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Impact of prevalence of gastrointestinal parasites in rhesus macaque (*Macaca mulatta* Zimmermann, 1780) in Chitwan-Annapurna landscape, Nepal

Bishnu Prasad Bhattarai[1], Jagan Nath Adhikari[2*], Dina Nath Dhakal[3]
[1, 2] Central Department of Zoology, Institute of Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2, 3] Department of Zoology, Birendra Multiple Campus, Tribhuvan University, Chitwan, Nepal

Abstract
The study of prevalence of Gastrointestinal (GI) parasites in Rhesus Macaque conducted by hotspot area search method and collection of fresh fecal samples from Devghat and Ramnagar, Chitwan that lies in Chitwan-Annapurna landscape. The GI parasites are highly prevalent (80%) in Rhesus Macaque. Among those parasites, helminths (72.2%) were more prevalent than protozoans (27.78%). Altogether nine helminths and seven protozoan species of parasites were identified. The infectious rate was more in summer season (protozoan 60%, helminths 75.5%) than winter (protozoan 46.6%, helminths 68.8%). The study reported *Taenia* sp. (about 8%) from rhesus monkey in Ramnagar area where the monkeys are mainly depended on the garbage and food produced by the hotels, meat shop and other wastes from local households. These empirical findings are useful for primatologists, veterinarians, ecologists, and also applicable to public health issues where such monkeys and human coexist.

Keywords: rhesus macaque, helminths, parasitic prevalence, Chitwan, gastrointestinal parasites

1. Introduction
Primates are the highest order of mammals with respect to taxonomic hierarchy that includes lemurs, monkeys, apes, human and other similar forms, typically having dexterous hands and feet, binocular vision as well as developed brain [30]. A total of five species of monkeys have been reported from Nepal: Assamese Macaque (*Macaca assamensis* Hodgson, 1840), Rhesus Macaque (*Macaca mulatta* Zimmermann, 1780), Terai Grey Langur (*Semnopithecus hector* Pocock, 1928), Nepal Grey Langur (*Semnopithecus schistaceus* Hodgson, 1840), and Himalayan Grey Langur (*Semnopithecus ajax* Pocock, 1928) [25]. The Rhesus Macaque *(Macaca mulatta)* is one of the well-known species of Old-World monkeys. Rhesus Macaques are locally known as Rato Bandar, Rhesus Monkey in English [31]. They have pale face with pointed and protruding ears, not heavily pigmented, fur brown olive or brown and yellow brown, large area of naked skin in buttocks, no marked menstrual swelling but skin of buttocks becomes red during oestrus period [8, 17].

Rhesus Macaque is the most common monkey species that is native to northern India, Bangladesh, Pakistan, Nepal, Burma, Thailand, Afghanistan, Vietnam, Southern China and some neighboring areas [10]. Rhesus Monkey exhibits appreciable adaptability within its wide range of distribution. They occur from low-lying flat lands to the foot of the Himalayas up to 4000m [8]. They exist in temperate coniferous, moist and dry deciduous forest, mangrove, scrub, rain forest, cropland, human habitation, temples, mixed and bamboo forest [8, 25]. In Nepal, they occur in lowland tropical forest of Terai to the valleys across the higher elevations of Makalu-Barun, Langtang and coniferous, alpine forest of Rara area too and found in larger number in religious jungles and temples like Pashupati, Swayambhu, Sankhu-Bajrajogini areas of Kathmandu valley [6, 7]. The total population of Rhesus Macaques is estimated to consist of approximately 100,000 individuals [25]. They can tolerate much type of climatic and vegetative zones of South and East Asia from Afghanistan to Pakistan, India, Nepal, Bangladesh, Myanmar, Thailand, Laos and Vietnam [40].

Parasites play a major role in ecosystems [15], host population growth and regulation [20, 21] and community biodiversity [22]. Parasites, the uninvited guest which entertained in the host body and caused harm. Both helminths and protozoan parasites are common in nonhuman primates [32]. Some of the parasites are considered to be nonpathogenic. However, a large number can result in physiologic disturbances, nutritional loss, or may produce lesions that result in serious debilitation, and can create opportunistic for secondary infections that may be fatal [45]. In monkeys, disease emergence can occur directly through human introduced diseases or indirectly through human induced changes in the disease ecology of wildlife. This study aims to determine the distribution of intestinal parasites of Rhesus Macaque (*Macaca mulatta*). A few studies of Rhesus Macaque have been done on the ecological basis [36, 39, 47] but, the information regarding the gastro- intestinal parasites of Rhesus macaque is still lacking.

The Rhesus Macaques (*Macaca mulatta*) come into frequent contact with humans and due to their habit of residing in the religious and park land of human proximity, there is possibility of zoonotic and anthroponotic disease transmission between them. Parasites play a central role in ecosystem affecting the ecology and evolution of species interactions [15], host population growth and regulation [20, 21] and community biodiversity [22]. Parasites are integral part of the natural history of mammals and are always of interest. They form an important, though usually overlooked,

component of the biodiversity of ecosystem. Parasite affects almost each and every group of living organisms and it is not unusual for monkeys also because of their habit. Rhesus Macaques (*Macaca mulatta*) constitute an integral part of biodiversity and cognizable link between humans and nature. Our ancient epics and plays also portray the relation of humans and monkeys which still exists. They are exceptionally adapted to co-existing with humans and thrive near human settlements in both urban and agricultural areas [5]. The Rhesus Macaque, in the capital city Kathmandu, live in commensalisms close to human fostered by religious beliefs and kindness. In Kathmandu valley, about 1000 Rhesus Macaque are inhabitants of major temple areas like Pashupatinath, Swyombhunath, Tripureshwor (Ram Mandir) etc. [6, 7]. Monkeys of temple areas not only share the food but also the parasites of the human inhabitants. Their close phylogenetic relationship with the human results the high potential pathogen exchange [9]. So, Rhesus Macaque population of Devghat, unexplored source of information regarding the zoonotic diseases, may provide recent status of intestinal parasites, both of zoonotic and anthropologic importance.

As the rhesus monkey and human are very close regarding their physiologic and genetic characters, they also share infectious agents like intestinal parasites besides their food. Evidences show that many emerging parasitic diseases in human are originated from primates on one side and on the other side; there is a great risk of human pathogen transmission to free ranging primates [26, 41]. Biologists have pointed out their deteriorating health conditions due to adulterated food, polluted water and habitat encroachment, thereby increasing threat to the lives of the monkeys.

This study helps to determine the threats caused by gastrointestinal parasitic infections among the temple rhesus monkeys of Devghat and Ramnagar areas of Chitwan-Annapurna landscape. Therefore, this study provides baseline information for primatologists, veterinarians, ecologists, and others interested readers on their health conditions.

2. Materials and Methods

2.1 Study area

Devghat Dham is the most sacred as well as an important religious pilgrimage site of Nepal. Located almost in the middle of Nepal, this shrine, which is a confluence of Kaligandaki and Trishuli Rivers, is regarded important not only in religious but also in natural, historical, cultural, archaeological and touristic point of view.

Devghat and Ramnagar are located in Chitwan, Tanahun and Nawalparasi districts in the Centre part of Nepal. Its geographical location is 85°22'30" to 84°30'00" east longitude and 27°42'30" to 27°47'30" north latitude. The geographical identity of Devghat area is the meeting point of Trishuli and Kaligandaki River and also meeting point of Tanahun, Chitwan and Nawalparasi district. This region covers inner Terai and hilly areas from 200m to 575m above sea level and occupies 54.34 km^2 [11]. For Rhesus Macaque, sites A, B, C and D were selected.

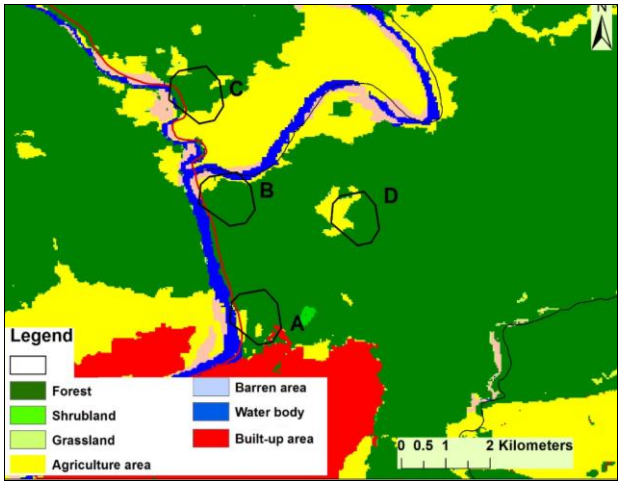


Fig 1: Location map of the study area (A- Bageshwari Area, B- Devghat Chitwan Area, C- Devghat Tanahun Area and D- Ramnagar Area).

2.3 Materials required

Apparatus

Vials	Camera	Gloves and masks
Weighing machine	Petri dish	Conical flasks
Test-tube stand	Glass- rod	Test tube
Wooden box	Tea strainer	Forceps
Beaker	Needle	Cover-slip
Slide	Centrifuge machine	Toothpick
Dropper	Pipette	Binocular- microscope

Chemicals

- Potassiu1m dichromate solution ($K_2Cr_2O_7$) (2.5%)
- Sodium chloride solution (NaCl)
- Lugol"s Iodine solution
- Methylene blue
- Distilled water

2.4 Field survey and data collection

Initially, on December 2016 preliminary survey was conducted to know the habitat, distribution and estimated troops (population) of Rhesus Macaque in Devghat area. We consulted with the Executive Director and other members of Devghat Area Development Committee and other local people about monkey. Eventually, Chitwan side of Devghat area was selected for the fecal sample collection.

Systematically troop followed method was applied for fresh fecal sample collection. Fecal samples were collected from January to May 2017 from monkeys during early morning (6:00am – 11:00am) and evening (3:00pm – 6:00pm). 90 fresh fecal samples were collected from 5 troops of Rhesus Macaque in village and temple area of Devghat. Before collection of the fresh fecal samples, color, consistency, worm, cestode segments, blood and mucus were carefully examined and noted. About 10 grams of fecal material was taken from the fecal mass with the help of wood spoon and placed in a vial containing 25ml Potassium dichromate solution ($K_2Cr_2O_7$) (2.5%). Finally, the sample was labeled according to species, serial number, location, date and time.

2.5 Microscopic examination of fecal samples

After collection and preservation, all fecal samples were examined at the laboratory of Department of Zoology, Birendra Multiple Campus. The fecal samples were microscopically examined for trophozoites, cysts, oocysts, eggs and larvae of gastrointestinal parasites by concentration method viz. floatation technique and sedimentation technique [42, 49].

Floatation technique

In the floatation technique, the fluid flotation medium i.e., saturated solution of Zinc Sulphate has higher specific gravity than parasitic forms. Higher specific gravity (SPG) of the floatation solution showed the greater variety of parasite eggs that would float.

All the helminth eggs and protozoan cysts float in such a solution except the eggs of *Ascaris lumbricoides*, *Taenia solium* and *Taenia saginata* and also the eggs of intestinal fluke larvae of *Strongyloides* do not float in salt solution [3].

It involved following processes:

- About 3 gm of fecal sample was taken.
- The sample was kept on the beaker and grinded with about 20 ml of water.
- Filtrate the fecal solution by tea strainer and poured into centrifuge tube up to 12 ml and centrifuge 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.
- The centrifuge tube was again filled with $ZnSO_4$ solution up to 12 ml and centrifuged at 1000 rpm for 5 minutes.
- The centrifuge tube was taken out and added more $ZnSO_4$ solution up to the tip of tube and a drop of methylene blue added upon it.
- A cover slip was placed over the top of the centrifuge tube so that the solution touched the cover slip and leaved for 5 minutes.

Then, cover slip was taken gently and placed on a microscopic slide and examined under 10X and 40X. Finally, photographs were captured.

Sedimentation technique

A sedimentation procedure is used to isolate eggs of flukes, acanthocephalans, some other

The eggs of tapeworms and nematodes are bit heavier than the others. For this technique, sediments of centrifuged contents were taken 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 examined the floatation.
- Remaining sediment content 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 second slide, added one drop of iodine solution for staining.
- The specimen was stained by Iodine solution and examined under 10X and 40X. Finally, photographs were captured.

In this way, two slides were prepared from one sample (one from floatation and one from sedimentation) were examined microscopically at 10X and 40X to detect eggs of helminths, trophozoites or cysts of protozoans and other gastro-intestinal parasites. The suspected samples were further stained with modified Zeihl Neelsen stain.

Staining Method

- Stool smear was prepared from floatation solution.
- Smear was dried in air and covered with Zeihl Neelsen stain.
- Smear was gently heat with flame.
- Smear was wash with1% H_2SO_4

Measurement of eggs, cysts and larva

By using ocular and stage micrometer, the length, breadth and diameter of parasites (eggs, cysts and larva) measured with calibration factor.

Identification of the eggs, cysts and larva

The identification of the eggs, cysts and larva were confirmed by comparing the structure, color and size of eggs, cysts and trophozoites of published literature, journals and books [16, 23, 42, 44].

2.6 Data analysis

For this study, prevalence was measured as the percentage of host individuals infected with a particular parasite [4, 29]. The collected data were encrypted and entered into Microsoft Excel spread sheet. Data were statistically analyzed using SPSS v. 20 [51] and Canoco software (CANOCO v 4.5) [50].

3. Results and Discussion

3.1 General prevalence rate of GI parasites

A total of 90 fecal samples were collected from the study area during two seasons, 45 samples were collected from winter and 45 from summer season. Out of these 90 samples, 72 fecal samples (80%) were found to be positive for one or more than one GI parasites. Hence, it revealed that there was a high prevalence rate of GI parasites in Rhesus Macaque. Same type of study was performed by Adhikari (2017) [2] found that 74.24% of samples were positive at Devghat area, the study about the intestinal parasites of Rhesus monkeys performed by Jha et.al (2011) [24] at Pashupatinath, found that 76.86% for all intestinal Parasites. This study showed more infectious rate of helminth parasites in temple monkeys at Devghat and Ramnagar area (Fig. 2).

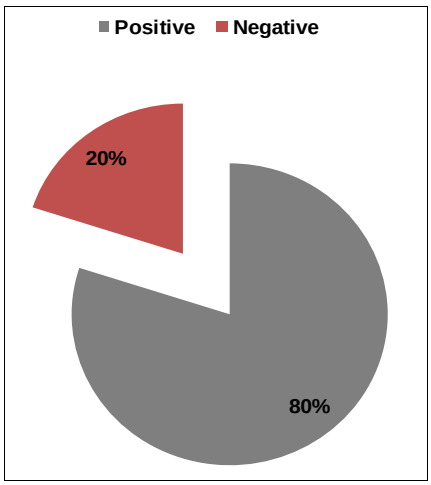


Fig 2: Overall general prevalence of GI parasite in monkeys

3.2 Infection status of GI parasites

Multiple gastro intestinal infection was more common in Rhesus at Devghat area. Out of 90 fecal samples, single,

double, triple and more than triple species of parasites were observed in 16, 20, 16 and 20 samples respectively. The infection status of GI parasites was not significantly difference (χ^2= 0.88, df=3 and P> 0.05). The seasonal variation in status of GI parasites was common in summer season than in winter season (Fig. 3). The study conducted by the Adhikari (2017) [2] was found that single, double, triple and multiple species of parasites were observed in 36.55%, 29.03%, 6.45% and 2.15% respectively. Similar that of study, Pokhrel (2014) [38] recorded 43.53%, 17.65%, 4.7% and 1.17% for Single, Double, Triple and Quadruple infection from Assamese Macaque. Dhoubhadel (2007) [14] observed 65.3%, 24.4%, and 7.08% for Single, Double and Multiple infections from Rhesus Macaque. But Nepal (2010) [34] documented 39.61% had a single infection and 60.39% were multiple infections from Rhesus Macaque due to categorized into only two status i.e., Single and multiple infections. From the above discussion, it was found that the multiple infections were common in Rhesus than in Assamese and Langur monkeys.

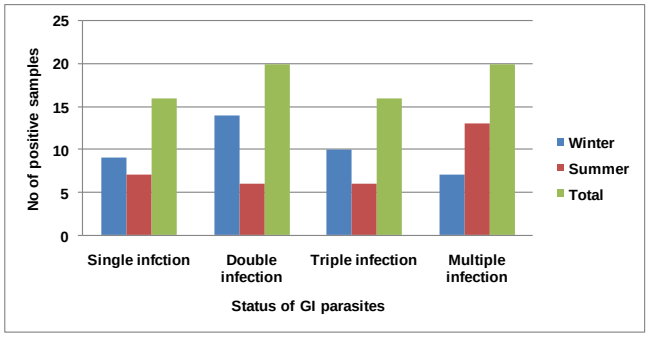


Fig 3: Ways of infection of GI parasites in winter and summer seasons.

3.3 Taxonomy-wise GI parasites infection

The common gastrointestinal parasites are protozoan and helminths parasites. Overall data shows that rhesus monkeys were more infected by helminths parasites than Protozoan parasites (Table 1). The infectious rate was more in summer season (protozoan 60%, helminths 75.5%) than winter (protozoan 46.6%, helminths 68.8%). Similar study in Assamese monkeys from Shivapuri Nagarjun National Park was reported high positive result in summer season (72.50%) than in winter (71.11%) [3, 38]. Rainy seasons and summer seasons were more prone for parasitic infection than in winter season due to more contamination of food and water and get chance to grow the parasites easy in hot days than in cold days [35].

Table 1: Overall prevalence of gastro-intestinal parasites (Positive cases) among monkeys in Devghat and Ramnagar area, CHAL.

Seasons	Sample size	Protozoa positive	Helminths positive
Winter	45	21 (46.6%)	31(68.8%)
Summer	45	27 (60%)	34 (75.5%)

3.4 Seasonal prevalence of specific Gastro-intestinal parasites

A total of 16 types of parasites were recorded in Rhesus monkeys of the study area, among them, 7 species were protozoan parasites and 9 species were helminths parasites (Table 2). Among the parasites reported, *Cryptosporidium* was found higher in both summer (44.44%) and winter (37.77%) seasons. Similarly, *Ascaris* was recorded more summer season than in winter season, with total prevalence rate of 22.22%. In case of helminths *Enterobius* sp. (34.44%) was high and *H. nana* (1.11%) was low in prevalence and reported only in the winter season (Table 2, Fig. 4).

Previous studies showed that there was no record of *Cryptosporidium* sp. in the Rhesus monkey of Devghat Area [2]; *Macaca assamensis* of Shivapuri Nagarjun National Park [38] and Rhesus monkeys of Pashupatinath, Kathmandu [24]. However, it is very much appealing that this study recorded the highest number of infections by *Cryptosporidium* in Rhesus monkeys (Table 2).

Ascaris sp. has been reported from *Macaca radiata*, *Cercopithecus aethiops sabaeus*, Colobus Monkeys and *Macaca mulatta* [19, 28, 33, 34, 46]. 34% prevalence rate of *Ascaris* sp. was reported from *Macaca radiata* [46] followed by 10.48% from *Macaca mulatta* [33] and 7.45% from the similar species [34]. The study conducted by Pokhrel (2014) [38] found that 10.58% *Ascaris* spp. was reported from *Macaca assamensis*. However, this study found 22.22% of the samples were infected by *Ascaris* spp.

Table 2: Seasonal prevalence of protozoan and helminths parasites.

Taxonomic Group	Parasites	Summer season		Winter season		Total infection	
		No of sample infected	%	No of sample infected	%	Total sample	%
1. Protozoan	*Giardia*	3	6.67	3	6.67	6	6.67
	Cyclospora	18	40	11	24.44	29	32.22
	Entamoeba	11	24.44	5	11.11	16	17.78
	E. coli	20	44.44	2	4.44	22	24.44
	Cryptosporidium	20	44.44	17	37.77	37	41.11
	Toxocara spp.	3	6.67	1	2.22	4	4.44
	I. beli	11	24.44	1	2.22	12	13.33
2. Helminthes	*Taenia* sp.	5	11.11	2	4.44	7	7.78
	Trichura trichuris	4	8.89	9	20	13	14.44
	Enterobius sp.	11	24.44	20	44.44	31	34.44
	Ascaris sp.	12	26.67	8	17.77	20	22.22
	Hook worm	3	6.67	3	6.67	6	6.67
	H. nana	0	0.00	1	2.22	1	1.11
	Paramphistomum	1	2.22	1	2.22	2	2.22
	Bunostomum	2	4.44	2	4.44	4	4.44
	Fasciola spp.	1	2.22	9	20	10	11.11

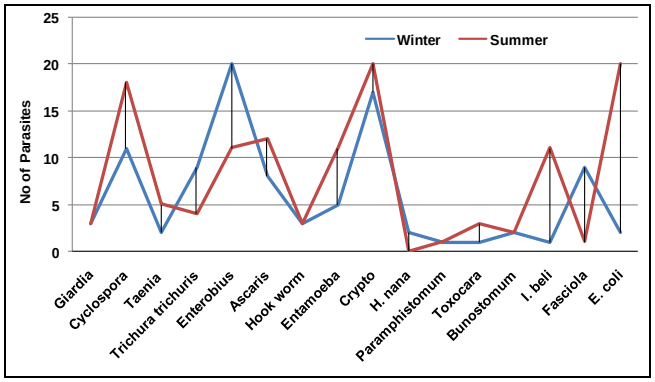


Fig 4: Seasonal occurrence of parasites.

The occurrence of *Trichuris* sp. has been observed among numerous primates including *Macaca sinica* and *Presbytis* spp., *Cercopithecus aethiops sabaeus, Papio cynocephalus* and *Papio anubis*, Guenons, Colobus Monkeys, OW monkeys, NW monkeys and apes, *Macaca mulatta*, *Macaca assamensis*, Cynomolgus and Rhesus monkey [13, 14, 18, 19, 27, 33]. Forty- six percent prevalence rate of *Trichuris* sp. was found in *Papio anubis* [33]. While in *Cercopithecus aethiops sabaeus* it was found to be 52.8% [33]. Similarly, in Golden langur and Goolock gibbon the prevalence rate of *Trichuris* sp. was found to be 33.33% [33]. In *Macaca mulatta* and old-world monkeys, new world monkeys and apes it was found to be 11.9% and 10% respectively [27,28]. Study conducted by Pokhrel (2014) [38] found that 9.41% of the total samples were infected by *Trichuris* in *Macaca assamensis*. Adhikari (2017) [2] reported that 20.54% of the samples were infected in Rhesus and 35% in Langur monkeys. This study found that only 14.44% of the samples were infected by *Trichuris* in Rhesus in and around the Devghat area. This difference in the prevalence rate could be attributed to the difference in climate, nature of food, distance between the village, waste management and topography.

In contrast, this study reported *Taenia* sp. (7.78%) from rhesus monkey in Devghat and Ramnagar area. The monkeys of Ramnagar and Bageshori area are mainly depended on the garbage and food produced by the hotels, meat shop and other households. The study conducted by Nepal (2010) [34] in Rhesus monkey (*Macaca mulatta*) from Swoyambhu area of Kathmandu Valley reported that 2 different types of cestodes, *Taenia* and *Dipylidium*, while Dewit *et al.*, (1991) [13] found one cestode, Hymenotepic. There was also a record of two species of cestodes from Japan Monkey Centre [48]. Gillespie *et al.*, (2005) [19] found one cestode (*Bertiella* sp.) from red colobus monkey of Uganda. Similarly, Malla (2007) [28] also reported only one cestode (i.e., *Taenia* sp.) from Rherus monkey of Pashupati Nath and Nilabarahi area of Kathmandu. Dhoubhadel (2007) [14] reported *Dipylidium* sp. for the first time from Rhesus monkey of Swoyambhu Nath, Kathmandu to be 7.08%.

3.5 Pattern of occurrence of Parasites

The numbers of intestinal parasites were found higher in summer season as compared to winter season. Canonical analysis showed distinct interrelationship among the parasites of the similar taxonomic groups (Fig. 5 and 6).

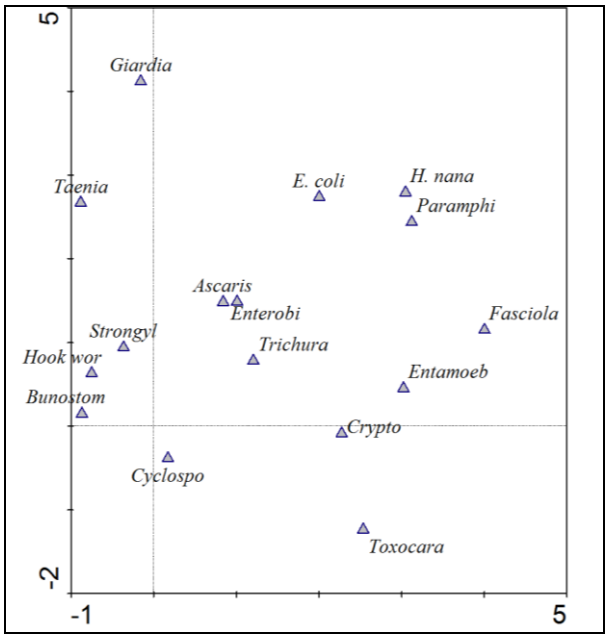


Fig 5: Simple Ordination plot through Canonical gradient analysis: Showing the pattern of presence and interrelationship among the parasites recorded in the collected stool samples of rhesus monkeys during winter season (CANOCO v 4.5) [50].

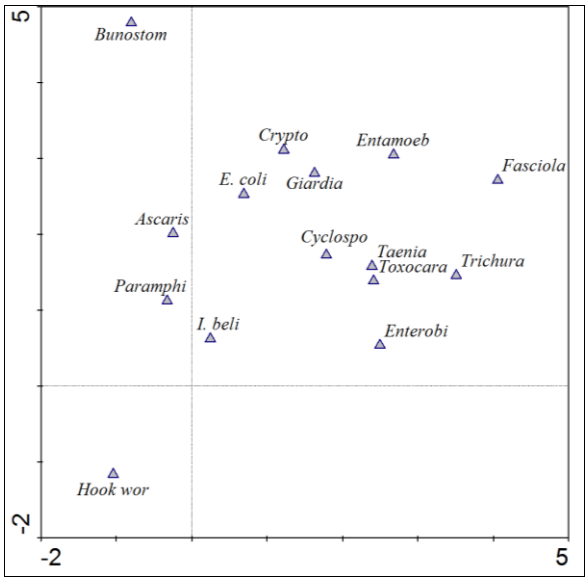


Fig 6: Simple Ordination plot through Canonical gradient analysis: Showing the pattern of presence and interrelationship among the parasites recorded in the collected stool samples of rhesus monkeys during summer season (CANOCO v 4.5) [50].

Fig 7: Some photographic representation of sample collection and processing (a) Visitors usually provides foods to the Rhesus monkey (b) Monkey, eating garbage (c) Rhesus: grooming (d) Fresh dropping of Rhesus.

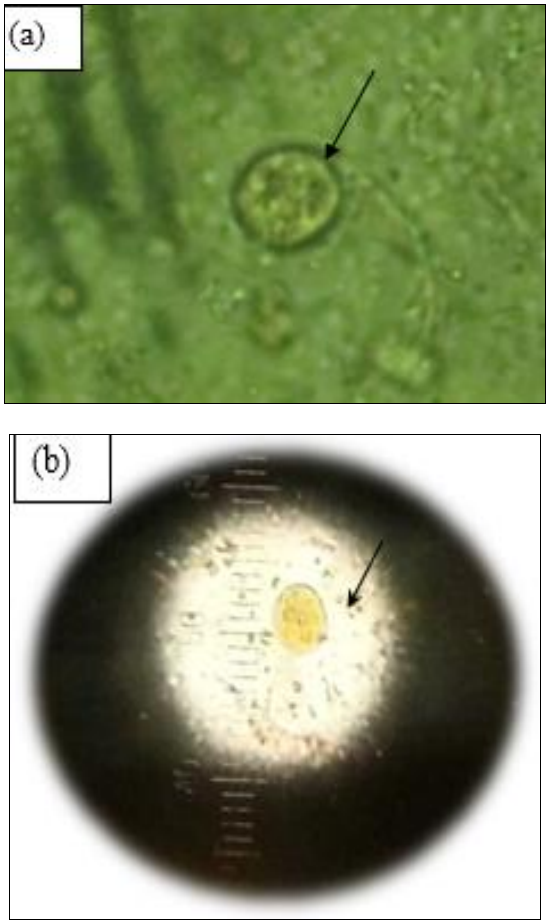
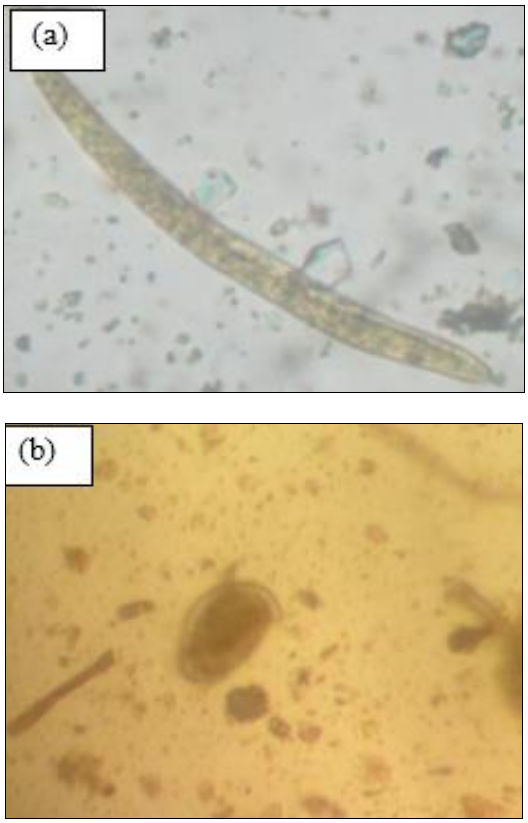


Fig 8: Protozoan Parasites recorded during stool examination of Rhesus monkey (a) Oocyst of *Cyclospora* (400X) (b) Cyst of *E. coli* (400X).



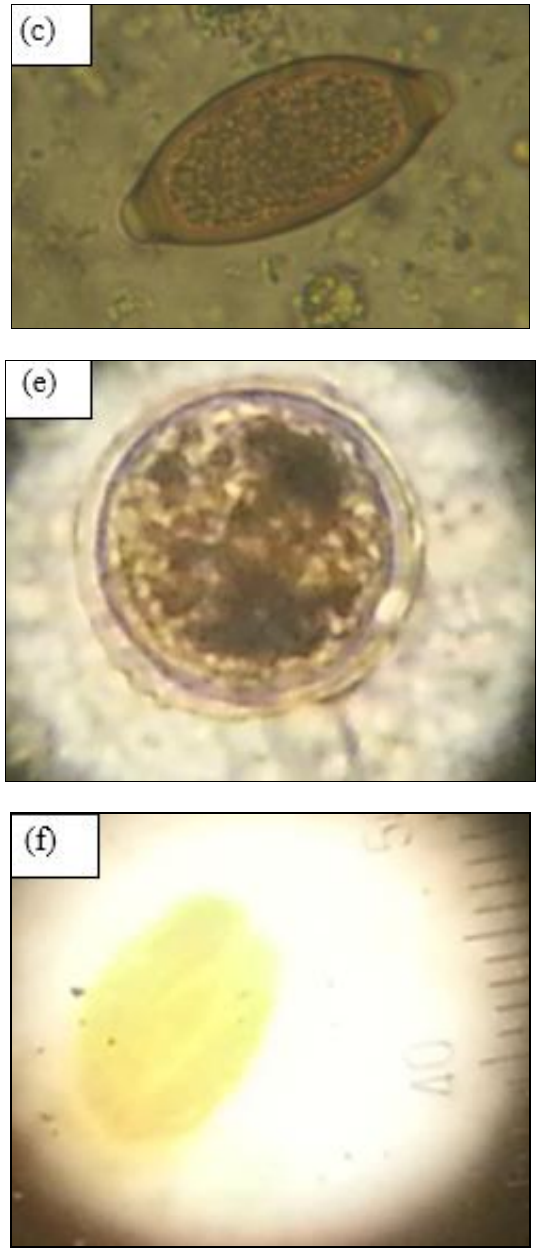


Fig 9: Helminth Parasites recorded during stool examination of Rhesus monkey at (400X) (a) Adult *Enterobius* sp., (b) Egg of *Enterobius* spp., (c) Egg of *Trituris* spp., (d) Larva of *Strongyloides* spp., (e) Egg of *Ascaris* spp., (f) Rhabditiform larva of *Ascaris* spp.

4. Conclusion

Present study concluded that the GI parasites are highly prevalent (80%) in Rhesus Macaque in Devghat and Ramnagar. Among the GI parasites, helminths (72.2%) were more prevalent than protozoans (27.78%). Altogether nine helminths and seven protozoan parasites were identified. The infectious rate was more in summer season (protozoan 60%, helminths 75.5%) than winter (protozoan 46.6%, helminths 68.8%). The study reported *Taenia* sp. (about 8%) from rhesus monkey in Ramnagar area where the monkeys are mainly depended on the garbage and food produced by the hotels, meat shop and other wastes from local households. Parasitic prevalence was found high in the monkeys near unmanaged dumping sites and temple areas as compared to natural areas. Better conservation of monkeys could be possible through developing Monkey Park in the study area.

5. Acknowledgements

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