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Prevalence of gastrointestinal helminth parasites in rhesus macaques and local residents in the central mid-hills of Nepal

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Summary

Rhesus macaques (*Macaca mulatta)* are distributed across Nepal in close association with humans and with a high probability of sharing of soil-transmitted intestinal helminth parasites. This study was carried out to determine the prevalence, richness and risk factors of gastrointestinal (GI) helminth parasites among rhesus macaques and humans in the Daunne Forest area, a community managed forest in the central mid-hills of Nepal. A total of 190 fecal samples, including 120 samples from rhesus macaques residing around the Daunne Devi Temple and in the surrounding forest, and 70 from local people, were microscopically examined by direct wet mount, floatation and sedimentation methods. Seasonal and age-sex based variations in helminth parasite prevalence were analyzed. Among the rhesus macaques, the total parasite prevalence was 39.2 %. *Strongyloides* sp. accounted for the highest prevalence (19.17 %) followed by *Ascaris* sp. (13.33 %), hookworm (10.83 %) and *Trichuris* sp. (4.17 %). Among the humans, *Ascaris lumbricoides* (11.3 %) was the only parasite detected. The Sorenson's coefficient of similarity of GI parasites between the macaques and local people at the generic level was 0.4. Mean parasite richness for the macaques was 1.21 ± 0.41 (SD) per infected sample. Parasite prevalence in the summer season (41.4 %) was higher than in the winter season (36 %). Adult macaques (41.67 %) had higher GI parasite prevalence than the young (30.77 %) and infants (27.27 %). Among the adult macaques, the prevalence rate was significantly higher (*P*=0.005) in females (52.46 %) than in males (22.86 %). Our results indicate that the temple rhesus macaques have a high prevalence of GI helminth parasites and could pose a potential zoonotic risk. As such, the need for routine monitoring and an effective management strategy is essential.
Keywords: Non-human primates; Temple monkeys; *Strongyloides*; Parasite richness; Daunne Devi Temple

Introduction

Gastrointestinal parasites like helminths and protozoans are ubiquitous in humans and non-human primates (NHPs) and are among the most prevailing infections worldwide (Sepahvand *et al.*, 2022). Gastrointestinal (GI) helminth parasites, also known as geohelminths or soil-transmitted helminths, include species like *Ascaris lumbricoides* (roundworm), *Trichiuris trichiuria* (whipworm), *Ancylostoma duodenale*, *Necator americanicus* (hookworms), etc. (Haque, 2007). These parasites are found in the gastro-intestinal tract of the host and heavy parasitic infections can lead to anorexia, cachexia and severe ascites (Loukopoulos *et al.,* 2007). Some

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of the parasites are non-pathogenic, however, large numbers of parasites have detrimental effects on the host which include physiological disturbances, nutritional loss and lesions. These effects impact the host's health and can lead to secondary infections (Toft & Eberhard, 1998). GI parasites in NHPs can be the major cause of gastro-enteritis, watery diarrhea, hemorrhage, dysentery and extra-intestinal infections such as liver abscess and even death (Akpan *et al.,* 2010).

NHPs are one of the major reservoirs of parasites and can transmit zoonotic diseases due to their close interactions with humans (Dawet *et al.,* 2013). There is a long history of interaction between humans and NHPs which has resulted in a complex web of behavioral, ecological, and epidemiological relationships. The spatial overlap and interactions between humans and NHPs create a shared environment which can result in co-mingling of infectious agents (Fuentes, 2006). It has been found that, particularly for viruses, protozoa and specialist parasites, human-NHP sympatry is positively correlated with parasite species richness (Nunn *et al.,* 2004).

Although pathogenic parasites may be the cause of diseases in the host individuals, nonpathogenic parasites are important as their presence in the host can provide evidence of the fecal-oral transmission route (Sarkari *et al.,* 2016). The transmission route of GI parasites within the human community includes uncooked and unwashed food, contaminated water, poor hygiene, and the fecal-oral route (Omalu *et al.,* 2013). The likelihood of interspecies exchange of GI helminths, such as *Ascaris* spp., *Oesophagostomum* spp., *Trichuris* spp. etc., between NHPs and humans depends in part on the degree and nature of anthropogenic disturbance to NHPs' habitats (Zanzani *et al.,* 2018; Medkour *et al.,* 2020). Human activities like habitat fragmentation may increase an NHP population's susceptibility to the risk of infection and in some cases can cause mortality (Chapman *et al.,* 2005). Major contributing factors responsible for parasitic transmission are soil and water pollution by waste food and garbage, especially during the festive and picnic programs, and the occasional open defecation by visitors in the forest areas and nearby water sources (Sapkota *et al.,* 2020).

Assessing parasite prevalence in a species is vital to an understanding of the species' health status, adaptation to the environment, and potential health risks. Among NHPs, rhesus macaques (*Macaca mulatta*) and humans share a range of infectious agents including gut parasites (Roberts *et al.,* 2020). Many of the diseases shared by humans and NHPs result from the exchange of parasites through the fecal-oral route, animal bites, nasal secretion, and water-mediated route (Chapman *et al.,* 2005). Therefore, an understanding of the intestinal parasites found in both rhesus macaques and the local the community is an important first step in

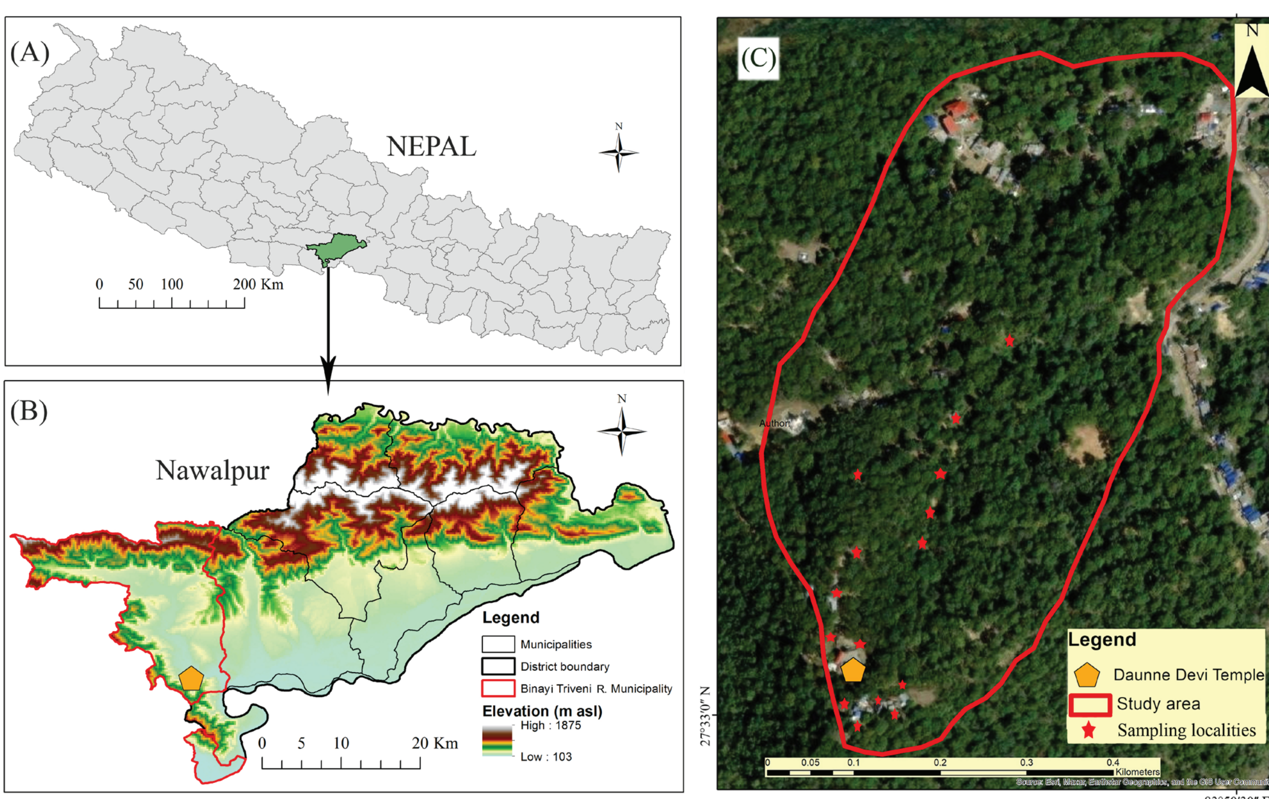

Fig. 1. Location of the study area; A - Map of Nepal with Nawalpur District highlighted. B - Nawalpur District indicating the Binayee Tribeni Rural Municipality. C - Daunne Devi Temple in the Daunne Forest area.

helping to establish proactive measures to mitigate disease risk.
Nepal has a large population of rhesus macaques living both in the wild as well as near human settlements, especially in the temple areas. In many locations, macaques raid crops from agricultural areas as well as storage facilities. In the temples and shrines, visitors and devotees frequently offer supplemental foods to the macaques (Adhikari *et al.,* 2018). As is the case at many temples and shrines around Nepal, human-macaque interaction is a regular occurrence at the Daunne Devi Temple area in Nawalpur District, Nepal. A recent study of the rhesus macaques in the Daunne Devi Temple area (Kshetri *et al.*, 2023) reported a higher dependence on supplemental food provisioning, and their daily path lengths were shortened when the number of visitors in the temple increased. Such interactions could result in the cross-species transmission of potentially zoonotic parasites. Given the potential health risks associated with parasite infection, the purpose of this study was to identify the prevalence, intensity, richness and associated risk factors of GI helminth parasites in the rhesus macaques and local people of the Daunne Forest area situated in the central mid-hills of Nepal.

Materials and Methods

Study area

This study was conducted at the Daunne Devi Temple (named for the Hindu Goddess Durga) along with Shivalaya (a temple of lord Shiva) in the Daunne Forest area (Fig. 1). The area lies in the Binayee Triveni Rural Municipality of the Nawalpur District, Gandaki Province in central Nepal and extends between 27°32'41" to 27°33'27" North latitudes and 83°50'10" to 83°50'36" East longitudes. The Binayi Triveni Rural Municipality covers an area of 288.06 km^2 and is inhabited by 32,943 people (BTRM, 2023). The temple sits at an elevation of around 1033 meters above sea level along the route from Bardaghat to Dumkibas. The local community has been taking care of the temple site since 1992. There are several buildings nearby the temple that provided temporary shelter for the caretakers and the hundreds of people who visit the temple every day.
The Daunne Forest has a subtropical to temperate climate. May and June are the hottest months, while December to February are the coldest months with an average annual rainfall of about 1500 mm. The temple is surrounded by a sub-tropical forest with variety of flora and fauna including mammal species such as the golden jackal (*Canis aureus*), leopard (*Panthera pardus*), rhesus macaque (*Macaca mulatta*), common langur (*Semmenopithecus hector*), jungle cat (*Felis chaus*), and common mongoose (*Herpestes edwardsii*) (Baral *et al.,* 2003, Upadhyay 2008). Dominant flora include sissoo (*Dalbergia sissoo*), sal (*Shorea robusta*), harro (*Terminalia chebula*), barro (*Terminalia bellerica*), saj (*Terminalia tomentosa*), simal *(Bombax ceiba*), and jamun (*Syzigium cumini*) (Subedi, 2008).

Field survey and sampling

A preliminary field survey was conducted during September 2021 to determine whether the research was feasible, and to observe the physiography of the study area, habitat and population status of rhesus macaques. Approximately 250 macaques in five different groups were counted in the Daunne Forest (Kshetri *et al.*, 2023). The human population in the core study area consisted of about 600 individuals in 125 households. Additional information related to the macaques' habitat was collected from informal interviews with residents of the study area.
Fecal samples were collected from October 2021 to May 2022. Macaque fecal samples (observed fresh drops) were collected opportunistically in the temple and surrounding forest area. During the collection, the macaque groups were followed to allow for the identification of the macaque's approximate age and sex (Table 1) following Chalise (2013). The fecal specimens were preserved in a 2.5 % potassium dichromate solution.
For the human fecal sample collection, sterile vials were distributed to the potential subject pool including priests, temple caretakers, shop owners near the temple, and local people visiting temple. Each individual was instructed on the use of the collection vial. The next morning the vials were collected, labeled, and immediately preserved in 2.5 % potassium dichromate solution. All the human samples (n=70) were obtained from adults (37 males; 33 females). Each individual was interviewed about their age, education, family size, availability of a toilet and other sanitation facilities at the household, recent medications for gastrointestinal disorders, etc. All the participants were informed about the purpose of the study and the measures to ensure their anonymity.
The fecal samples from both the rhesus macaques and humans were collected during winter (October and November 2021) and summer (May 2022). A total of 120 fecal samples were collected from rhesus macaques (50 during winter and 70 during summer). All fecal samples were taken to the Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu for processing and analysis.

Table 1. Age-sex categories and sample number of macaques in the study (n=120).

S.N.	Category	Description
1.	Adult male (61)	Full grown with adult morphology (e.g., pink scrotum and prominent testes)
2.	Adult female (35)	Full grown with adult morphology (e.g., elongated nipples, carrying baby)
3.	Young (13)	Individuals lacking secondary sexual characteristics and have near adult size
4.	Infant (11)	Small individuals nursed or are still in frequent proximity to a female

Laboratory processing and microscopic examination

For the identification of eggs and larval stages of GI helminth parasites, microscopic examinations of the fecal samples were carried out by direct wet mount and concentration method (floatation and sedimentation) as previously described (Zajac & Conboy, 2012; Dhakal *et al.,* 2023) with slight modification wherever applicable. For each sample, two slides were prepared (one with Lugol's iodine solution to stain protozoan cysts or oocysts, and other with normal saline) and examined under high power magnification (400×).

Sedimentation method

This method is commonly used to detect flukes and tapeworm eggs which do not float promptly in common flotation solutions (Zajac & Conboy, 2012). Approximately two grams of the fecal sample was filtered thoroughly and mixed with normal saline in a 15 ml centrifuge tube followed by centrifugation at 1200 rpm for five minutes. The supernatant was then discarded and the sediment was mixed with 10 ml of 10 % buffered formalin and 3 ml of ethyl acetate and centrifuged again. Using an applicator stick the plug of debris was removed and all the supernatant fluid was decanted and discarded. A drop of sediment was transferred onto a clean slide and examined.

Floatation method

This technique is used to float the protozoan cysts, oocysts and nematode eggs (Zajac & Conboy, 2012). Saturated salt solution was used as fluid floatation medium. About two grams of the fecal sample was mixed with normal saline in a 15 ml centrifuge tube, centrifuged at 1200 rpm for five minutes, and the supernatant was discarded. A saturated NaCl solution was then added followed by centrifugation at 1200 rpm for five minutes. The sediment solution was then poured into a tube until a convex surface was formed at the top where a clean coverslip was placed and left undisturbed for at least 10 minutes. The coverslip was gently transferred to a clean glass slide and examined.

Identification of egg, cyst and larva

The presence of diagnostic stages of parasites such as oocysts and cysts of protozoans, and eggs and larvae of helminths were carefully examined, photographed, and their size was measured using a calibrated oculomicrometer. The parasites were identified based on the morphological characteristics (shape, size, color, structure of cyst/ oocyst wall and shell, and the content within) as described in a number of reference books and research papers (Soulsby, 1968; Sapkota *et al.,* 2020; Zajac & Conboy, 2012).

Data analysis

The prevalence of parasites was calculated by dividing the number of fecal samples shedding parasite eggs by the total number of fecal samples examined. Richness of parasites was measured as the number of parasite species detected in each sample (Turgeon *et al.,* 2018). Seasonal variation and age-sex based variation in GI helminth parasite prevalence was tested for significance by Chi-squared test performed in "SPSS software". In all cases, the 95 % confidence interval (CI) and $p \leq 0.05$ were used as the criteria for a statistically significant difference. The GI helminth parasitic similarity between the macaques and humans was calculated by Sorenson's Coefficient of Similarity (SCS) as:

$$SCS = \frac{2c}{a + b + 2c}$$

where, c= number of common parasite species; a= parasite species present only in rhesus macaques; and b= parasite species present only in humans.

Ethical Approval and/or Informed Consent

The required permission for collection of the macaque fecal samples was obtained from the Department of Forest and Soil Conservation, Ministry of Forest and Environment, Government of Nepal (Permission Number 293/078/079). Human subject participation in the study was voluntary and verbal informed consent was obtained from each participant. Prior to their participation, the detailed purpose and procedures of the study were explained verbally to the participants in Nepali language. The participants were informed that they could end their participation in the study at any time. Additionally, in an effort to help protect participant anonymity no personal identifiers were associated with the samples.

Table 2. GI helminth parasite prevalence in rhesus macaques and local people.

Parasite	Rhesus macaques (n=120)		Local people (n=70)	
	Number of positive samples	Prevalence (%)	Number of positive samples	Prevalence (%)
Strongyloides sp.	23	19.17	0	0
Ascaris sp.	16	13.33	-	-
Ascaris lumbricoides	-	-	8	11.4
Hookworm	13	10.83	0	0
Trichuris sp.	5	4.17	0	0

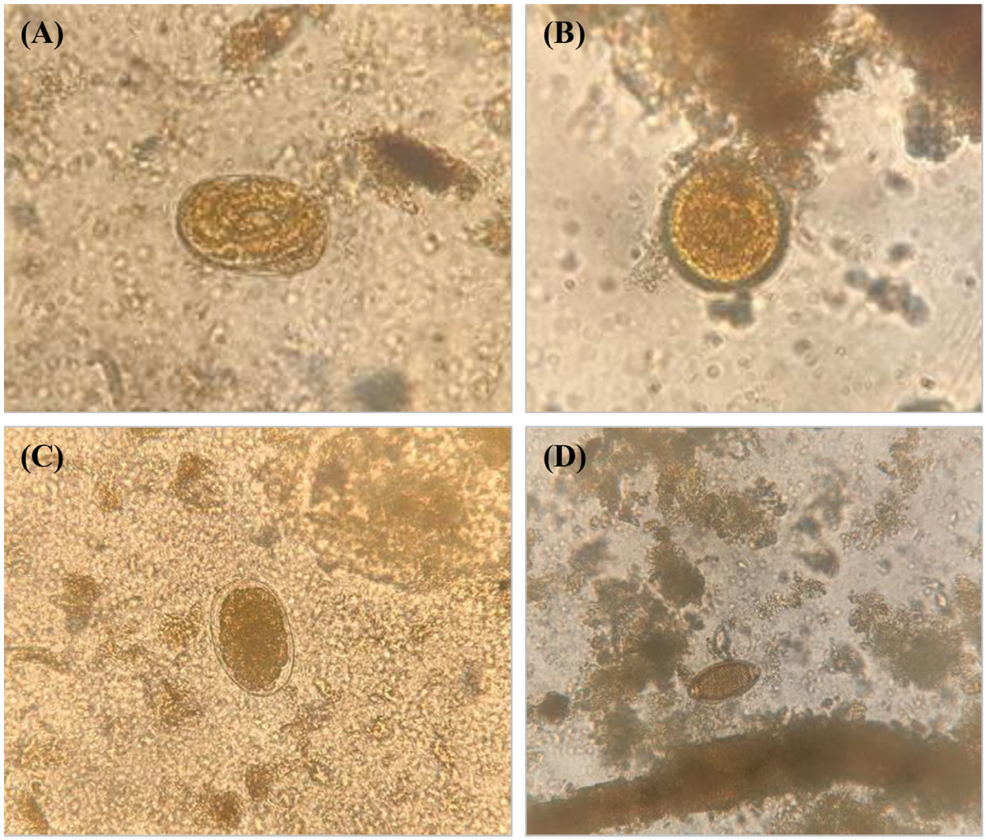

Fig. 2. Identified intestinal helminth parasites (400×). A - Egg of *Strongyloides* sp. (60µm×39µm); B - Egg of *Ascaris* sp. (52µm×55µm); C - Egg of hookworm (75µm×45µm); and D- Egg of *Trichuris* sp. (55µm×24µm).

Results

Of the 120 rhesus macaque fecal samples examined, 47 samples were found to be positive for gastro-intestinal helminth parasites (Table 2), for an overall prevalence of 39.2 %. Thirty-seven samples had a parasite richness of 1 and 10 other samples had a richness of 2. The mean GI helminth parasite richness was 1.21 ± 0.41 (SD). Four species of helminth parasites were detected in the rhesus macaques including *Strongyloides* sp., *Ascaris* sp., hookworm and *Trichuris* sp. (Fig. 2). Among the 70 human fecal samples, only 8 (11.4 %) were found to be infected, and only with *Ascaris lumbricoides* eggs. The eight positive samples included one sample from a caretaker of temple area and remaining were from the local people living around the temple stairway. The Sorenson's Coefficient of similarity for GI helminth parasites between the rhesus macaques and humans at the generic level was 0.4.

The prevalence of parasites in the macaques during the summer season (41.4 %, 29/70) was higher than in the winter season (36 %, 18/50) but the difference was not statistically significant (χ^2=0.361, df=1, *P*=0.575) (Fig. 3A). Based on the age category, the highest prevalence of GI helminth parasites was recorded in the adult macaques (41.67 %, 40/96) followed by young (30.77 %, 4/13) and infants (27.27 %, 3/11) (Fig. 3B). However, no significant differences were observed in the age-wise prevalence of GI helminth parasites in the rhesus macaques (χ^2=1.290, df=1, *P*=0.581). Among 96 adult monkey samples, 35 were from adult males and 61 were from adult females. Parasitic prevalence rate in the females (52.46 %, 32/61) was higher than in males (22.86 %, 8/35). Significant difference was observed in the parasitic prevalence rate between the male and female macaques (χ^2=8.018, df=1, *P*=0.005).

Discussion

This study explored the prevalence of GI helminth parasites in rhesus macaques and local people in Daunne Forest of Nawalpur District in the central mid-hills of Nepal. Four species of parasites were identified in the rhesus macaques including *Strongyloides* sp., *Ascaris* sp., hookworm and *Trichuris* sp. and a single species (*Ascaris lumbricoides*) was found in the humans. The overall parasite prevalence in the rhesus macaques (39.2 %) was considerably lower than reported for other temple sites in Nepal (Jha *et al.,* 2011; Adhikari & Dhakal 2018; Sapkota *et al.,* 2020). The highest prevalence of *Strongyloides* sp. (19.17 %) observed in this study was similar to the findings by Arunachalam *et al.* (2015) who recorded 43 % (26/60) prevalence of *Strongyloides* sp. in free

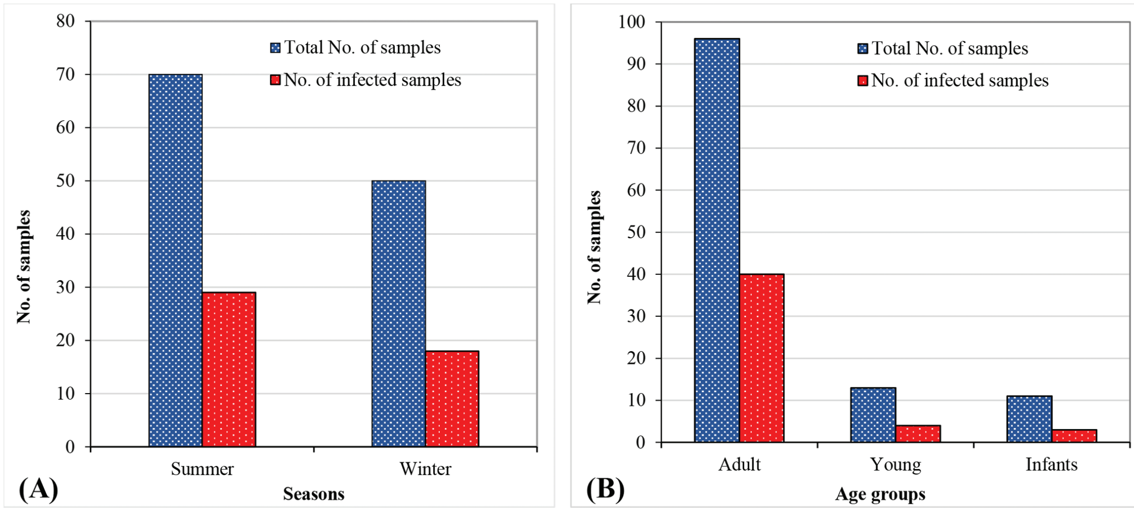

Fig. 3. Prevalence of GI helminth parasites in rhesus macaques. A- Seasonal prevalence, B- Age wise prevalence.

ranging macaques in Namakkal District of Tamil Nadu, India. Additionally, our findings were similar to those reported by Sapkota *et al.* (2020) who found a 21.4 % prevalence rate of *Strongyloides* sp. in urban rhesus macaques at a temple area in Kathmandu Valley. But a higher prevalence of *Strongyloides* sp. in macaques also has been reported by Mutani *et al.* (2003), Gillespie *et al.* (2005) and Malla (2007).

Of the 47 macaque samples with parasite infection, 78.7 % had parasite richness of one parasite; the remaining 21.3 % had a richness of two. Jha *et al.* (2011) and Sapkota *et al.* (2020) both reported higher parasitic richness, up to five parasite species in rhesus macaques at temples in the Kathmandu Valley. The parasite intensity and richness are managed in macaques by behavioral strategies like grooming techniques, licking, and consumption of medicinal plants (Dhakal *et al.,* 2018; Hart & Hart, 2018). Parasite infection rates are higher in populations of primates living in fragmented habitats than in continuous, protected forests as the fragmented habitats with human settlements create disturbances, and human and domestic animal interactions increase the chances of transmission (Trejo-Macías *et al.,* 2007). The community forest around Daunne Devi Temple is managed and protected by the local user groups. It has a variety of trees which provide substantial food sources for macaques in additional to other provisioned food supplied by the visitors in and around the temple. The availability of a natural forest and lower household densities (and in turn, reduced anthropogenic disturbance to macaques) may account for the lower parasite load observed in the rhesus macaques of Daunne Forest.

A noticeable difference in the seasonal prevalence of helminth parasites in the rhesus macaques was observed. The higher prevalence of intestinal parasites observed during the summer season compared to winter is consistent with Dhakal *et al. (*2018) for the rhesus macaques of Chitwan-Annapurna Landscape, and with Pokhrel and Maharjan (2014) for the Assamese macaques of Shivapuri Nagarjun National Park. During warmer weather, it is easier for parasites to reproduce resulting in greater contamination of food and water, and in turn, increased parasite infection (Gonzalez-Moreno *et al.,* 2013).

In addition to the seasonal difference in parasite infection, we also observed a higher parasite prevalence in the adult female macaques compared to the adult males and young. This finding is consistent with a study by Kumar *et al.* (2018) for bonnet macaques of India but is contrary to results reported by Tabasshum *et al.* (2018) and Kumar *et al.* (2019) who noted a higher parasite prevalence in male rhesus and lion-tailed macaques, respectively. The higher parasitic prevalence in adult rhesus macaques as compared to the young might be because of the socially mediated exposure of adult macaques to infective stages of helminth parasites (MacIntosh *et al*., 2012). A higher parasitic prevalence in females may be attributed to their greater dependence to human provisioned food and higher chances of infection through contaminated foods and water (Sapkota *et al.,* 2020).

Parasite prevalence in the humans (11.4 %) was found to be lower than in the macaques (39.2 %). The reason for this disparity may be related to the increased awareness and education regarding parasite infection, and the availability of modern health facilities (Schurer *et al*., 2019). *Ascaris* sp. was the only parasite observed in the humans in this study. It also is the most common intestinal helminth detected in other similar studies with humans (Gyawali, 2012; Shrestha & Maharjan, 2013; Chongbang *et al.,* 2016; Sapkota *et al.,* 2017), and is considered to be transmitted via a fecal-oral route and contamination of soil by macaque feces (Sapkota *et al.,* 2017).

The transmission of parasites between humans and macaques represents a serious one-health concern and deserves increased, long-term surveillance. Although we did not find the presence of shared helminth parasite species between the macaques and humans in this study, the risk of bi-directional transmission exists given the close interaction at the temple site. We strongly recommend further investigation (with larger sample sizes for both the macaques and local people) to more thoroughly assess parasite infection rates and the associated risk of disease transmission.

Conclusions

The prevalence of GI helminth parasites was identified in the rhesus macaques and humans in the Daunne Devi Temple area. The parasite occurrence was higher in the summer than in the winter season. The helminths detected in the rhesus macaques may pose a zoonotic risk to the local community and visitors. A comprehensive study, with a larger sample size of both the humans and non-human primates living in this shared environment, needs to be conducted to ascertain the potential zoonotic risk of enteric parasites.

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Conflicts of Interests

The authors declare that they did not have any conflict of interest in conducting this study. Moreover, the authors do not have any conflict of interest pertaining to this submission to Helminthologia.

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