Prevalence of gastrointestinal parasites in the Nicobar long-tailed macaque (*Macaca fascicularis umbrosus*) on the Nicobar Group of Islands, India

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Changes in the habitat can drive the species to adapt to the changing environment that may lead to a risk of infection and the emergence of diseases. The prevalence of gastrointestinal parasites (henceforth endoparasites) in a species is an indicator of changing habitat conditions, and the study of the same is important when the species is restricted to a few islands. Macaca fascicularis umbrosus, endemic to Great Nicobar, Little Nicobar and Katchal islands. The December 2004 tsunami destroyed much of its habitat and pushed them to agriculture fields, leading to a conflict. To study the endoparasites in these macaques, we collected 160 faecal samples from five groups of macaques on Great Nicobar, one group on Little Nicobar, and two groups on Katchal between 2014 and 2016. The endoparasite eggs and cysts were isolated from the faecal samples using flotation concentration and sedimentation techniques in the laboratory. The number and percent prevalence of endoparasites recorded in Great Nicobar, Little Nicobar and Katchal was 13, 5 and 3, and 69.38%, 60.00% and 39.39% respectively. The Campbell Bay group on Great Nicobar had 12, whereas other groups had 2-7 endoparasite taxa. The protozoan load was higher than the helminth load but the overall, helminth, and protozoan load did not differ between the islands. Ascaris sp., Oesophagostomum sp., Strongyloide ssp., Bunostomum sp. and Balantidium coli were the predominant endoparasites. The persistence of macaque with people probably has increased the richness and prevalence of endoparasites on Great Nicobar than in the other two islands.

Keywords: Changing habitat, faecal samples, gastroin-testinal parasites, islands, long-tailed macaque.

OWING to the concerns for human and wildlife health, especially in the habitats where they increasingly interact, endoparasites and their transmission have become an important issue. The alteration or loss of habitat can drive the species to adapt to the changing environment leading to a risk of emergence of diseases and infection of pathogens, including endoparasites¹. Endoparasites get their nourishment from the host organism and become pathogens to kill the host, or indirectly act on the host by reducing its immunity to fight against diseases. The prevalence, species richness and abundance of endoparasites in a species are among the indicators of changing habitat conditions. Thus a study of the same is important when the species is restricted to a few small and highly dynamic islands. One such species is the Nicobar long-tailed macaque Macaca fascicularis umbrosus, with a restricted distribution in the Nicobar Islands, India, viz. Great Nicobar, Little Nicobar and Katchal. It is one of the subspecies of the otherwise widespread long-tailed macaques^{2,3}.

Long-tailed macaques occupy a wide variety of habitats; vet they are commonly found along the seashore, mangrove forests and swamp forests⁴. Since the range of each subspecies is highly restricted to the largely isolated islands, their habitat is potentially susceptible to degradation on account of global warming, natural catastrophes and anthropogenic pressures^{5,6}. In many countries, shrimp farming, shipbuilding, agriculture and logging, among other anthropogenic activities, have also significantly reduced the extent of their habitat⁶. Consequently, the longtailed macaques have become commensal in most of their distribution range, with an increased conflict with people, resulting in a decline in the population in several countries⁷. Thus, the species is now considered widespread but rapidly declining⁸. The Nicobar long-tailed macaque is not an exception, as its habitat is also highly fragile facing many of the above-mentioned pressures. Thus, these

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Habitat features	Great Nicobar	Little Nicobar	Katchal
Location	93°38'05.6"–93°57'13.7"E and 6°44'7.8"–7°13'46.6"N	93°36'14.0"–93°46'17.4"E and 7°14'45.2"–7°26'33.7"N	93°28'32.9"–93°18'06.8"E and 7°52'24.2"–8°1'33.6"N
Island size (km ²)	895.48	138.25	139.39
Highest elevation (m amsl)	642	470	230
Inundated area (km ²) due to the 2004 tsunami	51.91	10.04	21.80
Human population	8069	298	2681
Human density/km ²	9.01	2.15	19.23
Encounter rate of Nicobar long-tailed macaque (groups/km)	0.30	0.35	0.48
Mean group size of Nicobar long-tailed macaque	39.83	-	43.50

Table 1. Habitat features of three islands inhabited by the Nicobar long-tailed macaque (source: ref. 3; District Census Hand Book 2011)

macaques are also categorized as 'vulnerable'⁹, and accorded the highest protection under 'Schedule-I' of the Indian Wildlife (Protection) Act-1972 (ref. 10).

The Nicobar long-tailed macaque has been associated with coastal vegetation, riverine forests or swamps². The December 2004 tsunami destroyed much of the coastal habitat and even the inland forests on some islands. The destruction of coastal forests was complete in a few islands like Katchal, and moderate to severe in the Great Nicobar and Little Nicobar^{3,11}. The habitat destruction caused a sharp decline in the population size and the age-sex ratio of these macaques was also found to be skewed¹². Later, the population showed a trend towards recovery and has even surpassed the pre-tsunami population size in recent years³. Minimal crop foraging by these macaques in some areas of the islands was reported during the pre-tsunami period². Due to the loss of habitat caused by the tsunami, several groups of these macaques were pushed to the villages and agriculture fields that increased the number of commensal macaque groups, leading to a high incidence of crop foraging and human-macaque conflict^{3,13}. They had adapted to live in the human-dominated landscape and with new food sources¹⁴⁻¹⁶. This may have exposed them to different pathogens, including endoparasites^{17,18}. The new pathogens or alteration in parasite composition can potentially alter the host's natural dynamics leading to difficulty in the survival of the individuals^{19–22}. Since there is no earlier documentation of endoparasites in the Nicobar long-tailed macaque, a direct comparison of the parasite species composition between pre- and post-tsunami periods is not possible. It has been observed in bonnet macaques of southern India, that the degree of provisioning was the most important determinant for the richness and load of the endoparasites²³. Similarly, in the lion-tailed macaque of the Western Ghats, a greater prevalence and number of endoparasite taxa were observed in groups closer to human settlements and domestic livestock than those away from human settlements²⁴. We, therefore, expected that the long-tailed macaque groups ranging in or close to human settlements would have more prevalence of endoparasites than the groups having lesser interactions with humans. The present study will provide baseline data for future research and management of these macaques in the Nicobar Islands.

Methods

Study site and study groups

The study was conducted on three islands, viz. Great Nicobar (GN), Little Nicobar (LN) and Katchal (KL) in the Andaman and Nicobar archipelago, India between 2014 and 2016 (Figure 1). Great Nicobar is the largest island in the Nicobar group (895.48 km²). Table 1 provides the habitat features of these islands. The main forest type of these islands is tropical evergreen, and all islands have more than 98% forest cover²⁵. There is a high degree of endemism in flora and fauna on these islands^{25,26}. The remoteness of these islands, coupled with high biodiversity occurring in small pockets, makes the archipelago vulnerable to natural disasters and exploitation. The December 2004 tsunami inundated the low-lying areas, including coastal swamp forests³. The human density is highest in Katchal, followed by Great Nicobar and Little Nicobar. However, the human population has been confined to a few villages on these islands. Great Nicobar being the largest island among the group of the Nicobar Islands, has the main port and a major market centre for these islands. This island, therefore, receives many ships and people. More groups of Nicobar long-tailed macaques have adapted to live in the human-dominated landscapes of Great Nicobar than in the Little Nicobar and Katchal³. We selected five groups of macaques (TR, PI, GN, MA and WT) from the Great Nicobar, one group (LN1) from the Little Nicobar and two groups (KT and OP) from Katchal for the collection of faecal samples. The macaque groups in the Great Nicobar had more ranging in and around human settlements than the groups in Little Nicobar and Katchal (Figure 1). During the survey of macaques in these islands³, we identified the groups based on the feasibility to follow them for sampling. We attempted to follow the selected groups of macaques between 2014 and 2016. Since large parts of these islands have impenetrable evergreen forests, we were able to follow and collect faecal samples from only a few groups; nevertheless we were able to collect faecal samples of these macaques from all three islands. We collected a total of 160 faecal samples from the study groups (TR – 59; PI – 11; GN – 15; MA – 5; WT – 8; LN – 29; KT – 15; OP – 18).

The group TR, located at Campbell Bay in the Great Nicobar, was selected for a long-term ecological and behavioural study, and all group members were individually identified. Although we attempted to collect one faecal sample per identified individual per month from the TR group, it could not be done due to rainfall, terrain and bush cover. However, the identity of the individuals helped avoid duplication of the faecal samples from the same individual in a month. Of the 160 faecal samples, 59 were from the TR group collected between January 2016 and December 2016. We pooled the faecal samples according to the wet season (35 samples collected between June and November) and dry season (24 samples collected between December and May).

Faecal sample collection

On observing fresh defecation while following a group, we collected 2 g of faeces and preserved it with 10% formalin.

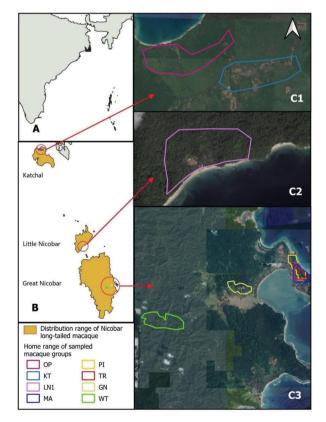


Figure 1. Location of the study groups and indication of ranging in human-dominated or largely forested areas. Groups MA, PI, and TR range primarily in the Campbell Bay township, Great Nicobar, India.

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We labelled each vial with a unique sample number, date, age–sex and individual identity when known, group and island identity.

Laboratory analysis of faecal samples

For each sample, 1 g of faecal sample was taken in a 15 ml Tarson centrifuge tube and 10 ml of distilled water was added to it. The content was homogenized using a glass rod and mixed thoroughly using a vortex for 10 min. The mixture was filtered using a cheesecloth. The volume of the filtrate was increased with distilled water up to 15 ml and centrifuged at 1800 rpm for 10 min. The supernatant was discarded and only the pellet was retained. The endoparasite eggs and cysts were isolated from the faecal samples using flotation concentration and sedimentation techniques in the laboratory^{27,28}. Both techniques were used to maximize the detection of all possible endoparasites in the samples. A McMaster's counting chamber was used to quantify the number of eggs per gram of each endoparasite species in the faeces²⁹.

Flotation concentration method: Ten millilitres of saturated sucrose solution (1.3 g/ml) was added to the pellet and thoroughly mixed. The volume of the mixture was increased with a sucrose solution up to 14.5 ml. The mixture was centrifuged at 4000 rpm for about 10 min. The upper layer of the mixture was separated and deposited in both chambers (0.3 ml) of the McMaster's counting chamber using transfer pipettes and allowed to settle for 5 min in order to allow the eggs float to the surface.

Sedimentation method: Ten millilitres of soap solution (specific gravity 0.002) was added to the pellet. The content was centrifuged at 5000 rpm for 5 min. The supernatant was discarded after leaving a few drops of suspension on the sediment pellet. This sediment mixture was deposited in one of McMaster's counting chambers. Finally, the eggs were identified using a 40× microscope and counted using a 10× objective under the light microscope (Lynx PH-100, LM-52-1804/SL. No. 100044). Each grid of the McMaster's slide was separately photographed and the images were stored in computer system with an ID using a microscope camera (ISH500) with the help of IS Capture 3.6.6 software³⁰.

Identification of endoparasite eggs/cysts: The endoparasite species were identified using appropriate taxonomic keys based on the morphology of their eggs and cysts^{31–36}.

Data analysis

Endoparasite richness is the number of endoparasite taxa recorded in the faecal samples. We pooled the number of endoparasite taxa in each sample and calculated the

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Ta	ble 2. Percent j	prevalence and end	oparasite taxa re	corded in the Nico	obar long-tailed m	acaque groups	
Group	No. of samples	Sample with endoparasite	Percent prevalence	No. of observed taxa	Helminth load	Protozoan load	Overall load
Great Nicobar (GN)							
TR	59	41	69.5	12			
PI	11	8	72.7	6			
GN	15	8	53.3	7			
MA	5	5	100.0	4			
WT	8	6	75.0	6			
Total	98	68	69.4	13	58.1 ± 155.3	221.2 ± 479.8	161.5 ± 388.0
Little Nicobar (LN)							
LN1	29	6	20.6	5	6.1 ± 9.3	145.3 ± 276.5	100.9 ± 225.0
Katchal (KL)							
KT	15	7	46.7	3			
OP	18	6	33.3	2			
Total	33	13	39.4	3	80.0 ± 190.3	84.6 ± 155.2	92.4 ± 182.0
Overall					57.6 ± 154.3	196.2 ± 433.9	

TR, Temple troop; PI, Pirate; GN, Govinda Nagar; MA, Macho; WT, 13 km watchtower; LN1, Little Nicobar group; KT, OP, Groups in Katchal.

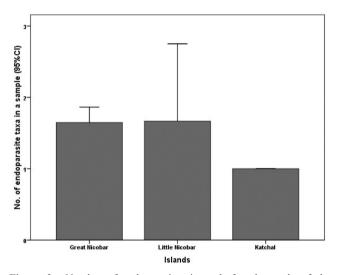


Figure 2. Number of endoparasites in each faecal sample of the Nicobar long-tailed macaque (number of faecal samples in Great Nicobar = 98, Little Nicobar = 29, Katchal = 33).

endoparasite richness for each macaque group. Endoparasite abundance is defined as the total number of eggs/cysts present in each sample. Endoparasite prevalence is the percentage of samples having endoparasite taxa of the total samples. We computed the endoparasite richness and abundance for each group.

We used χ^2 test for multiple proportions to compare the percent prevalence of endoparasites among the islands and to compare overall parasite prevalence between wet and dry seasons. We used ANOVA to test for differences in the number of endoparasite taxa, and to compare helminth, protozoan, and overall load in the samples among the islands. We used independent samples t-test to compare mean protozoan and helminth load, and mean endoparasite taxa in the samples between the dry and wet seasons. We used StatsToDo for chi-square tests and SPSS ver. 16.0 for ANOVA and t-tests (ref. 37).

Ethical statement

After screening the methodology and requirements of the study, it was funded by the Science and Engineering Research Board, Government of India. We have followed all national and international ethical guidelines during this study. The methodology followed in the study was approved by the Research and Ethical Committee of SACON, Coimbatore. Permission was obtained from the concerned Forest Department to conduct the study (permission letter number CWLW/WL/134/566 by the Forest Department of Andaman and Nicobar Islands).

Results

We collected 98, 29, and 33 faecal samples from five groups in the Great Nicobar, one group in the Little Nicobar and two groups in Katchal respectively (Table 2). The overall percent prevalence of endoparasites was 54.38. The percent prevalence of endoparasites significantly varied among the islands (GN: 69.38, LN: 20.60, KL: 39.39) $(\chi^2 = 53.92; P < 0.01)$, with a significant difference between GN and LN ($\chi^2 = 21.82$; P < 0.01) and GN and KL $(\chi^2 = 9.41; P < 0.01)$, but no difference between LN and KL ($\chi^2 = 2.54$; NS). The percent prevalence among the groups also differed significantly ($\chi^2 = 29.34$; P < 0.01), where two groups, viz. TR and MA of GN had higher values than LN1 of LN and OP of KL, with no differences among the other groups. The TR group in the Great Nicobar had 12 endoparasite taxa, whereas the other groups had 2-7 endoparasite taxa (Table 2). Although the number of endoparasite taxa recorded in the Great Nicobar (13 taxa) was higher than in the Little Nicobar (five) and Katchal (three), the number of endoparasite taxa in each sample among the islands was not different ($F_{2,80} = 2.296$, P = 0.107) (Figure 2).

		Great Nicobar	bar	I	Little Nicobar	ar		Katchal			Total	
	Number of positive	Pre- valence	Mean eggs/ cysts in infected	Number of positive	Pre- valence	Mean eggs/ cysts in infected	Number of positive	Pre- valence	Mean eggs/ cysts in infected	Number of positive	Pre- valence	Mean eggs/ cysts in infected
Endoparasite taxon	samples	(%)	samples (SD)									
Nematodes												
Spirurids	1	1.0	2.5							1	0.6	2.5
Strongyloides stercoralis	13	13.3	129.8 ± 238.5	ю	10.3	8.0 ± 10.4				16	10.0	107.0 ± 218.9
Trichuris sp.	4	4.1	12.0 ± 19.5							4	2.5	12.0 ± 19.5
Bunostomum sp.	11	11.2	7.2 ± 12.1							11	6.9	7.2 ± 12.1
Haemonchus sp.	1	1.0	2.0							1	0.6	2.0
Ascaris sp.	16	16.3	13.4 ± 12.8	2	6.9	0.1 ± 0.0	7	21.2	91.5 ± 202.6	25	15.6	34.2 ± 108.2
Oesophagostomum sp.	23	23.5	45.4 ± 142.2							23	14.4	45.4 ± 142.2
Toxocara sp.	1	1.0	0.2							1	0.6	0.2
Enterobius vermicularis	1	1.0	0.1							1	0.6	0.1
Trichostrongylus sp.	2	2.0	0.4 ± 0.4							2	1.3	0.4 ± 0.4
Protozoa												
Coccidia sp.	9	6.1	13.0 ± 18.7	2	6.9	4.3 ± 3.3				8	5.0	10.8 ± 16.4
Balantidium coli	26	26.5	290.8 ± 534.2	2	6.9	6.3 ± 0.4	1	3.0	30.5	29	18.1	262.2 ± 512.0
Entamoeba coli	7	7.1	14.4 ± 10.9	1	3.4	560.0	7	15.2	95.5 ± 171.0	10	6.3	87.6 ± 177.6

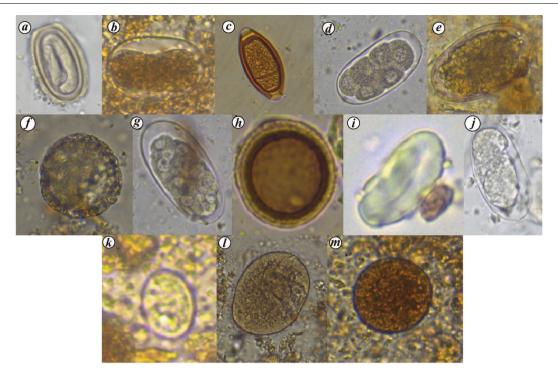


Figure 3. Eggs/cysts of endoparasites recorded in the Nicobar long-tailed macaque: (*a*) Spirurids (57.76 μ m × 44.08 μ m), (*b*) Strongyloides stercoralis (51.72 μ m × 29.78 μ m), (*c*) Trichuris sp. (56.86 μ m × 26.20 μ m), (*d*) Bunostomum sp. (78.72 μ m × 51.32 μ m), (*e*) Haemonchus sp. (77.71 μ m × 41.99 μ m), (*f*) Ascaris sp. (72.01 μ m × 65.80 μ m), (*g*) Oesophagostomum sp. (71.64 μ m × 41.49 μ m), (*h*) Toxocara sp. (84.92 μ m × 75.01 μ m), (*i*) Enterobius vermicularis (53.84 μ m × 26.21 μ m), (*j*) Trichostrongylus sp. (78.64 μ m × 41.50 μ m), (*k*) Coccidia sp. (23.9 μ m × 15 μ m), (*l*) Balantidium coli (62.11 μ m × 40.35 μ m), and (*m*) Entamoeba coli (34 μ m × 34.05 μ m).

Table 4. Prevalence of gastrointestinal parasites in TR group during the wet and dry season in the Great Nicobar Island

Details	Wet	Dry
Number of faecal samples	35	24
Number of samples with endoparasites	25	16
Percent prevalence of endoparasites	71.43	66.67
Mean number of endoparasite taxa (endoparasites/sample)	$1.69\pm0.94_{SD}$	$1.68\pm0.85_{SD}$

The overall mean protozoan load $(196.2 \pm 433.9_{\text{SD}})$ was significantly higher than the helminth load $(57.6 \pm 154.3_{\text{SD}})$ (t = 2.373, df = 108, P < 0.01). However, helminth, protozoan and overall load did not differ between the islands (helminth load: $F_{2,62} = 0.301$, P = 0.741; protozoan load: $F_{2,42} = 0.274$, P = 0.761 and overall load: $F_{2,82} = 0.243$, P = 0.785).

We recorded 13 endoparasite taxa in the Nicobar longtailed macaque, of which 10 were helminths and 3 were protozoans (Table 3; Figure 3). Among the helminths, *Ascaris* sp. was the most prevalent, followed by *Oesophagostomum* sp., *Strongyloides stercoralis*, and *Bunostomum* sp. *Ascaris* sp. was recorded in all the islands and *Strongyloides stercoralis* was recorded in the Great Nicobar and Little Nicobar. The other eight taxa were recorded only in the Great Nicobar. The mean egg load of *Oesophagostomum* sp. was higher than the other helminths. Among the protozoans, percent prevalence and cyst load of *Balantidium coli* were higher than the other two protozoans. The endoparasite prevalence during the wet (71.43%) and dry seasons (66.67%) did not differ in the TR group ($\chi^2 = 0.15$; NS) (Table 4). Similarly, the mean number of endoparasite taxa in a sample also did not differ between the wet (1.69 ± 0.94_{SD} endoparasites/sample) and dry seasons (1.68 ± 0.85_{SD} endoparasites/sample) (t = -0.026, df = 39, P = 0.979).

Discussion

A total of 13 endoparasite taxa were recorded from 160 faecal samples of the Nicobar long-tailed macaque. The number of endoparasite taxa recorded in the Great Nicobar was 13, Little Nicobar was 5 and Katchal was 3. The percent prevalence of endoparasites varied significantly between the islands. Two of the five groups in the Great Nicobar had a higher percent prevalence than one group at Katchal and one group in the Little Nicobar. The overall

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6	Entamoeba chattoni																	0	Ь								Ь			0
10	Entamoeba coli	Ь	Р													Ь		0	Р		Р						Ь			3
11	Entamoeba dispar																	0	Р	Р							Р		Ь	4
12	Entamoeba hartmanni																	0									Ь		Ь	0
13	Entamoeba histolytica																	0		Ч							Р	Р	Ч	4
4	Entamoeba polecki																	0			Ч									1
15	Entamoeba sp.				Р	Ρ	P F	Ь					Р				Р	9						Р						-
16	Giardia sp.				Р	P	P P	•		Ь	Ь		Р				Р	8								Р	Ч	Р		3
17	Iodomoeba butschlii				Р	Р	P P	•								Ь		5			Р						Р			0
18	Isospora sp.				Ь		РР	•					Ь					4									Р			1
19	Retortomonas sp.				Р													1												0
20	Trichomonas sp.				Ь		Ρ	•										0												0
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mean protozoan load was significantly higher than the helminth load, but the overall load, helminth load and protozoan load did not differ between the islands. *Ascaris* sp. *Oesophagostomum* sp., *S. stercoralis, Bunostomum* sp. and *B. coli* were the most predominant endoparasites recorded in these macaques. The endoparasite richness and their load did not differ between the seasons.

After the December 2004 tsunami, some coastal habitats and low-lying areas were inundated on all the three islands³. Due to the loss of coastal habitat and swamp forests along the coastline, several groups of the Nicobar long-tailed macaque were pushed towards villages and agricultural fields on these islands. Due to the loss of family members and agricultural fields, some people shifted their residence and activities, especially to the Little Nicobar and Katchal islands. However, Campbell Bay, Great Nicobar, continued to be the centre of major activity among these islands. Besides, the persistence of macaques in the villages and agricultural fields increased the interaction between macaques, humans and livestock, especially in the Great Nicobar¹³. Consequently, the endoparasite richness and percent prevalence were more in the TR and MA groups of the Great Nicobar, which had more interactions with humans, than in the other groups of the Great Nicobar and the other two islands.

The long-tailed macaque is one of the most studied species of macaques for infection of endoparasites (Table 5^{38-60} . These macaques were often captured and exported for laboratory research from Southeast Asia to the Western countries, especially after the 1977 ban on the export of rhesus macaques from India. During this process, screening and treating for infection was crucial to avoid crosstransmission of infection of endoparasites to the animal handler. Therefore, several studies on captive macaques have been reported (Table 5). Table 5 indicates that the free-living long-tailed macaques had higher endoparasite richness than the captive macaques. The highest endoparasite richness (18 taxa) was reported in macaques occupying different habitat conditions of Bali islands in Indonesia³⁹, followed by the present study where 13 endoparasite taxa have been reported. Also, 11 endoparasite taxa were reported in the Bali islands⁴⁴ (Table 5). Among the endoparasites reported in free-ranging long-tailed macaques, Trichuris sp. and Strongyloides sp. are the most common helminths, whereas B. coli and Giardia sp. are the most common protozoans (Table 5). Among these, Trichuris sp., Strongyloides sp. and B. coli were also recorded in the Nicobar long-tailed macaque. Further, among 13 endoparasite taxa that were recorded in macaques on the Nicobar Islands, the load of Strongyloide sp. and B. coli was very high. The percent prevalence of endoparasites in long-tailed macaques in Nicobar (54.38) was relatively less compared to many other islands, e.g. 78.60 protozoans in Bali, Indonesia³⁹, and 85.71 in Palawan, the Philippines⁶¹. Although endoparasite richness is very high in Nicobar, the prevalence is relatively less than in the other islands. This may be due to less exposure of several groups of long-tailed macaques to a human-dominated landscape, as in Katchal and Little Nicobar.

Lane et al.³⁹ reported that increased provisioning probably increased the immunity due to high nutrition in the provisioned macagues that could decrease the parasite prevalence. On the contrary, the high endoparasite richness and its prevalence in accordance with increased exposure to human-dominated landscapes were recorded in the Nicobar Islands. However, the major difference between these two study sites is the provisioning by tourists in Bali, whereas the macaques in the Nicobar Islands are not provisioned, but they range the entire village and human dwellings where domestic animals like dogs, cats, pigs, cattle and sometimes even people defecate. This has probably increased the chances of macaques getting infected with many endoparasite taxa. Similar results have also been reported for the bonnet²³ and lion-tailed²⁴ macaques of southern India. Although the consequence of such multiple infections is not known, the present study provides a documentation of endoparasites in these macaques, which can become an imperative research and management issue in these islands.

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ACKNOWLEDGEMENTS. We thank the Department of Science and Technology, Government of India (GoI) for financial assistance to H.N.K. (grant no. SR/SO/AS-49/2011), Rufford Small Grants, UK to S.K. (grant number: 16567-1, dated 7 November 2014), and the Science and Engineering Research Board (SERB), GoI for the award of SERB Distinguished Fellowship to M.S. (grant no. SB/DF-003/2019) for carrying out this study. We also thank the Principal Chief Conservator of Forests, Port Blair, Andaman and Nicobar Islands for granting permission to carry out the study (permit no. CWLW/WL/134/566) and Assistant Commissioner, Andaman and Nicobar Administration for providing the necessary permits to allow access to the tribal areas; the Andaman and Nicobar Forest Department, especially the Nicobar Division and the Nicobar Administration, for logistical support; Dr Michael Huffman (Kyoto University, Japan) and Prof. Hideo Hasegawa (Oita University, Japan) for help in the identification of endoparasites; V. R. Sheetal, Snehaa Sundaram, Dhee, Marishia Rodrigues, and Nikita Sarangdhar for their assistance in the collection of faecal samples and valuable inputs; Mahender Reddy for faecal sample analysis and Giza Rachel George, Ruby and A. Periyasamy for their support in the laboratory; K. Gowri Dhatri and K. Samhitha for their help and cooperation during the entire study, and Dr S. Babu for fruitful discussions.

Received 31 January 2022; revised accepted 6 April 2022

doi: 10.18520/cs/v122/i10/1199-1208