Prevalence and co-occurrence of gastrointestinal parasites in Nilgiri Langur (*Trachypithecus johnii*) of fragmented landscape in Anamalai Hills, Western Ghats, India

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Habitat fragmentation is known to alter species composition, influence infection risk and disease emergence in the native species of fragmented landscapes. This study aimed at understanding the prevalence of gastrointestinal parasite in Nilgiri langur, an endemic primate species of the Western Ghats, India. We collected 283 faecal samples from 8 rainforest fragments of Anamalai Hills, Western Ghats and examined gastrointestinal parasites using faecal flotation and sedimentation techniques. A total of 13 gastrointestinal parasite taxa were recovered, which are known to infect humans and livestock. Parasite species richness was higher in disturbed forest fragments than undisturbed ones. We found *Trichuris trichiura* to be the most prevalent parasite taxa followed by *Strongyloides* sp. A negative association between *Schistosoma* sp. and *Trichuris trichiura* was also observed. Fragment size, proximity to human settlements and other habitat variables such as tree density, canopy cover and tree height did not show any significant relationship with parasitism in Nilgiri langur, which might be attributed to their ability to survive in a disturbed landscape.

Keywords: Coccidia, forest fragmentation, gut parasites, Nilgiri langur, positive/negative association, strongyloides, Trichuris.

INCREASE in human population, agricultural expansion and urbanization are major reasons for habitat loss and forest fragmentation in tropical countries[1]. Forest fragmentation is known to impact species diversity, species composition, abundance, intra- and inter-specific interactions[2–4]. These changes augment the risk of acquiring parasite infection in native species including primates, which are more sensitive to parasitic infection resulting in high mortality and morbidity[5,6]. Further, the group living tendencies and social behaviour of primates increases their vulnerability to parasite infection[7,8]. Also, factors like environmental/microclimate conditions, dynamics of host parasite interaction and physiology/biology of both etiological agent and the host species govern the infection risks in primates[9]. Ecological factors like weather and habitat condition are known to influence enteric parasitism in olive baboons (*Papio anubis*) as evidenced by a higher prevalence of roundworm infection in forest dwellers than the populations of savanna[10]. A positive association has also been reported between host dominance hierarchy and nematode parasite transmission among females in a wild group of Japanese macaques[11].

Nilgiri langurs are endemic and listed in Schedule I of Indian Wildlife Protection Act (1972), primate species found in the Western Ghats between 8° and 12°N from Agasthyamalai in Kerala in the south to Kodagu in Karnataka in the north[12]. These folivores are known to live in groups with infrequent social interactions and form meta population by dispersing between forest fragments[12,13]. As a part of the research programme on host–parasite interaction in endemic and endangered animals of rainforest fragments in Western Ghats, India[14–16], the present study focused on identifying and quantifying gastrointestinal parasites of Nilgiri langurs in forest fragments of Anamalai Hills, Western Ghats. Additionally, plausible patterns of association between gastrointestinal parasites were also examined.

We carried out the study in fragmented rain forests of Anamalai Tiger Reserve (10°12′–10°35′N and 76°49′–77°24′E, Figure 1) and neighbouring Valparai plateau in Anamalai Hills, southern Western Ghats, India. The Valparai plateau (about 220 sq. km) was once covered with continuous tropical rainforest vegetation, which was clear felled between the 1890s and 1930s to develop the land

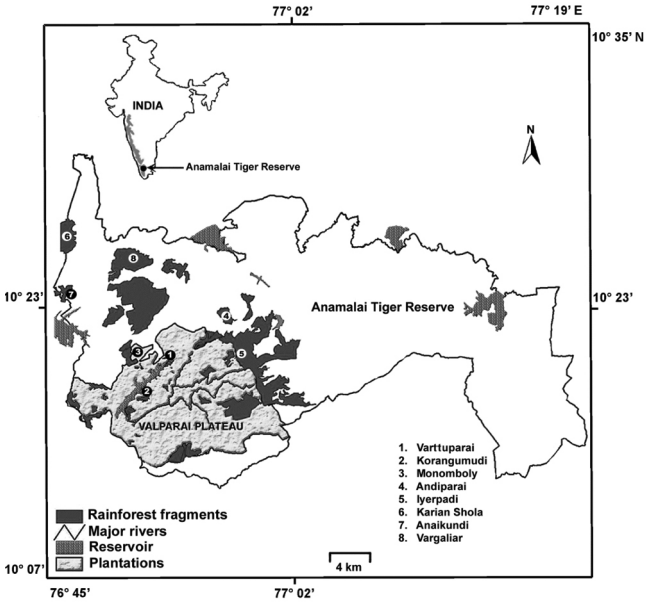

Figure 1. Rainforest fragments in Anamalai Tiger Reserve, Western Ghats, India.

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for tea, coffee, cardamom and Eucalyptus plantations[17]. Deforestation resulted in the creation of nearly 40 forest patches ranging from 2 ha to 2000 ha in the area which are interspersed with commercial plantations, the Valparai township and Pollachi-Chalakkudy road[2,15].

We collected fresh faecal samples by following Nilgiri langur groups between January 2014 and September 2015 in the rainforest fragments of Anamalai Hills, India. Approximately 5–10 g of faeces were sampled from the centre of faecal mass; an aliquot of which was concomitantly preserved in 10% formalin and remainder processed in 2.5% (w/v) potassium dichromate solution to facilitate sporulation of protozoan cysts[15,18]. Faecal samples were screened for the presence of gastrointestinal parasitic forms like helminth eggs, larvae and protozoan cysts by both faecal flotation and faecal sedimentation techniques[16,19]. We identified gastrointestinal parasite taxa by morphological characteristics like size, colour, wall structure, internal content and shape of various infective stages. If needed, a drop of Lugol's iodine solution was used to highlight the internal inclusions and facilitate the identification of protozoan cysts[20,21]. Except coccidia, the isolated gastrointestinal parasite taxa were identified at genus and/or species level.

We estimated the number of Nilgiri langur groups in each forest fragment by using line-transect method. Each forest fragment was surveyed at least 3–6 times along the existing trails between 0700 h and 0900 h when the langurs were more active[22]. The Nilgiri langur density (the number of groups per square kilometer) was estimated using King's method [$D = n/(l \times 2d)$, where n is the number of groups sighted, l the length of transect (in km) walked, and d the mean sighting distance (in km)][12]. However, in small forest fragments like Varattuparai (24 ha) and Korangumudi (35 ha), the Nilgiri langur groups were counted by traversing the entire forest fragment.

We estimated habitat variables like tree density (>15 cm GBH), basal area, canopy height, percentage canopy cover, percentage shrub cover and stump density (number of cut trees per ha) using 5 m circular plot method as described earlier[2]. We also collected information on human presence and settlement in all forest fragments.

We defined prevalence as the percentage of samples with any gastrointestinal parasite taxa and species richness as the number of unique gastrointestinal parasite taxa recovered from a sample[14,15]. We used Mann–Whitney U (M–W U test) statistics to test for differences between 2 samples and Spearman rank correlation coefficient (r_s) to examine the association between the two variables. Also, χ^2 test of independence was used to compare the prevalence of each parasite taxon[15].

We used non-metric multidimensional scaling (NMDS) at infrapopulation levels to elucidate the degree of overlap among the populations between forest fragments based on the occurrence of gastrointestinal parasite taxa and Jaccard's index as a similarity measure[23,24]. The variables used in NMDS analysis include the presence/absence of parasite taxa, presence/absence of humans, season and habitat variables of forest fragments. The existence of these clusters by fragments was further evaluated by one-way ANOSIM. We used PAST software for both NMDS and ANOSIM analyses[25] and SPSS, version 17.0 (SPSS, Inc., Chicago, IL) for other statistical analyses. We used sample-based rarefaction curves to determine the adequacy of sampling in landscape level in detecting the parasite species richness of langurs and comparing the same at infrapopulation levels using Estimate S 9.1.0 (ref. 26).

We collected 283 faecal samples of Nilgiri langurs from 8 forest fragments in Anamalai Hills, Western Ghats. 77.03% of these samples had at least 1 gastrointestinal parasite taxa and 60.09% of these positive samples had multiple gastrointestinal parasitisms (Table 1). A total of 13 gastrointestinal parasite taxa were recorded, which include 8 nematodes (*Ascaris* sp., *Trichuris trichiura*, *Strongyloides* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Enterobius* sp., *Bunostomum* sp., *Gongylonema* sp.); 1 each of cestode (*Hymenolepisnana*) and trematode (*Schistosoma* sp.); and 3 protozoa (*Neobalantidium* sp., *Cyclospora* sp., Coccidia; Table 2).

The number of parasite taxa recorded in forest fragments ranged from 4 to 11; the lowest was recorded in Anaikundi, an undisturbed forest fragment and the highest in Andiparai, a moderately disturbed forest fragment with human settlement on the periphery (Table 1). Owing to the difference in the number of samples collected from forest fragments, we analysed the data using sample-based rarefaction procedure and found the parasite species richness to be the lowest in Anaikundi and the highest in Varagaliar (Table 1). We did not find any significant difference in the number of parasite taxa or the percentage prevalence of these parasites in Nilgiri langurs inhabiting forest fragments with or without human settlement (M–W $U = 3.500$, $P = 0.219$; M–W $U = 2.000$, $P = 0.099$ respectively). Further, the number of parasite taxa, parasite prevalence and multiple parasite infections did not differ between dry and wet seasons among the fragments (M–W $U = 20.5$, $P = 0.22$, M–W $U = 25$, $P = 0.462$ and M–W $U = 19.5$, $P = 0.172$ respectively). Also, neither did the number of gastrointestinal parasite taxa ($r_s = -0.135$, $P = 0.750$) nor the percentage prevalence of these parasites ($r_s = 0.479$, $P = 0.230$) correlates with the area of forest fragments. Additionally, there was no significant correlation between the number of gastrointestinal parasite taxa or the percentage prevalence with habitat attributes namely tree density, basal area, canopy cover, shrub cover and stump density (Table 3). Surprisingly, the percentage prevalence of *Ascaris* sp. infection was higher in Nilgiri langurs inhabiting larger forest fragments as compared to those inhabiting smaller fragments ($\chi^2 = 7.696$, $P = 0.006$). Although Nilgiri

Table 1. Details of forest fragments, the number of parasite taxa observed/expected and percentage prevalence of gastrointestinal parasite taxa in Nilgiri langur in eight forest fragments of Anamalai Tiger Reserve, Western Ghats, India

Fragments	Area (ha)	Human presence	Total no. of faecal samples	No. of gastro intestinal parasite taxa (S_{obs})	Estimated gastrointestinal parasite taxa (S_{est})	Prevalence (%)	Multi parasites in infected samples (%)
Vargaliar	2000	N	36	07	13.00	80.56	55.17
Karian Shola	1520	N	45	09	11.54	91.12	75.61
Anaikundi	225	N	15	04	08.00	80.00	41.67
Monomboly	200	Y	38	09	12.43	76.31	55.17
Andiparai	185	Y	43	11	09.96	65.12	64.29
Iyerpadi	100	Y	40	08	10.89	72.50	62.06
Korangumudi	35	Y	26	09	12.04	80.77	52.38
Varattuparai	24	Y	40	07	12.75	72.50	55.17

Table 2. Percentage prevalence of gastrointestinal parasite taxa in Nilgiri langur in eight forest fragments in Anamalai Tiger Reserve, Western Ghats, India

Phylum	Gastrointestinal parasite taxa	Varagaliar	Karian Shola	Anaikundi	Monomboly	Andiparai	Iyerpadi	Korangumudi	Varattuparai	No. of fragments recorded
Nematoda	*Enterobius* sp.	0	0	0	0	03.57	0	0	0	1
	Bunostomum sp.	0	0	0	0	03.57	0	0	0	1
	Gongylonema sp.	17.24	0	0	03.44	07.14	0	14.28	0	4
	Trichostrongylus sp.	10.34	07.31	0	03.44	07.14	06.89	14.28	03.44	7
	Oesophagostomum sp.	06.89	09.75	25.00	06.89	14.28	06.89	0	06.90	7
	Ascaris sp.	34.48	41.46	0	03.44	25.00	20.69	33.34	20.69	7
	Strongyloides sp.	31.03	39.02	25.00	48.27	25.00	31.03	23.81	44.82	8
	Trichuris trichiura	68.96	85.36	83.34	82.75	82.14	82.75	71.43	72.41	8
Cestoda	*Hymenolepis nana*	0	09.75	0	0	0	06.89	0	0	2
Trematoda	*Schistosoma* sp.	0	0	0	10.34	0	0	09.52	0	2
Protozoa	*Cyclospora* sp.	0	04.87	0	03.44	07.14	0	04.76	0	4
	Neobalantidium sp.	0	02.44	83.34	0	07.14	03.45	04.76	17.24	6
	Coccidia	03.44	04.87	0	03.44	14.29	10.34	09.52	10.34	7

Table 3. Analysis of similarities (ANOSIM) among the forest fragments *R* values (lower left half) and *P* values (upper right half) with reference to habitat variables, parasite prevalence, presence of human settlement and seasonal variation

	Anaikundi	Andiparai	Iyerpadi	Karian Shola	Korangumudi	Monomboly	Varattuparai	Varagaliar
Anaikundi		0.0001	0.0001	0.2295	0.0001	0.0001	0.0001	0.4257
Andiparai	0.6457		0.9053	0.0001	0.4920	0.0745	0.8824	0.0001
Iyerpadi	0.6616	–0.0176		0.0001	0.1291	0.0112	0.7816	0.0001
Karian Shola	0.0447	0.5490	0.5104		0.0001	0.0001	0.0001	0.0083
Korangumudi	0.5361	–0.0024	0.0378	0.4087		0.0736	0.2377	0.0001
Monomboly	0.6376	0.0282	0.0512	0.4782	0.0492		0.0785	0.0001
Varattuparai	0.6279	–0.0166	–0.0131	0.5046	0.0208	0.0284		0.0001
Varagaliar	0.0026	0.5765	0.5474	0.0675	0.4616	0.5920	0.5489	

langur density ranged from 10 groups per sq. km (Andiparai) to 28 groups per sq. km (Anaikundi), no significant correlations between Nilgiri langur density and the number of gastrointestinal parasite taxa ($r_s = –0.246$; $P = 0.558$) and the percentage prevalence of these parasites ($r_s = 0.419$, $P = 0.301$) were observed.

NMDS analysis based on factors like the presence/absence of gastrointestinal parasite taxa, presence/absence of humans, season and habitat variables of forest fragments did not show any clustering pattern (Figure 2). Interestingly, there was a statistically significant difference in parasitism of langurs inhabiting undisturbed forest fragments compared to disturbed forest fragments ($R = 0.06751$ to 0.6616; $P < 0.05$; Table 3).

We found *Trichuris trichiura* to be the most dominant parasite taxa recovered in 78.89% of all the positive samples, followed by Strongyloides sp. in 34.86% of the samples. Both *Trichuris trichiura* and *Strongyloides* sp. were recorded in all the 8 forest fragments (Table 2). Interestingly, 33.72% of *Trichuris trichiura* positive

samples had *Strongyloides* sp. and 18.60% had *Ascaris* sp. infection respectively. None of the *Schistosoma* sp. positive samples had *Trichuris trichiura* eggs, while *Trichostrongylus* sp. was absent in *Strongyloides* sp. positive samples, indicating a plausible negative relationship between these parasite pairs (Table 4). NMDS analysis revealed three close associations between parasites namely *Trichuris trichiura* – *Strongyloides* sp.; *Ascaris* sp. – *Trichostrongylus* sp. – Coccidia and *Enterobius* sp. – *Bunostomum* sp.

This is the first study on gastrointestinal parasitism of Nilgiri langur populations inhabiting fragmented landscape of Anamalai Hills, Western Ghats, India. The present study showed 77.03% of all samples to be positive for at least 1 parasite taxa and multiple gastrointestinal parasitisms in 60.09% of these positive samples. In all, 13 parasite taxa were found to infect Nilgiri langurs with possible positive/negative interactions amongst themselves. All the parasite taxa recorded in this study are known pathogens of humans and nonhuman primates[6,10,14,27]. A similar parasite profile was also reported in other primate species of the fragmented forest ecosystem; 17 enteric parasites were reported in red colobus monkeys of forest patches along Tana River, Kenya[28], 14 gastrointestinal parasites in red colobus monkeys of fragmented forests in western Uganda[29] and 10 parasite species in red tail guenon of Kibale National Park, Uganda[29]. Of the 8 forest fragments studied, the lowest number of parasite taxa was recorded in Anaikundi, an undisturbed forest fragment away from human settlement, whereas the highest number of parasites was recorded in Andiparai forest fragment which is moderately disturbed and has a human settlement on the periphery. Sharing of water sources is known to increase the parasite load in primates[14], which is especially true in the case of Andiparai forest fragment where both wild animals and human/cattle share stream water frequently. Similarly, the numbers of parasite species infecting red tail guenons inhabiting logged forests have been found to be higher than primate population of unlogged forests[29]. Also, the presence of humans in the periphery of forest fragments is known to increase parasite species richness in Lion-tailed macaque[14]. Trejo-Macías *et al.*[30] also reported higher parasite prevalence in *Alouatta palliatamexicana* and *Alouatta pigra* populations of fragmented forests as compared to individuals inhabiting continuous/protected forests of Los Tuxtlas, Mexico. In this study, we did not find any significant difference in the percentage prevalence of parasite infection between Nilgiri langurs inhabiting disturbed and undisturbed forest fragments suggesting that habitat disturbance did not influence parasite prevalence in Nilgiri langur populations of Anamalai Hills, which is in accordance with earlier findings in red colobus and black and white colobus monkeys of Kibale National Park, Uganda, where no significant difference in parasite prevalence was found among populations inhabiting logged and unlogged forests[29]. Also, Nilgiri langurs may have an exploratory foraging behaviour (reported in colobus monkeys) and ability to disperse between forest fragments to form a meta population[12,31]. These explorers may act as a potential reservoir and intra- or inter-specific dispersers of gastrointestinal parasites and other etiological agents as reported in *Alouatta palliate* and *A. pigra* in forest fragments of Mexico[32].

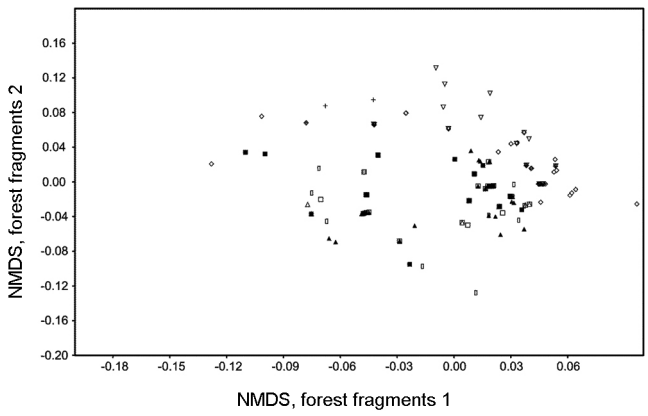

Figure 2. Non-metric multidimensional scaling analysis of habitat variables, presence/absence of parasite taxa and human settlement on the forest fragments.

Interestingly, the percentage prevalence of *Ascaris* sp. was higher in Nilgiri langurs of large and undisturbed forest fragments as compared to those inhabiting small and disturbed forest fragments which might be attributed to the interplay of local factors. Thus needs further investigation. However, there was no significant difference in the percentage prevalence of other parasite taxa observed among the fragments which vary in their size and disturbance level. A similar observation was made in studies on chimpanzees of Budongo Forest, Uganda, where the prevalence of all the 13 parasite taxa was similar across sites[33] and there was no difference in the percentage prevalence of parasite infection of black and white colobus monkeys in fragmented and unfragmented forests of Kibale National Park, Uganda[34]. In earlier studies on meta populations of red colobus monkeys inhabiting fragmented forests adjoining Kibale National Park and Ruwenzori mountains of Uganda, nematode infection risk positively correlated with the stump density in forest fragments[35]. However, in this study, we did not find any correlation between parasitism and various habitat attributes indicating the ability of Nilgiri langurs to adapt in fragmented forests of Anamalai Hills, Western Ghats.

Host density is known to affect gastrointestinal parasitism in many social animals[36] including primates. Nilgiri langur density which ranged widely (10 groups per sq. km to 28 groups per sq. km) among the forest fragments did not show any significant relationship with the number of parasite taxa present or the percentage prevalence of these parasites. This finding is against the

Table 4. Co-occurrence of gastrointestinal parasite taxa in Nilgiri langurs of the forest fragments in Anamalai Tiger Reserve, Western Ghats, India

Parasite taxa	Prevalence (%)	*Ascaris* sp.	*Trichuris trichiura*	*Strongyloides* sp.	*Neobalantidium* sp.	*Trichostrongylus* sp.	Coccidia	*Gongylonema* sp.	*Hymenolepis nana*	*Schistosoma* sp.	*Cyclospora* sp.	*Oesophagostomum* sp.	*Enterobius* sp.
Ascaris sp.	24.77												
Trichuris trichiura	78.89	√											
Strongyloides sp.	34.86	√	√										
Neobalantidium sp.	05.04	√	√	√									
Trichostrongylus sp.	06.88	√	√	x	x								
Coccidia	07.34	√	√	√	x	√							
Gongylonema sp.	05.04	√	√	√	x	√	√						
Hymenolepis nana	02.75	√	√	√	x	x	x	x					
Schistosoma sp.	02.29	x	x	√	x	x	x	x	x				
Cyclospora sp.	02.75	√	√	x	x	√	x	x	x	x			
Oesophagostomum sp.	08.71	√	√	√	√	x	x	x	x	x	√		
Enterobius sp.	00.45	x	x	x	x	x	x	x	x	x	x	x	
Bunostomum sp.	00.45	x	√	x	x	x	x	x	x	x	x	x	x

√, Indicates co-occurrence; x, Indicates absence.

epidemiological theory that host density influences both the parasite species richness and prevalence of directly transmitted parasite, which is due to the ability of Nilgiri langurs to move between forest patches using surrounding matrix and form a metapopulation. Dispersing individuals may act as parasite transmitters between individuals of different forest fragments[12,37].

Further analyses to understand association between parasite taxa revealed three sets of association, namely *Trichuris trichiura* – *Strongyloides* sp.; *Ascaris* sp-*Trichostrongylus* sp. – Coccidia and *Enterobius* sp.-*Bunostomum* sp. in Nilgiri langurs. *Trichuris trichiura*, a human parasite is also found commonly in free ranging primates[38] and like most nematodes, has a direct life cycle. It is transmitted through ingestion of embryonated eggs or first-stage infective larvae, which develop into adult worms, the anterior ends of which are threaded in the mucosal epithelium of the ascending colon or cecum[39]. In contrast, *Strongyloides* sp. has a complex life cycle, where the infective larvae develop into adult worms that reside in inter-epithelial tunnels or lumina of intestinal glands in the small intestine of the host species[14,39]. *Trichuris trichiura* and *Strongyloides* sp. occupy different locations and derive nutrition from the same host. Thus they seem to co-occur in the host species. Similarly, *Ascaris* sp., *Trichostrongylus* sp. and Coccidia occupy a different location in the gut of the host species. Adult worms of *Ascaris* sp. live in the lumen of the small intestine, and the infective larvae invade mucosal lining and migrate to the lungs via systemic circulation. On the other hand, adult worms of *Trichostrongylus* sp. burrow themselves superficially in the crypts of mucosa and most coccidian parasites are intracellular pathogens that raid the epithelial cells of the intestine[39]. Except Coccidian parasites that exist in both the small and large intestine of host species, *Ascaris* sp. and *Trichostrongylus* sp. are present in the small intestine[40]. These parasite taxa occupy a different niche in the host tissue, unlike *Trichuris* sp. and *Strongyloides* sp. which inhabit distinct organs. Not much is known about the pathogenesis of *Enterobius* sp. and *Bunostomum* sp. in non-human primates. However, *Enterobius* sp. is known to inhabit the colon in primates and *Bunostomum phlebotomum*, a parasite of ruminants is located in the abomasum and duodenum[39,41]. It may be possible that *Bunostomum* sp. that infects primates is present in the small intestine, thereby occupying a different location as compared to *Enterobius* sp. Interestingly, not all the parasites occupying a similar niche were recovered from a faecal sample. However, a detailed study is required to understand this co-occurrence and their relationships to understand parasitism in primates.

Additionally, negative associations were found between *Trichuris trichiura* – *Schistosoma* sp. and *Strongyloides* sp. – *Trichostrongylus* sp. pairs. Curry *et al.*[42] have demonstrated that co-infection of laboratory mice with *Schistosoma mansoni* and *Trichuris muris* eggs initiates a TH_2 mediated immune response in the mice to *Schistosoma* eggs, which in turn leads to the elimination of *Trichuris muris* infection. The antigenic molecular mimicry between these 2 species might be a possible explanation for the absence of *Trichuris trichiura* eggs from faecal samples positive for *Schistosoma* sp. in Nilgiri langur's. In the case of *Strongyloides* sp. – *Trichostrongylus* sp. negative association, these parasite taxa are known to infect the glands in small intestine, which might lead to a competitive interaction for space and nutrition between them[39]. Furthermore, pathologic changes of strongyloidiasis in the host gut-like shortening of villi or the loss of villi in severe infection may lead to a decrease in surface area and thereby the availability of luminal glands for infection by *Trichostrongylus* sp.[39]. Besides, interference or resource competition *Strongyloides* sp. and *Trichostrongylus* sp. may be antigenically similar parasite taxa which need further study.

Although 13 parasitic taxa were recorded in Nilgiri langur the fragmentation of habitat did not influence significantly on the parasitism in langur as they are capable of forming a meta-population. This study also records many negative and positive associations among the parasite taxa of Nilgiri langur. However a detailed study is required that involves more samples from known individuals and identification of parasite taxa at species level to understand parasitism in Nilgiri langur in fragmented rainforest landscape.

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