OCCURRENCE OF GASTROINTESTINAL PARASITES IN RED PANDA (*Ailurus fulgens*) IN THE COMMUNITY FOREST OF SURYODAYA MUNICIPALITY ILLAM, NEPAL



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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has been not submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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This is to recommend that the thesis entitled "Occurrence of gastrointestinal parasites in red panda in the community forest of Suryodaya Municipality Illam, Nepal" has been carried out by Darwin Niroula for the partial fulfillment of Master's Degree of science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best knowledge, this thesis work has been not submitted for any other degree in any institution.

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LIST OF ABBREVIATIONS

| Abbreviated forms | Details of abbreviations/acronyms |
|-------------------|-----------------------------------|
| CCF | Chhipchhipe Community Forest |
| CHCF | Chitrehile Community Forest |
| ft | Feet |
| GI | Gastrointestinal Parasites |
| gm | Gram |
| km | Kilometer |
| m | Meter |
| ml | Milliliter |
| Mm | Millimeter |
| OLM | Ocular Larva Migrans |
| RNP | Rara National Park |
| RPN | Red Panda Network |
| rpm | Revolutions per minute |
| SCF | Singhadevi Community Forest |
| VLM | Visceral Larva Migrans |

ABSTRACT

This study aimed to assess the prevalence and distribution of gastrointestinal parasites in Red Pandas (*Ailurus fulgens*) within three community forests in Suryodaya Municipality, Ilam, Nepal. A total of 36 fecal samples were collected and examined for parasites using concentration method. The results showed that 86.11% of the samples tested positive for gastrointestinal parasites, with protozoans being the most prevalent (69.44%), followed by nematodes (64%) and cestodes (13.89%). Among the protozoans, *Eimeria* sp. and *Entamoeba* sp. were identified. Different community forests exhibited variations in prevalence rates, with Singhadevi Forest showing the highest prevalence. Altogether seven types of helminth parasites were recorded; including Ascarid, Strongyle and *Moniezia* sp. Multiple infections were observed in 74.19% of the parasitic burden and highlight the importance of monitoring and managing gastrointestinal parasites in Red Panda populations for their conservation and overall well-being.

1. INTRODUCTION

1.1. Background

Nepal has the unique topography, it is rich in biodiversity and natural resources, ranging from lowlands with subtropical forests to frigid conditions in the Himalayan peaks. Geographically, it is 80⁰4" to 88⁰12" East longitude and 26⁰22" to 30⁰27" North latitude. It is approximately 885 kilometers long and 193 kilometers wide on average. The majority of Nepal's land mass sits along the Himalayas, and as a result, the country experiences huge altitudinal fluctuations ranging from 60m along the southern border to 8848m in Sagarmatha. As a result, it contains nearly all of the climatic zones found on Earth. As a result, Nepal has been endowed with a diverse range of climatic and biological zones that support a diverse range of fauna and plants (Shrestha 2015).

Parasites and infectious diseases are now key concerns in endangered species preservation because they can cause mortality, drastic population decreases, and even trigger regional extinction occurrences (Mir et al. 2016). Animals often get lost from their natural habitat for a variety of causes, including habitat degradation, natural disasters, lack of food and so on. To safeguard them in their natural or to restrict their haphazard use by people, each government has a wildlife conservation policy that includes the establishment of protected areas such as national parks, wildlife reserves, conservation areas, and so on (Shrestha & Maharjan 2008).

The Red Panda (*Ailurus fulgens*) is an endangered species that primarily lives in temperate forests of China, Bhutan, India, Myanmar, and Nepal (Yang et al. 2019). For starters, the red panda is taxonomically distinct, after much research, it is still uncertain whether it is most closely related to bears, procyonids, or the Giant Panda *Ailuropoda melanoleuca*. Most biologists would classify it as a monotypic subfamily. Second, numerous aspects of its biology and ecology indicate that it may be on the verge of extinction, at least in the Himalayan region of its range. Third, it is an incredibly beautiful animal that might serve as a flagship species to galvanize global public support for responsible natural resource management throughout its range (Yonzon & Hunter 1991).

The Red Panda is smaller than the Giant Panda, has a longer tail, a distinct body color and the soles of its feet are covered in a dense layer of wool. Although visitors to zoos are familiar with this lovely creature, its status and range in the wild are unknown (Choudhury 2001).

1.2 Distribution, Habit and Habitat

The Red Panda is confined to the southern slopes of the Himalayas (Roberts & Gittleman, 1984) and in China is endemic to the Hengduan and Himalayan Mountains. This species is distributed primarily in the temperate forests from central Nepal eastward along the Himalayas through Bhutan, India, Sikkim and Burma, northward into China (Roberts & Gittleman 1984; Wei et al. 2000). Both sub-species, *A. f. fulgens* and *A. f. styani* are to be found in China. However, the *styani* subspecies is more typical of the region. *Ailurus fulgens* is found throughout the Himalayas, in Tibet and the Gongshan Mountains of northwestern Yunnan province as well as to the southern Nujiang River (Patterson-Kane et al. 2009).

Red Pandas primarily inhabit fir Abies densa forests with bamboo undergrowth, typically at altitudes between 2,400 to 3,700 meters. They can be observed in several national parks and interconnected biological corridors within the protected areas of Bhutan. However, their habitat overlaps with rural human settlements that are undergoing increasing socio-economic development. Despite being culturally revered, red pandas face numerous threats in this shared environment. These threats include road construction, timber and bamboo harvesting, gathering of minor forest products, livestock grazing, inadequately regulated tourism practices, and the presence of domestic dogs (Dorji et al. 2012). Although the red panda is not classified as endangered like the giant panda, it encounters comparable challenges such as habitat fragmentation and loss. These challenges arise due to the expanding local human population engaging in agricultural activities and pursuing other needs, which directly impact the red panda's habitat (Wei et al. 2000; Zhao et al. 2020).

1.3. Characterstics and conservation status of Red Panda in Nepal.

Red panda is generally known as "Habre" in Nepal, "Naututoo" in the local dialects, and "Pude Kundo" in other areas. *Ailurus fulgens fulgens and Ailurus fulgens styani* are the two subspecies of the genus. *Ailurus fulgens fulgens* is the smallest and lightest of the two species. The Red Panda is a Himalaya native and a showcase species (Roberts & Gittleman 1984) which threatened with extinction worldwide (Wang et al. 2008).

The Panda measures approximately 42 inches (79-120cm) in length, which includes a lengthy and thick tail spanning from 30 to 60cm. This tail exhibits six alternating yellowish red transverse yellowish-brown rings. In size, it is slightly larger than a typical domestic cat, and their offspring are only slightly bigger than domesticated kittens. Male

pandas weigh between 4.5 to 6.2kg, while females weigh between 3.7 to 4.5kg. The upper parts of the panda's body are covered in long and soft reddish-brown fur, while the lower parts have blackish fur. The face is light in color and features tear markings, along with a tail adorned with light rings. The light face also bears white badges reminiscent of those seen on a raccoon, though each panda can have unique markings, totaling 17 in some cases. The panda's round head displays medium-sized upright ears, a black nose, and very dark eyes that are almost pitch black.

The Red Panda's legs are black, short, and resemble those of a bear. They have thick soles that serve as insulation against the cold on snowy or icy surfaces. Their claws are curved, sharp, and semi-retractable. Similar to the Giant Panda, they possess a unique feature called a "false thumb," which is an extension of the wrist bone. This false thumb enables them to grip and handle fruits effectively. Additionally, the red panda has thick fur on the soles of its feet, providing protection from the cold and hiding scent glands. With their large teeth and strong jaws, red pandas are capable of consuming rigid bamboo leaves. Apart from the mating season, the red panda typically prefers a solitary lifestyle and establishes its own territory. It is known to be both nocturnal, active during the night, and crepuscular, active during the twilight hours. During the daytime, the Red Panda rests by sleeping on tree branches or finding shelter in tree hollows. However, as the day progresses into the late afternoon and early evening, it becomes more active.

In hot weather, the Red Panda adopts a unique sleeping position by sprawling out on a branch, allowing its legs to swing freely. On the other hand, when it gets chilly, the red panda curls up, using its tail to cover its face and provide additional warmth. Red Pandas are great climbers and spend most of their time foraging in trees. They primarily consume bamboo, but may also consume small mammals, birds, eggs, flowers, and berries. They were observed in captivity eating birds, flowers, maple and mulberry leaves, as well as the bark and fruits of maple, beech, and mulberry (Roberts & Gittleman 1984).

It is extremely heat sensitive, preferring temperatures between 100° C. and 25° C. It cannot handle temperatures beyond 25° C. As a result, Red Panda rests in the shady uppermost branches of trees during the hot noontime, frequently sleeping spread out on forked branches or folded up in tree caves with its tail covering its face. It hunts for food at night, racing with speed and agility along the ground or through the woods, and once

found, it uses its front paws to transfer the meal into its mouth. Red Pandas drink by dipping their paws into water and licking them.

Upon awakening, Red Pandas engage in grooming behaviors akin to cats. They commence by licking their front paws, followed by scratching various parts of their bodies such as the back, belly, and sides. Additionally, they rub their backs and bellies against trees or rocks. They navigate the terrain, either dashing across the ground or maneuvering through wooded areas in search of sustenance. Red Pandas may utilize their front paws to convey food to their mouths or directly ingest it (Roberts & Gittleman 1984).

1.4 Parasitic Infection of Red Panda

Parasites, apart from their significance in terms of sanitation and economy, play a fundamental role as a component of the biosphere. They are an essential part of ecosystems, contributing to ecological processes and interactions within various organisms (Raga et al. 2009). In nature, practically no animal is free from parasites (Das et al. 2018). Parasites depend on their hosts for metabolic nourishment, establishing a mutually exclusive relationship. Infections caused by parasites can lead to illness and mortality in wild animals, and they can also serve as a source of infection for domesticated animals (Allwin 2015). In natural environments, wild animals inhabit vast territories, resulting in lower genetic resistance to parasitic infections due to reduced exposure compared to domestic herbivores. Consequently, wild ruminants are also susceptible to various internal parasites as they share the same pastures (Chaudhary 2014; Lim et al. 2008). The focus of this study is on protozoa and helminths, however parasites also include bacteria, viruses, and fungi. Endoparasites inhabit the internal regions of the host's body, such as the gut, body cavity, liver, lungs, gall bladder, pus, or other internal cavities, tissues, or cells, and ectoparasites reside on the host's outer surface or in the host's superficial tissues. Intestinal parasites are internal parasites that inhabit the host's digestive tract and can cause harm. Common parasites in this category include Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides and Strongyloides stercoralis. These parasites have a propensity for the alimentary canal and can result in various health issues (Sherchan et al. 2012).

The Red Panda itself can be infected trans-cutaneously by larval forms when in search of food and water on ground contaminated with feces. Pandas can also be infected through the consumption of vegetation and water contaminated with parasitic stages such as cysts, oocysts, eggs and larval forms. Infections can also occur while ingesting birds, insect and molluscs infected with trematodes or nematodes (Lama et al., 2015). These studies highlight the high prevalence and diversity of gastrointestinal parasites in red pandas, particularly nematodes and protozoans. The presence of multiple parasite infections also suggests the potential for interactions among parasites, which may have implications for the health and survival of red pandas.

1.4.1 Intestinal protozoan parasites

Protozoa are single-celled organisms that are widespread and commonly exist as freeliving entities, posing no harm. Intestinal protozoan parasites are a specific category of single-celled organisms that invade the intestines of both animals and humans. These parasites thrive and multiply within the gastrointestinal system, giving rise to various intestinal diseases that can weaken the host's immune response and adversely impact their overall physical well-being (Thawait et al. 2014).

1.4.2 Intestinal helminth parasites

Helminths are multicellular organisms with bilateral symmetry, elongated bodies that can be flat or round in shape. They are classified into three main categories based on their morphology: (1) nematodes or roundworms, which include hookworms, pinworms, whipworms, threadworms, and roundworms, (2) trematodes or flukes, and (3) cestodes or tapeworms.

Nematodes, often known as roundworms, have cylindrical long bodies tapering at both ends. Adult members of this group range from 5 mm-50 cm. They have a fully developed system of digestion as well as a robust and elastic exterior coat called the cuticle. Nematode mouth regions might be adapted for adhering to or feeding on their host. Some species, such as giant strongyles, have mouths capsules with tooth tailored for their particular use. Male nematode species have a feature called a bursa located near the back of their body. The bursa, which has a bell-shaped or funnel-shaped enlargement, is aided by the rays, that have finger-like extensions. Males use the bursa to attach to females while mating. Spicules, which are structures utilized by males to grab and widen the female's vaginal entrance, also aid in the coupling procedure. The form and configuration of the male bursa and spicules vary between nematode species, and are frequently used for determining their species (Shrestha 2015).

1.5 Statement of problems

As Red Panda are mammals, we can easily expect that the majority of the parasites present in them might be common to wild or domesticated mammals found around. Intestinal parasitic infections are prevalent in numerous developing countries across Asia and Africa (Tiwari et al. 2013). Several studies have revealed that the gastrointestinal parasites are highly responsible for the mortality of the Red Panda. The autopsy of a Red Panda in a UK zoo revealed large numbers of *Angiostrongylus vasorum* nematode larvae and eggs present in the lungs. The presence of these nematodes likely contributed to the respiratory distress and death of the animal (Patterson-Kane et al. 2009). This show, the Red Panda are at high risk of being affected by the endo-parasites especially protozoa and helminthes. Moreover, Limited studies were carried out regarding their health status and parasitic invasion. Though this is the severe issue, no national effort has been followed for the conservation of this endangered species. So, the major intention of conducting this research is to highlight the current status of the parasitic prevalence and parasitic burden in Red Panda.

This study puts aid in the conservation of the red panda as we are concerned with the health status and conservational issues. Moreover, we will also be focused on the various conservational issues and strategies for the conservation of Red Panda in this region and around the country.

1.6 Justification of the study

As parasitic transmission in between red pandas have huge possibilities with other animals in the wild. Their populations are in the risk due to parasitic load and cross infection in between. The available field data on wildlife diseases generally does not provide sufficient information to accurately evaluate the future risks of transmission between wildlife and coexisting livestock populations (Morgan et al. 2006). This study aid and provide the reliable information regarding the GI parasite prevalence in red panda from the particular study area. Also the status of parasitic burden and its impact on conservation of the red panda can be easily determined. This study mainly aids in the conservation of such species of higher importance.

1.7. Objectives of the study

1.7.1. General Objective

• To determine the occurrence of gastrointestinal parasites in red panda in the community forest of Suryodaya Municipality Ilam, Nepal.

1.7.2. Specific Objectives

- To analyze the prevalence of gastrointestinal parasites in red panda from community forest
- To assess the parasitic intensity and concurrency in red panda from the community forest

2. LITERATURE REVIEW

2.1. Scenario of Gastrointestinal Parasites in global context

Gastrointestinal parasites are a major concern globally, affecting a wide range of animal species, including humans. The prevalence and impact of these parasites vary between different animal species and regions. The following is a literature review on selected studies regarding different gastrointestinal parasites.

Unfortunately, there is a significant lack of available data regarding gastrointestinal parasites in wild mammals, as highlighted by Hewavithana et al. (2022). A recent survey revealed that 30% of the European red panda population (*Ailurus fulgens*) was infected with a newly discovered metastrongyloid nematode. Consequently, a follow-up study was conducted, which involved comparing four captive-bred red pandas that were naturally infected with two uninfected control animals. The study found that the infected red pandas had significantly higher levels of eosinophils, globulin, and fibrinogen than the uninfected control animals. The infected red pandas also had significantly lower levels of albumin, urea, and creatinine (Willesen et al. 2012).

Angiostrongylus vasorum is a nematode that is known to infect dogs and foxes, but it can also infect other mammals, including red pandas. In a study conducted in a UK zoo, a male red panda that was observed to have breathing difficulties and weight loss was found to have extensive mineralization and granulomatous inflammation in the lungs. The autopsy revealed large numbers of *A. vasorum* nematode larvae and eggs present in the lungs. The presence of these nematodes likely contributed to the respiratory distress and death of the animal (Patterson-Kane et al. 2009).

Raccoons are frequently infected with *Eimeria nuttalli* and *E. procyonis*, two species of protozoan parasites, as well as with *Sarcocystis*, a genus of parasitic coccidian protozoa. A study conducted in Illinois found that 58% of raccoons were infected with *E. nuttalli*, 25% with *E. procyonis*, and 17% with *Sarcocystis sp* (Adams et al. 1981).

Baylisascaris procyonis is a roundworm parasite that is primarily found in raccoons (Procyon lotor) in North America. In a study conducted in raccoons of eastern Tennessee, the overall prevalence of *B. procyonis* was 12.7%. The prevalence rates did not differ significantly between males and females or between adults and juveniles. *B. procyonis* has been found to infect over 100 species of other animals, including dogs, cats, foxes,

and rodents. The parasite has a complex life cycle that involves multiple hosts, including raccoons as the definitive host and various other animals as intermediate hosts (Souza et al. 2009). According to a study conducted in Texas by Long et al. (2006) on raccoons, the predominant parasite identified was *Macrocanthorhynchus ingens*, observed in 11 out of 19 raccoons. Additionally, the most abundant parasite found was *Atriotaenia procyonis*, with a total count of 63 occurrences.

Researchers investigated the potential of *Baylisascaris transfuga* larvae to cause ocular larva migrans (OLM) in mice. They concluded that the larvae have the capacity to cause OLM in mice through oral infection and therefore, could potentially cause OLM in other animals and humans (Papini et al. 1996). The process of *Baylisascaris tasmaniensis* larvae developing into the adult stage was examined using laboratory mice and Tasmanian devils as experimental hosts. The first moult occurred in the egg, and the second moult occurred in the tissues of mice 1-2 weeks after ingesting embryonated eggs. The larvae migrated to different organs (Sprent & MoKeown 1973).

2.2. Scenario of gastrointestinal parasites in context of Nepal

The Red Panda (*Ailurus fulgens*) is an endangered species and its survival is threatened by various factors including habitat loss, poaching and disease. Parasitic infections are among the diseases that can affect the health and survival of red pandas. Wild ruminants can contract the intestinal parasite from domestic ruminants when they share the same pasture, and vice versa, the parasite can be transmitted from wild ruminants to domestic ones in similar circumstances (Airee 2018). Several studies have investigated the prevalence and diversity of gastrointestinal parasites in red pandas in different regions of Nepal.

In national context, the study on the prevalence of Gastrointestinal parasites in red pandas are relatively less in number than any other mammals despite being an endangered species. The majority of research on red pandas has focused on their ecology, behavior, and population status across various national parks, conservation areas, and hunting reserves. Several studies, such as Yonzon's (1989) and Yonzon and Hunter's (1991) research, as well as Sharma and Belant's (2009, 2010) work, have contributed to this understanding. Shrestha & Maharjan (2017) conducted a study that examined 55 fecal samples (red pandas, n=14; cattle, n=41) from a community forest, all samples were found to be positive for protozoan or helminth parasites. The recorded protozoan parasites

were *Eimeria* sp., *Entamoeba* sp. and *Balantidium* sp. with prevalences of 64.28%, 57.14%, and 14.28% in red pandas and 60.97%, 21.95% and 7.31% in livestock, respectively. Among helminth parasites, seven nematodes were recorded in red pandas with various prevalence rates: *Oxyuris* (100%), *Ascaris* (57.14%), *Trichostrongylus* (50%), *Strongyloides* (50%), *Trichuris* (42.8%), *Crenosoma* (42.85%), and Hook Worm (35.7%). No trematode or cestode were found. On the other hand, in livestock, six nematodes were recorded: *Oxyuris* (87.8%).

In a study done in Rolpa, among 23 samples, all the samples were found to be positive for gastrointestinal parasites (100%) where the occurrence rates for different groups of parasites were as follows: protozoa 100.0%, nematodes 52.2%, trematodes 13.0%, and cestodes 4.3%. Out of the 23 samples positive for protozoa, 16 samples contained protozoan species of a size ranging from 10–14 μ m diameter, which we were unable to identify. Five other samples contained coccidian parasites of various oocyst characters. One sample contained oocysts from 4–5 μ m in diameter resembling *Cryptosporidium*, one sample contained oocyst from 8–9 μ m in diameter resembling *Cyclospora*. Out of 12 samples positive for nematodes, *Trichuris* spp. were found in six samples and *Baylisascaris* spp. were found in three samples. Larval forms resembling the first stage of *Angiostrongylus* larvae were found in two samples (Lama et al. 2015).

Other several studies have investigated the prevalence and diversity of gastrointestinal parasites in red pandas. Shrestha (2015) found that 93% of fecal samples of red panda from Rara National Park contained parasites, with 12 different parasite species identified, including two protozoan and ten helminth species. The most prevalent parasite was *Eimeria*, followed by *Entamoeba* and *Oxyuris* sp. where multiple parasite infections were common.

Similarly, Bista et al. (2017) collected 272 fecal samples from the red panda distribution range in Nepal and found that 90.8% of the samples had parasites, with nematodes being the most common (87.62%). The most prevalent parasite was *Ancyclostoma duodenale*, followed by *Entamoeba histolytica* and *Ascaris lumbricoides*. Seven different species from three genera of Protozoans, Cestodes, and Nematodes were identified. These studies highlight the high prevalence and diversity of gastrointestinal parasites in red pandas, particularly nematodes and protozoans. The presence of multiple parasite infections also

suggests the potential for interactions among parasites, which may have implications for the health and survival of red pandas.

Another study by (Sharma & Achhami, 2022) in Rara National park and Langtang National Park, nematode and coccidian prevalence were high in both livestock and red pandas in two national parks (prevalence range 0.93-1 and 0.67-1 respectively), while trematode prevalence was low (0-0.27) and cestode prevalence was intermediate with high variability across hosts and sites (0.07-0.87). Livestock generally had higher prevalence than red pandas, except for a few taxa in each park. Eight parasite taxa were shared between livestock and red pandas, while *Ancylostoma* spp. and *Moniezia* spp. were unique to livestock and *Fasciola* sp. was unique to red pandas.

A study conducted in the Langtang National Park in musk deer identified four types of nematodes, one cestode, one trematode, and one protozoan parasite, with *Ascaris sp.* being the most prevalent (88.89%). Heavy infestations were found in most samples, and multiple parasite infections were observed in some individuals (Langtang et al. 2016). Another study conducted in Langtang National Park found that the prevalence of gastrointestinal parasites in wild ruminants and chauri was 85.92%, with seven species of parasites identified. The study included 71 fecal samples from Himalayan Tahr, Barking Deer, Musk Deer, and Chauri. The parasites found included one protozoan, four nematodes, one cestode, and one trematode. There was no significant difference in parasite types between the two groups (Achhami 2016). There is huge possibilities of cross infection of red panda with other wild mammals as well as other domestic animals because they may share the same habitats with Red Panda.

3. MATERIALS AND METHODS

3.1. Study Area

Suryodaya Municipality, which derives its name from the Nepali translation of "Rising Sun," was established in 2013 through the amalgamation of various Village Development Councils such as Fikkal, Panchakanya, and Kanyam. Following the promulgation of the constitution of Nepal in 2015, additional Village Development Councils were merged, including Gorkhe, Pasupatinagar, Sriantu, Samalbung, Laxmipur, and Jogmai, thereby expanding the municipality's area to 225.52 sq. km. It is centrally located in the Ilam District, bordered by Darjeeling in the west, Ilam Municipality in the west, Rong Rural Municipality in the south, Maijogmai Rural Municipality in the north, and Mai Municipality in the southwest. The municipality features three transit points with India, namely Pasupaitinagar, Chhabise, and Manebhanjang. Geographically, it is situated in the hilly region known as the Mahabharat range, characterized by its natural beauty and pleasant climate.

A forest refers to an extensive expanse primarily characterized by the presence of trees. Across the globe, numerous specific definitions of forests are employed, taking into account various factors such as the density and height of trees, land utilization, legal status, and ecological role. The relationship between human society and forests involves mutual impacts, encompassing both positive and negative aspects. Forests offer valuable ecosystem services to humans and serve as popular destinations for tourists. However, forests can also influence human well-being, including potential effects on health. It is important to note that human activities, such as the extraction of forest resources, can have adverse consequences on forest ecosystems.



Figure 1: Nepal showing Koshi province, Ilam district, Suryodaya municipality, Illam and Gorkhe in the Suryodaya Municipality.

Gorkhe lies in the Suryodaya municipality ward no.1 of Ilam District of Eastern Nepal. It is about 48km in the eastern part of Ilam bazar where 5358 peoples in habitate. Gorkhe cover an area of 25.27 sq.km. Most of its land lies in the Mahabharat range. Gorkhe has three major community forest they are Singhadevi community forest (2.063 sq.km), Chhipchhipe community forest (0. 24 sq.km) and Chitrehile community forest (2.22 sq.km). Red panda were recorded in these forest in limited number. This site is barely selected under the study area of different researchers due to which the most valuable and endangered species of our country is being underrated. Currently, the RPN (Red Panda Network), a famous organization is working actively on the conservation of this species in Nepal, has kept limited forest rangers for the surveillance of the red panda of these community forest.

3.2. Materials

3.2.1. Materials in the field

- i. Measuring tape
- ii. Vials
- iii. Gloves

3.2.2. Materials in the laboratory

| i. Gloves | xi. Centrifuge tubes (15ml) |
|--------------------|--|
| ii. Glass slides | xii. Centrifuge machine (Remi) |
| iii. Cotton | xiii. Electronic weighing machine |
| iv. Tooth picks | xiv. Motor and pestle |
| v. Cover slips | xv. Refrigerator |
| vi. Strainer | xvi. Glass rod |
| vii. Filter Stand | xvii. Compound microscope (Swift) |
| viii. Test tubes | xviii. Petri dishes |
| ix. Beakers | xix. Droppers |
| x. Test tube Stand | xv. Occulo and stage micrometer (Erma Inc) |

3.2.3 Chemical reagent

| i. 2.5% potassium dichromate | iv. Lugol's Iodine solution (1%) |
|---------------------------------------|--------------------------------------|
| ii. Normal saline solution (0.9% w/v) | v. Saturated salt solution (45% w/v) |
| iii. Distilled water | vi. Methylene blue |

3.3. Study Design

The study was designed so as to assess the gastro-intestinal parasitic infection in the Red Panda (*Ailurus fulgens*) in the community forest of Suryodaya Municipality (Gorkhe), Illam by the collection of fecal pellets using the line transect method and the analysis of the parasitic eggs and oocyst using standard methods.

3.3.1. Sample size

A total of 36 fecal samples from red panda were collected from three different community forest from the East-West and North-South Slope of Hills during the month of December, 2022.

3.3.2. Sampling technique

East-West and North-South slope of the hills from three different community forest of Suryodaya municipality viz. Singhadevi, Chitrehile and Chhipchhipe was selected for the collection of faecal samples of Red Panda. These three sites were marked first and each sites were laid with four vertical or horizontal line transect of 500m each from top of the hills to downward. The samples were collected from three different community forest in order to prevent their repetition. Samples were collected altogether from the three different site and was preserved in 2.5% of potassium dichromate solution in an airtight sterile urine vials.

For the first site i.e. Singhadevi community forest, East-West facing hill was selected and four baseline points were marked on trail at the top of hill. Baseline points were marked at the distance of 100m. The transects were laid and the samples were searched thoroughly at the 5m distance on each side of the transect down the slope. Similarly for the second site i.e. Chhipchhipe community forest, the samples were collected in similar fashion as first site. For the third site, i.e., Chitrehile community forest, the vertical line transects were laid along the hill facing North-South and the samples were collected following the same technique as mentioned above.

The Samples were gathered in the airtight sterile urine vials. The necessary information was clearly marked, such as the date of faecal pellet collection, altitude, transect and plot and so on. Inorder to preserve the protozoan cysts and helmiths egg the samples were stored in the vials with 2.5% potassium dichromate and was transported to the parasitology laboratory of the Central Department of Zoology.

3.4. Examination of fecal sample

The obtained samples were stored in sterile vials with 2.5% potassium dichromate solution and transported to the laboratory, where they were refrigerated at 4° Centigrade. In the mortar, 5 gram of the sample was placed, and 10 mL of saline (0.9% NaCl) was added. The mixture was strained using a tea strainer. The filtrate was investigated using three different techniques (Ghimire & Bhattarai, 2019) in the lab of the Central Department of Zoology. Samples were inspected macroscopically to confirm the presence of nematodes, cestodes, and/or parasite fragments, and microscopically by flotation test employing a low-specific-gravity solution and sedimentation (Fagiolini et al. 2010).

The laboratory techniques followed are mentioned below:

3.4.1. Floatation technique

This approach detects nematode and cestode eggs in the feces of Red Pandas and other animals. It also insures that the eggs float in the floatation liquid, which aids in the identification of the eggs (Shrestha & Maharjan 2017).

The flotation reagent utilized in this procedure is a saturated salt mixture. It was made by combining 500 g of NaCl solution with 1 liter of distilled water. Following the making of the saturated salt solution, 2 g of fecal samples were mixed thoroughly in a 13 milliliter (mL) solution of normal saline (0.9% w/v) and filtered through a tea strainer. The solution was centrifuged for 5 minutes (1200 revolutions per minute, rpm) in a 15mL centrifuge tube. Following supernatant separation, 12mL of 45% w/v salt solution was added and centrifuged (1200 rpm for 5 minutes). After that, a few drops of salt solution (45% w/v) were added to the tubes to create a convex surface at the very top, and a coverslip was placed over the mouth. After ten minutes, the coverslip was carefully removed and placed on the glass slide, either with or without Lugol's iodine, for microscopic examination at 10X and 40X magnifications (R.B. Adhikari et al. 2020).

The photographs of cysts, eggs and parasites were obtained and identified with the help of the physical characteristics like; color, shape and size of the eggs. Measurements of egg were taken with an ocular micrometer attached to a compound microscope. To determine the size, the calibration factor was used to multiply the number of divisions on the ocular micrometer that were spanned by the parasite oocysts or eggs (Dhakal et al. 2023).

3.4.2. Sedimentation technique

A sedimentation approach was used to detect trematode eggs. Trematode eggs are often heavier than others helminth eggs, therefore sedimentation is the ideal approach for identification of trematodes.

For this method approximately 2-3 grams of the fecal samples were taken in a mortar and pestle and was minced finely. Then the sample was mixed in a normal saline and was filtered with the help of tea strainer and kept in a conical centrifuge tube. After which the filtrate was centrifuged at 1200 rpm for about 5 minutes in a centrifuge machine. Then the supernatant was discarded and the residue or pellets in the centrifuge tubes were taken for the further examination where the samples were examined under a compound microscope .The slides were prepared by placing a drops of Lugol's Iodine solution and the samples in a clean glass slides with the help of dropper and mixed thoroughly with the help of a toothpick and was spread placing clean coverslip on to it. The slide of the fecal sample smear was then observed under the compound microscope at 10x and 40x.

Iodine wet mount is mostly used to examine protozoan cysts, Pale refractile nuclei, yellowish cytoplasm, and brown glycogen material are visible in iodine stained cysts. Helminthic eggs and larvae are demonstrated using a standard saline wet mount. It is also used to treat intestinal protozoa motile trophozoites. Methylene blue is used to show nuclei in protozoan trophozoites (Shrestha 2015).

3.4.3. Determination of concurrency and intensity

3.4.3.1. Concurrency

The host harbors one or more parasites with low numbers; in such circumstances, the host does not develop sickness yet might continue to be a source of infection for an extended length of time. Single, double, triple, quadruple, quintuple, and many infections of gastrointestinal parasites were classified. A single infection was defined as the presence of only one egg/cyst/larva of the parasite per field, whereas double, triple, quadruple, quintuple, and multiple infections were characterized as the presence of two, three, four, five, or six or more egg/cyst/larva of the parasite per field.

3.4.3.2. Intensity

The total amount of eggs/oocysts and larvae detected per field was used to calculate the intensity of parasite infestation. The level of gastrointestinal parasite infection was

divided into four categories. The presence of less than two eggs/cysts/larva of the same species per field was used to assess light infection. In a similar manner the presence of 2-3 egg/cyst/larva, 4-5 egg/cyst/larva, and 6 or more egg/cyst/larva of the same species per field characterized mild, moderate, and heavy infections.

3.5. Data analysis

- Data entry was done in Microsoft Excel Sheet for the statistical analysis.
- Chi-square test was used for testing of significance, p≤0.05 at 95% considered as statistically significant.
- Statistical analysis was done in SPSS V22.

4. RESULTS

The coprological study was conducted from the of fecal samples of Red Panda (*Ailurus fulgens fulgens*). A total of 36 faecal samples were collected from three different community forests (viz. Singhadevi, Chhipchhipe and Chitrehile) of Suryodaya Municipality (Gorkhe), Illam from December 23,2022 to December 27, 2022, where as 15, 13 and 8 fecal samples of red pandas were collected from Singhadevi, Chhipchhipe and Chitrehile community forests, respectively.

4.1. Prevalence of gastrointestinal parasites in Red Panda

Among the 36 samples examined, 31 (86.11%) were found to be positive for gastrointestinal parasites, and the remaining 5 (13.89%) were found to be negative. Among them, 25(69.44%), 23(64%) and 5 (13.89%) were found positive for protozoan, nematode and cestode respectively.



Figure 2: Class wise prevalence of gastrointestinal parasites in Red Panda of Gorkhe Community Forest.

Forest specific class wise gastrointestinal parasitic distribution indicated that, Singhadevi Community forest showed the highest prevalence rate with 93.33% followed by Chitrehile with 87.50% and Chhipchhipe (76.92%). Similarly coming to class, Singhadevi forest showed the highest prevalence rate of Nematoda followed by Chitrehile and Chhipchhipe 100%, 62.50% and 53.84% respectively. The prevalence rate of class

Cestoda was comparatively low than other classes in all the community forest. Here, Singhadevi and Chitrehile both showed the identical prevalence rate of this class with 25% each followed by the prevalence rate of Chhipchhipe forest which was 8%.



Figure 3: Prevalence on the basis of Classes of Gastrointestinal parasites in Red Panda in different community forest of Gorkhe.

| S.N | Class | Name of species | Forest spe | Forest specific class wise Prevalence | | | |
|-----|----------|----------------------|------------|---------------------------------------|----------|---------------|--|
| | | | of Species | of Species | | | |
| | | | SCF, | CCF, n=13 | CHCF, | species(among | |
| | | | n=15 | | n=8 | 36 samples) | |
| 1 | Protozoa | <i>Eimeria</i> spp. | 8(53.33%) | 7(53.84%) | 3(37.5%) | 18(50%) | |
| 2 | | Entamoeba spp. | 6(40%) | 4 (30.76%) | 4(50%) | 14(38.89%) | |
| 3 | Nematoda | Strongyloids sp. | 2(13.33%) | 2(15.38%) | 0(0%) | 4(11.11%) | |
| 4 | | Capillaria spp. | 2(13.33%) | 0(0%) | 1(12.5%) | 3(8.33%) | |
| 5 | | Trichostrongylus | 1(6.67%) | 0(0%) | 1(12.5%) | 2(5.56%) | |
| | | spp. | | | | | |
| 6 | | Strongyle | 2(13.33%) | 2(15.38%) | 1(12.5%) | 5(13.89%) | |
| 7 | | Trichuris spp. | 1(6.67%) | 1(7.69%) | 2(25%) | 4(11.11%) | |
| 8 | | Ascarid | 7(46.67%) | 2(15.38%) | 0(0%) | 9(25%) | |
| 9 | Cestoda | <i>Moniezia</i> spp. | 2(13.33%) | 1(7.69%) | 2(25%) | 5(13.89%) | |

Table 1: Forest specific class wise Prevalence of Gastrointestinal Parasites in Red Panda

The association between three different community forest with the general prevalence of Gastro-intestinal parasites showed that there is no significant difference between them (χ^2 =2.00, d.f =2, p>0.05).

4.1.1. Prevalence of protozoan parasites in Red Panda

Out of the total 36 samples examined, two genera of Gastrointestinal protozoan parasites were obtained. The protozoan parasites were identified as *Eimeria* sp. and *Entamoeba* sp. Among 36 samples 18(58%) samples were positive for *Eimeria* sp. and 14 samples were positive for *Entamoeba* sp.

As we have determined that the protozoan parasites had the highest prevalence rate among all the parasites in total sample .The two genera of protozoan parasites i.e. *Eimeria* sp. and *Entamoeba* sp. also showed same kind of similar prevalence rate in the forest level. Prevalence of *Eimeria* sp. In both Singhadevi and Chhipchhipe community forest were found similar but in Chitrehile prevalence was comparatively less.

The association between the two different kinds of protozoan parasites showed there is no significant difference with their prevalence ($\chi^2 = 0.500$, p>0.05, d.f. =1).



Figure 4: Forest wise prevalence of protozoan parasites

4.1.2. Prevalence of helminth parasites in Red Panda

Altogether, 7 different types of helminthes parasites were recorded from Red Panda. Where only one genera of Cestode and among 7 genera, 6 types of parasites were found to be intestinal worms (*Strongyloides* sp., *Capillaria* sp., *Trichostrongylus* sp., Strongyle, *Trichuris* sp. and Ascarid).

Among all the 36 samples, Ascarid showed the highest prevalence (9 i.e. 25%) followed by Strongyle and *Moniezia* sp. (5 each; 13.89%), *Strongyloides* sp. and *Trichuris* sp. (4 each; 11.11%), *Capillaria* sp.(3, i.e., 8.33%), *Trichosatrongylus* spp.(2, i.e., 5.56%).

In Singhadevi forest, the most prevalent parasite was Ascarid (7 i.e. 46.67%) followed by *Strongyloides* sp., *Capillaria* sp., Strongyle and *Moniezia* sp. (2 each i.e. 13.33%), *Trichostrongylus* sp. and *Trichuris* sp. (1 each; 6.67%). Similarly, in Chhipchhipe forest, the prevalent rates of three helminth parasite i.e. *Strongyloides* sp., Strongyle and Ascarids was identical (2, i.e., 15.38%) followed by *Trichuris* sp. and *Moniezia* sp. (1each; 7.69%). Here, *Capillaria* sp. and *Trichostrongylus* sp. showed no prevalence. In the Chitrehile community forest, the highest prevalence was of *Trichuris* sp. and

Moniezia sp. (2 each; 25%) followed by *Capillaria* Spp., *Trichostrongylus* spp., Strongyle and *Strongyloides* sp.

The association between the different kinds of helminth parasites with their prevalence showed that there is no any significant difference ($\chi^2 = 0.224$, d.f = 6, p>0.05). Similarly, the association between protozoan and helminth parasites showed that there is significant difference in between these parasites ($\chi^2 = 0.001$, d.f. =8.p<0.05).

4.2. Concurrency and intensity of gastrointestinal parasites in Red Panda

Infections of the gastrointestinal tract in animals from the wild, including the Red Panda, are a regular occurrence. The parasite's high intensity is required to create a pathogenic consequence in the host. Many hosts have one or more parasites in low numbers; in such circumstances, the hosts do not develop disease but could remain a source of infection for an extended length of time.

4.2.1. Concurrency of gastrointestinal parasites in Red Panda

In the current study, the multiple infections were observed in Red Panda. Out of 31 (86.11%), positive samples 23 samples were found to have mixed infection with 2 to 5 Species in each microscopic field. Among 31 positive samples, single and double infections were found to be the highest where 16(51.61%), 8(25.80%), 5(16.12%) and 2(6.45%) samples were infected with Double, Single, Triple and Quadruple infection respectively.

| S.N | Concurrency | Occurency | Percentage of | |
|-----|---------------------|-----------|---------------|--|
| | | | occurrence | |
| 1 | Single Infection | 8 | 25.80% | |
| 2 | Double Infection | 16 | 51.61% | |
| 3 | Triple Infection | 5 | 16.12% | |
| 4 | Quadruple Infection | 2 | 6.45% | |

Table 2: Table showing concurrency of parasites in Red Panda

4.2.2. Intensity of infection of gastrointestinal parasites in Red Panda

The intensity of Gastro-intestinal parasite was obtained by counting the total number of eggs or cyst per microscopic field. Among 18 positive samples for *Eimeria* sp., 3(16.67%), 9(50%), 5(27.78%) and 1(5.56%) showed light, mild, moderate and heavy infection respectively. Out of 14 positive samples for *Entamoeba* sp. 9(64.28%), 4(28.57%) and 1(7.14%) showed light, mild and moderate infection respectively. Similarly, *Strongyloides* spp., *Trichostrongylus* spp. and Strongyle showed 4(100%), 2(100%) and 5(100%) of light infection. Other heminthes, *Capillaria* spp. 2(66.67%) and 1(33.33%), *Trichuris* spp. 3(75%) and 1(25%), Ascarid 6(66.67%) and 3(33.33%), *Moniezia* spp. 4(80%) and 1(20%) samples showed mild and moderate infection respectively.

| | | | Light | Mild | Moderate | Heavy |
|------|----------|------------------------|----------|----------|----------|---------|
| S.N. | Class | Name of species | (+) | (++) | (+++) | (++++) |
| | | | 3 | 9 | 5 | 1 |
| 1 | Protozoa | <i>Eimeria</i> spp. | (16.67%) | (50%) | (27.78%) | (5.56%) |
| | | | 9 | 4 | 1 | |
| 2 | | Entamoeba spp. | (64.28%) | (28.57%) | (7.14%) | - |
| | | Strongyloides | 4 | | | |
| 3 | Nematoda | spp. | (100%) | - | - | - |
| | | | 2 | 1 | | |
| 4 | | <i>Capillaria</i> spp. | (66.67% | (33.33%) | - | - |
| | | Trichostrongylu | 2 | | | |
| 5 | | s spp. | (100%) | - | - | - |
| | | | 5 | | | |
| 6 | | Strongyle | (100%) | - | - | - |
| | | | 3 | 1 | | |
| 7 | | Trichuris spp. | (75%) | (25%) | - | - |
| | | | 6 | 3 | | |
| 8 | | Ascarid | (66.67%) | (33.33%) | - | - |
| | | | 4 | 1 | | |
| 9 | Cestoda | <i>Moniezia</i> spp. | (80%) | (20%) | - | - |

Table 3: Table showing intensity of parasites in Red Panda

| + = Light infection i.e. less than 2 ova per field |
|---|
| ++ = Mild infection i.e. 2-3 Ova per field |
| +++ = Moderate infection i.e. 4-5 Ova per field |
| ++++ = Heavy infection i.e. 6 or more ova per field |

5. DISCUSSION

The present study revealed two genera of gastrointestinal protozoan parasites and helminthes parasites. Altogether, 7 different types of helminthes parasites were recorded from Red Panda. Out of 14 fecal samples in a study conducted by (Shrestha & Maharjan 2017) in the Illam community forest 100% of the sample showed prevalence for gastrointestinal parasites which was similar to this study(86.11%). This prevalence was also almost similar to the study done by (Lama et.al, 2015; Shrestha, 2015) where their study showed 93.02% and 100% in Rara National Park and KBCA Rolpa (Lama et al. 2015). But were higher than the study done in different mammals and primates in Italian zoo; 61.5% (Fagiolini et al. 2010).

This study also showed the similar prevalence rate, i.e., 90.8% from the Red Panda samples of Nepal. In a study done by (Bista et al. 2017) the prevalence rate seems quite higher than the study done by (Sharma & Achhami 2022) done in Rara National Park and Langtang National Park which was 67% in Red Pandas but was higher in livestocks; 93%. The result in this study also showed the identical prevalence with the study done in a Musk deer and Chauris in Langtang National Park (Achhami 2016; Langtang et al. 2016) which was 88.89% and 85.92% respectively which shows that the cross infection of gastrointestinal parasites and their diet is the major factor for the higher prevalence. Specially, the grazing mammals are at high risk of getting infected.

The class wise prevalence in this study showed highest prevalence of protozoan(69.44%) followed by Nematoda (74%) and Cestode (16.12%). Here, the Cestoda showed relatively low prevalence which is similar to the result of (Shrestha 2015). It also showed that the prevalence of protozoan parasites are common. The low prevalence of *Moniezia* sp. might be due to its food habit as Red panda do not graze on pasture and there is possibility for cross infection between other mammals is quite less.

This study also showed forest specific class-wise gastrointestinal parasite distribution where among three forest Singhadevi Community Forest had the highest prevalence (93.33%) followed by Chitrehile Community Forest (87.50%) and Chhipchhipe Community Forest (76.92%). This study showed the prevalence of Protozoan parasites in all the community forest are almost similar. Alike, Singhadevi Community Forest (100%) had the highest prevalence of class Nematoda followed by Chitrehile Community Forest (62.50%) and Chhipchhipe Community Forest (53.84%) respectively. Chitrehile and

Chhipchhipe Community Forest had the low prevalence as compared to Singhadevi Community Forest. And for the class Cestoda Singhadevi Community Forest (25%) and Chitrehile Community Forest (25%) had the identical prevalence rate and Chhipchhipe Community Forest (8%) had the least prevalence.

Forest wise prevalence showed SCF had the highest prevalence rate among other forest which might be due to the abundance of food and water resources, forest territory and enough pasture land for other domesticated mammals which increases the probability of cross transmission. The prevalence of protozoan parasites in Red Panda in Illam community forest and Rara National Park was done where Illam community forest and RNP showed the prevalence of *Eimeria* sp. to be 67.44% and 64.28% respectively. Similarly, *Entamoeba* sp. 62.79% and 57.4% in Illam community forest and RNP respectively. It was almost similar to the result of the study. (Sharma & Achhami 2022) also showed the prevalence of coccidian parasite to be the highest in their study which was 67%.

The forest-wise prevalence of protozoan parasites in this study showed Singhadevi and Chhipchhipe Community Forest had the highest prevalence for *Eimeria* sp (i.e. 53.3% and 53.84%) than Chitrehile Community Forest (37.50%). Chitrehile Community Forest (50%) had the highest prevalence for *Entamoeba* sp. than the Singhadevi Community Forest (40%) and Chhipchhipe Community Forest (30.76%).

In a study in RNP and Illam community forest recorded ten and seven helminthes parasites respectively which is in the similar ratio to this study which is seven. The highest prevalent helminth parasite was *Oxyuris* sp. With 48.84% and 100% in RNP and Illam community forest respectively (Shrestha 2015; Shrestha & Maharjan 2017). In the similar study of RNP, Ascarid had the highest prevalence among the helminthes recorded which was 25%.

There might be the higher chances of occurrence of various types of infection in Red Panda. Once they are infected by the parasitic eggs or cysts it is certain that the infection ascends day to day progressively. In wild no any specific treatment are received by the infected animals due to which the various types of parasites get the favorable environment for the persistency of their eggs, cysts or adult in their host species. Among total of 36 samples, double and single infections were found to be highest with 51.61% and 25.80% respectively, followed by triple (16.12%) and quadruple (6.45%). Double

infection was seen in 16 samples and Single infection was seen in 8 samples. The Concurrency of parasites in the fecal samples of red panda revealed that the double infection was higher (51%) which was different than the result of (Shrestha 2015) in Red Pandas of RNP where multiple infection was recorded to be the highest of all. As we know that the eggs or oocysts could serve as reliable indicators of infection intensity. The findings of the study indicated that both protozoans were heavily infected, suggesting a high level of intensity. It is important to consider that several factors can influence the intensity of parasitic infections, including the host's immune response, environmental conditions, and the life cycle of the parasites. Analyzing and quantifying the eggs or oocysts present in the host's feces provides valuable insights into the extent of the infection and its potential impact on the host's well-being.

Out of all 36 samples, the intensity of the parasite was determined by counting the total number of eggs or cyst per microscopic field. Here, the intensity of parasites were classified into four groups on the basis of density of eggs or cyst present per microscopic field. Here, in this study the intensity is determined as: less than 2 ova per field i.e. light infection, 2-3 Ova per field i.e. mild infection, 4-5 Ova per field i.e. moderate infection and 6 or more ova per field i.e. heavy infection.

Among the total samples 50% of sample were mildly infected and only 5.56% were infected heavily by *Eimeria* sp. Similarly, 64.28% of samples had light infecton of *Entamoeba* sp. and 28.57% was mildly infected. In helminthes, no any samples had moderate and heavy infection. Maximum number of samples showed light infection followed by mild infection. Here, 100% of samples positive for *strongyloides* spp. were lightly infected. Similarly, *Capillaria* (66.67%), *Trichostrongyle* (100%), Strongyle (100%), *Trichuris* (75%), Ascarid (66.67%) and *Moniezia* spp. (50%) were lightly infected. Among helminthes, double infection is seen only in *Capillaria* (33.33%), *Trichuris* (25%), Ascarid (33.33%) and *Moniezia* (1%). This shows the light infection are asymptomatic and one not likely to cause serious infections but heavy infection may cause serious health issues in Red Panda.

The present study has few limitations, for instance; some parasites have not been identified to their species level and the number of fecal sample was low. Despite these limitations, the study was conducted using two different concentration techniques to assure the cysts or eggs of the parasites.

6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Based on the results of the coprological study conducted on Red Pandas (*Ailurus fulgens*), several key findings have emerged. A total of 36 fecal samples, 31 (86.11%) tested positive for gastrointestinal parasites, while the remaining 5 (13.89%) were negative. Protozoan parasites were the most prevalent, with 69.44% of samples testing positive, followed by nematodes (64%) and cestodes (13.89%).

Multiple infections were observed in the Red Panda samples, with 23 out of 31 positive samples (74.19%) showing mixed infections with 2 to 5 species in each microscopic field. The majority of infections were either single or double, accounting for 51.61% and 25.80% of the positive samples, respectively. The intensity of gastrointestinal parasites varied, with different levels of infection observed. The prevalence of infection ranged from light to heavy, depending on the parasite and species.

In conclusion, the coprological study conducted on Red Pandas revealed a high prevalence of gastrointestinal parasites, particularly protozoans and helminths. The study highlighted variations in prevalence rates across different community forests and identified specific parasites with varying levels of intensity. These findings contribute to our understanding of the health and parasitic burden in Red Pandas, which can help in building up the various conservational strategy for the protection of the Red Panda.

6.2 RECOMMENDATIONS

1. Different national or international organizations should get focused on developing the veterinary hospitals and modern research centers for protecting and conserving the endangered animals by consistently monitoring the health conditions in the wild.

2. Further research on probable source of infection is mandatory to reduce parasitic burden in Red Panda.

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PHOTOGRAPHS





Strongyle egg (136 μm in length) at iodine ×400

Egg of Ascarid (52 μm in length) at iodine×400



Oocyst of *Eimeria* spp. with micropyle (22 µm)×400



Oocyst of *Eimeria* sp. at methylene blue



Strongyle egg (54 \times 32 µm) at Iodine \times 400 Oocyst of *Eimeria* sp. without micropyle (26 µm)×400



Egg of *Capillaria* spp. (85 μm in length) at iodine

Egg of *Trichostrongylus* sp. (92×50µm)

Egg of *Trichuris* spp. $(52.5 \times 22 \mu m)$ in 400



Feces of leopard in the forest

Livestock captured in the forest area

Common water resource (pond)



Fecal samples of Red Panda being collected

Microscopic analysis of the samples in the laboratory

Habitat of red panda (bamboo forest)