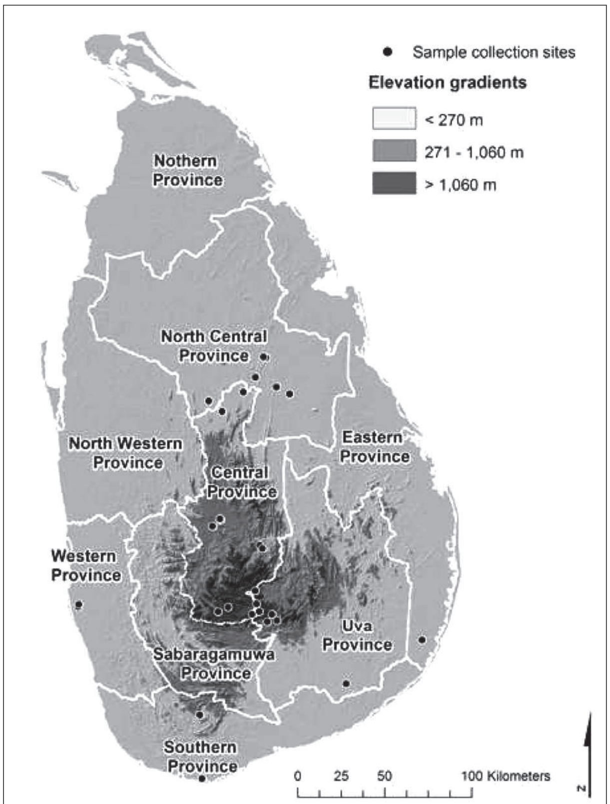

Figure 1: Sites of faecal sample collection

Zoonoses potentially have a significant impact on the health and well-being of both human populations and the co-existing primate populations (Jones-Engel *et al*., 2004; Pederson & Fenton, 2006; Gasser *et al*., 2009). Given the growing degree of contact between humans and monkeys in Sri Lanka due to deforestation, human population growth, expansion of various rural development projects (Nahallage *et al*., 2008; Nahallage & Huffman, 2012), and the limited information on the parasites of Sri Lankan primates, there is an urgent need to better understand the status of potential zoonotic infections in monkeys through the monitoring of primate populations across the country. This is important for both human health and primate conservation efforts (Daszack *et al*., 2000; Patz *et al*., 2000). To address this need, the occurrence of four typical intestinal parasite, species of zoonotic importance to humans (*Entamoeba coli*, *E. histolytica* / *dispar, Trichuris* sp. and hookworm), known to be shared by many primate species (Huffman & Chapman, 2009; Lane *et al*., 2011; Cooper *et al*., 2012) was investigated by surveying three non-human primate species in Sri Lanka across a range of altitudinal and climatic zones.

METHODS AND MATERIALS

Study area

The faecal samples were collected from 32 sites from 49 different primate troops (Table 1, Figure 1). The sites reasonably represent the different altitudinal / climatic zones across the country where these primate species naturally occur. These altitudinal / climatic zones partition the country by altitude into three peneplains [lowland (0 − 270 masl), upland (270 − 1060 masl) and highland (1060 − 2420 masl)] (Crusz, 1986; Vitanage, 1970), and by trends in rainfall (wet zone: > 1900 mm/ year; intermediate zone: between 1250 – 1900 mm / year; dry and arid zone: < 1250 mm/year) (Beenaerts *et al*., 2010).

Table 1: Collection sites, elevation, altitudinal / climatic classification and the number of samples collected from three primate species across Sri Lanka

Province	District	Site	Elevation (masl)	Altitudinal / climatic zone	Toque macaque (N = 58)	Grey langur (N = 21)	Purple - faced langur (N = 14)
Central	Kandy	Kadugannawa	373	Upland / Wet	3		
		Kandy	523	Upland / Wet	8		
	Matale	Dambulla	186	Lowland / Intermediate	6		
		Jathika Namal Uyana	600	Lowland / Intermediate		2	
	Nuwara Eliya	Edition Patana	1450	Highland / Wet	1		
		Hakgalla Gardens	1704	Highland / Wet	3		3
		Horton Plains	2300	Highland / Wet			3
		Keerthibandarapura	405	Lowland / Dry		3	
		Ohiya	1786	Highland / Wet	4		1
		Pathana Kovil	1134	Highland / Wet	3		
		Pattipola	1990	Highland / Wet			1
		Peradeniya	492	Upland / Wet	4		
		Ratkarawwa	1145	Highland / Wet	1		
		Sita Eliya Kovil	1760	Highland / Wet	1		
		Wewakele Temple	690	Upland / Wet	1		
North Central	Polonnaruwa	Kaudulla NP	75	Lowland / Dry	1	1	
		Minneriya NP	131	Lowland / Dry	3	2	
		Giritale	106	Lowland / Dry	4	4	
		Polonnaruwa	56	Lowland / Dry			1
		Sigiriya Junction	300	Lowland / Dry		2	
		Galoya	97	Lowland / Dry		2	
Subaragamuwa	Ratnapura	Uda Walawa	97	Lowland / Dry	4	3	
		Venture Estate	1281	Highland / Wet	1		
Southern	Hambantota	Bundala NP	8	Lowland / Arid	1	2	
	Matara	Matara Dhamhalla	2	Lowland / Wet			1
		Matara Thihagoda	2	Lowland / Wet			1
Uva	Badulla	Haldummulla	1038	Highland / Wet	1		
	Monaragala	Kataragama Town	150	Lowland / Arid	1		
		Kataragama Vadehitikanda	391	Lowland / Arid	2		
		Yala Paranagantota	100	Lowland / Arid	5		
Western	Colombo	Boralasgamuwa	12	Lowland / Wet			1
		Univ. of Sri Jayawardenepura	20	Lowland / Wet		2	

Study Subjects

Four parasite species of zoonotic potential, *E. coli*, *E. histolytica* / *dispar*, *Trichuris* sp. and hookworm in groups of toque macaque (Sinhalese: rilawa, Tamil: kurangu), grey langur (S: wandura / konda wandura, heli wandura, T: mundi / mundi-kurangu) and purple-faced langur (S: kalu wandura, T: mundi) were the target species of this investigation. These primate species were easily identified by their distinct morphological characteristics (Figure 2). The distribution of each primate species is particular to its ecologically influenced habitat preference. While the toque macaque and purple-faced langur can be found in most of the altitudinal / climatic zones, the grey langur is restricted to lowland dry and arid regions, mainly in the north, east and southeast areas of the country (Nahallage *et al*., 2008). The toque macaque and grey langur spent much of their time on the ground when moving between feeding sites and / or when resting. In contrast, the purple-faced langur rarely came to the ground, mostly travelling, feeding, resting and sleeping in the trees.

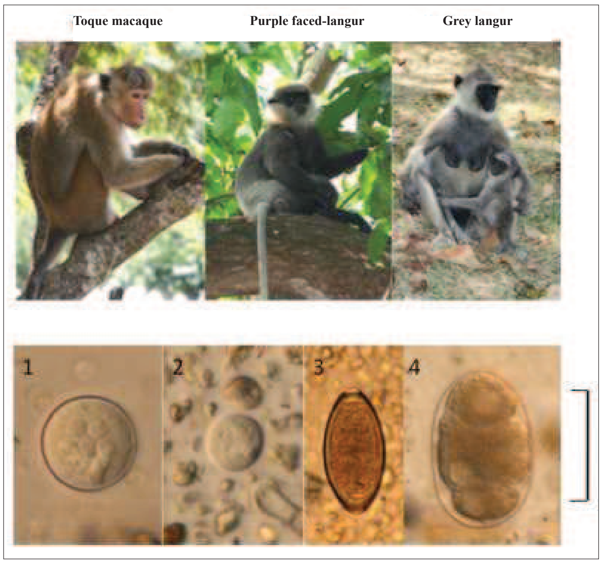

Figure 2 : The three primate host species (top)
Parasites identified from the feacal samples. 1. *Entamoeba coli*. 2. *Entamoeba histolytica / dispar*. 3. *Trichuris* sp. 4. hookworm (Scale: 25 µm for 1 and 2; 50 µm for 3 and 4) (bottom)

The substrate preferences will influence the host's susceptibility to being infected, depending on the transmission and life-cycle characteristics of the infecting organism. All four species of zoonotic potential investigated here have direct transmission routes, i.e. no intermediate host is required for development to the infective stage. Hookworm transmission occurs through penetration of the skin (feet), making travel on the ground the primary substrate of contact (Marquardt *et al*., 2000). *E. coli*, *E. histolytica / dispar*, *Trichuris* sp. occurs through oral ingestion of cysts or eggs *via* faecal-contaminated water, food or hands (Marquardt *et al*., 2000). In this case, infection can occur with or without direct contact with the ground.

Sample collection

Fresh faecal samples were collected opportunistically during the rainy season months of February - March in 2007 and 2009 and in December 2010. The rainy season was selected to avoid seasonal bias across the study periods, ensuring the best possible comparison of parasite species prevalence. The samples were typically collected while following a group, most often with no other primate species in the area. Two to three samples were collected from each faecal material, and one sample was used for a separate primate phylogenetic study. Based on these genetic data, in only two cases was our on-site assignment of primate species incorrect; and this was corrected for the current analysis based on the genetic data.

Samples were collected fresh, as noted by consistency and moistness. The top layers (without soil) of the faecal samples (2 − 8 g) were collected immediately after defaecation, placed in a sealed vial to prevent contamination and filled up to the top with 10 % formalin and mixed thoroughly, typically within 5 h after collection. Samples were stored in a dark place in the laboratory until examined.

Examination of faecal specimens

The faecal specimens were analyzed in the Department of Parasitology, Faculty of Medical Sciences, University of Sri Jayawardenepura, Nugegoda and the Department of Biology, Faculty of Medicine, Oita University, Oita, Japan. Both laboratories used a modified version of the formol-ether concentration method (Ritchie, 1948). Contents of the sediments of the concentrated samples were examined. Identification of cysts of protozoans and eggs of helminths were carried out under the light microscope based on their morphology.

Quantitative analysis

Since monkeys could not be individually identified, calculation of individual prevalence was not possible. Although it was highly unlikely that the same individual was sampled more than once in a group since the number of samples collected for any one group was usually small, and were collected within a short period of time, the sample prevalence and species richness was calculated. Prevalence was calculated as the percent of samples positive for species X / total number of samples collected. Prevalence was analyzed with respect to host species and altitudinal / climatic distribution. The level of statistical significance was set at $p > 0.05$. Fishers exact test was used for all statistical comparisons.

Permission to conduct this research and for sending the primate faecal samples on a one time basis to Japan for comparative research purposes was granted by the Department of Wildlife Conservation, Sri Lanka. The research was conducted according to local laws and conformed to the ethical standards set out in the Guidelines of Research on Primates in the Wild established by the Primate Research Institute of Kyoto University, Japan (2008).

RESULTS

Parasite species prevalence

In total, 93 faecal samples were collected from toque macaques, grey langurs and purple-faced langurs in their natural habitats from 49 different troops at 32 sites in the Central, North Central, Subaragamuwa, Southern, Uva and Western Provinces (Figure 1). Due to the opportunistic nature of the sampling, there was wide variation in the number of samples obtained from a troop (mean 2.1; range, 1 – 6; Table 1). Figure 2 shows the sample prevalence of cysts of *E. coli* and *E. histolytica* / *dispar* and the eggs of *Trichuris* sp. and hookworm detected in the three primate species. The most prevalent of the potentially zoonotic species found in the pooled data were *Trichuris* sp. (28 %), hookworm (26 %) and *E. coli* (25 %; bottom line of Table 2). The prevalence of *E. histolytica* / *dispar* (10 %) was less than half of the other species. While *Trichuris* sp. was most prevalent in the two langur species, hookworm was totally absent from the arboreal purple-faced langur. In contrast, purple-faced langurs had the highest prevalence for *E. histolytica* / *dispar*, being 4 and 6 times more prevalent than in toque macaques and grey langurs, respectively. By primate species, the most prevalent potentially zoonotic species was the hookworm for toque macaques, and *Trichuris* sp. for grey langurs and purple-faced langurs.

Altitudinal / climatic zone prevalence variation in toque macaques

For the analysis of altitudinal and climatic zone variation in prevalence, adequate data was available only for the toque macaque. Overall trends between the three lowland zones (intermediate / dry / arid) were similar

Table 2: Sample prevalence of four potentially zoonotic species in three primate hosts

Host	*E. coli* No. positive (%)	*E. histolytica/ dispar* No. positive (%)	*Trichuris* sp. No. positive (%)	Hookworm No. positive (%)	Samples No. examined	Troops No. examined
Toque macaques	15 (25)	4 (7)	13 (22)	19 (33)	58	28
Grey langurs	4 (19)	1 (5)	8 (38)	5 (23)	21	10
Purple-faced langurs	4 (29)	4 (29)	5 (36)	0	14	11
Pooled data	23 (25)	9 (10)	26 (28)	24 (26)	93	49

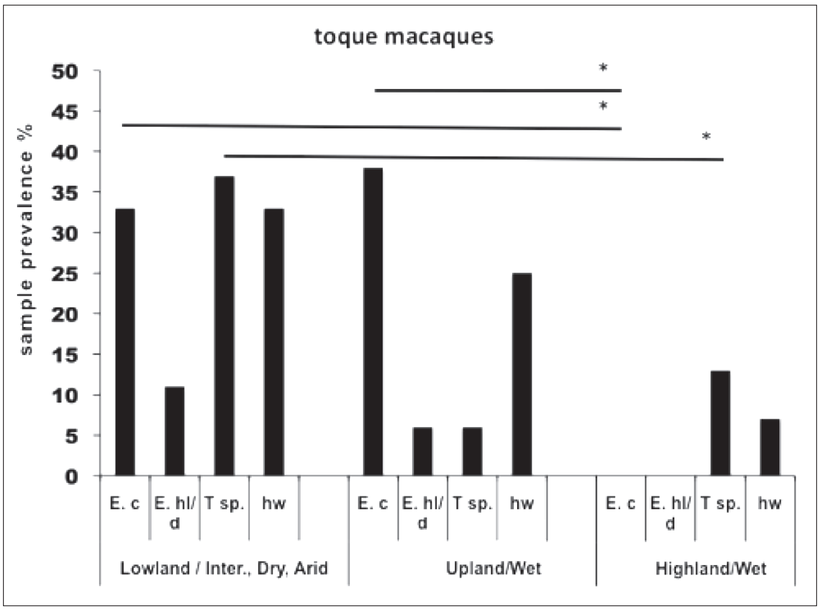

Figure 3 : Inter-altitudinal/climatic variation in prevalence levels of four potential zoonotic species in the toque macaque

and the data were therefore pooled together into one zone and compared with upland/wet and highland / wet zones, respectively. The overall group prevalence values decreased with increasing altitude (Figure 3). The highest prevalence values were noted in the intermediate to arid lowland zones, decreasing in the upland wet zone, with only *Trichuris* sp. and hookworm found in the highland / wet zone. Significant differences were noted for *E. coli* (highland < lowland, $p = 0.02$; highland < upland, $p = 0.02$, Fisher's exact test), *Trichuris* sp. (upland < lowland, $p = 0.03$) and hookworm (highland < lowland, $p = 0.07$, significant trend). No significant differences were noted for *E. histolytica* / *dispar*, but it should be noted that amoeba cysts were not detected in any of the samples collected from the highland wet zone. Compared to the other three species, *E. histolytica* / *dispar* prevalence was consistently low across all zones (Figure 3).

DISCUSSION AND CONCLUSION

The knowledge on potentially zoonotic or otherwise parasitic species in Sri Lankan primates comes mainly from Polonnaruwa in the North Central Province, a lowland, arid habitat (Dewit *et al*., 1991; Ekanayake *et al*., 2006; 2007). This is the first extensive multiple site survey of potentially zoonotic species in non-human primates to be conducted in Sri Lanka. While the primate hosts' distribution across the country is influenced by altitudinal and climatic factors (Nahallage *et al*., 2008), at least one, but often two or even all three primate hosts, when found together, were infected with these four species at multiple sites across the country. This reflects the need for a systematic survey of the status of zoonoses of primate origin in the human populations of these areas where human-primate interactions are high (Jones-Engel, 2008; Nahallage *et al*., 2008; Nahallage & Huffman, 2012).

As expected, the prevalence of potentially zoonotic parasite infections described for the three primate species was partially explained by the hosts' behavioural habits and the modes of transmission of each potentially zoonotic species (Marquardt *et al*., 2000). Most noticeable was the prevalence of hookworm, where infections were not detected in the highly arboreal purple-faced langurs, in contrast to the two other more highly terrestrial primate species. The remaining three species, *E. coli*, *E. histolytica*/*dispar* and *Trichuris* sp. were found in all three primate host species. Interestingly, however, *E. histolytica*/*dispar* was least prevalent among the more terrestrial toque macaque and grey langur groups surveyed. Detection of *E. histolytica* infections among humans is also reportedly on the decrease in Sri Lanka (de Silva *et al*., 1994). Neither the sample size nor host behaviour alone can easily explain this difference. On the other hand, Ekanayake *et al*. (2006) reported a much higher prevalence for *E. histolytica*/*dispar* in toque macaques in Polonnaruwa. In particular individuals in troops exposed to a greater degree of soil substrates (54 %) are more likely to promote infection of geohelminths. At Polonnaruwa, contamination from local livestock was demonstrated in the case of *Cryptosporidium* (Ekanayake *et al*., 2007). In future, intensive sampling of each troop and molecular comparisons will be necessary to better understand the patterns observed in the present study. With a larger sample size from each troop as well as concurrent sampling from livestock and the local human population, it will be possible to determine whether the levels of potentially zoonotic species prevalence are mitigated (Lane *et al*., 2011) or increased (Nunn & Altizer, 2006) by the level of contact with humans and their livestock.

Altitude and climatic factors strongly influence the survivability and infectivity of parasitic and commensal organisms with direct transmission life cycles (Anderson, 1992; Marquardt *et al*., 2000). The statistically significant relationships between higher levels of prevalence and altitudinal and climatic factors found for the toque macaque reflects this, and suggests that the most likely areas for zoonotic transmission in this host species is in warmer drier areas in the lowland zones. Future research should be focused in this aspect. With increasing altitude, the potential for transmission decreased dramatically for these four potentially zoonotic species. The small number of samples currently available for grey langurs and purple-faced langurs precludes meaningful analyses of these trends in their cases. Further work is needed to accurately determine not only the prevalence but also the intensity of infection of these parasites to identify risk areas for zoonotic infection between humans and primates. While the parasitological analyses were restricted to four species of potential zoonotic importance, this analysis clearly shows that a potential for zoonotic transmission between humans and non-human primates exists. Hence, an expanded analysis of a wider range of potential zoonotic agents is warranted. Verification of cross-species transmission, whether it is between different monkey species and/ or between monkeys and humans will require molecular typing of the parasites recovered from monkeys and humans in these areas to clearly determine the potential threat of zoonoses (Gasser *et al*., 2009).

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