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GASTRO-INTESTINAL PARASITES OF RED PANDA (*Ailurus fulgens fulgens* Cuvier, 1825) IN RARA NATIONAL PARK, MUGU, NEPAL

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ABSTRACT

A total of 22 faecal samples of Red panda were collected in the month of May/June 2011 and 21 faecal samples from the same site during the month of May/June 2012. All the samples were microscopically examined by faecal floatation and sedimentation techniques. Out of 43 faecal samples, 40 samples (93.02%) were found positive for gastro-intestinal parasites. Altogether, 12 different species of gastro-intestinal parasites were recovered from Red panda. Among them, two species of protozoan parasites and 10 species of helminthes were observed. In protozoan parasites, *Eimeria* (67.44%) was more prevalent as compared to *Entamoeba* (62.79%). Among helminthes, *Oxyuris* sp. showed the highest prevalence rate followed by *Toxoascaris* sp. (48.84%), Hookworm (44.19%), *Baylisascaris* sp., *Crenosoma* sp. (34.88%), *Strongyloides* sp., *Moniezia* sp. (18.60%), *Trichuris* sp., *etastrongylus* sp. (4.65%) and *Angiostrongylus* sp. (2.33%). Only one species of cestoda i.e. *Moniezia* sp. was found but trematodes and acanthocephalans were not found in Red Panda of Rara National Park.

Keywords: prevalence, *Eimeria*, *Entamoeba*, *Oxyuris*, *Angiostrongylus*, *Trichuris, Crenosoma*

INTRODUCTION

Red panda (*Ailurus fulgens fulgens* Cuvier, 1825) is one of the poorly known small-bodied mammalian carnivores which belong to the order, Carnivora and the family Ailuridae. It is the only living species of the genus *Ailurus* and family Ailuridae (Flynn *et al.,* 2000).

Globally, it is found in mountainous regions of India, China, Bhutan, Myanmar, Laos including Nepal (Glatston, 1994). It is currently distributed in Nepal, Bhutan, India, Myanmar and China, but was once widely distributed across Eurasia (Roberts & Gittlemans, 1984).

It occurs in coniferous, deciduous, and mixed forests with dense understory (Choudhary, 2001; Pradhan *et al.,* 2001; Roberts & Gittlemans, 1984; Wei *et al.,* 1999; Yonzon *et al.,* 1991). Although, it is a carnivore, it has adapted to an almost completely herbivorous diet. It feeds upon bamboo leaves throughout the year and bamboo shoots in the spring (Wei *et al.,* 1999).

Globally, it is estimated that there are fewer than 10,000 mature individuals, and populations continue to decline (Wang *et al.* 2008). It is categorized as vulnerable species (IUCN, 2015) and listed in Appendix I by CITES. In Nepal it is placed in the protected species by Government to conserve the species under National Parks and Wildlife Conservation Act (1973).

MATERIALS AND METHODS

Study area

Rara National Park is located at the north-western high mountains (81º 59'54" to 82º 08'27" E, 29º 26' 28" to 29º 33'11" N) of Nepal. It is the smallest national park with an area of approximately 106 km^2 including the lake Rara which is situated at an altitude of 2990 m. Most of the area of the park is located in Mugu District with a small portion in Jumla District of Karnali zone. Majority of the park landscape is dominated with conifers which is suitable habitat of about 51 species of different mammal species (DNPWC, 2012)

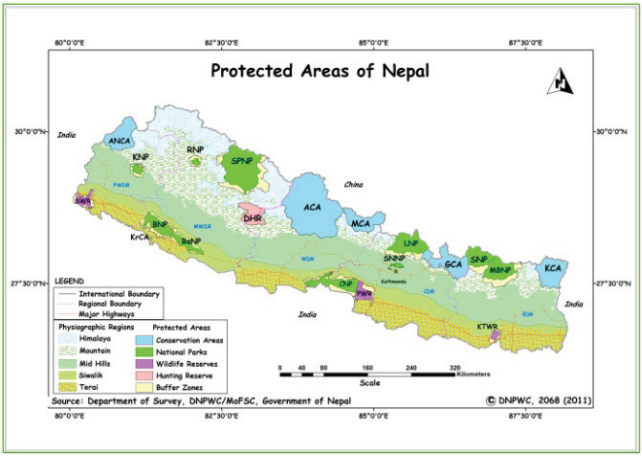


FIG. 1a. Map of Nepal showing the location of the Rara National Park.

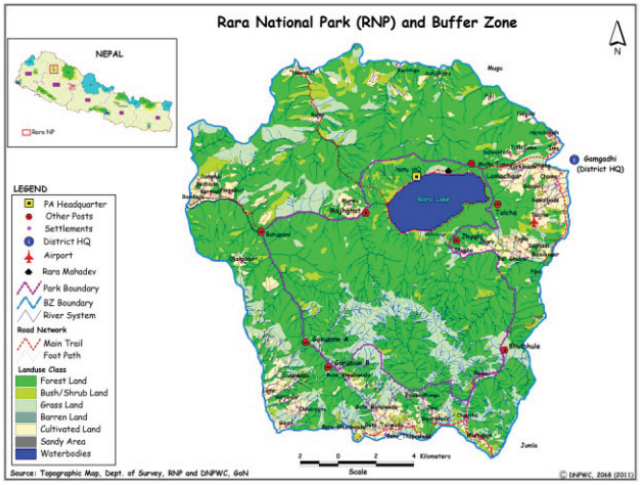


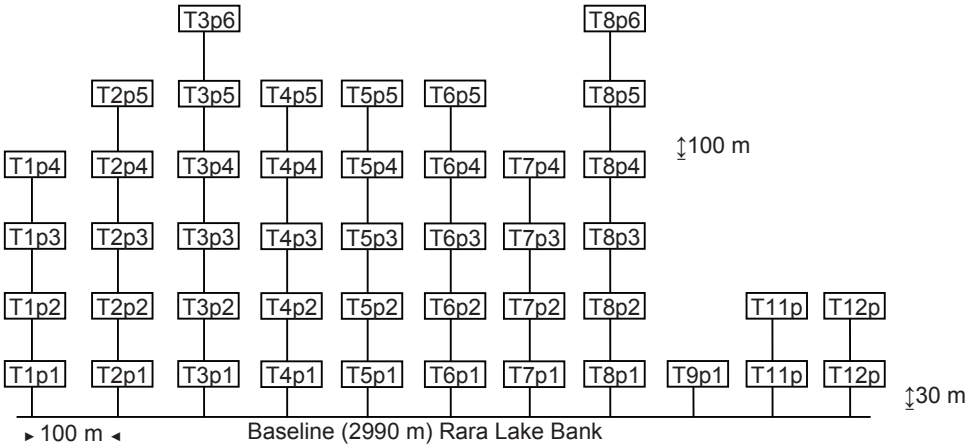
FIG. 1b. Map showing the study area Rara National Park.

Sample size and study design

A total of 22 faecal samples of Red Panda were collected from the east-west facing slope of mountain surrounding the Rara Lake during the month of May/June 2011 and 21 faecal samples from the same site during the month of May/June 2012. The present study was designed to assess the gastrointestinal parasitic infection in Red panda (*Ailurus fulgens*) of Rara National Park by collecting faecal pellets using line transect method and analyze those using standard methods of eggs and oocyst detection.

Sampling technique

The site was divided into 12 line transects. First transect was marked at western end of mountain. Sample collection method was designed as shown in flow chart below. Briefly 30m above the baseline (bank of Rara Lake) first plot was marked (T1P1). Each plot was of 10m ×10m in size searched thoroughly for faecal pellets. Second plot was 100 m above the first plot and marked (T1P2) and so on. Faecal pellets were collected in the plot as well as in transect. Fresh faecal pellets were collected in sample collection plastic bags. Necessary information were marked clearly, such as faecal pellet collection date, altitude, transect and plot etc. Faecal pellets were preserved in Zipper plastic bags, brought to the Central Department of Zoology and filled with 2.5% potassium dichromate for preserving both helminthic eggs and protozoan cysts.



Flow Chart showing sample collection design.

Examination of faecal samples

Microscopic examination of collected faecal samples was carried out for the demonstration of cysts, eggs and larvae. Two concentration techniques (floatation and sedimentation techniques) and Stoll's Counting Method were followed. The faecal pellets were examined under microscope at the laboratory of Central Department of Zoology, Tibhuvan University, Kirtipur, Kathmandu.

Red Panda in the field.

Faecal sample collection.

Faecal sample on Petridish.

Faecal sample preserved in 2.5% K2Cr2O7.

RESULTS AND DISCUSSION

General prevalence of gastrointestinal parasites in Red panda

The prevalence of gastrointestinal parasites in Red panda was observed to be 93.00% (40/43). (fig. 2)

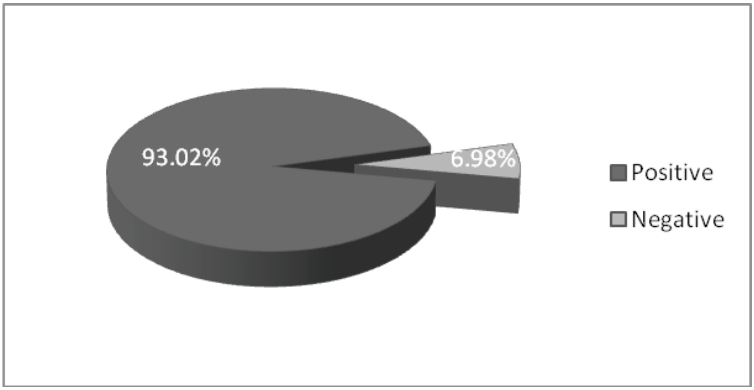


FIG. 2. Prevalence of gastrointestinal parasites in Red panda.

Class-wise prevalence of gastrointestinal parasites

Among 43 samples examined, 33 samples (76.74%), 37 samples (84.05%), and 8 samples (18.60%) were found to be positive for protozoan, nematode and cestode infections respectively. Nematodes showed the highest prevalence rate with 84.05% and cestode showed the least prevalence rate with 18.60% (fig. 3)

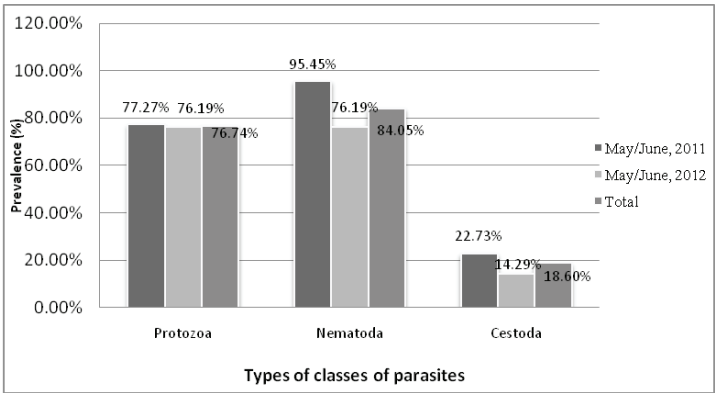


FIG. 3. Class-wise prevalence of gastrointestinal parasites in Red panda.

Prevalence of protozoan parasites in Red panda

Eimeria and *Entamoeba* species were identified by the examination of morphological shape, size and internal structure. Regarding coccidian parasites, two types of *Eimeria* were identified on the basis of presence or absence of micropyle. The higher prevalence rate was observed in the coccidian parasite, *Eimeria* as compared to *Entamoeba,* 67.44% against 62.79% (fig. 4)

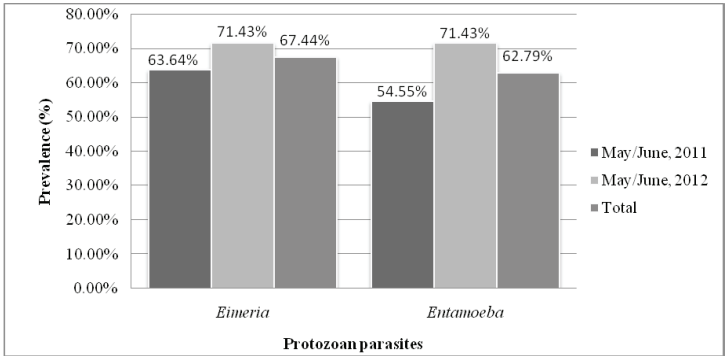


FIG. 4. Prevalence of protozoan parasites.

The size of the oocyst of *Eimeria* found in Red panda were greatly varied. The size of the oocyst ranges from 7.89µm-34.19µm in diameter and the size of the cyst of *Entamoeba* ranges from 21.04µm-28.93µm in diameter.

Photographs showing different protozoan parasites at 400X magnification.

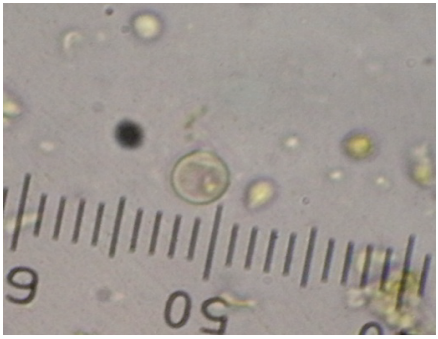


Plate 1. Oocyst of *Eimeria* with micropyle.

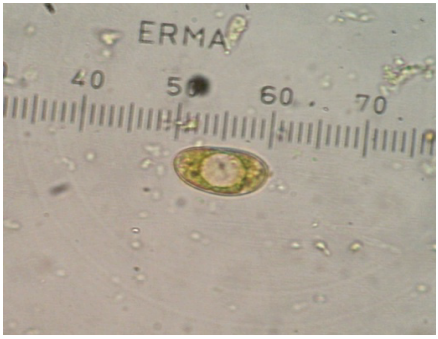


Plate 2. Oocyst of *Eimeria* without micropyle.

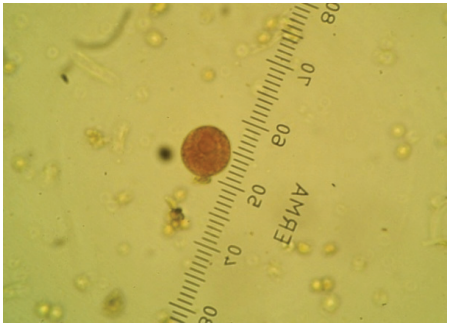


Plate 3. Cyst of *Entamoeba*.

Prevalence of helminth parasites in Red panda

Altogether 10 different types of helminthes were observed in Red panda, only one genera belonging to cestode and among 9 genera, 6 types of parasites were found to be intestinal worms (Hookworm, *Toxoascaris* sp., *Baylisascaris* sp., *Strongyloides* sp., *Trichuris* sp., *Oxyuris* sp.) and 3 genera belong to lungworm nematode (*Crenosoma* sp., *Angiostrongylus* sp., *Metastrongylus* sp.) but trematodes were not observed during the study.

Out of 43 samples, the most prevalent helminth parasite was *Oxyuris* sp. (25 i.e.58.14%) followed by *Toxoascaris* sp. (21 i.e. 48.84%), Hookworm (19 i.e. 44.19%), *Baylisascaris* sp. and *Crenosoma* sp. (15 i.e. 34.88%), *Strongyloides* sp. and *Moniezia* sp. (8 each i.e. 18.60%), *Trichuris* sp. and *Metastrongylus* sp. (2 each i.e. 4.65%), *Angiostrongylus* sp. (1 i.e. 4.65%) (table 1).

TABLE 1. Prevalence of helminth parasites in Red panda.

Types of parasites	Positive samples from 2011	Positive samples from 2012	Total
Hookworm	10 (45.45%)	9 (42.86%)	19 (44.19%)
Trichuris	2 (9.10%)	0 (0.00%)	2 (4.65%)
Strongyloides	6 (27.27%)	2 (9.52%)	8 (18.60%)
Baylisascaris	8 (36.36%)	7 (33.33%)	15 (34.88%)
Toxoascaris	12 (54.45%)	9 (42.86%)	21 (48.84%)
Moniezia	5 (22.73%)	3 (14.29%)	8 (18.60%)
Oxyuris	14 (63.64%)	11 (52.38%)	25 (58.14%)
Crenosoma	6 (27.27%)	9 (42.86%)	15 (34.88%)
Angiostrongylus	1 (4.55%)	0 (0.00%)	1 (2.33%)
Metastrongylus	1 (4.55%)	1 (4.76%)	2 (4.65%)

Characteristics of helminths eggs

Bean shaped and oval shaped eggs of *Oxyuris* sp. were observed ranging the size from 26.30µ – 71.01µm in length. The eggs of *Trichuris* were barrel shaped with transparent mucous plug at either pole containing unsegmented embryo with average length 55.23µm – 68.38µm. *Strongyloides* eggs were ellipsoidal, thin walled, embryonated with 44.71µm in size. Eggs of *Toxoascaris* were observed ranging the size from 21.04µm-52.60µm while *Baylisascaris* eggs are typical ascarid eggs with thick, finely pitted shells; they are slightly smaller than *Toxocara canis* eggs. The eggs are oval in shape, with average, approximated dimensions of 80×70µm. Eggs of *Baylisascaris* were observed ranging from the size from 23.67µ-39.45µm. Similarly, the eggs of *Moniezia* were triangular in shape containing a pyriform apparatus with the size ranging from 15.78µm-28.93µm in diameter.

Concurrency of gastrointestinal parasites in Red panda

In the present study, the multiple infections were observed in Red panda. Among 40 positive samples, 39 samples were found to have mixed infection with 2 to 9 species in each microscopic field. Among total of 43 samples, multiple and quintuple infections were found to be highest with 20.93% followed by quadruple (18.60%), triple (16.28%), double (13.95%) and single infections (2.33%) (table 2).

TABLE 2. Multiple infection of gastrointestinal parasites in Red panda.

Concurrency	Occurrency (2011)	Occurrency (2012)	Total
No Infection	0 (0.00%)	3 (13.64%)	3 (6.98%)
Single Infection	1 (4.55%)	0 (0.00%)	1 (2.33%)
Double Infection	5 (22.73%)	1 (4.76%)	6 (13.95%)
Triple Infection	3 (13.64%)	4 (19.05%)	7 (16.28%)
Quadruple Infection	4 (18.18%)	4 (19.05%)	8 (18.60%)
Quintuple Infection	5 (22.73%)	4 (19.05%)	9 (20.93%)
Multiple Infection	4 (18.18%)	5 (23.81%)	9 (20.93%)

Intensity of infection of gastrointestinal parasites in Red panda

Intensity of parasitic infection has been calculated based upon the number of eggs/oocyst and larvae found per field. Among protozoans, the high intensity of lightly infected cases was observed due to *Eimeria* sp. with 9 (20.93%) samples and mildly infected cases was due to *Eimeria* sp. with 14 (32.56%) samples while high intensity of moderately infected cases and heavily infected cases were due to *Entamoeba* sp. with 5 (11.63%) samples and 3 (6.98%) samples respectively. Among helminthes, the high intensity of lightly infected cases and mildly infected cases were observed due to *Oxyuris* sp. with 11 (25.58%) samples and high intensity of moderately infected cases was due to *Toxoascaris* sp. with 4 (9.30%) samples. Similarly, high intensity of heavily infected cases was due to the *Baylisascaris* spp. and *Trichuris* spp. with 2 (4.65%) samples followed by *Oxyuris* sp. with 1 (2.33%) sample (table 3).

TABLE 3. Overall intensity of infection of gastrointestinal parasites in Red panda.

SN	Class	Name of species	+	++	+++	++++
1	Protozoa	*Eimeria* spp.	9	14	4	2
2		*Entamoeba* spp.	7	12	5	3
3	Nematoda	Hookworm spp.	10	7	2	-
4		*Strongyloides* spp.	4	3	1	-
5		*Toxoascaris* spp.	7	10	4	-
6		*Baylisascaris* spp.	4	6	3	2
7		*Oxyuris* spp.	11	11	2	1
8		*Crenosoma* sp.	5	8	2	-
9		*Angiostrongylus* sp.	-	1	-	-
10		*Metastrongylus* sp.	-	2	-	-
11		*Trichuris* spp.	-	-	-	2
12	Cestoda	*Moniezia* spp.	3	3	2	-

+= less than 2 ova per field i.e. light infection

++= 2-4 ova per field i.e. mild infection

+++= 4-6 ova per field i.e. moderate infection

++++
= 6 or more ova per field i.e. heavy infection

Photographs showing different protozoan parasites at 400X magnification.

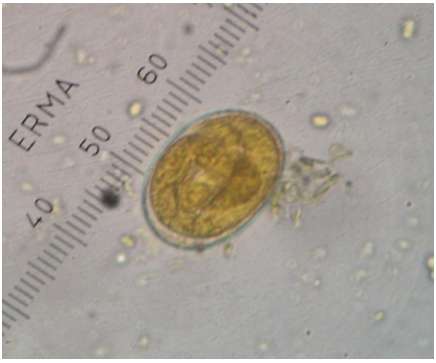


Plate 4. Egg of *Strongyloides* sp.

Plate 5. Egg of *Trichuris* sp.

Plate 6. Egg of *Oxyuris* sp.

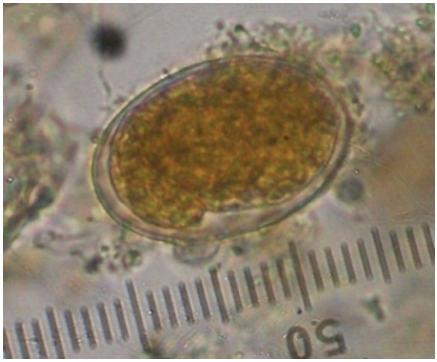


Plate 7. Egg of Hookworm.

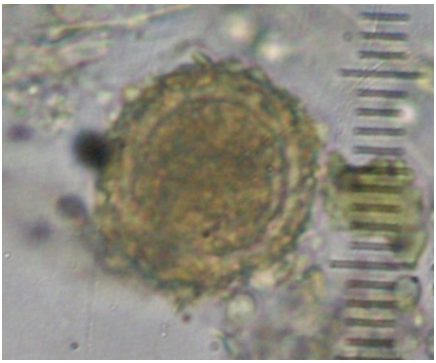


Plate 8. Egg of *Baylisascaris* sp.

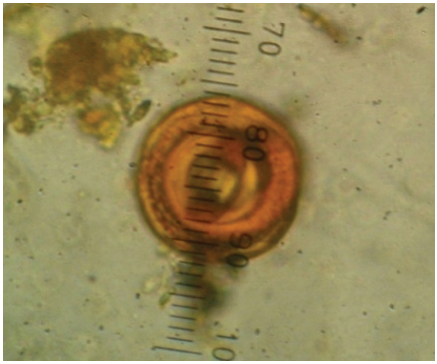


Plate 9. Egg of *Toxoascaris* sp.

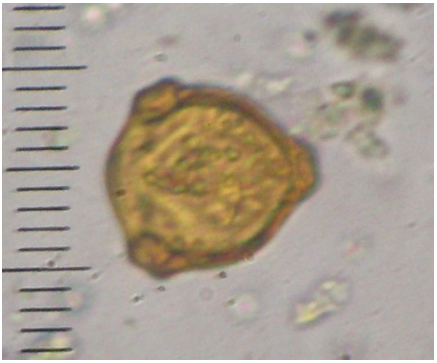


Plate 10. Egg of *Moniezia* sp.

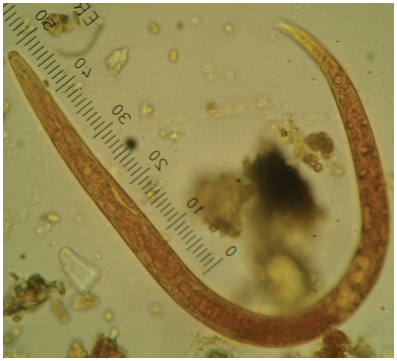


Plate 11. Larva of *Strongyloides* sp.

Plate 12. Larva of *Crenosoma* sp.

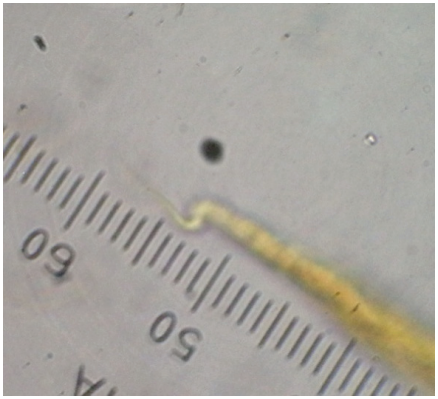


Plate 13. Larva of *Angiostrongylus* sp.

Plate 14. Larva of *Metastrongylus* sp.

No organism is free of parasites and parasites are cosmopolitan in distribution. Gastrointestinal parasites are found in human, wildlife and domestic animals. Wild animals harbour a wide range of parasites as definitive and reservoir hosts. This study attempts to document parasitic fauna of Red panda (*Ailurus fulgens fulgens*) in Rara National Park, Nepal. In the present study, altogether 12 different genera of parasites were reported from 40 out of 43 faecal samples of Red pandas. This high rate of gastrointestinal parasites may be due to different factors such as feeding behaviour, habitat, defecating openly on the ground and on trees, etc. Besides, parasites can also be easily transmitted through mechanical vectors like flies, rats, birds, lizards, cockroaches and beetles from one faecal matter to another. The pandas can also be infected by consuming the contaminated water and vegetation with infective stages of the parasites such as cysts, eggs, oocysts and larval forms. This may be the reason why 93.02% samples were found to be infected with at least one type of gastrointestinal parasites. Out of 43 samples, 33 samples (76.74%), 37 samples (84.05%), and 8 samples (18.60%) were found to be positive for protozoan, nematode and cestode infections respectively. In general, the parasites having direct life cycle have high prevalence rate e.g. coccidian and gastrointestinal nematodes while the parasites having indirect life cycle such as cestodes e.g. *Moniezia* sp. and trematodes have low prevalence rate. This is why nematodes were highly prevalent (84.05%) and cestode was least prevalent (18.60%). Both the protozoan parasites, *Eimeria* sp. and *Entamoeba* sp. have also high prevalence rate of 67.44% and 62.79% respectively. Thus, the prevalence rate of the coccidian parasite, *Eimeria* was found to be slightly higher as compared to *Entamoeba*. A recent study, from 23 faecal samples of Red panda in Nepal also revealed that the occurrence rate of protozoan parasites such as coccidians, *Cryptosporidium* and *Cyclospora* was 100% following nematodes 52.2%, unidentified species trematodes 13.0% and unidentified species of cestodes 4.3% (Lama *et al.,* 2015). This showed that *Eimeria* and *Entamoeba* were commonly found in Red panda and had not been reported in any previous studies. This is the first case to report the *Eimeria* and *Entamoeba* in Red panda. A study carried out in Padmaja Naidu Himalayan Zoological Park, Darjeeling, India showed infection by *Trichomonas* sp. as protozoan parasite (Pradhan *et al.,* 2011).

Altogether 10 different types of helminthes were reported in Red panda from Rara National Park. Helminthes included only one genus of cestode and 9 different types of nematodes among which 6 types of nematodes belonged to intestinal worms and 3 genera belonged to the lungworm nematodes. But trematodes and acanthocephalans were not observed during the study. However, the trematode *Ogmocotyle ailuri* was previously described from the Red panda, *Ailurus fulgens*, at a zoo in the United States (Price, 1954; Price, 1960). This trematode was also reported from the small intestine of Taiwanese monkeys, *Macaca cyclopis* (Yoshimura *et al.,* 1969) and in the small intestine of Japanese monkey, *Macaca fuscata*, captured in Sendai City, Miyagi Prefecture, Japan (Iwaki *et al.,* 2012). This shows that *Ogmocotyle ailuri* has a wide range of hosts. A study carried out in PNHZP, Darjeeling, India showed a trematode, *Schistosoma* sp. was reported in Red Panda (Pradhan *et al.,* 2011) and similarly an unidentified trematode species were also recently reported in Red panda in Nepal (Lama *et al.,* 2015). Only one genus, *Moniezia* was found in this study and the prevalence was found to be 18.60%. This is the first record of the genus *Moniezia* from Red panda in the global context. However, an unidentified cestode species were recently reported in Red panda in Nepal (Lama *et al.,* 2015).

In this study, the prevalence of *Oxyuris* sp. was found to be the highest (58.14%) among nematodes followed by *Toxoascaris* (48.84%), Hookworm (44.19%), *Baylisascaris* (34.88%), *Crenosoma* (34.88%), *Strongyloides* (18.60%), *Trichuris* (4.65%) *Metastrongylus* (4.65%) and *Angiostrongylus* (2.33%). Among these, *Oxyuris* spp., *Toxoascaris* sp., Hookworms, *Strongyloides* sp., and *Metastrongylus* sp. are reported for the first time in the global context. But *Crenosoma* spp. and *Angiostrongylus* spp. had been reported from Red Panda in the previous studies from different places (Grondahl *et al., 2005; Patterson-Kane et al., 2009;* Bertelsen *et al.,* 2010) and more recently *Angiostrongylus* spp. was reported in 2 of 23 faecal samples, *Trichuris* in 6 of 23 faecal samples and *Baylisascaris* in 3 of 23 samples in Red panda in Nepal (Lama *et al.,* 2015). *Baylisascaris spp.*, a parasite favouring humans, utilizes more than 100 species of birds and mammals as hosts. These nematodes are pathologically very important because they appear as ocular, visceral and neural larvae migrans, resulting in blindness, loss of muscle control, hapatomegaly and coma. *Trichuris* spp. was not common in the samples but the parasite has been reported from humans, pets, livestock and wild animals such as foxes and dogs (Traversa, 2011).

Among total of 43 samples, multiple infections and quintuple infections were found to be highest with 20.93% followed by quadruple (18.60%), triple (16.28%), double (13.95%) and single infection (2.33%). Single infection was observed only in one faecal sample while double infection in 6 samples. The intensity of infection of gastrointestinal parasites was studied in this study in which both protozoans were found to be heavily infected. Similarly, among helminthes, *Baylisascaris*, *Oxyuris* and *Trichuris* were found to be heavily infected. Light infection may be asymptomatic but heavy infections cause diseases.

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