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Amrit Research Journal December 2023, Vol. 4, Issue 1, 79-90
ISSN: 2738-9553
RESEARCH ARTICLE

Gastro-Intestinal Parasites in Monkeys of Shivapuri Nagarjun National Park and Temple Areas of Kathmandu Valley

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(Received: May 15, 2023, Received in revised form: November 17, 2023, Accepted: December 7, 2023, Available Online)

DOI: https://doi.org/10.3126/arj.v4i1.61217

Highlights

- 100% prevalence of GI parasites with all the samples positive for one or more than one type of GI parasites
- 18.57% of the samples were infected with more than five parasite species
- First report on the presence of Nematodirus spp. in macaques of Nepal

Abstract

The study intended to assess the prevalence of gastro-intestinal parasites in free-ranging macaques of Bajarayogini temple, Bajrabarahi temple and areas of Shivapuri Nagarjun National Park (SNNP). A total of 70 fresh fecal samples of Macaca sps, were collected from October 2022 to May 2023 and processed by direct wet mount method and concentration method. The result disclosed the distribution of total 5 species of protozoan parasites and 8 species of helminth parasites, indicating 100% prevalence rate where Entamoeba coli (67.14%) showed the highest prevalence followed by Trichostrongylus spp. (1.43%) with the lowest prevalence. It was observed that the helminth parasite, Nematodirus spp. was identified for the first time from the samples of monkeys in Nepal. Human-primate transmission of infectious disease is one of the most important emerging threats that's why, emphasizing public awareness for control of parasitic infection to reduce the zoonotic transmission of the pathogenic parasites was suggested.

Keywords: *Gastro-intestinal (GI) parasites, Shivapuri Nagarjun National Park (SNNP), Nematodirus spp., concentration methods, zoonotic transmission*

Introduction

Primates are a assorted arrange of warm blooded animals that emerged 85-55 million a long time prior to begin with from little earthly warm blooded creatures, which adjusted to dwell within the trees of tropical timberlands: numerous primate characteristics speak to adjustments to life in this challenging environment, counting huge brains, visual sharpness, color vision, a bear support permitting a large degree of development within the bear joint, and able hands. There are 376-524 species of living primates, depending on which classification is used. Modern primate species proceed to be found: over 25 species were depicted within the 2000s, 36 within the 2010s, and three within the 2020s. Of all the primates, monkeys have been successful next to humans to adapt best to widely diverse environmental conditions. They are found in tropical timberlands, dry savannas, mountains, towns, sanctuaries and indeed in expansive cities. In total, five species of monkeys have been reported from Nepal that include Rhesus macaque *(Macaca mulatta),* the Assamese monkey *(Macaca assamensis),* Terai grey langur (Semnopithecus hector), Nepal grey langur *(Semnopithecus schistaceus)* and Himalayan grey langur *(Semnopithecus ajax)* (44).

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Parasites play a central portion within the biological system as they influence the ecology and species interactions, host population and regulation and community diversity (37, 38, 49). They share disease-causing pathogens like gastrointestinal (GI) parasites, for illustration from long time, these primates have been related with the flare-up of rise of parasitic illnesses in people (9, 10, 27). Gastrointestinal parasites may influence the wellbeing of macaques straightforwardly or in a roundabout way, in this manner uncovering primates to conservational dangers. Multiple parasitic infections can cause physiological disturbance, nutritional loss or may cause lesion that result in genuine debilitation, and can create opportunistic for the secondary infection which may lead to fatal conditions, while some of the parasites maybe non-pathogenic too. Gut parasitism is a proof that primates including macaques and humans are prone to high morbidity and mortality around the world (6, 10, 19, 21, 50).

By ethicalness of hereditary, physiologic, and behavioral similitude of non-human primates to people, they are especially likely sources of rising irresistible specialists with the capacity to contaminate people, and primate-to-human cross-species transmission of irresistible specialists has ended up a center of logical request. As human-primate contact is common in Asia, the continent provides greater opportunities to pursue research in such areas. In Nepal, study regarding emergence of parasitic infections from primates is still deficient. Moreover, there is no any statistical information regarding the status of monkeys. Meanwhile, the proposed study didn't focus on obtaining any statistical information rather it studied the intestinal infections and harms caused by them to both human and non-human primates. Hence, the study was done to provide information related to distribution of protozoan and helminth parasites of *Macaques* of Bajarayogini, Bajrabarahi and Shivapuri Nagarjun National Park.

Methods

Study Area:

The study was conducted in temples (Bajarayogini and Bajrabarahi) and areas of Shivapuri Nagarjun National Park of Kathmandu Valley. Important from religious, archaeological and historical point of view, both Bajarayogini (located in Shankharapur Municipality, Kathmandu) and Bajrabarahi (located in Chapagaun Village, Lalitpur), is home to most of the macaques. Both temples reside in the centre of the dense forest which also describes the natural beauty of the temple. Due to the religious significance of the temples, many religious people, tourists, and local people usually visit these site which show that macaque-human interaction is typical in these areas. Considering about national park, it is the only protected area that falls entirely within the middle mountain range of Nepal and represents its flora, fauna and ecosystem (SNNP, 2017). The area includes religious sites, army barracks (scattered within the area), and a convent. Human settlements are located around the boundary of the national park. The Nagarjun area of the park is one of the best locality to observe the Assamese macaque (SNNP 2017). It is estimated that more than 200 Rhesus monkeys inhabit around SNNP and with a maximum number of 64 individuals in a troop of Sundarijal (33, 34)

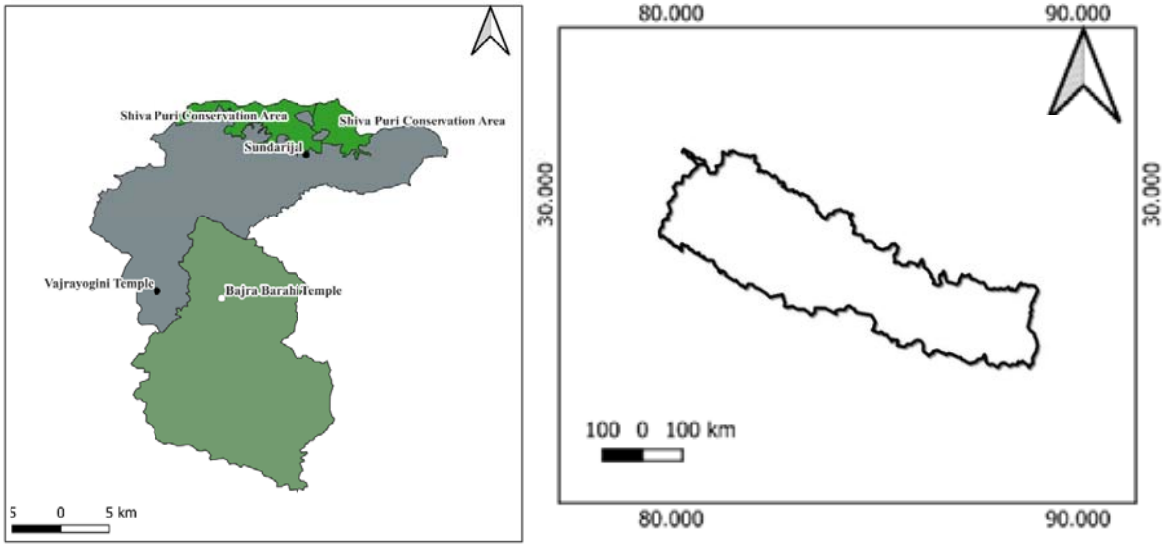

Fig 1. Map showing fecal sample collection areas

Field Survey And Data Collection:

From October 2022 to May 2023, a total of 70 fresh fecal samples of macaques, were collected non-invasively from the study area including Bajrayogini temple, Bajrabarahi temple, and Nagi Gumba, Nagarjun forest areas and Sundarijal area of Shivapuri Nagarjun National Park. Fecal samples were collected without causing any harm or disturbance to the monkeys. The samples were collected during early morning hours (7a.m.-9a.m.) to avoid human intervention. About 10gm of sample was collected from the fecal mass with the help of ice-cream stick and was immediately preserved in 2.5% potassium dichromate solution in 20mL sterile vials. All the samples collected were labelled properly as per season, place and date and then brought to laboratory of zoology, Amrit Campus for further processing.

Laboratory examination of fecal samples

The fecal samples were macroscopically examined for the presence of adult helminth and segments of tapeworms and then microscopically examined for the presence of trophozoites, cysts, oocysts of protozoans and eggs and larvae of helminths gastro-intestinal parasites through wet mount and concentration method (Floatation technique and Sedimentation technique) (5, 13)

Wet mount method (Saline and Iodine)

Saline wet mounts and iodine wet mounts were prepared by distinctly mixing a small volume of stool sample with one drop of saline and one drop of iodine solution (diluted in 1:5 distilled water), subsequently, on a clean, grease-free glass slide and setting a coverslip over the smear. The smear was then examined under an electric microscope at low power (10X) and then under high power (40X) (13).

Concentration Methods

Floatation Technique

This technique shows the presence of all the helminth eggs and protozoan cysts in the prepared solution except the eggs of *Ascaris lumbricoides,* Taenia sps, and also the eggs of *Strongyloidesas* they don't float in salt solution (1). It involves following process:

- About 3gm of fecal sample was taken.
- The sample was kept on beaker and grinded with about 20mL of water. The sample solution was filtrated with the help of cotton gauze and poured into centrifuge tube upto 12mL and centrifuged at 1000 rpm for 5 minutes.
- The centrifuge tube was taken out and upper part of the water was removed with the help of pipette and the tube was again filled with ZnSO4 solution upto 12mL and centrifuged at 1000 rpm for 5 minutes.
- The centrifuge tube was taken out and more ZnSO4 was added so as to form upper meniscus and a drop of methylene blue was added in it.
- A cover slip was put over the top of the centrifuge tube so that the solution touched the cover slip and it was then left for 5 minutes. Then cover slip was taken out gently and placed on a microscopic slide and examined under 10X and 40X. Finally, photographs were taken.

Sedimentation Technique

A sedimentation procedure is used to isolate eggs of flukes, acanthocephalons and some other eggs of tapeworm and nematodes which are bit heavier. This technique involves taking out sediments of centrifuged contents for eggs detection.

- The centrifuge tube was taken out and upper part of the saturated Zinc sulphate solution was removed with the help of pipette, after examining the floatation sample. Then remaining sediment content was poured into the watch glass and stirred gently.
- A small drop of sediment mixture was taken with the help of pipette and placed on the slide and one drop of iodine solution was added for staining.
- The stained specimen was examined under 10X and 40X and photographs were captured. (5)

Laboratory analysis of egg, cysts and larva

By using ocular and stage micrometer, the length, breadth and diameter of parasites (eggs, cysts and larva) were measured with calibration factor. Then they were identified by comparing the structure, color and size of eggs, cysts and trophozoites with the help of published literature, journals and books. In this study, we followed Veterinary Clinical Parasitology by (5) for identification of protozoans and helminths. Additionally, the taxonomy has been updated by changing the nomenclature from *Balantidium* coli to *Balantioides* coli, in accordance with the revisions proposed by Li & Ponce-Gordo (14).

Data analysis

Data were expressed as numbers of positive samples as well as prevalence rates in the table using Microsoft Word 2007. Total prevalence rates were calculated by dividing the number of parasite positive samples (total or particular species) by the total number of samples observed Statistical analysis was performed using Excel software and data were analyzed by SPSS software. The likelihood ratio test was used for statistical analysis. In all cases, 95% confidence interval (CI) and $P<0.05$ were considered for a statistically significant difference.

Results

Prevalence of GI parasites

All the samples were found positive for one or more than one GI parasites, which shows 100% prevalence rate. The prevalence of protozoan was higher, that is, (92.86%) as compared to that of helminths, (81.43%) as shown in Fig 2. A total of 13 species of GI parasites were reported which included 5 species of protozoa and 8 species of helminth parasites.

Among the protozoan parasites, *Entamoeba* coli (67.14%) showed the highest prevalence and Coccidia spp. was present in least number (1.42%) as represented in Fig 2. Meanwhile the helminth parasites included *Strongyloides* spp. (52.85%) with the highest prevalence followed by *Trichostrongylus* spp. with the least one (1.43%). (Fig 2)

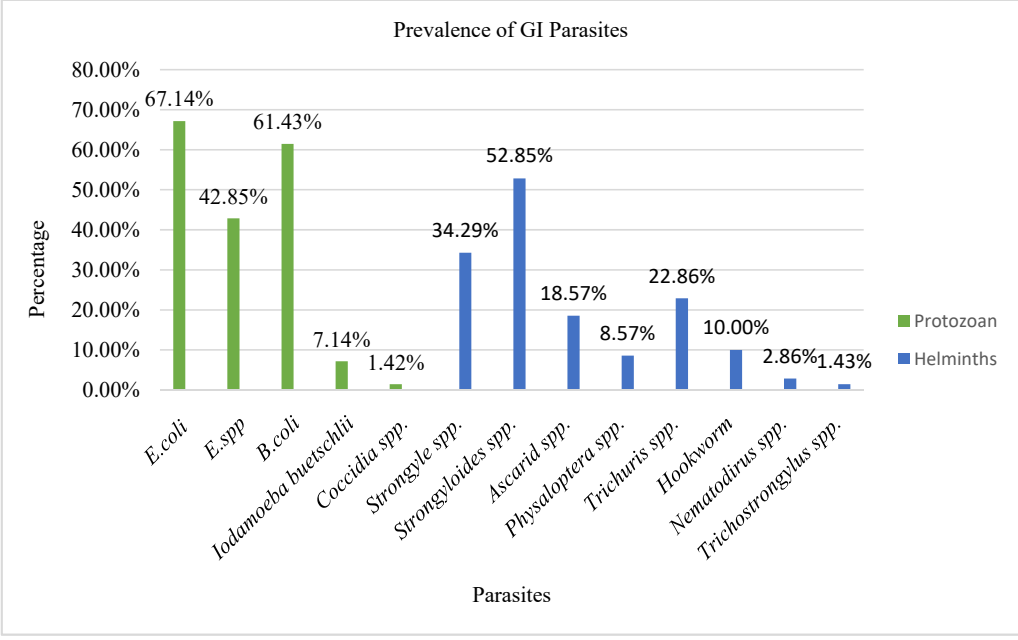

Fig 2. Prevalence of GI parasites

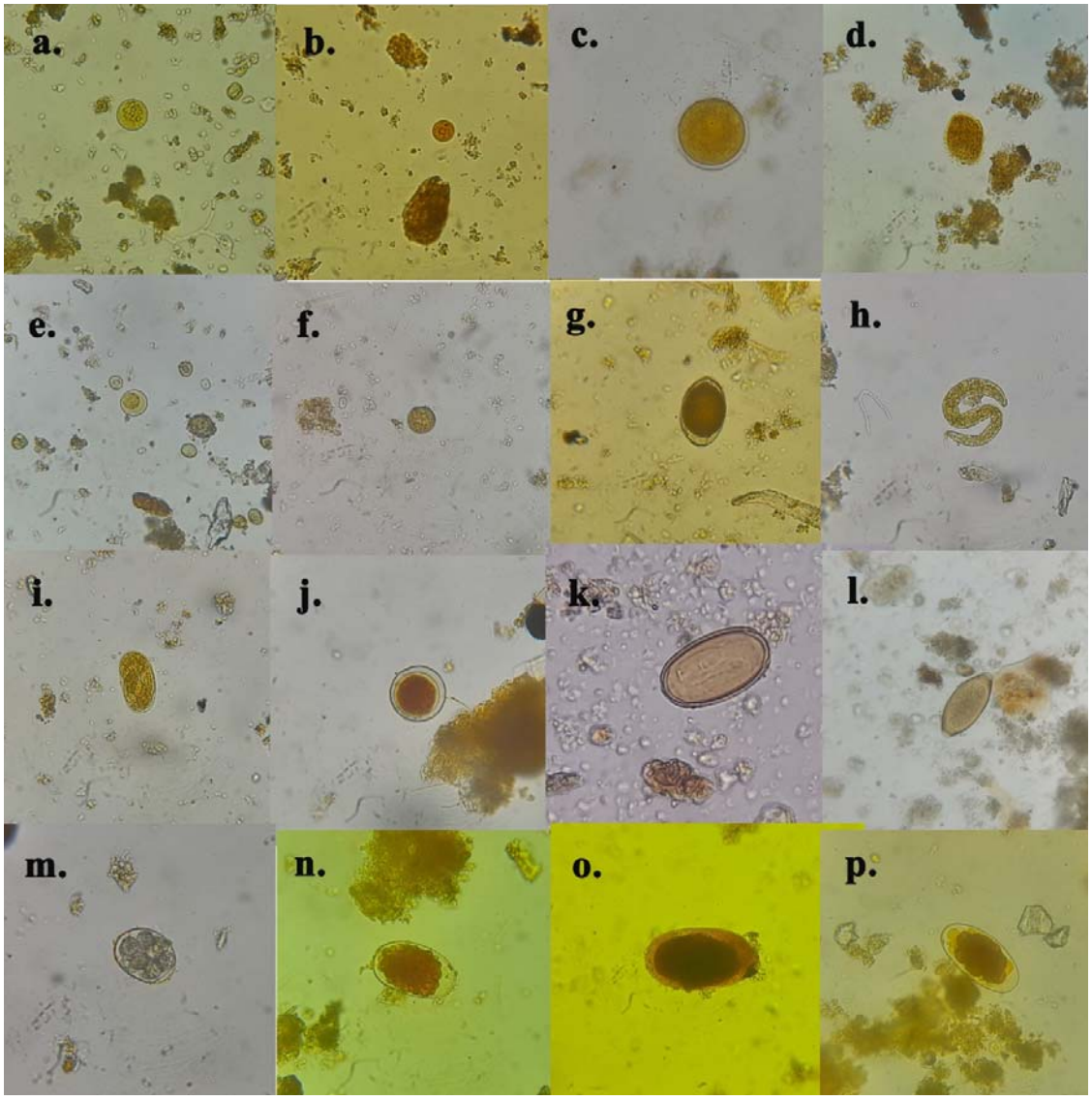

Fig 3. ***Photographs of various GI parasitic species*****. (a)** Cyst of *E.coli*, **(b)** Cyst of *E.spp*., **(c)** Cyst of *B.coli*, **(d)** Trophozoite of B.coli, **(e)** Cyst of *Iodamoeba buetschlii*, **(f)** Oocyst of *Coccidia* spp., **(g)** Egg of *Strongyle* spp., **(h)** *Strongyloides* larvae **(i)** Egg of Strongyloides spp.**, (j)** Egg of *Ascarid* spp., **(k)** Egg of *Physaloptera* spp., **(l)** Egg of *Trichuris* spp., **(m)** Egg of Hookworm, **(n)** Egg of Hookworm, **(o)** Egg of *Nematodirus* spp**.,** and **(p)** Egg of *Trichostrongylus* spp.,

Location wise prevalence of GI parasites

The study showed that protozoan prevalence was somewhat similar in Bajrayogini and SNNP area that is, 92% while Bajrabarahi showed prevalence of 94.44% which is more as compared to both areas. Among protozoan parasitic infection, *E.coli* (80%) had the highest prevalence reported in samples of Bajrayogini while *Coccidia* spp. had the least prevalence rate, 3.70%, only present in the samples of SNNP.

Considering the helminth parasitic infection, it was found that Bajrayogini (96%) showed the highest prevalence of helminth parasites followed by 81.48% in SNNP and 61.11% in Bajrabarahi. Among all, *Strongyloides* spp. (68%) had the highest prevalence, reported from Bajrayogini while *Trichostrongylus* spp. (4%) was least prevalent, only in Bajrayogini as shown in Fig 4.

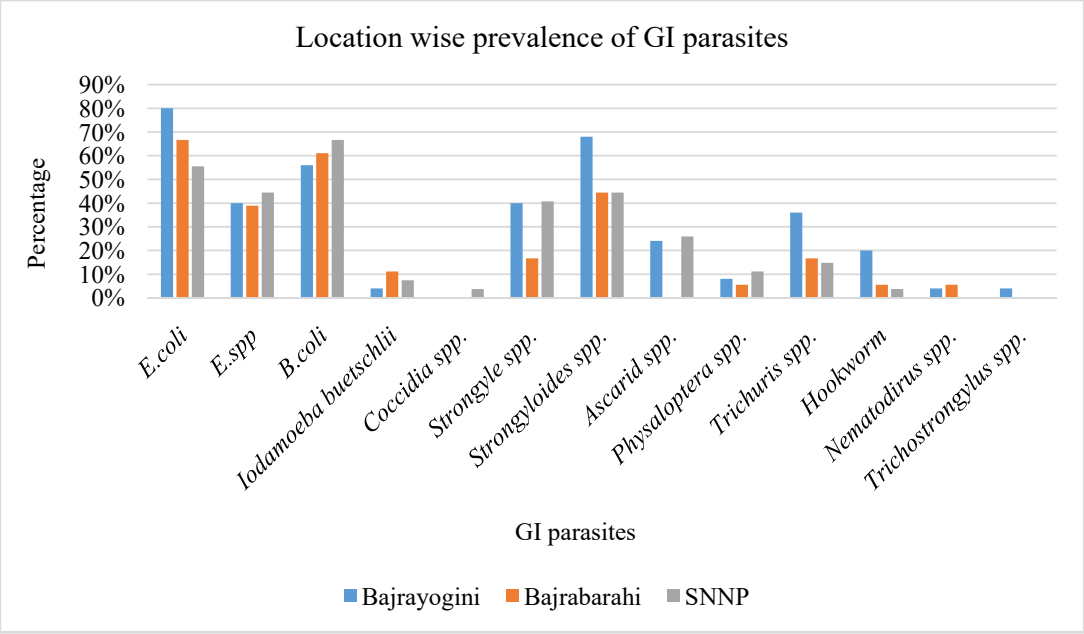

Fig 4. Location wise prevalence of GI parasites

Likelihood ratio test between location and presence of parasites

The significant value of Likelihood test is less than $\alpha = 0.05$ so, the distribution of all the GI parasites do not depend on the location as their value exceeds the number 0.05. The obtained value of Likelihood ratio test is shown below in Table 1. and the data analysis is shown in Appendix III (a).

Table 1. Test of significance between the location and presence of parasites

Location vs Parasites				
Parasites	**Value**	**df**	**Likelihood ratio**	**Status**
E.coli	3.518	2	3.612	Insignificant
E.spp.	0.170	2	0.170	Insignificant
B.coli	0.624	2	0.626	Insignificant
Iodamoeba buetschlii	0.803	2	0.811	Insignificant
Coccidia spp.	1.616	2	1.928	Insignificant
Strongyle spp.	3.342	2	3.638	Insignificant
Strongyloides spp.	3.579	2	3.642	Insignificant
Ascarid spp.	5.558	2	8.736	Insignificant
Physaloptera spp.	0.442	2	0.452	Insignificant
Trichuris spp.	3.831	2	3.713	Insignificant
Hookworm	4.362	2	4.213	Insignificant
Nematodirus spp.	1.384	2	2.042	Insignificant
Trichostrongylus spp.	1.826	2	2.085	Insignificant

Species richness of GI parasites

The result shown that greatest number of monkeys endured from triplet parasitic disease, either protozoan or helminth parasites appearing the most elevated prevalence rate of 28.57% while 18.57% were infected with more than five parasite species (fig 4). Most extreme tests with numerous diseases were due to overwhelming prevalence of both protozoan and helminth parasites.

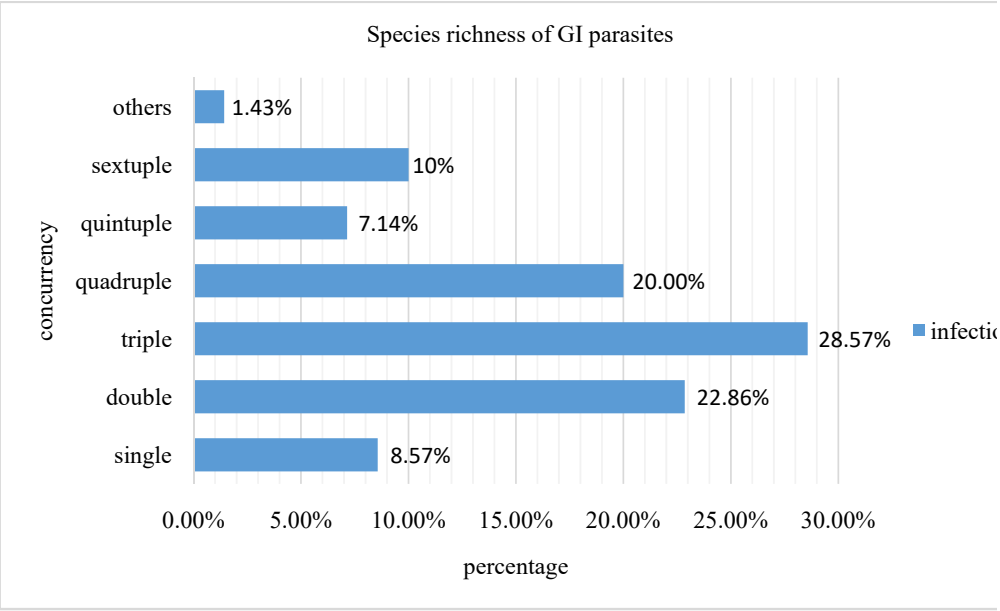

Fig 5. Species richness of GI parasites

Discussion

The current study showed prevalence rate of 100%. All the samples were positive for one or more than one GI parasites which was similar to the result from Bangladesh (100%) (48), higher than the findings from Nepal (61.9% – 86%) (2, 7), and India (40%–66.5%) (3, 42, 43). Meanwhile, the study conducted by Bikram Sapkota in 2020, also recorded 100% prevalence of gut parasites in urban macaques with higher prevalence of protozoan parasites (90.5%) than compared to that of helminth parasites (47.6%). The current study also showed the prevalence rate of protozoan higher (92.85%) than compared to that of helminths (81.43%), and hence the result coincided with that of, Sapkota *et al*., 2020. However, this result contrasted with the previous findings (7) that recorded higher prevalence rates in helminths.

Five protozoan genera and eight helminth genera of zoonotic importance were detected in the NHPs examined in this study. Among the protozoans, *Entamoeba coli* (67.14%) showed the highest prevalence followed by *Balantioides coli* (61.43%), *Entamoeba spp.* (42.85%), *Iodamoeba buetschlii* (7.14%) and *Coccidia spp.* (1.42%). All recorded protozoan show zoonotic transmission and are human pathogens. In common, protozoan contaminations are profoundly prevalent due to the capability of blisters to outlive indeed in unforgiving situations, and their one host-life cycle. These recorded protozoans can transmit specifically through the fecal-oral course by means of sullied nourishment or water sources.

With respect to protozoa, the prevalence of E.coli within the current study was 67.14% which was higher than the findings from Nepal (9.52% – 24.44%) (2, 7, 9), India (10% – 26.92%) (3, 43) and China (42%) (52). Although this species is typically asymptomatic in primates (10), its nearness ought to be taken as the sign of other pathogens interior the intestine (46).

The prevalence of Entamoeba spp. within the current study was 42.85% which was lower than the findings from China (89.96%) (39), from Nepal (13.97% – 66.7%) (2, 7, 9, 15) and higher than that recorded from India (10% – 23.07%) (3, 43). A few species of these pseudopodial amoebas like Entamoeba histolytica, E.nuttalli, E.dispar, E.moshkovskii, E.hartmanni, E.chattoni, and E.polecki have as of now been detailed from macaques all over the world; be that as it may, most of them are considered safe and don't exhibit pathologic ailment within the macaques (18, 39, 52).

Within the same way, Balantioides coli, a ciliate protozoan had a prevalence rate of 61.43% which was higher than the findings from Nepal (27.95% – 60%) (2, 7, 9, 15) and India (8.7% – 19%) (32, 42). *Balantidium sp.* is the only ciliated protozoan that commonly contaminates people and several animals. It can be pathogenic to both macaques and people by causing clinical side effects of Balantidiasis which incorporate the dysentery and diarrhea. Critically, it can compromise the development of newborn children which can cause a few wellbeing suggestions to macaque populaces.

Similarly, *Iodamoeba buetschlii* had a prevalence rate of 7.14% as reported in current study which was lower than the findings from Senegal (16.5%) (24), from Côte d'Ivoire (53.37%) (41)

It was eminent that, the rate of prevalence of Coccidian sp. was 1.42%, which was lower than the discoveries from Nepal (11.9%) (9), (41.1%) (7), India (26.92%) (3), China (10.94%) (22). This coccidian parasite causes profoundly deadly sorts of intestinal and extraintestinal pathologies and could be transmitted among people and primates zoonotically (22, 35).

Regarding helminth parasites, *Strongyloides* spp. (52.85%) showed the highest prevalence followed by *Strongyle* spp. (34.29%), *Trichuris* spp. (22.86%), *Ascarid* spp. (18.57%), Hookworm (10%), *Physaloptera* spp. (8.57%), *Nematodirus* spp. (2.85%) and *Trichostrongylus* spp. (1.43%). Nematode diseases posture high potential for zoonotic transmission with their coordinate and basic life cycles whereas the contaminations by cestodes and acanthocephalan are regularly uncommon and as it only causes diarrhea and stomach torment in primates (11, 25)

Helminth parasite named *Strongyloides* spp. was recorded the highest among macaque species inhabiting both religious and non-religious habitats with the prevalence rate of 52.85% that was lower than the findings from England (55.6%) (21), Nepal (10% – 34%) (2, 7, 9), India (26.66% – 57%) (32, 42, 43), and Thailand (2%) (20). Mellow to serious strongyleoidiosis caused by transitory hatchling as well as the grown-up of this zoonotically noteworthy parasite may lead to passings, particularly in youthful macaques (10, 19, 21) Strongyloidiasis in people is depicted as a potential zoonotic infection. Neurotic impacts of this disease in primates change from spewing, loose bowels, and serious drying out (25) whereas fibrosis of the intestine results in people.

Strongyleid nematode is one of the critical nematode groups representing diverse species. Its current prevalence rate, 34.29%, was higher than the discoveries from India (33%) (23) and lower than the findings from Nepal (9.5%) (9). *Strongyleids* are zoonotic and can cause genuine ailments coming about within the passings of macaques (10, 19, 21). It in the long run postures a risk to macaque populaces as this disease causes tissue harm, impeded development, iron deficiency, and aggravation. More critically, macaques in devout territories make human-macaque interfacing that aggravate the elements of hookworm disease and contaminated macaques can be a danger to closely collaboration people.

Another helminth parasites, *Ascarid* spp. was recorded with the prevalence rate of 18.57% which was lower than the findings from Bangladesh (90.90%) (48), Malaysia (49.7%) (30), India (25.5% – 26.66%) (42, 43), and Nepal (20% - 22.22%) (7, 9) and higher than the findings from Nepal (10.58% – 11.82%) (7, 15), India (5%) (23), Republic of Congo (2%) (17), and Thailand (1%) (20). *Ascarid* spp. could be a common nematode parasite in people and has been related with intestinal pathology, respiratory indications and lack of healthy sustenance in children from endemic regions.

Physaloptera spp. was recorded with a prevalence of 8.57% that was higher than the findings from China (1.79%) (35), Nepal (1.17%) (15), and China (0.8%) (26) and lower than the findings from Africa (57%) (17). Nine species of *Physalopterids* have been reported to occur in the upper gastrointestinal tract of non-human primates. It has been found within the esophagus, stomach and small intestine of the rhesus macaques, primates and orangutans. This parasite can be transmitted to humans, but total life-cycle isn't distinguished until presently. It has been detailed from people in Brazil, Colombia, Congo Republic (Zaire), India, Indonesia, Israel, Namibia, Panama, Zambia and Zimbabwe. In one case, in an Indonesian lady, adult worms were recuperated from the bile conduit, where they had caused biliary torment, jaundice and fever (25)

Trichuris spp. was reported with a prevalence rate of 22.86% that was higher than findings from Nepal (14.05% – 14.44%) (2, 7, 9), Thailand (19.6%) (20), (3.7% – 12%) (32, 42) and England (11.2%) (21), and lower than the reports from Nepal (23.65%) (7) and Bangladesh (50%) (48). High prevalence of *Trichuris* spp. was also observed in NHPs in Côte d'Ivoire (41), Malaysia (4), and China (26), signifying its global distribution among NHP population. Trichuriasis in macaques lead to intestinal clutter went with by rectal prolapse and may indeed actuate passing (19, 21). *Trichuris* spp. disease happens orally, and ova can survive for a longer period exterior the have, in serious natural conditions. Serious whipworm diseases can create clinical signs such as anorexia, diarrhea resulting sometimes in death.

While the prevalence rate of *Nematodirus* spp. was found to be 2.85% in present study but the study didn't support findings from other research as there was no traces of *Nematodirus* spp. found from NHP in Nepal rather it was reported from cattles like goats, sheeps, and elephants, rhino including ruminants as well. Since *Nematodirus* species are primarily known to infect ruminants,

it's not uncommon to find some parasites crossing over to different host species. Parasites can sometimes adapt and infect other animals, including monkeys, through mechanisms like 'host switch' or 'host range expression'. The presence of *Nematodirus* spp. in monkeys may not necessarily result in a full infection or disease. Sometimes, parasites can be present in a host without causing significant harm or clinical symptoms. Overall, the presence of this parasite in the study highlights the complexity of host-parasite interactions. Conducting further research would help in better understanding the specific mechanisms behind this phenomenon.

The current prevalence of *Trichostrongylus* spp. was found to be 1.42% among the helminth parasites which was supported by Hilser *et al*., (2011) from Red Langur. The result was lower than the findings from South Africa (71%) (40), Borneo (48.5%) (4) and Nepal (11.57%) (2). The significance of *Trichostrongylus* spp. in monkeys lies in its potential impact on their health and well-being. This parasite can cause damage to the intestinal lining and impair nutrient absorption, leading to nutritional deficiencies and monkeys can experience diarrhea, weight loss, anemia and overall poor condition (45)

Intensity of gastro intestinal infection was high in monkeys in the current study, 91.43% of the monkeys had more than one infection, whereas 28.57% of them had triple infections which was most common followed by 18.57% having more than 5 parasitic species. Hence, the findings make clear that monkeys are harbored with more than one parasite which conclude that the high rate of transmission may well be conceivable either due to high populace thickness or due to favorable natural conditions for parasites. Fecal samples showing 100% prevalence rate of both protozoan and helminth parasites indicate that monkeys are living in close proximity to each other, such as in social groups or in captivity. As this increased proximity increases the chance of parasites spread within the population, leading to a higher prevalence rate in fecal samples.

A few ponders to recommend that the ceaseless human unsettling influences and infringement, the capacity of a few parasites to contaminate different host species, and high level of hereditary homology between people and primates, increment the development and transmission of parasitic infections from primates to people and vice versa (27, 28, 29). It is important to note that the survival of gastrointestinal parasites in nature is influenced by factors such as environmental conditions, host availability, and interactions with other organisms. Additionally, the ability of parasites to adapt and exploit different ecological niches contributes to their survival. They can exploit various resources and host species, increasing their chances of finding suitable hosts and continuing their life cycle (12). Such intuitive give cause for genuine concern on primate preservation as well as on open wellbeing. In this way, it would be sound to consider these monkey's populaces as the reservoir hosts of several intestinal parasites of humans.

Conclusions

Present study concluded that the GI parasites are highly prevalent (100%) in both temple areas (Bajarayogini and Bajarabarahi) and SNNP. Among the GI parasites, protozoans (91.42%) were more prevalent than helminths (82.85%). Altogether 5 protozoan and 8 helminth parasites were identified. Besides, resulting diseases up to seven different species of parasites within the fecal tests may show that these pathogens may pose negative impacts on the intestine of macaques. Most of the parasitic species recognized in this study are zoonotically significant, and hence, the macaques may improve the hazard of transmitting the parasites to humans. The study highlights the significance of further research work with respect to intestine diseases of monkeys and affiliation with their survivability in order to conserve them. Thus, attention of concerned authorities must be drawn toward making the NHPs free from GI parasites and there's a require of improved mindfulness among nearby individuals and guests towards receiving clean safety measures and diminishing the human macaque interaction in order to control the zoonotic transmission of the pathogens.

Acknowledgements

The author expresses her most extreme appreciation to her Supervisor, Mrs. Babita Maharjan for her continuous support, guidance, and cooperation for completion of this research work. I am appreciative to Department of National Park and Wildlife Conservation (DNPWC) and Shivapuri Nagarjun National Park (SNNP) for giving consent to conduct the research inside the National Park. I express my sincere thanks to Department of Zoology of Amrit Campus, Kathmandu for providing lab facilities.

Ethical Approval

The authors proclaim that the study was conducted on actually contaminated Macaque species. No exploratory disease was set up amid this research work. The specified authorization for the collection of the fecal samples was issued by Department of National Park and Wildlife Conservation (DNPWC) (Permission number: 079/80, 633).

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