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Research Article

Diversity and prevalence of gut parasites in urban macaques

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Abstract: Rhesus macaques (*Macaca mulatta*) are commonly found to inhabit various religious sites and cities in Nepal. Similar to other nonhuman primates, they are also the natural or reservoir host of several gut parasites. However, the status of gut parasitism, particularly in the urban dweller macaques, remains largely unexplored in the country. This study aimed to assess the prevalence and diversity of gut parasites in the monkeys inhabiting Bajrabarahee, an urban temple area in Lalitpur District, Nepal. A total of 42 fresh fecal samples of macaques belonging to five different troops, were collected and preserved in 2.5% (w/v) potassium dichromate solution. The fecal samples were processed by direct wet mount, concentration, and acid-fast techniques and examined under an optical microscope. All the fecal samples were positive with gut parasites. The parasites detected were Ascarid spp., *Balantidium coli*, *Cryptosporidium* sp., *Eimeria* sp., *Entamoeba coli*, *Entamoeba* spp., *Giardia* sp., hookworm, *Strongyloides* sp., Strongylid spp., *Trichomonas* sp., and *Trichuris* sp. Cent percent prevalence rate and high species richness with 12 parasites may indicate that they have impact on the gut health of these monkeys. This suggests the need of deworming the macaque population and enhancing public awareness for pro-active control of parasitic infection as well as of adopting the preventive measures to lessen the zoonotic transmission of the pathogenic parasites.

Keywords: *Cryptosporidium, Entamoeba, gastro-intestinal (GI), Macaca mulatta, zoonosis.*

सारांश : रातो बाँदरहरू प्रायः नेपालका विभिन्न धार्मिकस्थल र शहरहरूमा पाइन्छन् । अन्य गैर-मानव प्राइमेटहरू जस्तै तिनीहरू पनि पेटको धेरै परजीवीहरूको प्राकृतिक आश्रयस्थलहरु हुन्, यद्यपि, खासगरी नेपालको सहरवासी रातो बाँदरको पेटमा परजीवीको स्थिति भने अझै अन्वेषण गर्नुपर्ने रहेको छ । यसैले नेपालको ललितपुरस्थित शहरीक्षेत्रको मन्दिर बज्रबाराहीमा रहेको बाँदरको आन्द्रामा पाइने परजीवीहरूको व्यापकता र विविधताको आँकलन गर्नु यस अध्ययनको लक्ष्य हो । फरक-फरक पाँच प्रकारका समूहमा रहेका बाँदरहरुबाट कुल ४२ वटा ताजा दिसाका नमूनाहरू जम्मा गरि २.५% पोटाशियम डाइक्रोमेटमा संकलन गरी संरक्षित गरिएको थियो । उक्त नमूनाहरु डाइरेक्ट, कन्सनट्रेसन, र एसीड-फास्ट तरिकाहरुबाट एउटा अप्टीकल माइक्रोस्कोपद्वारा विश्लेषण गरिएको थियो, जसले आन्द्राहरुमा पाइने १२ वटा परजीबीहरु देखायो, जस्तै एस्कारिड, बालाण्टिडियम, क्रिप्टोस्पोरिडियम, आइमेरिया, एन्टअमिबा कोली, एन्टअमिबा इस्पेसिज, जिआर्डिया, अंकुशे जूका, स्ट्रङ्गाइलिड, स्ट्रङ्गाइलोइडिस, ट्राइकोमोनस, र ट्राइचुरिस थिए । धेरै परजीबीहरुको उच्च दर र ब्यप्तताले गर्दा यी बाँदरहरुको आन्द्राको स्वास्थ्यमा नकरात्मक असर पर्न सक्छ र मानवमा परजीबीहरु प्रसारणको जोखिममा उल्लेखनीय वृद्धि गर्दछ । यसले परजीबीहरुको अग्रिम नियन्त्रणको लागि बाँदरहरुको जनसंख्यालाई औषधी खुवाउनु पर्ने र जनचेतना कार्यक्रमहरुको आवश्यकता र रोगजनक परजीवीहरूको जुनोटिक प्रसारण कम गर्नका लागि रोकथामको उपायहरू अनुकूलन गर्न सुझाव दिन्छ ।

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1. Introduction

The Rhesus macaques, *Macaca mulatta* (Zimmermann, 1780) (*Rato bander* in Nepali) are well-known Old-World nonhuman primates. They are physiologically and genetically similar to humans as both are believed to share a common ancestor that diverged about 25 million years ago and developed independently (Kumar and Hedges 1998; Gibbs et al., 2007). They are listed as 'Least Concern' by IUCN Red List (https://www.iucnredlist.org/species/12554/3356486, accessed on May 22, 2020). Compared with other macaques *M. mulatta* have a high level of adaptation and are well-adapted to co-exist with the human in urban settlements (Rathoure 2014; Kumar et al., 2013).

Monkeys have an important status in mythology and religion particularly, in Hinduism and Buddhism (Jokinen 2014; Fuentes 2017; Ale et al., 2020) and are commonly found in religious sites like temples, monasteries, and many urban areas (Ale et al., 2020). There is an extensive, unregulated, and close contact of macaques with humans because the religious sites are always full of local people, worshippers, and visitors (Fuentes 2005). Their feeding ecology and habitat are more or less similar to that of humans; thus, in the nearby areas, they often invade the houses, gardens, or agricultural fields for sharing the niches. They are also known to share disease-causing pathogens like the gut or gastrointestinal (GI) parasites, for example, from many years, these mammals have been linked for the outbreaks of emerging parasitic diseases in humans (Chapman et al., 2005; Jones-Engel et al., 2006; Ghimire et al., 2020). Gut parasitism has been evidenced to result in the high morbidity and mortality in nonhuman primates including many types of macaques (Fremming et al., 1955; Remfry 1978; Toft 1986; Chapman et al., 2005) as well as in human (Stauffer and Ravdin 2003; Haque 2007; WHO 2020) around the world. Thus, it is crucial to know the status of gut parasitism in the monkeys, especially in the anthropogenic ones, to reduce the possible health consequences in macaques as well as humans. The current study was conducted to assess the prevalence and diversity of gut parasites in the monkeys inhabiting Bajrabarahee, an urban temple area with increasing human–macaque interactions in the Lalitpur district, Nepal.

2. Materials and Methods

2.1. Study area

The study had been conducted in Bajrabarahee, a religious Hindu temple area located in Godawari Municipality, Lalitpur, Nepal. The area (27.60610 N and 85.32930 E) is covered by a sacred forest and is a typical habitat of several species of birds, reptiles, and mammals, including the *M. mulatta*. By direct counting methods, we assessed 50 monkeys in the forest. The area is surrounded by agricultural land to the south and east, and human settlement to the north and west. Besides the religious importance, it is also a bird-watching and recreational spot, and thus many religious people, tourists, and local people usually visit the site. It has also been developed as a picnic spot, so macaque-human interaction is typical in the study area.

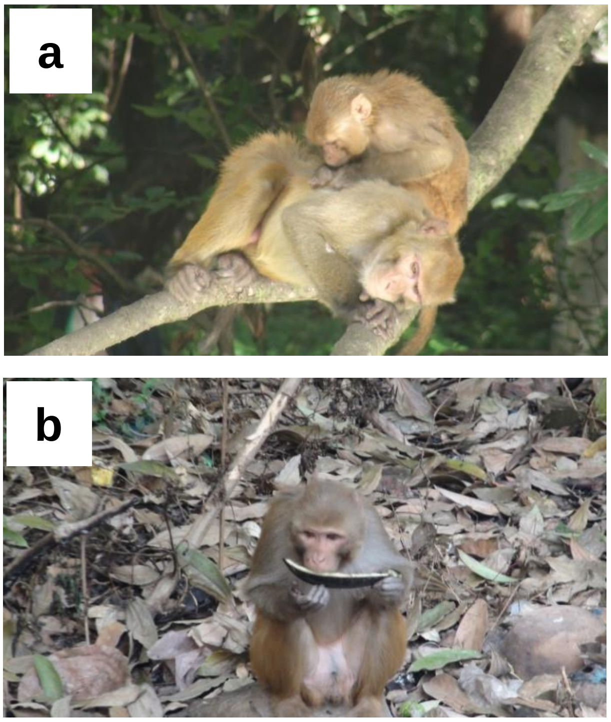

Figure 1. *M. mulatta* in the study area. (**a)** Close-contact scenario. **(b)** Feeding on garbage.

2.2. Sample collection, preservation, and transportation

From June to August 2019, a total of 42 fresh fecal samples of *M. mulatta* belonging to five different troops were collected non-invasively from various sites in the study area (Figure 1). The fecal samples were immediately preserved in 2.5% (w/v) potassium dichromate solution in 20 mL sterile vials. Then, the samples were transported to the Animal Research Laboratory (ARL) of the Nepal Academy of Science and Technology (NAST) and stored in the refrigerator (4^{o} Celsius) for further analysis.

2.3. Laboratory processing and examination

The fecal samples stored in 2.5% (w/v) potassium dichromate were microscopically examined by four different techniques - direct wet mount, sedimentation, saturated salt (45% w/v NaCl) flotation, and modified acid-fast techniques as previously described (Ghimire and Bhattarai 2019).

2.4. Parasite identification

All the fecal samples were observed under an optical microscope (Optika Microscopes Italy, B-383PLi) at X100, X400, and X1000 total magnifications. Parasitic images were taken by the camera (SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013-2014). The micrometry of parasitic bodies was assessed using ImageJ 1.51k (National Institute of Health, USA) and identification was carried out as previously described (Petrášová et al., 2010; Soulsby 2012; Li et al., 2017)

2. 5. Data analysis

Data were expressed as numbers of positive samples as well as prevalence rates in the table using Microsoft Word 2007. Prevalence rates were calculated by dividing the number of parasite positive samples (total or particular species) by the total number of samples observed (Ghimire and Bhattarai 2019).

3. Results

In the current study, we reported a 100% prevalence rate of gut parasites. The prevalence of protozoa was higher (90.5%) compared to that of the helminths (47.6%). Furthermore, a total of 12 gut parasitic species were detected. They were protozoa - *Entamoeba* spp. (66.7%), *Balantidium coli* (59.5%), *Entamoeba coli* (57.1%), *Cryptosporidium* sp. (11.9%), *Eimeria* sp. (7.1%), *Giardia* sp. (4.8%), and *Trichomonas* sp. (2.4%) and helminths such as Ascarid spp. (21.4%), *Strongyloides* sp. (21.4%), hookworm (19%), *Trichuris* sp. (14.3%), and Strongylid spp. (9.5%) (**Figure 2**) (**Table 1**).

All samples were found to be mixed infections with two or more gut parasitic species. Triplet infection was the highest (57.1%) followed by the duplet (26.2%), and pentuplet (4.8%) infections were the least (**Table 1**). Further, two morphotypes of Ascarid eggs were detected. Some of these eggs were similar to human *Ascaris* (size range: 54–58x40–44µm), and others were similar to animal *Toxocara* (size range: 72–84x61–68µm). Similarly, based on the morphology and micrometry, three morphotypes of Strongylid eggs were detected (size range: 72–111x38–65µm) in the current study **(Figure 2)**.

4. Discussion

The current study explores the status and diversity of the gut parasites in monkeys inhabiting an urban temple area situated in between human settlements in Nepal. In this study, the overall prevalence of the gut parasites was 100% which was similar to the result from Bangladesh (100%) (Tabasshum et al., 2018), higher than the findings from Nepal (61.9% – 86%) (Jha et al., 2011; Adhikari and Dhakal 2018; Bhattarai et al., 2019), and India (40%–66.5%) (Parmar et al., 2012; Jaiswal et al., 2014; Kumar et al., 2018). Besides, cent percent concomitant infections with maximum triplet co-infection rate suggested the parasitic richness in the gut of the macaques.

Table 1. Gut parasitic species, their concurrency, and prevalence in *M. mulatta.* *N represents total samples collected and n represents number of positive sample/s.*

Infecting Parasitic species	Overall Prevalence (nX100/N) (N=42)
Protozoa	
Entamoeba spp.	28 (66.7%)
Entamoeba coli	24 (57.1%)
Eimeria sp.	3 (7.1%)
Cryptosporidium sp.	5 (11.9%)
Giardia sp.	2 (4.8%)
Balantidium coli	25 (59.5%)
Trichomonas sp.	1 (2.4%)
Helminths	
Ascarid spp.	24 (21.4%)
Strongylid spp.	4 (9.5%)
Hookworm	8 (19%)
Strongyloides sp.	9 (21.4%)
Trichuris sp.	6 (14.3%)
Total Protozoan infection	**38 (90.5%)**
Total Helminth infection	**20 (47.6%)**
Total Mixed infection	**42 (100%)**
Duplet Infection	11 (26.2 %)
Triplet Infection	24 (57.1 %)
Quadruplet Infection	5 (11.9%)
Pentuplet Infection	2 (4.8%)

The higher parasitic prevalence might be related to the applied study design, sampling techniques, laboratory-based techniques, sampling geography, including the climatic conditions and the lifestyle pattern of the existing macaque population. In the current study, we used a purposive sampling technique and collected the fresh fecal samples immediately after defecation by the macaques. The sampling period was warm and wet, the period favorable for the survival of larva, cysts, oocysts, and trophozoites of gut parasites (Zvinorova et al., 2016). Also, laboratory techniques like direct wet mount, concentrations (sedimentation and flotation), and acid-fast staining techniques were used to enhance the detection of the parasites from each fecal sample.

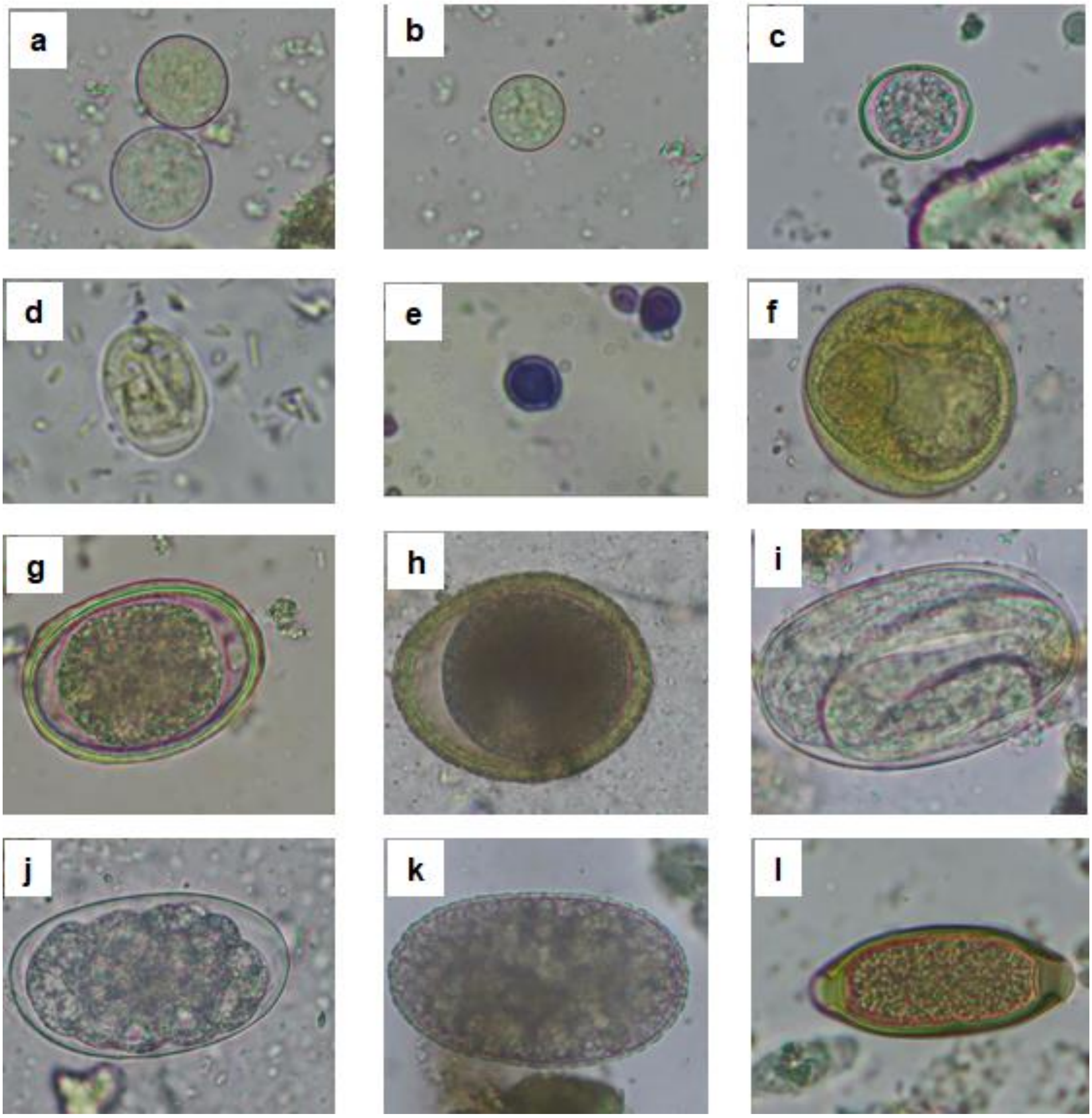

Figure. 2. Photomicrographs of various gut parasitic species. (a) Cysts of *Entamoeba coli*, 19µmx19µm, X400. **(b)** Cyst of *Entamoeba* sp., 13µmx13µm, X400. **(c)** Oocyst of *Eimeria* sp., 20µmx14µm, X400. **(d)** Cyst of *Giardia* sp., 14µmx9µm, X1000. **(e)** Oocyst of *Cryptosporidium* sp., 6µmx6µm, X1000. **(f)** Cyst of *Balantidium coli*, 54µmx51µm, X400. **(g)** Egg of Ascarid sp. 56µmx41µm, X400. **(h)** Egg of Ascarid (*Toxocara* sp.), 82µmx67µm, X400. **(i)** Egg of *Strongyloides* sp., 69µmx43µm, X400. **(j)** Egg of hookworm, 66µmx39µm, X400. **(k)** Egg of Strongylid sp., 111µmx65µm, X400. **(l)** Egg of *Trichuris* sp., 55µmx24µm, X400.

Macaques are the natural and reservoir hosts of many gut parasites. They live in a group and spend a semi-nomadic life that might massively increase the parasite dispersal (Macpherson 1994; Swedell 2012). The increasing soil and water pollution by waste food and garbage, especially during the festive and picnic programs, and the occasional open defecation by visitors/outsiders in the forest areas and nearby water sources, are the risk factors of parasite transmission. In this scenario, macaques are usually in contact with contaminated soil and water, and consumption of garbage foods may lead to the acquisition and transmission success of the gut parasites in them, which explains their species richness.

The current prevalence of gut protozoa was higher than that of the helminthes; however, this result contrasts with the previous findings (Adhikari and Dhakal 2018; Bhattarai et al., 2019) that recorded higher prevalence rates in helminths. Regarding protozoa, the prevalence of *Entamoeba* spp. in the current study was 66.7% which was lower than the findings from China (89.96%) (Zhang et al., 2019) and higher than those reported from Nepal (13.97% – 32%) (Jha et al., 2011; Pokhrel and Maharjan 2014; Adhikari and Dhakal 2018; Bhattarai et al., 2019) and from India (10% – 23.07%) (Parmar et al., 2012; Jaiswal et al., 2014). Several species of these pseudopodial amoebas like *Entamoeba histolytica*, *E nuttalli*, *E. dispar*, *E. moshkovskii*, *E. hartmanni*, *E. chattoni*, and *E. polecki* (Tachibana et al., 2007; Jiang et al., 2008; Tachibana et al., 2013; Guan et al., 2016; Zhang et al., 2019) have already been reported from macaques all over the world; however, majority of them are considered harmless and do not exhibit pathologic illness in the macaques. Pathologically, *E. histolytica and E. nuttali* are critical because they induce fatal intestinal and extraintestinal amebiasis (Fremming et al. 1955; Loomis et al., 1983; Haq et al., 1985; Beaver et al., 1988; Pang et al., 1993; Verweij et al., 2003; Tachibana et al., 2007; Jiang et al., 2008; Levecke et al., 2010). In our study, *Entamoeba coli* showed 57.1% prevalence rate which was higher than the previous findings from Nepal (9.52% – 24.44%) (Jha et al., 2011; Bhattarai et al., 2019), India (10% – 26.92%) (Parmar et al., 2012; Jaiswal et al., 2014), and China (42%) (Guan et al., 2016). Although this species is typically asymptomatic in primates (Chapman et al., 2005), its presence should be taken as the indication of other pathogens inside the gut (Ghimire 2014).

It was notable that, the rate of prevalence of *Cryptosporidium* sp. was 11.9%, which was lower than the findings from Nepal (41.1%) (Bhattarai et al., 2019) and India (26.92%) (Jaiswal et al., 2014), and higher than the findings from China (10.94%) (Ye et al., 2012) and Thailand (1%) (Sricharern et al., 2016). This coccidian parasite causes highly fatal types of intestinal and extraintestinal pathologies (Kuhn et al., 1997; Kaup et al., 1998) and might be transmitted among humans and primates zoonotically (Ye et al., 2012; Zhao et al., 2019). Another coccidian parasite *Eimeria* sp. was reported in 7.1% of the fecal samples. This rate was lower than the findings from Nepal (16.12%) (Adhikari and Dhakal 2018) and higher than the results from India (3%) (Arunachalam et al., 2015). *Eimeria* sp. can cause severe pathologic consequences, especially in the young monkeys compared to the old ones (Burrows 1972).

Giardia and *Trichomonas* are the two flagellated parasites reported in the current study. The prevalence rate of *Giardia* sp. was 4.8% which was lower than the findings from India (31%) (Debenham et al., 2017), China (8.51%) (Ye et al., 2012), Nepal (6.67%) (Bhattarai et al., 2019), Thailand (7%) (Sricharern et al., 2016), and higher than the reports from India (1.2%) (Kumar et al., 2018). *Giardia* causes enteritis in macaques (Toft 1986; Chapman et al., 2005) and is a zoonotically critical parasite for the public and veterinary health (Ye et al., 2012, 2014). The prevalence rate of *Trichomonas* sp. was 2.2% when examined in the fecal samples. Reports of this species are found in *M. mulatta* following histopathologic studies in USA (Blanchard and Baskin 1988) and Germany (Kuhn et al., 1997; Kondova et al., 2005). This parasite is associated with mild to moderate gastritis (Blanchard and Baskin 1988; Blanchard 1993; Kaup et al., 1998), including many other severe GI pathologic consequences in immunocompromised macaques (Kondova et al., 2005).

In the same way, *Balantidium coli*, a ciliate protozoan had a prevalence rate of 59.5% which was higher than the findings from Nepal (27.95% – 36%) (Jha et al., 2011; Pokhrel and Maharjan 2014; Adhikari and Dhakal 2018; Bhattarai et al., 2019) and India (8.7% – 19%) (Knezevich 1999; Kumar et al., 2018). This zoonotic parasite can also cause severe pathology in the intestinal tract of macaques, including diarrhea and rectal prolapse (Burrows 1972; Toft 1986; Kuhn et al., 1997).

Regarding helminths, the prevalence rate of Ascarid spp. was 21.4% which was lower than the findings from Bangladesh (90.90%) (Tabasshum et al., 2018), India (25.5% – 26.66%) (Parmar et al., 2012; Kumar et al., 2018), and Nepal (22.22%) (Bhattarai et al., 2019) and higher than the findings from Nepal (10.58% – 11.82%) (Pokhrel and Maharjan 2014; Adhikari and Dhakal 2018), India (5%) (Arunachalam et al., 2015), and Thailand (1%) (Schurer et al., 2019). The presence of *Toxocara* sp. in macaques, was also explained in various research findings from Nepal (Jha et al., 2011; Bhattarai et al., 2019) and Bangladesh (Tabasshum et al., 2018). Ascarid infection is associated with zoonosis (https://www.cdc.gov/parasites/ascariasis/index.html, Accessed on: June 2, 2020). It is one of the leading causes of high morbidity and mortality in macaques (Weiszer et al., 1968; Richards et al., 1983; Toft 1986; Chapman et al., 2005). Another nematode species *Strongyloides* was detected with the prevalence rate of 21.4% that was lower than the findings from England (55.6%) (Remfry 1978), Nepal (30% – 34%) (Jha et al., 2011), India (26.66% – 57%) (Knezevich 1999; Parmar et al., 2012), and higher than the findings from Nepal (10.75%)

(Adhikari and Dhakal 2018), India (13%) (Kumar et al., 2018), and Thailand (2%) (Schurer et al., 2019). Mild to severe strongyloidiosis caused by migratory larva as well as the adult of this zoonotically significant parasite may lead to deaths, especially in young macaques (Remfry 1978; Toft 1986; Toft and Eberhard 1998; Chapman et al., 2005).

Strongylid nematode is one of the critical nematode groups representing different species. The species cannot be easily distinguished by the morphology of the eggs or without performing larval cultures and development (Ghimire and Bhattarai 2019). Its current prevalence rate 9.5% was lower than the findings from India (33%) (Arunachalam et al., 2015). Strongylids are zoonotic and can cause serious illnesses resulting in the deaths of macaques (Remfry 1978; Toft 1986; Chapman et al., 2005).

Trichuris sp. was reported with a prevalence rate of 14.3% that was in accordance with the findings from Nepal (14.05% – 14.44%) (Jha et al., 2011; Bhattarai et al., 2019) and lower than the reports from Bangladesh (50%) (Tabasshum et al., 2018), Thailand (19.6%) (Schurer et al., 2019), Nepal (23.65%) (Adhikari and Dhakal 2018), and higher than the findings from India (3.7% – 12%) (Knezevich 1999; Kumar et al., 2018) and England (11.2%) (Remfry 1978). Trichuriasis in macaques leads to intestinal disorder accompanied by rectal prolapse and may even induce death (Thienpont et al., 1962; Remfry 1978; Toft and Eberhard 1998)

The current prevalence 19% of hookworm was lower than the findings from Thailand (23%) (Schurer et al., 2019), Bangladesh (22.72%) (Tabasshum et al., 2018) and was higher than the findings from India (15.38%) (Jaiswal et al., 2014) and Nepal (6.67%) (Bhattarai et al., 2019). This zoonotic nematode may cause mild to severe clinical pathologies in the macaques, including anemia and diarrhea (Toft 1986; Toft and Eberhard 1998).

5. Conclusions

In conclusions, the Rhesus macaques are heavily infected with ameboid, flagellate, ciliate, coccidian, and nematode parasites. Besides, concomitant infections up to five different species of parasites in the fecal samples may indicate that these pathogens may pose negative impacts on the gut of macaques. Most of the parasitic species detected in this study are zoonotically significant, and thus, the macaques may enhance the risk of transmitting the parasites. Thus, practical control actions like deworming of the macaques should be conducted by the concerned authorities. There is a need of enhanced awareness among local people and visitors towards adopting hygienic precautions and decreasing the human-macaque interaction in order to control the zoonotic transmission of the pathogens.

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Conflicts of Interest: The authors declare no conflict of interest.

Ethical approval

The authors declare that the study was conducted on naturally infected Rhesus monkeys. No experimental infection was established during this research work. The required permission for the collection of the fecal samples was issued by Government of Nepal, Ministry of Forests and Environment, Department of Forestry (Permission number: 202/2018).

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Competing interests

None declared.

Availability of data and materials

All data generated or analyzed during the research work are included in this article

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