I
INTRODUCTION

1.1 Background

Nepal is rich in biodiversity and natural resources due to its unique topography which ranges from lowlands with subtropical forests to arctic conditions in the Himalayan highlands. This makes Nepal one of the most biodiverse countries in the world, having 4.2% of all mammals, 8.5% of all birds and 2.2% of all flowering plants on Earth (Shrestha et al. 2001) within its small area of 147,181km^2 which is less than 1% of the world's total land mass.

Geographically, it is 80^{0}4” to 88^{0}12” East longitude and 26^{0}22” to 30^{0}27” North latitude. It is approximately 885km in length and its mean width is 193km. The majority of Nepal's land mass lies along the Himalayas and as a result, within a small latitudinal range of approximately 200 km, the country undergoes vast altitudinal changes from 60m along the southern border, up to 8848m, of Sagarmatha. So, it has almost all climatic zones found in Earth. As a result, Nepal has been gifted with various climatic and ecological zones providing a home for a large variety of fauna and flora.

Red panda *Ailurus fulgens fulgens* (Cuvier 1825) is one of the poorly known small-bodied mammalian carnivores which are adapted to the herbivore diet. Red Panda belongs to the order, Carnivora and the family, Ailuridae. According to the molecular phylogeny, Red Panda is relatively close to the American Racoon and may be either a monotypic family or a subfamily within the procynonid family (Mayr 1986, Zhang and Ryder 1993, Slattery and O’Brien 1995). They have no close living relatives, and their nearest fossil ancestors, *Parailurus*, lived 3-4 million years ago (Roberts and Gittleman 1984). On the basis of the fossil record, Red Panda diverged from its common ancestor with bears about 40 million years ago (Mayr 1986). The Red Panda is the only living species of the genus *Ailurus* and the family Ailuridae (Flynn et al. 2000).

1.2 Distribution, Habit and Habitat

Globally, The Red Panda *Ailurus fulgens fulgens* (Cuvier 1825) is found in mountainous regions of India, China, Bhutan, Myanmar, Laos including Nepal (Glatston 1994). It is an arboreal mammal and endemic species of south-eastern Himalayas in Nepal, India and southern China and has a range that extends from eastern Nepal through Bhutan, India, and Myanmar to southern Tibet and Western Yunnan Province of China (Chaudhary 2001, Wang et al. 2008, Wei et al.1999). Red Pandas are currently distributed in Nepal, Bhutan, India, Myanmar and China, but were once widely distributed across Eurasia (Roberts and Gittlemans 1984). The two subspecies of Red Panda, *Ailurus fulgens fulgens* and *A. f. styani*, are geographically separated by the Nujiang River: the former subspecies inhabits the bamboo-dominated temperate forests of Nepal, India, Bhutan, Myanmar and parts of China, while the latter occurs in south western China in Sichuan and Yunan provinces (Roberts and Gittleman 1984, Glatston 1994). The Western Red Panda has lighter pelage, especially in the face, while the Styans Red Panda has more dramatic facial markings.

In Nepal, it is distributed to a narrow altitudinal range of 1500 to 4000 m in the Himalaya and is reported in the eight protected areas namely Kanchanjunga Conservation Area, Manaslu Conservation Area, Makalu Barun National Park, Sagarmatha National Park, Langtang National Park, Annapurna Conservation Area, Dhorpatan Hunting Reserve and Rara National Park (Yonzon 1989, Jackson 1990, Yonzon et al. 1991, Yonzon and Hunter 1991a, Yonzon and Hunter 1991b, Karki 1999, Karki and Jendrzejewski 2000, Shrestha and Ale 2001). The Red Panda has also been reported from community-managed and national forest land in the villages of Jamuna and Mabu of Ilam in eastern Nepal (Williams 2004).

The Red Panda occur in coniferous, deciduous, and mixed coniferous and deciduous, forests with dense understory (Choudhary 2001, Pradhan et al. 2001, Roberts and Gittlemans 1984, Wei et al. 1999, Yonzon et al. 1991). It prefers *Abis-Thamnocalamus* fir-jhapra) forests from 2800 to 3900m (Yonzon 1989).

1.3 Characteristics and Conservation Status of Red Panda in Nepal

Red Panda is about 42 inches (79-120cm) long, including a long, bushy tail (30 to 60cm) with six alternating yellowish red transverse yellowish-brown rings. It is slightly larger than a domestic cat and their cubs are little bigger than domesticated kittens. Male weigh 4.5 to 6.2kg: female 3.7 to 4.5kg. It has long and soft reddish-brown fur on upper parts, blackish fur on lower parts, light face with tear markings, and a light-ringed tail. The light face has white badges like that of a raccoon, but each individual can have distinctive markings. Its round head has medium-sized upright ears, a black nose, and very dark eyes: almost pitch black.

The legs are black, short and bear-like with thick soles which serve as heat insulator on snow-covered or ice surfaces; and curved, sharp semi-retractable claws and, like the Giant Panda, has a 'false thumb' which is really an extension of the wrist bone, permitting them to seize fruit. Thick fur on the soles of the feet offers protection from cold and hides scent glands. Red Panda have wide teeth and powerful jaws that help them to chew tough bamboo leaves.

The red panda is territorial; it is solitary except during mating season. It has been reported to be both nocturnal and crepuscular, sleeping on tree branches or in tree hollows during the day and increasing its activity in the late afternoon and early evening hours. It sleeps stretched out on a branch with legs dangling when it is hot, and curled up with its tail over the face when it is cold (Roberts and Gittleman 1984). It is very heat sensitive with the most favourable temperature between 10^{0}C and 25° C., and cannot tolerate temperatures over 25 º C (77^{0}F) at all. As a result, Red Panda sleeps during the hot noontime in the shady topmost branches of trees, often lying stretched out on forked branches or rolled up in tree caves with its tail covering its face. It searches for food at night, running along the ground or through the trees with speed and agility and, after finding food, use its front paws to place the food into its mouth. Red Panda drinks by plunging its paws into the water and licking it.

Shortly after waking, red pandas clean their fur like a cat, licking their front paws and then rubbing their backs, stomachs and sides. They also rub their backs and bellies along the sides of trees or rocks. Then they patrol their territories, marking with urine and a weak musk-smelling secretion from their anal glands. They search for food running along the ground or through the trees. Red pandas may alternately use their forepaws to bring food to their mouths or place food directly into their mouths (Roberts and Gittleman 1984).

Red pandas are excellent climbers, and forage largely in trees. They eat mostly bamboo, and may eat small mammals, birds, eggs, flowers and berries. In captivity, they were observed to eat birds, flowers, maple and mulberry leaves, and bark and fruits of maple, beech and mulberry (Roberts and Gittleman 1984).

Red panda diet is largely vegetarian, and consists chiefly of young leaves and shoots of bamboo, yet also includes fruit, roots, succulent grasses, acorns, lichens, bird eggs, insects, and grubs (Choudhury 2001). It eats bamboo leaves throughout the year and bamboo shoots in the spring (Wei et al. 1999) which together can constitute more than 95% of their annual diet (Wei et al. 2000). Although, it is a carnivore, has adapted to an almost completely herbivorous diet and has digestive system more suitable to a carnivorous diet, yet, survive primarily on bamboo. In order to survive, it relies on young tender bamboo shoots and leaves. Like Giant Panda it cannot digest cellulose, hence it must consume a large amount of bamboo to survive. It poorly processes bamboo, especially the cellulose as microbes does not play a significant role in its digestion. The bamboo passes through the digestive tract of Red Panda very rapidly (~2–4 hours). In order to survive on this poor-quality diet, it has to select high-quality sections of the bamboo plant such as the tender leaves and shoots in large quantities (over 1.5 kg of fresh leaves and 4 kg of fresh shoots daily) (Wei et al. 1999). This food passes through the digestive tract fairly rapidly (about 2–4 hr) so as to maximize nutrient intake (Wei et al. 1999).

The biodiversity of Nepal is of very important in the world due to its richness in fauna and flora due to diverse topography to a wide altitudinal range and climatic zones. Globally, about 5000 species of mammals are found. Among them, 208 different species have been recorded from Nepal, which makes about 5% of the global population of mammals (Baral and Shah 2008). Geographically these mammals are distributed from the range of 63m in Terai to 5500m in the mountainous region. Such a huge mammalian diversity is due to the diverse land topography, climatic variation and floral diversity. Mammals of Nepal are included in 12 orders and 33 families, among which order Carnivora includes 8 families viz: Canidae, Ursidae, Procynidae/Ailuridae, Mustelidae, Viveridae, Herpestidae, Hyaenidae, and Felidae.

It is estimated that the number of Red Pandas in Nepal is 314 on the basis of the ecological density of one panda per 2.9 sq.km (Yonzon et al. 1997, Chaudhary 2001) and globally it is estimated that there are fewer than 10,000 mature individuals, and populations continue to decline (Wang et al. 2008). It is listed by The World Conservation Union (IUCN) in its vulnerable category "VU" (a species facing a high risk of extinction in the wild) (Wang et al. 2008) and by Convention on International Trade in

Endangered Species of Wild Fauna and Flora (CITES) in its Appendix I (species threatened with extinction which are or may be affected by trade). In Nepal, *Ailurus fulgens fulgens* is protected by the National Parks and Wildlife Conservation Act (1973). It is a threatened (IUCN 2012) and endangered carnivore mammal species and occurs in the Himalayan mountain range (Roberts and Gittlemans 1984).

The Department of National Park and Wildlife Conservation (DNPWC) presently works with network 10 national parks, 3 wildlife reserves, one hunting reserve and 6 conservation areas and 11 buffer zones covering an area of 34,186.62 sq. km, that is, 23.23 percent of the total area of the country.

1.4 Parasitic infection of Red Pandas

Parasites are defined as an animal or plant which lives in or upon another organism and draws its nutrient directly from it (Parija 2000). Parasites are said to be metabolically dependent on their hosts, and it is the dependency that makes the relationship an obligatory one (Cheng 2010). According to this definition parasites includes the bacteria, viruses, fungi as well as protozoa and helminthes but protozoa and helminthes are only studied in this research. They are broadly classified into ectoparasites which live on the outer surface or in the superficial tissues of the host and endoparasites which lives within the host such as the gut, body cavity, liver, lungs, gall bladder and blood or within the internal cavities, tissues or cells of the host. Endoparasites which live in alimentary canal of the host cause the damage in the host are called intestinal parasites. The major parasitic infection includes protozoa, nematodes, trematodes and cestodes.

1.4.1 Intestinal protozoan parasites

The protozoan parasites are microscopic, unicellular organism which have complex internal structure and perform various complex metabolic activities such as digestion, reproduction, respiration, excretion, etc. Some protozoan parasites found in the intestine of carnivore include *Eimeria* and *Sarcocystis* (Adams et al. 1981); *Cryptosporidium* (Synder 1988).

1.4.2 Intestinal helminth parasites

The helminthes are multicellular, bilaterally symmetrical, elongated, and flat or round bodied organisms. They are broadly classified into: (1) nematodes or roundworms which include hookworms, pinworms, whipworms, threadworms and roundworms (2) trematodes or flukes (3) cestodes or tapeworms. Parasites are assigned to these categories according to their morphology, or structure.

Nematodes or roundworms are elongated, cylindrical and tapered at both ends. Adults of this class range from 5 millimetres to more than 50 centimetres in length. They have a complete digestive tract and tough, elastic, skin-like cuticle. The mouth area may be specialized for attaching to or feeding on the host. For example, the large strongyles (*Strongylus* species) have mouth capsules with teeth to perform such functions. Males of certain of species of nematodes attach to females for mating by using a structure called a bursa. This is a posterior expansion of the cuticle or skin, which is bell-shaped or funnel-shaped and supported by finger-like projection called rays. Mating is also assisted by structures called spicules, used by the male to hold and open the genital orifice of the female. The shape and arrangement of the male bursa and spicules vary from species to species and are frequently used to identify different nematodes.

Helminthes are the most important endoparasites which mostly cause the infections in the intestine and also other various organs such as liver, lungs, blood, lymphatic system and skin. *Dirofilaria immitis* is usually found in right ventricle of heart, pulmonary artery, venacava and occasionally in lungs when the infestation is heavy. The disease has been reported from a wide range of wild carnivores, namely Golden Cat, Jackal, Fox, Wild dog (*Cuon alpinus*) and Wolf (*Canis lupus*) at Nandankanan Zoo (Rao and Acharjyo 1993); Asiatic Lion (*Panthera leo persica*) at Guwahati Zoo (Nashiruddullah and Chakraborty 2001); and Lioness (*Panthera leo*) at Ranchi Zoo (Haque 1998). Hookworm infection has also been recorded from different species of wild carnivores from India like Wild Dog, Wolf, Jackal, Fox, Jungle Cat, Civet Cat, Fishing Cat, Sloth Bear. The various hookworm species reported from small intestine of different species of wild carnivores from India are *Ancylostoma caninum*, *A. brazilliense*, *A. ceylanicum*, *A. duodenale*, *A. paraduodenale*, *Anthrocephalus gambiensis*, *A. herpestis*, *Uncinaria felidis*, *U. Stenocephala* (Chowdhury 2001).

Flukes or trematodes are characteristically flat, unsegmented worms. Suckers are located at the front and back of the fluke and are used as organs of attachment to the host. The most common species encountered in Indian wild carnivores is *Paragonimus westermanii*

(Acharjyo 2004). *Heterobilharzia americana* was found in raccoons (*Procyon lotor*) of north central Texas (Kelley 2010).

Tapeworms, or cestodes are flat, ribbon-like organisms that live most often in the small intestine of their host. The head, or scolex, of the tapeworm has suckers, hooks or a combination of suckers, and hooks used to attach the worm to the wall of the intestine. Proglottids (tapeworm segments) are generated from the scolex. In some species, the strobila or body of the worm may become several metres long. Each mature proglottid is a complete functional unit, incorporating a digestive system, organs of both sexes, and other organs. This phenomenon of both sexes in one body is known as hermaphroditism. Cestodes absorb nourishment directly through their tegument from the gut contents of the host animal.

1.5 Statement of the problem

As Red Panda populations tend to live in isolated pockets, the risk of inbreeding and local extinction is high. "Although the Red Panda is classified as a carnivore, it has adapted to an almost completely herbivorous diet. In order to survive, the Red Panda relies on young tender bamboo shoots and leaves; a viable Red Panda population, therefore, is an indicator of a healthy forest." The Red Panda is classified as an endangered species. There is an estimated population of less than 10,000 mature individuals worldwide (Wang et al. 2008). This is likely to decline even more in the coming years. No animals are free of parasites. Parasites of large number of animals are being studied but study of parasites of Red Panda has found least.

1.6 Justification of the study

No organism is free of parasites and parasites are cosmopolitan in distribution. Most of the researches regarding the GI parasites are focused among human and domestic animals while the researches in wildlife are neglected in Nepal. This study attempts to document parasitic fauna of Red Panda (*Ailurus fulgens fulgens*) in Rara National Park, Nepal. Very little study has been done about the intestinal parasite of animals like Red Panda which have biological or ecological value. Red Pandas are endangered animals whose number is declining in the world due to the habitat loss and parasitic infections, so, they should be protected. They are not known to transmit diseases but may act as reservoir host for many zoonotic pathogens including parasites that pose a health risk to humans. Also, they frequently come in contact with humans and transmission of parasites may cause. The threat caused by the intestinal parasites to Red Panda and transmitting such parasite to human are least studied so still many more such studies have to be conducted. So from above reasons, it is necessary to target *Ailurus fulgens fulgens* for research work. The study of parasites of wildlife is ignored or least studied. But study is focussed more on the parasites of domestic animals.

1.7 Objectives of the study

1.7.1 General objectives

To find out the distribution of gastro-intestinal parasites of Red Panda (*Ailurus fulgens fulgens*) from Rara National Park.

1.7.2 Specific objectives

i. To determine the prevalence of gastro-intestinal parasites of Red Panda
ii. To determine the concurrency and intensity of gastro-intestinal parasites of Red Panda

II

LITERATURE REVIEW

2.1 Scenario of gastrointestinal parasites in world context

Scenario in Zoo and Captivity

The metastrongyloid nematode *Angiostrongylus vasorum* has been reported in Red Pandas (*Ailurus fulgens fulgens*) from European zoos (Jensen et al. 2005, Grondahl et al. 2005). In the similar study, this species was also reported in a 9 year old male, captive Red Panda from Bristol Zoo Gardens (Patterson-Kane et al. 2009). Similarly, in a coprological survey of 115 Red Pandas from 54 zoos, 3 of 115 Red Pandas (2.6%) were found to be infected with *A. vasorum*, 5 of 115 with *Crenosoma* (4.3%) and 32 of 115 Red Pandas with previously unidentified metastrongyloid species, similar to, but morphologically distinct from *A. vasorum* as the most prevalent (27.8%) (Bertelsen et al. 2010). But Willesen et al. (2012) examined four naturally infected captive-breed Red Pandas in Aalborg Zoo, Denmark infected with a newly discovered metastrongyloid nematode and compared with two uninfected control Red Pandas housed in Copenhagen Zoo, Denmark.

A study carried out in Padmaja Naidu Himalayan Zoological Park (PNHZP), Darjeeling, showed infection of protozoan *Trichomonas* sp., trematode *Schistosoma* sp. and nematode *Ascaris* sp. in Red Pandas. Out of 115 red panda, only seven animals tested positive for the parasite out of which five animals (33%) were interestingly old animals above the age of 11 years (Pradhan et al. 2011). Another trematode *Ogmocotyle ailuri* was previously reported from Red Panda (*A. fulgens*) at National Zoological Park in the United States (Price 1954).

Scenario in different regions of the world

Other cases of *Angiostrongylus* sp. and Crenosomatidae had been reported from 52 Red Pandas from the National Zoos (Montali et al. 1984). Chowdhary (2001) reported *Toxocara transfuga* and hookworm species from red pandas from Indian Subcontinent. Similarly Neiffer et al. (2002) confirmed heartworm *Dirofilaria* sp. infection, presumably with *D. immitis* in Red Panda (*A. fulgens fulgens*) from North America and 10 of 48 red pandas in the Chengdu Research Base of Giant Panda Breeding, Sichuan province, China (Lan et al. 2012). *Toxoplasma gondii* has also been reported in red panda (*A. fulgens fulgens*) (Sikarskie et al. 1991, Juan-Salles et al. 1997).

Baylisascaris spp., are widely distributed in the giant panda *Ailuropoda melanoleuca*), red panda (*Ailurus fulgens*), raccoon (*Procyon lotor*), Ursid species (*Ursus maritimus, Ursus arctos pruinosus, Selenartos thibetanus mupinensis* and *Ursus arctos lasiotus*) and other mammals including humans and can lead to severe clinical visceral (VLM), ocular (OLM) and neural larva (NLM) migrans in these definitive or intermediate hosts (Sato et al. 2004, Zhang et al. 2011, Xie et al. 2011, Xie et al. 2013). Among them, *B. schroederi* is the most common parasite in wild and captive giant pandas and cause severe baylisascariasis in both wild and captive giant pandas (Yang 1998, Zhang 2008, He 2009, Xie et al. 2011, Yang and Zhang 2013). In the same study, Xie et al. (2011) reported *B. ailuri* in Red Panda. Similarly, *B. procyonis* was also found in raccoon (Stefanski and Zarnowski 1951, Sprent 1968, Kidder et al. 1989, Kerr et al. 1997, Valle 1999, Kazacos 2001) and in human (Huff et al. 1984, Park et al. 2000).

Zhang et al. (2011) studied parasite fauna load in wild giant panda (*Ailuropoda melanoleuca*) across six mountain ranges in China. They found 57.9% overall prevalence of helminth infections including 5 species namely *B. schroederi*, *Ogmocotyle sikae*, *Toxoascaris seleactis*, *Ancylostoma ailuropodae* and *Strongyloides* sp. but the prevalence of these helminths was not homogenous and the prevalence of single parasite *B. schroederi* infection was 54.0%. In another report, Zhang et al (2012) showed 54% prevalence of *B. shroederi* in 91 faecal samples examined and 48% in the faecal samples of 31 identified individual wild giant pandas in China. Zhang and Wei (2006) and Hu (2001) reported *B. schroederi* in the intestine of giant pandas in China. Zhou et al. (2013) investigated the infection of *B.* s*chroederi* in 88% of faecal samples which was 30% higher than the conventional floatation technique in Giant panda (*Ailuropoda melanoleuca*) in China using PCR.

Xie et al (2011) investigated *B. procyonis* in raccoons using PCR in China. In Canada, the prevalence of *B. procyonis* was estimated in 37.1% of the urban raccoon population of Winnipeg (Manitoba) as assessed through an analysis of fresh raccoon faeces deposited at latrine sites and in 53.5% of the urban raccoon population of the Winnipeg through post-mortem examination of nuisance raccoons (Sexsmith et al. 2009). Another study carried out in Portland showed that the prevalence of *B. procyonis* in raccoons was 58% (Yeitz et al. 2009).

Hernandez et al. (2012) reported *Baylisascaris procyonis* in 9 of 74 (i.e. 12%) raccoons (*Procyon lotor*) from North Carolina but previously *B. procyonis* were reported in eastern Tennessee (Souza et al. 2009). The higher prevalence rates of *B. procyonis* in raccoons have been reported in the north eastern, Midwestern, mid-Atlantic, some western states (California, Washington, Oregon and Colorado) and some regions of Texas (Kazacos 2001, Long et al. 2006, Chavez et al. 2012). The prevalence of *B. procyonis* infection is high in wild raccoons in Germany and those kept in zoos or as pets in Japan (Baeur and Gey 2002, Kazacos 2001, Miyashita 1993, Sato et al. 2001). In areas where *B. procyonis* is common in raccoons, it has much higher prevalence in juvenile raccoons (> 90%) than in adults (37%–55%). Average parasite intensity ranges from 43 to 52 worms, with juvenile raccoons having a higher mean intensity (48–62, range 1–480) than adult raccoons (12–22, range1–257) (Snyder and Fitzgerald 1985, Ermer and Fodge 1986, Kazacos 2001). In United States *B. procyonis* roundworms are most prevalent in the Midwestern, northeastern, and Pacific western states. Numerous surveillance studies have been conducted in the southeastern United States, and parasite are most common in the mountainous regions of Virginia, Kentucky, and West Virginia (Kazacos 2001, Souza et al. 2009).

A study carried out in Illinois showed that *Eimeria nuttalli* oocysts were found in 58% (21/36) and *E. procyonis* oocysts in 25% (9/36) of raccoons (*Procyon lotor*) and sporocysts of *Sarcocystis* sp. in 17% (2/12) of other raccoons in Illinois (Adams et al. 1981). Similarly, Dubey (1982) surveyed 28 raccoons from Columbus, Ohio for intestinal parasites. He found *Baylisascaris procyonis* was found in 7 of 28 raccoons, trichurid eggs in 2 of 28 raccoons, capillarid eggs in 8 of 28 raccoons, trichostrongyloid eggs in 9 of 28 raccoons, and *Eimeria procyonis* oocysts in 23 of 28 raccoons. In the similar study, *Eimeria procyonis*-like oocysts were reported in 15 of 15 captive raccoons and endogenous coccidian stages (large and small schizonts) in 6 of 6 juvenile wild-caught raccoons (Dubey et al. 2000). Foster et al. (2004) reported *Eimeria procyonis* (84%), *Eimeria nuttalli* (10%), 1 unidentified species of *Eimeria* (3%) and an unidentified species of *Sarcosystis* (3%) from adult raccoons (*Procyon lotor*) in U.S.A.

Kelley (2006) reported 2327 cestodes (prevalence 72.2%) from 36 raccoons (*Procyon lotor*) from Texas including *Atriotaenia procyonis*, *Mesocestoides* spp., and the immature species of Taeniidae. In the similar study, Kelley et al. (2008) reported 2280 cestodes from 35 raccoons (*Procyon lotor*) in North Central Texas, including *Atriotaenia procyonis*, *Mesocestoides* sp. and immature *Taenia pisiformis*. *Atriotaenia procyonis* was the most prevalent cestode comprising 86.9% of all cestodes found in *P. lotor*. Another study carried out in North Central Texas, showed, other species of trematode parasites in raccoons (*Procyon lotor*), the mammalian schistosome *Heterobilharzia americana* with prevalence rate of 47.2% (i.e. 17 of 36) (Kelley 2010). Among 19 raccoons captured, 8 species of helminths including five nematodes (*Physaloptera rara*, *Placoconus lotoris*, *Molineus barbatus*, *Baylisascaris procyonis*, *Toxoascaris procyonis*); one acanthocephalan (*Macrocanthorhynchus ingens*), and two cestodes (*Atriotaenia procyonis* and *Mesocestoides lineatus*) were reported in raccoon (*Procyon lotor*) from Duval County, Texas. The most common parasite found was *Macrocanthorhynchus ingens,* which occurred in 11 of 19 raccoons and the most abundant was *Atriotaenia procyonis* (Long et al. 2006). Iwaki et al. (2012) reported the trematode *Ogmocotyle ailuri* for the first time in Japanese monkey, *Macaca fuscata* that was captured in Sendai, Japan and from the small intestine of Taiwanese monkey, *Macaca cyclopis* from Taiwan (Yoshimura et al. 1969).

2.2 Scenario of gastrointestinal parasites in National Context

In the national context, no investigations have been done in gastro-intestinal parasites of Red Panda in Nepal. Most of the studies were performed in ecological basis like ecology, behaviour, and population status of red panda in different national parks, conservation areas and hunting reserves (Yonzon 1989, Yonzon and Hunter 1991, Sharma and Belant 2009 and Sharma and Belant 2010). Thapa (2012) carried out a survey of gastrointestinal infection of parasites in Himalayan Tahr and Barking Deer in Rara National Park. She reported altogether 10 genera of intestinal parasites including *Eimeria* (88.24%), *Oxyuris* (88.24%), *Strongyloides* spp. (64.71%), *Ascaris* sp. (52.94%), *Trichostrongylus* sp. (11.76%), *Dictyocalus* sp. (11.76%), *Mullerius* sp. (11.76), *Haemonchus* sp. (5.88%) and *Moniezia* (70.59%) in Himalayan Tahr while in Barking Deer, *Eimeria* sp. (73.53%), *Oxyuris* sp. (70.59%), *Ascaris* sp. (17.65%), *Trichuris* sp. (8.82%), *Dictyocalus* sp. (8.82%), *Haemonchus* sp. (2.94%) and *Moniezia* sp. (47.06%) were reported. Lama et al.

(2015) studied the intestinal parasitic infections in free-ranging Red Panda (*Ailurus fulgens*) in Nepal. They showed 100% of prevalence of protozoan parasites in 23 samples including unidentified protozoan species in 16 samples, coccidian parasites of various oocysts characters in 5 samples, oocysts of *Cryptosporidium* in one sample and oocysts *Cyclospora* in one sample. They also showed *Trichuris* spp. in 6 samples, *Baylisascaris* spp. in 3 samples, *Angiostrongylus* larvae in 2 samples, unidentified eggs resembling nematode eggs in one sample, and operculated eggs of trematodes in three samples and eggs of cestode in one sample.

2.3 Zoonosis and Risk Factors

Human demands on natural resources result in landscape changes that facilitate the emergence of zoonotic diseases which play role in the decline of vulnerable wildlife species. *Baylisascaris procyonis*, the common raccoon ascarid of raccoons (*Procyon lotor*), is a recognized zoonotic infection that has remarkable disease-producing capability in animals causing extinction. It is highly non-specific and frequently pathogenic with regard to paratenic hosts, which contact eggs of *B. procyonis* at raccoons latrines and over 130 species of vertebrates have been identified with clinical larval infections (Kazacos 2001, Kazacos 2013). *B. procyonis* is capable of producing clinical larva migrans in animals and affects a wide variety of wild and domestic species. Infection with *B. procyonis* is best known as a cause of fatal or severe neurologic disease (NLM, cerebrospinal nematodiasis), which has been reported in > 90 species of mammals and birds in North America (Kazacos 2001). It is also an important zoonosis, causing severe neurological disease (cerebrospinal nematodiasis) and often damage visceral and ocular tissues (Kazacos 2001). Infection has important health implications for free-ranging and captive wildlife, zoo animals, domestic animals, and human beings, on an individual as well as population basis.

Strikingly, being nonspecific in their infection *B. procyonis* larvae commonly undergo somatic migration in intermediate or paratenic hosts and enter various internal organs and tissues where they become encapsulated and persist, for later transmission to carnivores (Sprent 1952, Sprent 1953a, Sprent 1953b, Tiner 1953a, Tiner 1953b, Sprent et al. 1973, Sheppard and Kazacos 1997). Thus, ascarids of carnivores and *B. procyonis* in particular, are excellent examples of helminthes that produce larva migrans. The possibility of human infection was anticipated by Beaver (1969) and later by Kazacos and Boyce

(1989). More recently, the zoonotic potential of *B. procyonis* has become evident. The first confirmed cases of NLM in humans were described to have occurred in two young boys, in 1984 and 1985 (Huff et al. 1984, Fox et al. 1985). Since first recognized in the 1980s, there have been at least 30 documented human NLM cases. Most diagnosed cases have been severe (usually fatal) with all but two survivors having mental or physical disabilities (Sorvillo et al. 2002, Haider et al. 2012, Peters et al. 2012, Hung et al. 2012). OLM results in mild sight loss to blindness (Goldberg et al. 1993). Epidemiologic studies suggest that pica or geophagia and exposure to infected raccoons or environments contaminated with their faeces are the most important risk factors for human infection (Wise et al. 2005, Kazacos et al. 2000). The first recognized human case was reported in 1984 in a 10-month-old child in Pennsylvania (Huff et al. 1984)

The primary risk factors for human *B. procyonis* infection include contact with raccoon latrines, pica/geophagia, young age (<4years), and male sex. Another contributing risk factor appears to be developmental delay (Kazacos 2001, Evans 2002, Cunningham et al. 1994, Boschhetti and Kasznica 1995, Gavin et al. 2002, Kazacos et al. 1985). Raccoons harbouring *B. procyonis* in major urban areas are a potential source of zoonotic spread to humans. Raccoons adapt readily to human habitation and may defaecate in close proximity to homes, potentially putting large numbers of infective eggs in the immediate environment of children and others playing or working in yards, parks, playgrounds, and similar environments (Murray 2002). Heavily infected raccoons may shed millions of eggs daily. This is of particular importance because human exposure to *Baylisascaris* is primarily through the faecal-oral route and risk of infection is proportional to the number of eggs in the environment (Sorvillo et al. 2002, Gavin et al. 2005). *B. procyonis* infection is widespread within raccoon population. There has been demonstration of patent infection in dogs (Averbeek et al. 1995, Bowman et al. 2005). Adaptation of *B. procyonis* to dogs would likely provide a greatly enhanced zoonotic risk and contact between dogs and raccoons.

III

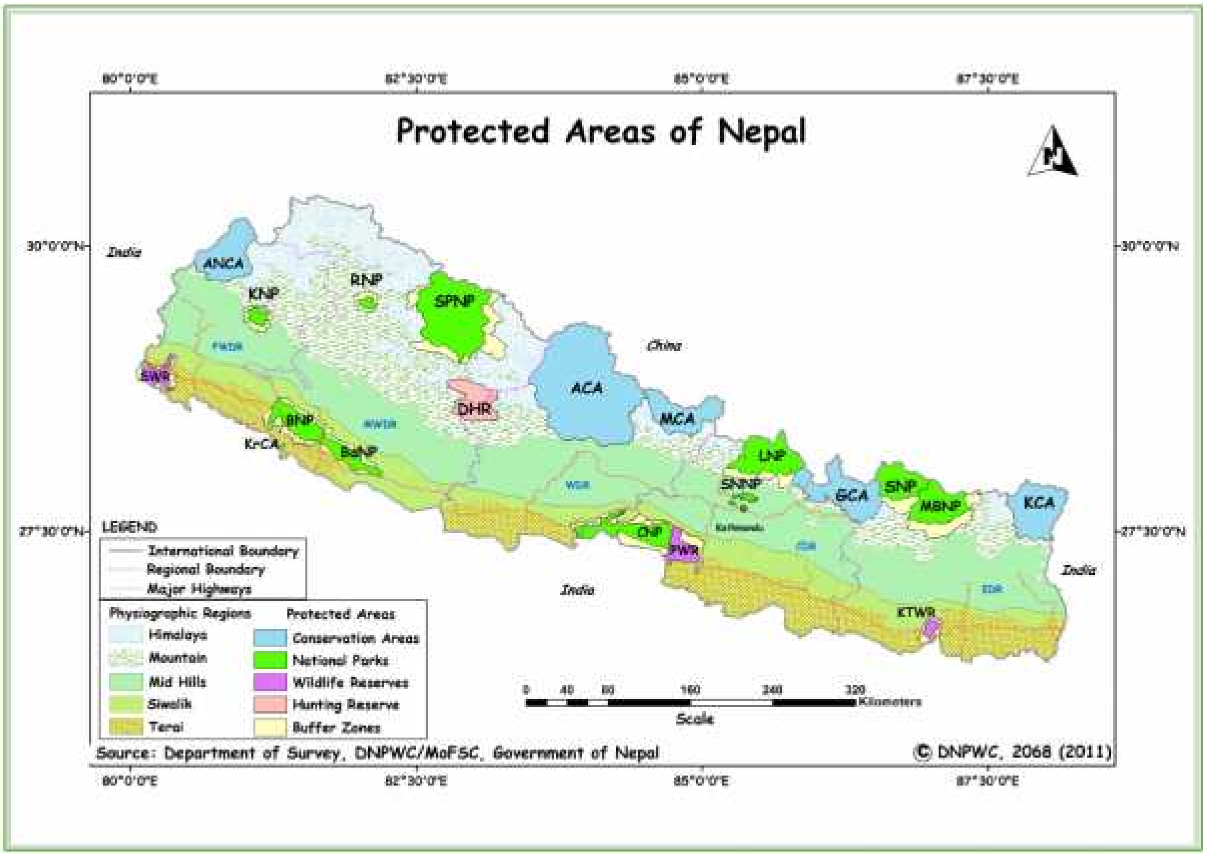
MATERIALS AND METHODS

3.1 Study Area

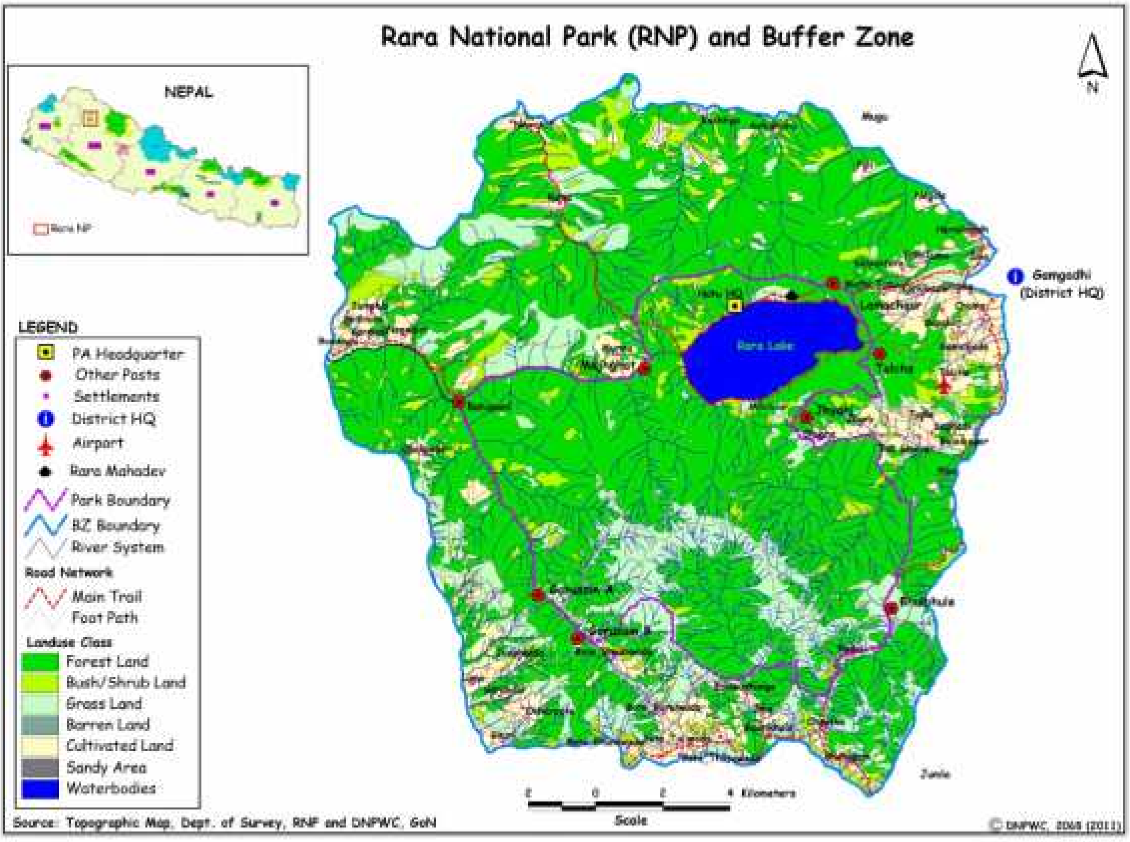
The study has been carried out in Rara National Park (RNP) which is located in the North-Western high mountains of Nepal. It is the smallest national park with an area of approximately 106 km^2 and was established in 1976 to conserve its biodiversity and maintain the unique landscape. The important feature of this park is the lake Rara (also known as Mahendra Tal) which covers 10.8 km^2, area and is situated at an altitude of 2990m (9810ft.). The lake, in 2007, has been declared as a wetland of international importance, i.e. the Ramsar site. The highest point of the park is Chuchemara (4038m). Summer season is pleasant but winter is cold with heavy snowfall (upto one metre). Most of the park including Lake Rara is in Mugu District with small area in Jumla District of Karnali zone.

Most of the parks landscape is dominated with conifers. The area around the lake is dominated by Blue Pine (*Pinus wallachiana*) up to 3200m. Rhododendron (*Rhododendron arboretum*), Black Juniper (*Juniperus indica*), West Himalayan Spruce (*Picea smithina*), Oak (*Quercus semecarpefolia*) and Himalayan Cypress (*Cupressus torulosa*) are other species. Above this elevation, the vegetation is replaced by mixed coniferous forest of Pine, Spruce and Fir. At about 3350m Pine and Spruce give way to Fir, Oak and Birch forest. Other deciduous tree species found in the park are Indian Horse-chestnut, Walnut and Himalayan poplar. Faunal diversity includes about 51 species of mammals, 272 bird species and snow trout. The park serves as an ideal habitat for the endangered Musk Deer (*Moschus moschiferous*), Himalayan Tahr (*Hemitragus jemlahicus*), Himalayan Black Bear (*Ursus thibetanus*), Leopard (*Panthera pardus*), Himalayan Ghoral (*Nemorhaedus goral*), Jackal (*Canis aureus*), Yellow-Throated Marten (*Martes flavigulla*), Wild Dog (*Cuon alpinus*), Wild Boar (*Sus scrofa*), Common Langur (*Presbytis entellus*), Rhesus Macaque (*Macaca mulatta*) and Common Otter (*Lutra lutra*) (DNPWC 2012).

Map of the study area



Map 1: Nepal showing the location of the Rara National Park



Map 2: Rara National Park

3.2 Materials used

3.2.1 Materials for Laboratory:

i. Gloves
ii. Slides
iii. Cotton
iv. Volumetric flask
v. Electronic weight machine
vi. Pasteur pipette
vii. Test tube rack
viii. Motor and pestle
ix. Refrigerator
x. Glass rod
xi. Cover slips
xii. Compound microscope
xiii. Tea strainer
xiv. Petri dishes
xv. Centrifuge machine
xvi. Centrifuge tubes
xvii. Test tubes
xviii. Droppers

3.2.2 Materials for Field:

i. 40m long nylon rope
ii. 1m long stick
iii. Zipper plastic bags

3.2.2 Chemical Reagents Used:

i. 2.5% potassium dichromate
ii. Normal saline solution
iii. Iodine solution
iv. Methylene blue
v. Saturated solution of sucrose
vi. Distilled water

3.3 Study Design

The present study was designed to assess the gastro-intestinal parasitic infection in Red Panda (*Ailurus fulgens fulgens*) of Rara National Park by collecting faecal pellets using line transect method and analyze using standard methods of eggs and oocyst detection.

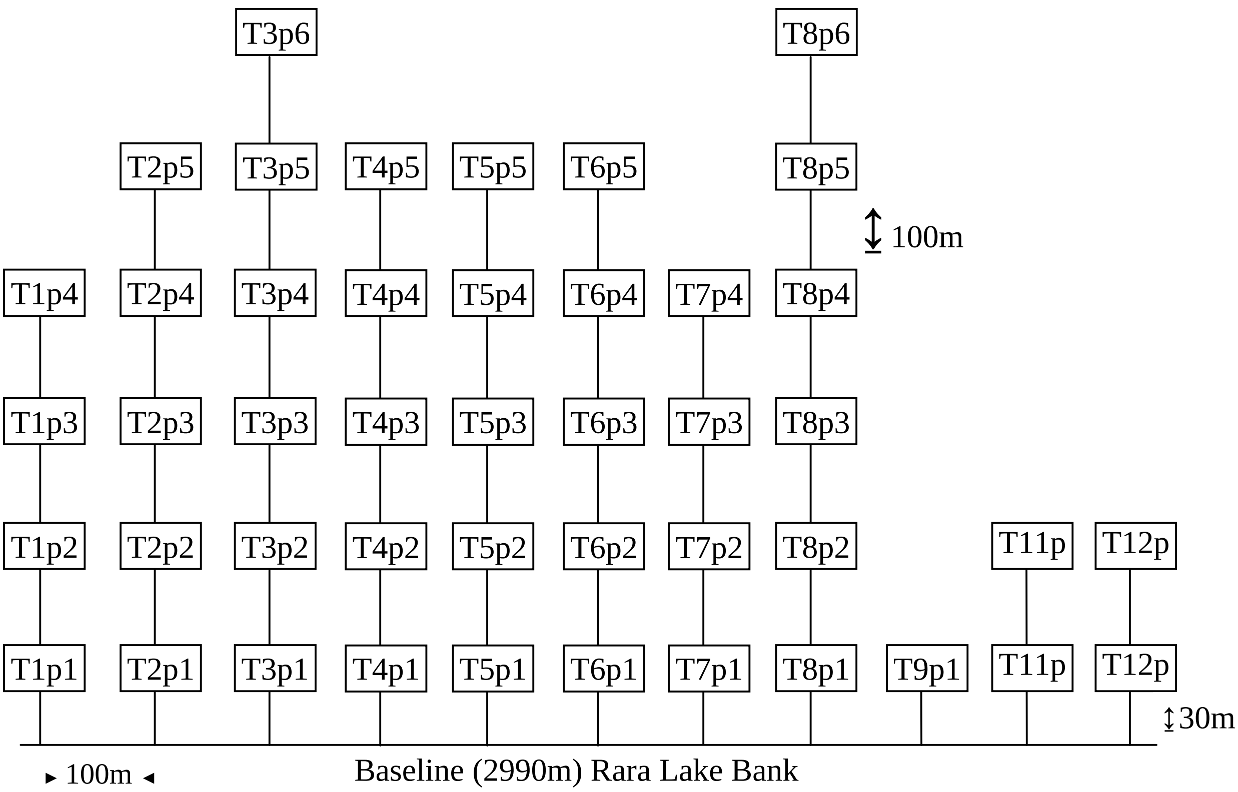
3.3.1 Sample Size

A total of 22 faecal samples of Red Panda were collected from the East-West 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.

3.3.2 Sampling Technique

East-West facing slope of the mountain surrounding the Rara Lake was chosen to collect faecal pellets of Red Panda. This site had been divided into 12 transects. Twenty two and twenty one samples were collected by using line transect in May/June, 2011 and May/June, 2012 respectively. These samples were collected from different sites to prevent the repetition of the faecal. Overall forty three samples were collected from Rara National Park and preserved in 2.5% potassium dichromate solution.

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

3.4 Examination of Faecal Samples

Microscopical 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.

3.4.1 Floatation Technique:

In this technique, the saturated sucrose solution was used as it has higher specific gravity than the parasitic forms which helps in rise of helminth eggs from the faecal matter to the surface. Except the unfertilized egg of *Ascaris,* eggs of *Taenia*, operculated eggs of trematodes and larvae of *Strongyloides*, all the eggs of nematodes and cestodes float on saturated sucrose solution as they are relatively small and light (Parija 2000). It provides good results among other floatation technique and is one of the easiest and short ways for identifying and counting the eggs. (Source: Veterinary Lab. Techniques 2003)

Three grams of faecal sample was taken in a beaker and 42 ml of water was added and the sample was grinded lightly with the help of motor and pestle, and then filtered with a tea strainer. The filtrate was poured into a centrifuge tube of 15 ml and centrifuged at 2000 rpm for 10 minutes. The tube was taken out and suspension was removed with the help of pipette. Initially a few drops of saturated sucrose solution was added to the sediment left and mixed thoroughly to make it a fine emulsion. More sucrose solution was added to the tube till it is filled up completely up to the brim so that convex surface is formed at the top of the tube. A cover slip was placed on the top of the tube so that sucrose solution comes in contact with the cover slip. This was allowed to be kept for 10 minutes. The cover slip was then removed carefully and placed on a slide and examined under the microscope at 10X and 40X. Photographs were taken, parasite were counted and identified on the basis of eggs colour, shape, content and size. Measurements were made using an ocular micrometer fitted to the compound microscope.

3.4.2 Sedimentation Technique:

In this technique, the helminth eggs and protozoan cysts are recovered in the sediment using centrifugation. This method is easy to perform and can be done with minimum technical error and comparatively more sensitive than floatation technique. Relatively, more helminthic eggs and protozoan cysts are concentrated and morphology of the eggs and cysts are preserved. (Source: Veterinary Lab. Techniques 2003)

Three grams of faecal sample was taken in a beaker and 42 ml of water was added and the sample was grinded lightly with the help of motor and pestle, and filtered with a tea strainer. The filtrate was poured into a centrifuge tube of 15 ml and centrifuged at 2000 rpm for 10 minutes. The tube was taken out and suspension was removed with the help of pipette. The sediment was taken out from the tube with the help of pipette and placed in the slide, added a drop of methylene blue into it and examined under the microscope at 10X and 40X. Iodine wet mount is mainly used for protozoan cysts. Iodine stained cysts shows pale refractile nuclei, yellowish cytoplasm and brown glycogen material. Normal saline wet mount is used to demonstrate helminthic eggs and larvae. It is also used for motile trophozoites of the intestinal protozoa. Methylene blue is used for demonstration of nuclei in the trophozoites of protozoa (Parija 2000).

3.4.3 Stoll's Counting Technique:

It is the easiest quantitative method to count the number of eggs present in the microscopic field without the help of McMaster (According to Dr. Tom Nola, University of Pennsyvania, 2004). The number of eggs of trematode, cestode, nematode was detected and counted. The total number of eggs determines the number of eggs present per gram of faeces. (Source: Veterinary Lab. Techniques 2003)

Three grams of faecal sample were taken in a beaker and 42 ml of water was added. Using a tongue depressor or 3 gm of faeces was pushed through a sieve into the water. Then the sieve was fitted and holds over the dish. The remaining water was then pushed out from the faeces. After stirring the water-faeces mixture, 0.15 ml of the suspension was taken and spread over two slides. Each slide was covered with a long cover slip. Then both slips were examined for eggs. The total amount of eggs counted multiples with 100 represent the number of eggs per gram of faeces.

3.4.4 Determination of concurrency and intensity:

3.4.4.1 Concurrency

The host harbour one or many parasites in low number in such cases hosts don't develop disease but can remain source of infection for long period of time. The concurrency of gastrointestinal parasites was categorized into single, double, triple, quadruple, quintuple and multiple infections. The single infection was determined by the occurrence of only one egg/cyst/larva of the parasite per field while double, triple, quadruple, quintuple and multiple infections were determined by the occurrence of 2, 3, 4, 5 and 6 or more egg/cyst/larva of the parasite per field respectively.

3.4.4.2 Intensity

Intensity of parasitic infection has been calculated based upon the number of eggs/oocysts and larvae found per field. The intensity of infection of gastrointestinal parasites was categorized into 4 groups, i.e. light infection, mild infection, moderate infection and heavy infection. Light infection was determined by the occurrence of less than 2 egg/cyst/larva of the same species per field. Similarly mild, moderate and heavy infections were determined by the occurrence of 2-4 egg/cyst/larva, 4-6egg/cyst/larva, 6 or more egg/cyst/larva of the same species per field respectively.

IV

RESULTS

The coprological study was conducted by collecting faecal samples of red panda (*Ailurus fulgens fulgens*) of Rara National Park for two consecutive years 2011 and 2012. A total of 43 samples, 22 faecal samples and 21 samples were collected during the year 2011 and 2012 respectively following the methodology in materials and method section.

4.1 Prevalence of gastrointestinal parasites in Red Panda

Among 43 samples examined, 40 samples (93.00%) were found to be positive for gastrointestinal parasites. In 2011, all samples i.e. 22 samples were found to be positive with single, double, triple, quadruple, quintuple or multiple infections of gastrointestinal parasites, hence positive percentage was found to be 100%. Similarly, in 2012, out of 21 samples, 18 samples (85.71%) were found to be positive for intestinal parasites.(Figure 1)

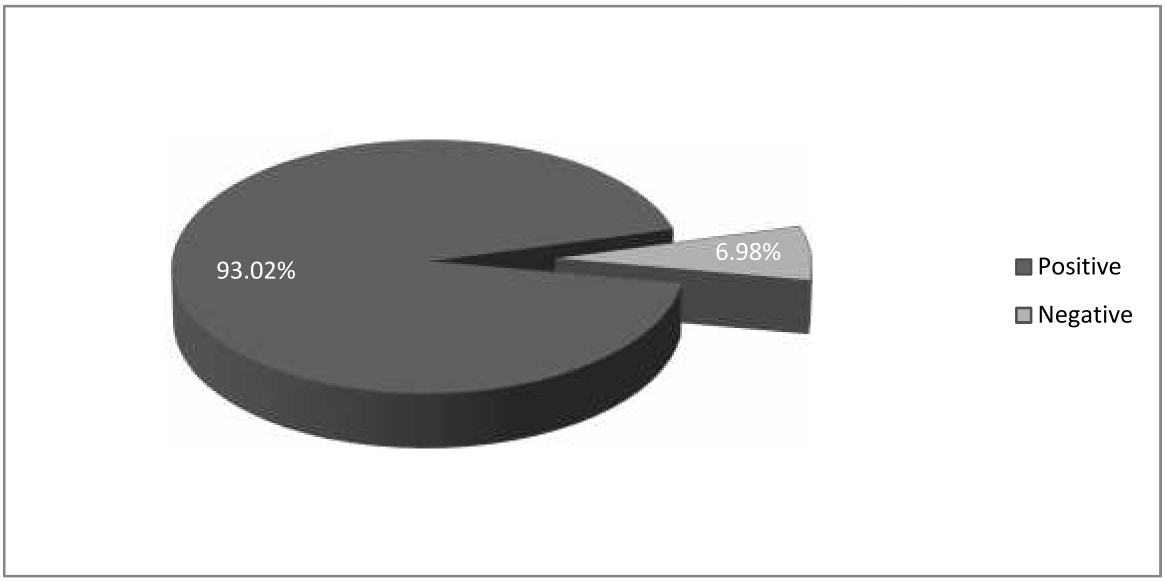


Figure 1: Prevalence of gastrointestinal parasites in Red Panda

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. In 2011, out of 22 samples, 17 samples (77.27%) were found to be positive for protozoan parasites, 21 samples (95.45%) for nematodes, 5 samples (22.73%) for cestodes. Trematodes and acanthocephalans were found to be nil. Nematodes were highly prevalent (95.45%). Similarly, in 2012, out of 21 samples, 16 samples (76.19%) were found to be positive for both protozoan parasites and nematodes and 3 samples (14.29%) for cestode infection. Protozoans and nematodes were equally prevalent (76.19%). (Figure 2)

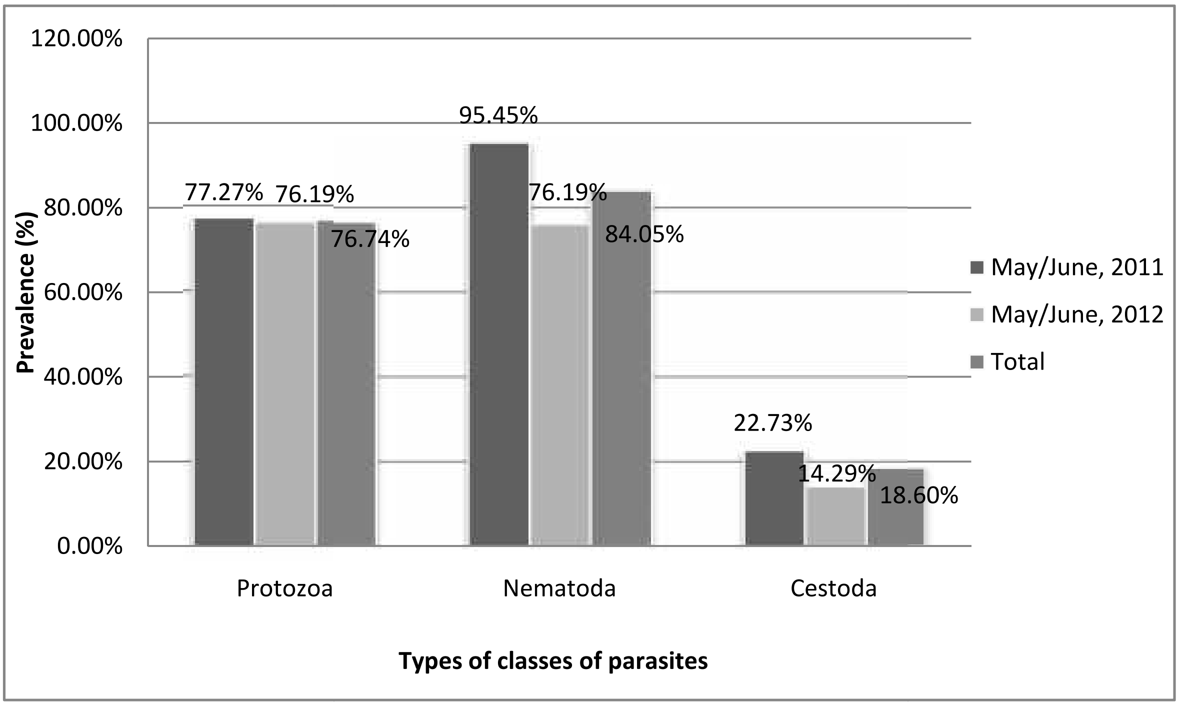


Figure 2: Class-wise prevalence of gastrointestinal parasites in both years

4.1.1 Prevalence of protozoan parasites in Red Panda

Altogether 2 genera of protozoan parasites were recovered from 43 faecal samples examined. The protozoan parasites were identified as *Eimeria* sp. and *Entamoeba* sp. Among 43 samples, 29 samples (67.44%) and 27 samples (62.79%) were found to be positive for *Eimeria* and *Entamoeba* respectively. Thus, *Eimeria* coccidian parasite was found to be the most prevalent protozoan parasite.

In 2011, the most prevalent protozoan parasite was *Eimeria* sp. 14 samples (63.64%) followed by *Entamoeba* sp. 12 samples (54.55%). In 2012, both protozoan parasite *Entamoeba* sp. and *Eimeria* sp. were equally recovered 15 samples (71.43%). (Figure 3)

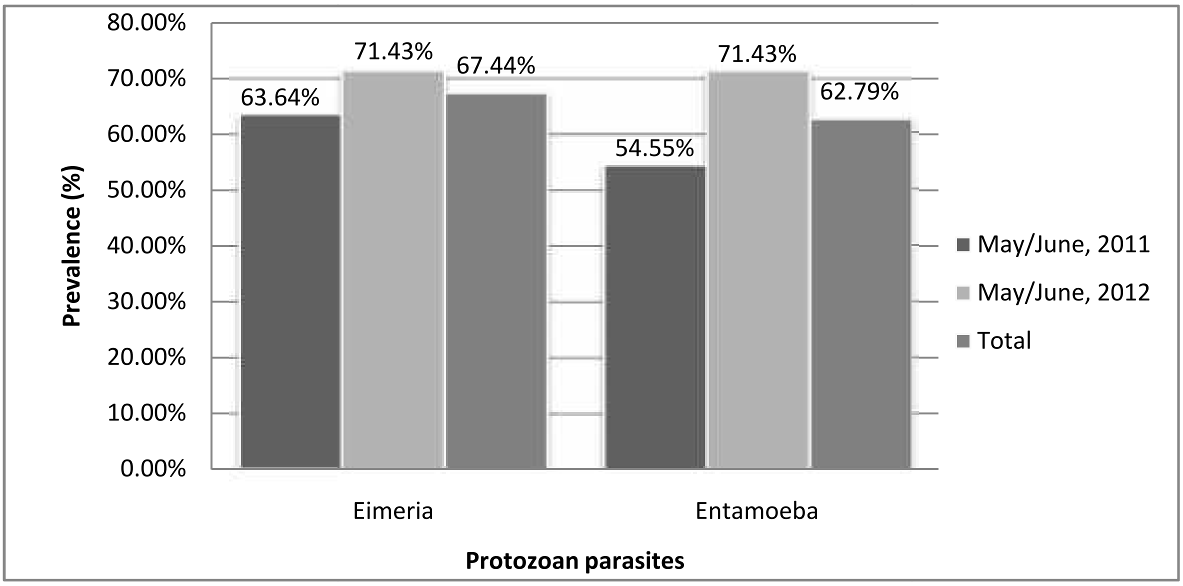


Figure 3: Prevalence of protozoan parasites

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 (Photo No. 8 and 9) and the size of the cyst of *Entamoeba* ranges from 21.04µm-28.93µm in diameter. (Photo No. 10)

4.1.2 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 lungworms (*Crenosoma* sp., *Angiostrongylus* sp., *Metastrongylus* sp.) but trematodes were not observed during the study.

In 2011, out of 22 samples, the most prevalent helminth parasite was *Oxyuris* sp. (14 i.e. 63.64%) followed by *Toxoascaris* sp. (12 i.e. 54.45%), Hookworm (10 i.e. 45.45%), *Baylisascaris* sp. (8 i.e. 36.36%), *Strongyloides* sp. and *Crenosoma* sp. (6 each i.e. 27.27%), *Moniezia* sp. (5 i.e. 22.73%), *Trichuris* sp. (2 i.e. 9.10%), *Angiostrongylus* sp. and *Metastrongylus* sp. (1 each i.e. 4.55%).

In 2012, out of 21 samples, the most prevalent helminth parasite was *Oxyuris* sp. (11 i.e. 52.38%) followed by Hookworm, *Toxoascaris* sp. and *Crenosoma* sp. (9 each i.e. 42.86%), *Baylisascaris* sp. (7 i.e. 33.33%), *Moniezia* sp. (3 i.e. 14.29%), *Strongyloides* sp. (2 i.e. 9.52%), *Metastrongylus* sp. (1 i.e. 4.76%). *Trichuris* sp. and *Angiostrongylus* sp. were found to be nil. (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%)

4.1.3 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 (Photo No. 11). The eggs of *Trichuris* were barrel shaped with transparent mucous plug at either pole containing unsegmented embryo with average length 55.23µm – 68.38µm (Photo No. 12). *Strongyloides* eggs were ellipsoidal, thin walled, embryonated with 44.71µm in size (Photo No. 14). Eggs of *Toxoascaris* were observed ranging the size from 21.04µm-52.60µm (Photo No. 13) 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 (Photo No. 15). Similarly, the eggs of *Moniezia* were triangular in shape containing a pyriform apparatus with the size ranging from 15.78µm-28.93µm in diameter (Photo No. 17).

4.2 Concurrency and intensity of gastrointestinal parasites in Red Panda

Gastrointestinal parasite infections in wild animals including Red Panda are common phenomenon. To cause the pathological effect by the parasite in host needs high intensity of the parasites. In many cases many hosts harbour one or many parasites in low number, in such cases those hosts don't develop disease but can remain source of infection for long period of time.

4.2.1 Concurrency of gastrointestinal parasites in Red Panda

In the present study, the multiple infections were observed in Red Panda. Among 40 positive samples (93.00%), 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%). In 2011, all samples were found to be infected with at least one intestinal parasite. Out of 22 samples, 1 sample (4.55%) with single infection, 3 samples (13.64%) with triple infections, 5 samples (22.73%) with double and quintuple infection, 4 samples (18.18%) with quadruple and multiple infections were observed.

In 2012, out of 21 samples, 3 samples (14.29%) were non-infected with intestinal parasites. Only 1 sample (4.76%) with double infection, 4 samples (19.05%) with triple, quadruple and quintuple infections, 5 samples (23.81%) with multiple infections were observed. Single infection was not observed. (Figure 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%)

4.2.2 Intensity of infection of gastrointestinal parasites in Red Panda

Intesity 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 *Entamoeba* sp. with 4 (18.18%) samples and mildly infected cases was due to *Eimeria* sp. with 9 (40.91%) samples while high intensity of moderately and heavily infected cases were due to *Entamoeba* sp. with 3 (13.64%) samples each. Among helminthes, the high intensity of lightly infected cases was observed due to *Oxyuris* sp. with 7 (31.82%) samples and mildly infected cases was due to *Toxoascaris* sp. with 8 (36.36%) samples. Similarly, high intensity of moderately infected cases was due to the Hookworms and *Oxyuris* sp. with 2 (9.09%) samples and the heavily infected cases due to *Baylisascaris* sp. and *Trichuris* sp. with 2 (9.09%) samples. (Table 3)

Table 3: Intensity of infection of gastrointestinal parasites in Red Panda in May/June 2011

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

Among protozoans, the high intensity of lightly infected cases was found in *Entamoeba* sp. with 4 (19.05%) samples but high intensity of mildly infected cases was found in *Eimeria* sp. with 9 (42.86%) samples and high intensity of moderately and heavily infected cases were found in *Entamoeba* sp. with 3 (14.29%) samples. Among helminthes, the high intensity of lightly infected cases was found in Hookworm species with 5 (23.81%) samples but the high intensity of mildly infected cases was found in *Oxyuris* with 6 (28.57%) samples. Similarly, the high intensity of moderately infected cases were found in *Toxoascaris* sp. and *Baylisascaris* sp. with 3 (14.29%) samples and and the high intensity of heavily infected cases was found only in *Oxyuris* sp. with 1 (4.76%) sample. (Table 4)

Table 4: Intensity of infection of gastrointestinal parasites in Red Panda in May/June 2012

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

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 5)

Table 5: Overall intensity of infection of gastrointestinal parasites in Red Panda

S.N.	**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

V

DISCUSSIONS

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 GI parasites may be due to different factors such as feeding behaviour, habitat, defecating openly on the ground and on trees. etc. Parasites can 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 GI 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). Similarly, in the previous study, from 61 adult raccoons live trapped on Key Largo, Florida intestinal coccidia, *Eimeria procyonis* (84%), *Eimeria nuttalli* (10%), 1 unidentified species of *Eimeria* (3%) and an unidentified species of *Sarcocystis* (3%) were observed in faecal samples (Foster et al. 2004). *Eimeria nuttalli* and *Eimeria procyonis* also observed in raccoon (*Procyon lotor*) (Wright and Gompper 2005). *Eimeria procyonis*, *Isospora* sp. and *E. nuttalli* were observed from American raccoon (*Procyon lotor*) (Inabnit, Chobotar and Ernst 1972) and oocysts of *Cryptosporidium parvum* in the faeces of naturally infected raccoons (Synder 1988). *Eimeria* infection was also observed in raccoons in America (Dubey 1982). *Eimeria procyonis* like oocysts were observed in 15 of 15 captive raccoons and 6 of 6 juvenile wild caught raccoons (Dubey et al. 2000). *Eimeria* and *Sarcocystis* were also observed in raccoons in Illinois (Adams et al. 1981). This showed that *Eimeria* spp. were commonly found in raccoons and Red Panda. However, *Eimeria and Entamoeba* sp. 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). In the present study, trematodes were not reported. This may be due to absence of intermediate hosts. A study carried out in Archer and Wichita counties of North Central Texas, showed that overall prevalence of adult fluke, *Heterobilharzia americana* in raccoons (*P. lotor*) was 17 of 36 or 47.2% (Kelley 2010). Wright and Gompper (2005) observed three different genera of trematodes, *Alaria* sp. *Digenea* sp. and *Eurytrema procyonis* from raccoon.

The 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). Similarly, Long et al. (2006) reported one acanthocephalon (*Macrocanthorhynchus ingens*) and two cestodes (*Atriotaenia procyonis* and *Mesocestoides lineatus*) in raccoon (*P. lotor*) from Duval County, Texas. Among them they found *Macrocanthrorhynchus ingens* was the most common parasite which occurred in 11 or 19 raccoons and *Atriotaenia procyonis* was the most abundant. Similarly, three genera of tapeworms were reported from 35 raccoons in Archer and Wichita counties of north central Texas including *Atriotaenia procyonis*, *Mesocestoides* sp., and immature *Taenia pisiformis*. In the same study, *Atriotaenia procyonis* was the most prevalent cestode comprising 86.9% of all cestodes found in raccoon, *P. lotor* and *Mesocestoides* sp. was most abundant in spring and winter whereas *A. procyonis* was the most abundant in summer (Kelley and Horner 2008).

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). Wright and Gompper (2005) also observed the different genera of nematodes *Baylisascaris procyonis*, *Capillaria acrophilia*, *Capillaria plica*, *Capillaria procyonis*, *Capillaria putorii*, *Crenosoma* sp., *Placoconus lotoris* and *Strongyles* sp. from two populations of 59 known raccoons and 14 unknown raccoons in southern New York. Similarly, *B. procyonis* was also reported in 9 of 74 (12%) raccoons from western North Carolina (Hernandez 2012). Another studies conducted in the north-eastern, Midwestern, mid-Atlantic, some western states (California, Washington, Oregon, and Colorado), and some regions of Texas (Kazacos 2001, Long et al. 2006, Chavez et al. 2012), the highest prevalence rates for *B. procyonis* in raccoons have been reported. Recent reports in the south-eastern USA suggest that this parasite is either spreading to new regions or has been present in unrecognized locales (Eberhard et al. 2003, Blizzard et al. 2010a, Blizzard et al. 2010b).

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. Intensity of parasitic infection can be defined as the number of adult worms infecting a host and many factors affect the number of eggs expelled in host faeces. Though in the present study, the eggs of all parasite species recovered were counted and analyzed as their intensity relating to that parasitic eggs or oocysts load could be same indication of intensity. 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.

The present study has few limitations. For example, the parasites have not identified to species level and the number of faecal samples was low. Yet, the study was conducted using two different concentration techniques to assure the cysts, eggs or larvae of the parasites.

VI

CONCLUSIONS

The present study contributes baseline data on the gastrointestinal parasites of Red Panda (*Ailurus fulgens fulgens*) providing a step toward an index of population health and diseases risk assessment for conservation and management plans of endangered Red Panda. The information collected in this study provides the information about the parasite burden, patterns of infection and their intensity in Red Panda. Therefore, a de-worming program should be considered in this area. Avoiding cattle grazing near the Red Panda habitat would prevent transmission of zoonotic diseases.

A total of 43 faecal samples of Red Panda from Rara National Park were examined by using coprological techniques. Among them, 40 (93.02%) samples were found to be positive for gastrointestinal parasites. Altogether 12 genera of parasites were recovered from Red Panda, which includes two genera of protozoa belonging to class sporozoa (*Eimeria* sp.) and rhizopoda (*Entamoeba* sp.) and 10 genera of helminths including one genera belonging to cestode and nine genera belonging to nematode. Among nematodes, 6 genera belongs to intestinal parasites (Hookworm, *Toxoascaris* sp., *Baylisascaris* sp., *Strongyloides* sp., *Trichuris* sp., *Oxyuris* sp.) and 3 genera belong to lungworms (*Crenosoma* sp., *Angiostrongylus* sp., *Metastrongylus* sp.). 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 but trematodes and acanthocephalans were not recovered during this study.

The prevalence of protozoan parasite, *Eimeria* sp. was found to be 67.44% while that of *Entamoeba* sp. was found to be 62.79%. Thus, the prevalence rate of the coccidian parasite, *Eimeria* was found to be slightly higher as compared to *Entamoeba*. Similarly, the prevalence of *Oxyuris* was found to be the highest (58.14%) among the helminthes followed by *Toxoascaris* (48.84%), Hookworm (44.19%), *Baylisascaris* and *Crenosoma* (34.88%), *Strongyloides* and *Moniezia* (18.60%), *Metastrongylus* and *Trichuris* (4.65%) and *Angiostrongylus* (4.65%).

The concurrency of gastrointestinal parasites showed that the 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 (2.33%). It was observed that most of the samples were infected with *Eimeria*, *Entamoeba*, *Strongyloides*, *Baylisascaris*, *Oxyuris*, *Crenosoma* , Hookworm and *Toxoascaris*. Eight (18.60%) faecal samples showed combination of four species in Red Panda while seven (16.28%) and six samples showed combinations of three and two species but single infection was observed only in 1 (2.33%) faecal sample.

Intensity of parasitic infection was found to be different in different faecal samples. Some samples were highly infected with parasites and some are lightly infected. Among protozoan, both protozoans, *Eimeria* and *Entamoeba* were found to be heavily infected with 4.65% and 6.98% respectively while among helminthes, *Oxyuris*, *Baylisascaris* and *Trichuris* were found to be heavily infected with 2.33%, 4.65% and 4.65% respectively.

VII

RECOMMENDATIONS

1. Parks, Conservation Areas and Wildlife Reserves should establish National veterinary laboratory unit for regular monitoring of disease diagnosis and treatment.

2. Research should be conducted on parasitic diseases prevalent in Red Panda.

3. Concerned authorities must have regular programmes of wild animal census to know the animal population along with disease status, records maintenance.

4. Domestic animals should be strictly prohibited from entering the protected areas since most of the parasites are shared in between domestic animals and wildlife.

VIII

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