



Prevalence and diversity of gastrointestinal parasites in free-ranging *Macaca mulatta* and *Semnopithecus hector* in Dharan, eastern Nepal

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Abstract

Gastrointestinal (GI) parasites pose a crucial health risk to non-human primates (NHPs), particularly in areas with frequent human-wildlife interaction. This study assessed the prevalence and diversity of GI parasites in free-ranging *Macaca mulatta* (Rhesus macaque) and *Semnopithecus hector* (Hanuman langur) in Dharan, eastern Nepal, in the year 2024. The collected fecal samples (n=89) were examined using direct wet mount and flotation techniques. Overall, the parasitic prevalence (74.15%), with helminths (65.15%), was found to be more than protozoans (33.70%). Notably, *Trichuris* sp. was the most prevalent parasite (48.63%). In addition, *M. mulatta* showed higher parasite diversity (Shannon H'=1.96) and protozoan prevalence (43.63%) compared to *S. hector* (H'=1.72; 17.64%), likely due to their synanthropic behavior. Moreover, the co-infections were found to be more frequent, particularly in macaques. These results highlight the zoonotic risks associated with gastrointestinal parasites and underscore the role of human activities, such as improper waste disposal and habitat disturbance, in facilitating parasite transmission. This calls for targeted habitat management strategies, improved waste management practices, and public health interventions to reduce disease risks in urban areas where human and primate populations coexist.

Keywords: Gastrointestinal parasites, *Macaca mulatta*, Parasite diversity, *Semnopithecus hector*, Zoonotic threats

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Introduction

Gastrointestinal (GI) parasites are among the most prevalent pathogens affecting non-

human primates (NHPs), with significant impacts on their health and behaviour (Blersch *et al.*, 2021; Wren *et al.*, 2021;

Agostini *et al.*, 2023). Free-ranging primates, particularly those near human settlements, are especially vulnerable to parasitic infections due to habitat fragmentation, human disturbances, and increased interactions with wildlife (Kowalewski and Gillespie, 2009; Islam *et al.*, 2022; Mason *et al.*, 2022; Adhikari *et al.*, 2023). The most common NHPs, *Macaca mulatta* (Rhesus Macaque) and *Semnopithecus hector* (Hanuman langur), are found throughout South Asia, including Nepal (Wada, 2005; Rai and Rai, 2024) and frequently interact with humans, making them potential zoonotic parasite reservoirs (Debenham *et al.*, 2017; Tandan *et al.*, 2023). Despite their ecological and epidemiological importance, research into GI parasites in these animals, particularly in Nepal, remains limited. Therefore, additional studies are necessary to determine infection prevalence and associated risks.

Parasitic infections in primates can have profound health implications, such as diarrhoea, malnutrition, decreased reproductive success, and even death (Chapman *et al.*, 2006; Mapagha-Boundoukou *et al.*, 2024). Furthermore, many GI parasites of primates are zoonotic, suggesting public health risks in areas where human-NHPs contacts are common (Banda *et al.*, 2024). Notably, *Strongyloides*, *Giardia*, and *Entamoeba* spp. have been documented in both monkeys and humans, indicating the possibility of cross-species transmission (Kouassi *et al.*, 2015; Adhikari *et al.*, 2023). In Nepal, the parasites are more likely to be transmitted in macaques and langurs due to their foraging behaviour in urban environments, such as cities, temples, and garbage (Adhikari *et al.*, 2023).

The available literature on primate parasitology in Nepal focuses mainly on the central regions, with little attention paid to the eastern lowlands (Adhikari *et al.*, 2023; Pokhrel and Maharjan, 2014; Tandan *et al.*, 2023). Dharan, a rapidly urbanizing city in

eastern Nepal, hosts large populations of free-roaming *M. mulatta* and *S. hector*, yet systematic studies of their parasite diseases are scarce. In addition, the previous research in various locations has found that the prevalence of nematodes, cestodes, and protozoans in these species varies depending on potential factors such as food, troop size, and environmental contamination (Gotoh, 2000; Adhikari and Dhakal, 2018; Fernando, Udagama and Fernando, 2022; Islam *et al.*, 2022). However, regional changes in climate, human activity, and habitat structure demand site-specific research to better understand parasite ecology and transmission risks.

Understanding the prevalence and diversity of gastrointestinal parasites in free-ranging primates is thus critical for both animal and public health, especially in areas where humans and primates interact frequently (Chapman, Gillespie and Goldberg, 2005; Salyer *et al.*, 2012). Furthermore, environment and land-use changes may affect parasite distribution patterns, requiring baseline data for future monitoring (Chakraborty *et al.*, 2019; Morales-Castilla *et al.*, 2021). Given these concerns, assessing the incidence and prevalence of gastrointestinal parasites in primate hosts is essential for reducing zoonotic risks and informing wildlife management strategies.

In Nepal, the rhesus macaque (*Macaca mulatta*) and the hanuman langur (*Semnopithecus hector*) closely foraged near the human settlement areas, increasing the risk of pathogen transmission between primates and humans. Despite their ecological and zoonotic importance, detailed studies on their parasitic infections remain limited. In this context, we aimed to (1) measure the prevalence and diversity of GI parasites in *M. mulatta* and *S. hector* in Dharan, Nepal, and (2) compare infection rates between these species to assess host-specific susceptibility. Furthermore, we hypothesized that both species would have high parasite frequency

due to their human associated behavior, with *M. mulatta* having greater parasite diversity due to its terrestrial and opportunistic feeding habits.

Our study provides a detailed assessment of gastrointestinal (GI) parasites in these primate species from eastern Nepal. It provides essential baseline data for wildlife health and zoonotic disease prevention. These findings contribute to broader conservation initiatives and public health interventions aimed at reducing cross-species disease transmission in changing habitats.

Materials and Methods

Study area

We conducted this study in Dharan Sub-metropolitan City, which is located in Sunsari District of Koshi Province in eastern Nepal. It lies at an elevation of 349 m above sea level (Fig. 1) and is situated at the foothills of the Churia Range. This region covers an area of 192.6 km and lies at 26.7944°N latitude and 87.2817°E longitude. The region experiences a tropical climate and temperatures from 5°C in winter to 35°C in summer. The forest in this region is predominantly covered by *Shorea robusta*, *Terminalia alata* and *Dillenia pentagyna*. In addition to this, the area is also culturally important due to the presence of religious sites like Budhasubba, Dantakali, and Bishnupaduka. The large mammals such as *Muntiacus muntjak* (barking deer), *Sus scrofa* (wild boar), and *Axis axis* (Spotted deer), are known to inhabit the Churia foothills of the study area. The non-human primate species, such as Rhesus Macaques (*Macaca mulatta*) and Hanuman Langurs (*Semnopithecus hector*), are primarily found in these habitats and were selected for fecal sampling during the study.

Table 1. Comparative prevalence of GH parasites among monkey species

Host species	Sample size	Protozoa positive	Helminth positive	Total positive (%)
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		(%)	(%)	
Macaca mulatta	55	43.63	63.63	76.36
Semnopithecus hector	34	17.64	67.64	70.58
Total	89	33.70	65.15	74.15

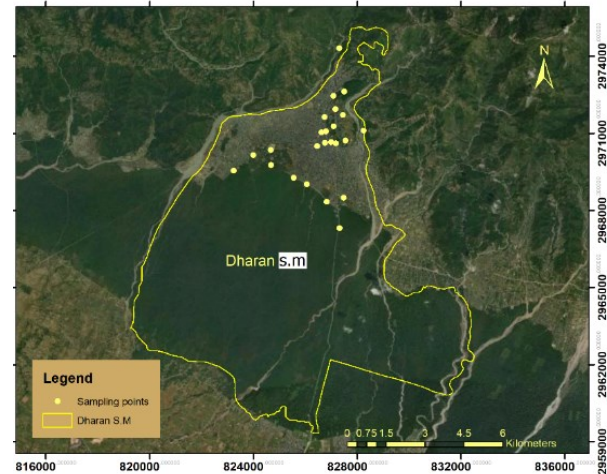
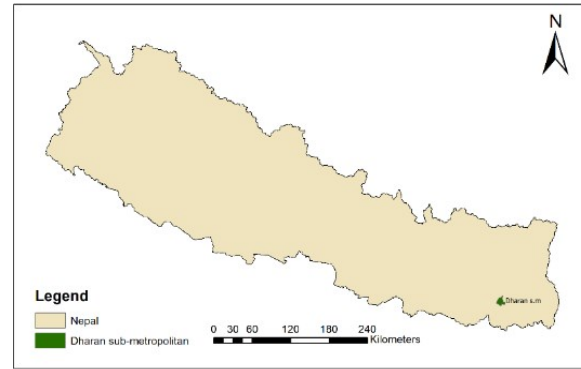


Figure 1. Faecal sample collection point in the study area

Sample collection and preservation

Faecal samples were collected at twenty-four potential sampling points immediately after defecation events between 07:00 and 11:00 AM to minimise contamination risks. Sampling focused on troops inhabiting human-primate conflict zones, particularly urban areas. A total of 89 fresh samples were collected, 55 from *Macaca mulatta* (Rhesus macaques) and 34 from *Semnopithecus hector* (Hanuman langurs). For each sample, approximately 10 g of faecal matter was collected in sterile vials containing 2.5%

potassium dichromate ($K_2Cr_2O_7$) for preservation. The vials were labelled with species identifiers and location code, then stored at 4°C until laboratory analysis.

Laboratory examination

The laboratory examination, including microscopic analyses, was done at the Central Campus of Technology, Hattisar, Dharan. Parasite detection was carried out utilizing both qualitative and quantitative approaches, including direct wet mount and flotation (Soulsby, 1982; Zajac *et al.*, 2021). In direct mounts, faeces samples were emulsified in Lugol's iodine and 0.85% saline and directly observed. In the flotation process, about 3 g of faecal sample was emulsified in water and poured into a 15 ml centrifuge tube, centrifuged at 1200 rpm for five minutes, filtered, and re-suspended in saturated NaCl (SPG 1.20). Lugol's iodine was used to investigate leftover silt containing heavier parasite eggs that were not recovered during flotation and observed under 10× and 40× magnification. Images of several parasite phases were photographed and identified through their shape, size, and structure of cyst/oocyst, as described in reference books and journal papers (Chatterjee, 1976; Soulsby, 1982; Zajac *et al.*, 2021).

Data analysis

Parasite prevalence was compared between host species using Fisher's exact tests (for low-frequency parasites) and χ^2 tests (for prevalence >5%), with Bonferroni correction for multiple comparisons ($p < 0.05$ considered significant) (McDonald, 2014). We employed the Shannon-Wiener index (H') and Simpson's index (1-D) to assess overall parasite diversity in each host species. These indices were calculated based on pooled parasite occurrence data for *Macaca mulatta* and *Semnopithecus hector* separately. The Shannon index captures both richness and evenness of species, while the Simpson index emphasizes dominance and

common species, providing a more comprehensive assessment of parasite assemblages. The assessment was done by using the *vegan* package (Oksanen *et al.*, 2013), with significance tested via permutation (9999 iterations) to account for unequal sample sizes (Anderson, 2017). Sørensen's similarity coefficient (SCS) quantified assemblage overlaps between species (Koleff, Gaston and Lennon, 2003). Median intensity (parasites per infected host) was compared using Wilcoxon rank-sum tests due to non-normal distributions (Shapiro-Wilk $p < 0.05$). We used R (v4.3.0, R Core Team, 2023) software for statistical analyses.

Results

Overall parasite prevalence

A high overall prevalence of gastrointestinal parasites (74.15%, $n=66$) was observed among the sampled primates, with both *Macaca mulatta* and *Semnopithecus hector* showing substantial infection rates, with 76.36% of rhesus macaques and 70.58% of langurs testing positive for at least one parasite species (Fisher's exact test, $p=0.53$). Helminth infections were prevalent (65.15% of all samples), suppressing protozoan infections (33.70%). This pattern was especially noticed in *M. mulatta*, which showed significantly higher protozoan prevalence (43.63%) compared to *S. hector* (17.64%, $p=0.008$; Table 1). Despite these differences, Sørensen's similarity coefficient (0.82) indicated a substantial overlap in parasite species between the two primate species (Table S1).

Species-specific parasite assemblages

Our analysis revealed that the rhesus macaque (*Macaca mulatta*) exhibited significantly greater parasite diversity (Shannon $H' = 1.96$) compared to the langur (*Semnopithecus hector*, $H' = 1.72$; permutation test, $p = 0.022$), supporting our hypothesis about differential exposure risks associated with their distinct foraging ecologies.

Trichuris sp. emerged as the most prevalent parasite across both hosts (48.63% combined prevalence), though infection patterns differed substantially between species. In macaques, we observed a broader parasite assemblage (9 species total), including unique occurrences of *Balantidium* sp. (12.72%) and *Toxocara* sp. (3.63%) along with

significantly higher *Trichuris* infection intensity (4.23 vs 3.82; $p = 0.038$). Langurs, by contrast, showed a more focused parasite spectrum dominated by *Trichuris* sp. (50%) and hookworms (29.41% prevalence, compared to just 14.54% in macaques), with the unique presence of *Enterobius* sp. (2.94%) (Figure 1 and Figure 2).

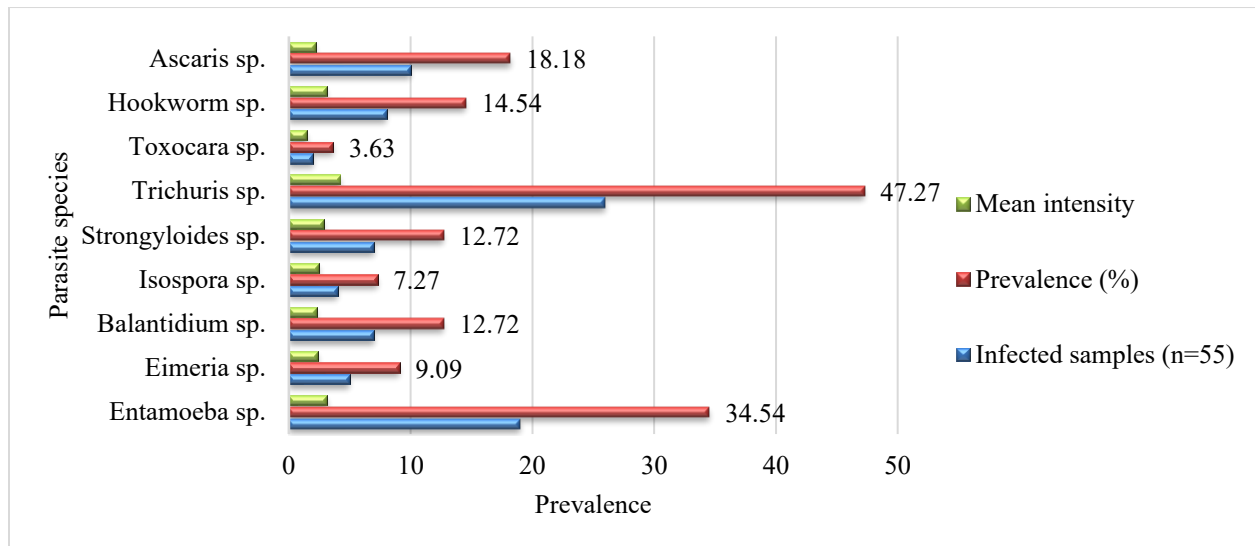


Figure 2. Prevalence (%) and mean intensity of gastrointestinal parasites identified in faecal samples from *Macaca mulatta*

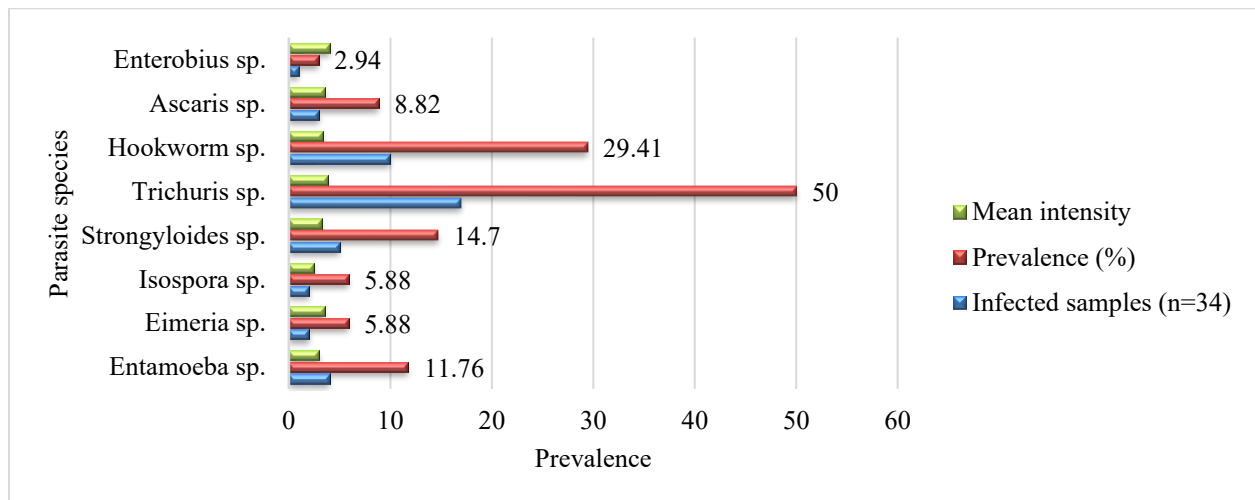


Figure 3. Prevalence (%) and mean intensity of gastrointestinal parasites identified in faecal samples from *Semnopithecus hector*

Infection patterns and host differences

We found distinct parasite infection patterns between the two host populations. In the

Macaca mulata (n=55), double infections were most prevalent (32.72%, 18/55), followed by single infections (30.90%, 17/55), with triple

and higher-order infections being comparatively rare (16.36% combined). This distribution significantly deviated from an equal expectation ($\chi^2=14.67$, $df=3$, $p=0.002$). The *Semnopithecus hector* ($n=34$) showed a markedly different pattern, with single infections dominating (41.18%, 14/34) and no more than triple infections observed. Its distribution differed significantly from expected ($\chi^2=14.47$, $df=3$, $p=0.002$). Notably, *Semnopithecus hector* exhibited higher

prevalence of single infections (41.18% vs 30.90%) and lower overall multi-infection rates (38.23% vs 49.08%) compared to the *Macaca mulatta*, suggesting potential host-specific differences in susceptibility to concurrent parasite infections (Table 2). These patterns may reflect variations in host immune competence, ecological exposure, or parasite competition dynamics between the two populations.

Table 2. Distribution of Single and Multiple Gastrointestinal Parasite Infections in *Macaca mulatta* and *Semnopithecus hector*

Infection Type	<i>Macaca mulatta</i> (no.of sample)	Percentage (%)	<i>Semnopithecus hector</i> (no.of sample)	Percentage (%)
Single infection	17	30.90	14	41.18
Double infection	18	32.72	9	26.47
Triple infection	5	9.09	4	11.76
Multiple (>3) infections	4	7.27	0	0
Total multi-infections	27	49.08	13	38.23
χ^2 (df = 3)	14.67	—	14.47	—
p-value	0.002	—	0.002	—

Note: Infection distribution patterns significantly differed from expected values in both species ($p < 0.05$).

Discussion

We found a high prevalence (74.15%) of gastrointestinal (GI) parasites in free-ranging *Macaca mulatta* and *Semnopithecus hector* in Dharan, eastern Nepal. Our study aligned with prior national and international studies that report significant parasitic burdens in non-human primates (NHPs), mainly due to their social behaviour, habitat overlap with humans, and environmental exposure (Adhikari *et al.*, 2023; Balasubramaniam *et al.*, 2019; Johnson *et al.*, 2024; Medkour *et al.*, 2020). Helminthic infections (65.15%) were notably more prevalent than protozoan infections (33.70%), which is consistent with results from (Adhikari *et al.*, 2023) and (Munene *et al.*, 1998), pointing out the dominance of helminths in the primates.

Detecting ten parasite species, six helminths, and four protozoans underscores the diversity of parasitic fauna affecting both

species. The *Trichuris* species from the helminth group showed the highest prevalence (48.31%), which is comparable to findings by Bhattarai and Adhikari (2019) and Adhikari *et al.* (2023), indicating that this nematode is a common parasite in Nepal and poses a significant threat to the primate population. Besides, Hookworm sp. (20.22%), *Ascaris* sp. (14.60%), and *Strongyloides* sp. (13.38%) are commonly observed species in primate. However, the less frequent, *Toxocara* sp. (2.24%) and *Enterobius vermicularis* (1.12%) were also identified, demonstrating the presence of both common and rare parasites.

Notably, *Entamoeba* sp. (25.84%) poses significant zoonotic risks due to its dominance and strong transmission potential, as in other places (Deere *et al.*, 2019; Liu *et al.*, 2022). However, particular parasite species, such as *Eimeria*, *Balantidium*, and

Isospora, represented a wide range of protozoan diseases in primates.

Infection richness varied among individuals: 34.83% exhibited single infections, 29.21% had dual infections, 8.98% showed triple infections, and 4.49% harboured multiple parasite species. These patterns suggest frequent co-infections, which can exacerbate health complications in host animals, as also documented by (Adhikari and Dhakal, 2018; Trejo-Macías *et al.*, (2007) . Co-infections likely result from shared foraging grounds, contaminated water sources, and poor waste management in the periphery of urban and temple areas, where human-primate interactions are common.

When comparing host species, *M. mulatta* exhibited a higher infection rate (76.36%) than *S. hector* (70.58%). Rhesus macaques also showed a greater diversity of parasites (nine species vs. eight in langurs), with exclusive detection of *Balantidium* sp. and *Toxocara* sp. These differences may arise from behavioural and ecological variations. Rhesus macaques, being more synanthropic, have increased exposure to human waste and food, increasing their parasite burden. On the other hand, Hanuman langurs, though also susceptible, live in less densely populated areas and may benefit from less exposure to anthropogenic contaminants.

The Sorenson's Coefficient of Similarity (SCS = 0.82) suggests a significant overlap in parasite assemblages between the two species. This implies that due to their common shared resources, contaminated water, and faeces sites, they suffer similar environmental risks and may spread disease.

Our findings are consistent with Adhikari *et al.* (2023) and Adhikari and Dhakal (2018), who also reported high prevalence and helminth dominance in free-ranging rhesus macaques in Nepal, indicating similar transmission risks in urban environments. Additionally, our observed parasite diversity aligns with the patterns

reported by Islam *et al.* (2022) in Bangladesh, although our study recorded a slightly higher rate of protozoan co-infections, possibly due to differences in habitat disturbance and human-primate interaction intensity.

It also reinforces the concern about potential zoonotic transmission, especially from species like *Entamoeba* and Hookworm, which have been documented in both human and primate populations.

Conclusion

This study highlights a high prevalence and notable diversity of gastrointestinal parasites in free-ranging *Macaca mulatta* and *Semnopithecus hector* in Dharan, eastern Nepal, with *Trichuris* sp. emerging as the most common parasite in both species. The greater parasite diversity and co-infection frequency observed in rhesus macaques likely result from their close proximity to human settlements and opportunistic feeding behavior. These findings underscore the potential zoonotic risks posed by human-primate interactions, especially in urban and peri-urban settings. To address these risks, there is an urgent need to improve habitat sanitation, regulate waste disposal around religious and urban monkey habitats, and promote awareness about disease transmission. Furthermore, regular health surveillance of urban primate populations and molecular identification of parasites are recommended to better understand transmission dynamics. Future research should also consider seasonal patterns, host immunity, and longitudinal monitoring to support evidence-based management strategies for both wildlife conservation and public health.

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