

**PREVALENCE OF GASTROINTESTINAL HELMINTH PARASITES
OF BARKING DEER (*Muntiacus vaginalis* Boddaert, 1785) IN
SHIVAPURI NAGARJUN NATIONAL PARK, KATHMANDU,**

Nepal



Entry 05

M.Sc. Zoo Dept. Parasitology..

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degree of Master of Science in Zoology with special paper Parasitology.**

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Kirtipur, Kathmandu, Nepal

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been 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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LETTER OF APPROVAL

On the recommendation of supervisor “**Professor Dr. Mahendra Maharjan**” this thesis submitted by **Mr. Saroj Thapa** entitled “**PREVALENCE OF GASTROINTESTINAL HELMINTH PARASITES OF BARKING DEER IN SHIVAPURI NAGARJUN NATIONAL PARK, KATHMANDU, NEPAL**” is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master’s Degree of Science in Zoology with special paper Parasitology.

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LIST OF ABBREVIATIONS

CITES -	Convention on International Trade in Endangered Species of Wild Fauna and Flora
cm -	Centimetre
DNPWC -	Department of National Park and Wildlife Conservation
et al. -	And his associates
FAO -	Food and Agriculture Organization
GI -	Gastrointestinal
i.e. -	That is
IUCN -	International Union for Conservation of Nature
kg -	Kilogram
km ² -	Kilometre square
m -	Metre
Ms –	Micro soft
P value -	Probability value
rpm -	Revolutions per minute
sp. -	Species
UK -	United Kingdom
USA -	United State of America
WHO -	World Health Organization
WWF -	World Wildlife Found

ABSTRACT

Various infectious diseases of the animals directly or indirectly effects on the health and wellbeing of animals. Among them, GI parasites can be considered as emerging health problems of wild ruminants. This study was conducted to determine the prevalence of gastro-intestinal helminth parasites as well as concurrency of helminth parasites in barking deer of ShivapuriNagarjun National Park (SNNP). A total of 90 pellet samples of barking deer were collected in day time between May and September using purposive sampling as well as opportunistic sampling methods from three different locations. The collected fecal samples were placed in a sterile vial filled with 2.5% potassium dichromate. In the lab of Central Department of Zoology, the preserved fecal samples were processed for microscopic examination. Faecal samples were examined by direct smear and concentration methods. The eggs of parasites were identified on the basis of their morphological appearance in microscope (10X and 40X) and morphometric using ocular and stage micrometer. Altogether 77.78% deers of SNNP were found to be infected with gastrointestinal helminths parasites where the prevalence of GI parasites was recorded highest in Panimuhan side (82.50%) followed by Nagarjun (75%) and Sundarijal (73.33%). In SNNP, the prevalence of *Strongyloides* sp. (41.11%) was highest followed by *Haemonchus* sp. (25.56%), *Ascaris* sp. (15.56%), *Trichostrongylus* sp. (13.33%), *Muellerius* sp. (10%), *Capilaria* sp. (8.89%), *Fasciola* sp. (7.78%), *Trichuris* sp. (5.56%) and *Oxyuris* sp. (3.33%). Both single as well as double helminth parasitic infection was found almost equal. The result indicated that barking deer of SNNP were highly infected with helminth parasites.

1. INTRODUCTIONS

1.1 Background

1.1.1 Wild Ruminants

Ruminants are the herbivores having special four chambered stomach. Among four compartments, rumen is the largest compartment in which partially chewed grass is stored and broken down into balls of cud. They are even-toed ungulate mammal that chews the cud regurgitated from its rumen. They are worldwide in distributions which are kept in the suborder Rumentia under the order Artiodactyla. There are about 150 species of both wild and domestic ruminants such as buffalo, cattle, goat, sheep, tahr, deer, giraffe, goral, antelope etc. Different protected areas have been established in all around world for protection of wild animals and ^{their} habitat. Till date there are a total of 20 Protected Areas including 12 National Parks, 1 Wildlife Reserves, 6 Conservation Areas and 1 Hunting Reserve are declared by government of Nepal (DNPWC, 2019). To promote integrated and long term conservation management by improving co-operation among Protected Areas and Buffer Zone communities, on 2052 BS Buffer Zone Management Regulations was introduced (DNPWC, 2019). Among them Shivapuri Nagarjun National Park (SNNP) is major habitat of wild animals like ruminants.

A total of 208 species of wild mammals are recorded in Nepal, constituting 4.2% of the world's mammalian fauna (Jnawali *et al.*, 2011). Among them 22 are ruminants where one species from family Tragulidae, three from family Moschidae, five from family Cervidae, 13 from family Bovidae distributed all around Nepal (Baral, 2008). But Chiru (*Pantholops hodgsoni*), Indian Spotted Chevrotain (*Moschiola meminna*) and wild yak (*Bos mutus*) are regarded to be extinct from Nepal (Baral, 2008) but not confirmed. 32 wild ruminants are found in Nepal where seven ruminants are categorized as rare species by IUCN red list. There are nine ruminants which are declared by Nepal Government as protected animals (Baral, 2008). Among them some ruminants are isolated in fix area like Blackbuck (*Antilope cervicapra*) in Khairapur, Bardiya district, gaur (*Bos gaurus*) in Chitwan, swamp deer (*Cervus duvauceli*) in Suklaphata and wild water buffalo (*Bubalus arnee*) in Koshi tappu. Three species of musk deer, Himalayan tahr (*Hemitragus jemlahicus*) and blue sheep (*Pseudois nayaur*) distributes in high altitude range of 2500-5000m of Himalayan region. Chiru (*Pantholops hodgsoni*), wild yak (*Bos mutus*) and argali (*Ovis ammon hodgsoni*) are distributing above 4000m in protected area of Nepal. Similarly, some ruminants like nilgai (*Boselaphus tragocamelus*), four horned antelope (*Tetracerus quadricornis*), hog deer (*Axis porcinus*), chital (*Axis axis*), sambar deer (*Cervus unicolor*) are resident of terai region (Baral, 2008; Jnawali *et al.*, 2011).

Among wild ruminants, barking deer (*Muntiacus vaginalis* Boddaert, 1785) is one which belongs to order Artiodactyla and family Cervidae. Body of it is chestnut red coloured and brown black facial markings with small antlers (Jnawali *et al.*, 2011). They are distributed throughout south and south-east Asia, commonly known as Ratuwa Mirga. They are widely distributed from lowland to the high mountains and found in variety of habitats from dense forest (Pokharel and Chalise, 2010; Brodie and Brockelman, 2009;

Barrete, 1977). It is found up to the elevation of 3500m (Timmins *et al.*, 2008). The height of an adult ranges from 50 to 75cm and weighs about 22 to 23 kg (Shrestha, 2003). They are common in all national parks of Terai foothills (Shrestha, 1997). Antlers are small consisting of a short brow-tine and unbranched beam (Dey, 2007). Frontal glands on the forehead is believed to be scent glands which are activated during the mating period and serve as a means of attracting the hinds (female stag) (Shrestha, 2003).

They are found in Asian country like Bangladesh, Bhutan, Cambodia, China, Hongkong, India, Myanmar, Nepal, Pakistan, SriLanka, Thailand and Vietnam (Timmins *et al.*, 2008; Jnawali *et al.*, 2011). It is distributed in all protected areas of Nepal and occurs in dense tropical and subtropical forests, thickly wooded hills and prefers ravines, stream gorges, dried upstream beds and thick undergrowth for covers (Jnawali *et al.*, 2011). It feeds on fallen fruits, buds, small seeds, seed pods, tender leaves and young grass (Timmins *et al.*, 2008). Female becomes sexually mature within their first year (Dey, 2007). After gestation period of six months, It gives birth to usually single or two young (Dey, 2007; Jnawali *et al.*, 2011).It makes a barking sound when alarmed and in fight give out a series of short cackling barks (Shrestha, 2003)

It is widely traded and hunted for wild meat and antlers (Timmins *et al.*, 2008). Hunting, habitat loss and degradation due to human encroachment, clearing for agriculture and livestock grazing are the main threats (Jnawali *et al.*, 2011).The population estimated in Nepal is greater than 10,000 individuals which has declined rapidly over the past15 years (Timmins *et al.*, 2008; Jnawali *et al.*, 2011). The Government of Nepal has protected Barking Deer as vulnerable species under the National Park and Wildlife Conservation Act, 1973, CITES listed it in appendix I and the IUCN Red List of threatened species listed it as least concern.

1.2 Effect of GI helminth parasites in barking deer

Population of animals is determined by various factors and disease is one of them. Disease caused by parasites is familiar one. Infection and disease are important determinant of the health and well-being of animal populations (Scott, 1988). Infectious disease may cause considerable mortality in animal populations, especially at high population densities. Data on invertebrates suggest that this mortality may play a role in the regulation of their populations, but data on vertebrates suggest that most of this mortality is compensatory to other mortality and does not play any role in population regulation (Holmes, 1982).

Intestinal parasites cause significant morbidity and mortality throughout the world (Corry Jeb *et al.*, 2007). Most ruminants are infected by gastrointestinal parasites. Parasites might also reduce the probability of host reproducing or surviving (Hoberg *et al.*, 2001). Presence of those gastrointestinal parasites in animal especially in young alters the condition, reduces body weight and brings reproductive disorder. Gastrointestinal nematodes can have a significant effect on host condition and fecundity (Stien *et al.*, 2002).

Helminths are multicellular organisms mostly endoparasites causing the intestinal infection (Morariu *et al.*, 2012). Trematodes are commonly known as a flukes which reside in the bile duct causing minor to severe damage to host. These species play a vital role in the degradation of health of domestic as well as wild animals. Intermediate host plays important role in completion of their life cycle such as snail, Cray fish etc. Parasitic diseases infected by trematodes in ruminants can cause watery diarrhea, weakness, weight loss, secondary infection and even mortality of host (Soulsby, 1986). Fascioliasis and paramphistomiasis are caused by the infection of *Fasciola* sp. and *Paramphistomum* sp. respectively. These parasites have been reported from wild ruminants of Nepal (Pandey, 2017; Achhami, 2016; Chaudhary, 2014).

Cestodes are commonly known as tape worm, found in the gut of hosts. Host gets infected by ingestion of contaminated food or water. The major cestode parasites of ruminants are *Moniezia* sp. (Pandey, 2017; Achhami, 2016; Chaudhary, 2014), *Taenia* sp.

Nematode parasites are commonly known as round worms which are most prevalent parasites among other. Important nematode parasites of the ruminants are Strongyles (*Trichostrongylus* sp., *Strongyliodes* sp. and *Strongylus* sp.), Ascarids (*Ascaris* sp.), *Oesophagostomum* sp., *Nematodirus* sp. and *Haemonchus* sp. have the highest prevalence (Foreyt, 2001). *Trichostrongylus* sp. and *Strongyliodes* sp. resides on small intestine feeding on mucus and sucking blood whereas *Haemonchus* sp. on rumen. Normally nematodes are transmitted through contaminated food and water with feces containing eggs and larvae of parasite where as some species such as *Strongyliodes* sp. are transmitted by direct penetration by infective larvae or through oral route. Similarly, different GI parasitic nematodes such as *Trichostrongylus* sp. *Haemonchus* sp., *Strongyloides* sp., lungworm and others as well as cestode have been reported in wild ruminants from different parts of Nepal (Chaudhary, 2014; Thapa and Maharjan, 2015; Achhami, 2016; Pandey, 2017).

1.2 Objectives of the study

1.2.1 General objective

To determine the prevalence of gastrointestinal helminth parasites of barking deer of Shivapuri-Nagarjun National Park, Nepal.

1.2.2 Specific Objectives

- To determine the prevalence of gastrointestinal helminth parasites in different location of barking deer in SNNP.
- To determine the concurrency of parasitic infection of barking deer.
- Morphometry of eggs and larvae of helminth parasites.

1.3 Significance of the study

The study will be very much beneficial for developing knowledge about parasitic infection of barking deer in national park which will help the concerned authorities of the Shivapuri Nagarjun National Park to formulate the plans for conservation and management of barking deer. The result of the study will provide data of parasitic infection on barking deer and help to know the prevalence of gastrointestinal parasites on this species. In addition, the research will also provide necessary information related to barking deer. This study can be considered as baseline documentation on the gastrointestinal parasites of barking deer of SNNP. It will be helpful while designing control strategies of gastro-intestinal parasites in barking deer and also be useful to other ruminants animals found in national park.

2. LITERATURE REVIEW

Ruminants i.e. barking deer maintain ecosystem by providing the food or being food for the predator. To maintain healthy ecosystem most importantly the prey of ecosystem must be healthy. Parasitic infection directly as well as indirectly impact upon animal's health status. They get infected with different parasitic diseases and spread to their surrounding animals (Hutchinson 2009, Boomker *et al.*, 1989). Besides bacterial, viral and fungal infection, wild as well as domestic animals are highly susceptible to different diseases like nematodiasis, fascioliasis, schistosomiasis, fasciolopsiasis etc. (Hudson *et al.*, 1998). Intestinal parasites cause significant morbidity and mortality throughout the world (Corry Jeb *et al.*, 2007). Parasites might also reduce the probability of host reproducing or surviving (Hoberg *et al.*, 2001). Few published paper are available with regard to parasitic diseases on barking deer while most of work done on ruminants of captive and wild ruminants. Researches were restricted on the topic related to distribution, population status and conservation threats.

2.1 Scenario of gastro-intestinal parasites of barking deer

2.1.1 Global context

Barking Deer is a solitary, small, wild ruminant host of different types of intestinal parasites like *Eimeria* sp., *Moniezia* sp., *Fasciola* sp., *Paramphistomum* sp., *Ascaris* sp., *Trichuris* sp., *Haemonchus* spp., *Strongyloides* sp., *Trichostrongylus* sp., *Mullerius* sp. were recorded in different study (Kanungo *et al.*, 2010; Rahman *et al.*, 2014; Thawait *et al.*, 2014; Mir *et al.*, 2016; Aviruppola *et al.*, 2016).

Examination of pellet samples of Barking Deer in Bangladesh was carried by Kanungo *et al.* (2010) and reported barking deer were infected by (78.79%) helminth parasite, which included *Paramphistomum* sp., *Haemonchus* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Trichuris* sp., *Oesophagostomum* sp. and *Capillaria* sp. In addition to this, *Fasciola* sp. was recorded from Dhaka National Zoological Garden (Rahman *et al.*, 2014). Similarly, Thawait *et al.* (2014) studied on captive wild animals of Nandan Van Zoo of Raipur, Chhattisgarh and identified herbivores were infected with intestinal parasites and all barking deer were infected with *Ascaris* sp. Similarly, from Sri Lanka, *Moniezia* sp. was reported while corpological survey of GI parasites had carried out in Diwala Zoological Garden (Aviruppola *et al.*, 2016). Mir *et al.* (2016) carried out their works in Deer Park of Patiala in Punjab, India and identified *Strongyloides* sp. infection in a Barking Deer.

2.1.2 National context

Nationally, few studies related to gastro-intestinal parasites of barking deer have been conducted (Thapa and Maharjan, 2015; Achhami, 2016; Pun, 2018 and Kandel, 2018). Studies conducted on Himalayan Tahr and Barking Deer of Rara National Park (RNP) reported that Barking Deer (97.06%) were infected with seven genera of intestinal parasites (Thapa and Maharjan, 2015). However, Achhami (2016) reported that Barking

Deer (83.87%) had parasitic infection in Langtang National park (LNP). Barking Deer in RNP have been reported that infected with *Oxyuris* sp. (70.59%), *Moniezia* sp. (47.06%), *Ascaris* sp. (17.65%), *Trichuris* sp. (8.82%), *Dictyocaulus* sp. (8.82%) and *Haemonchus* sp. (2.94%) (Thapa and Maharjan, 2015). Whereas in LNP, Barking Deer harbored gastro-intestinal parasites predominately *Eimeria* sp. followed by *Ascaris* sp., *Strongyloides* sp., *Moniezia* sp., *Trichuris* sp., *Strongyle* sp. and *Paramphistomum* sp. (Achhami, 2016).

Likewise Pun (2018) studied on gastro-intestinal parasites in ruminants at Central Zoo, Kathmandu where barking deer were infected by *Haemonchus* sp., *Strongyloides* sp., and *Trichostrongylus* sp. And same year Kandel conducted research on prevalence of gastro-intestinal parasites of wild in Baghmara Buffer Zone Community Forest (BBZCF) of Chitwan National Park, where result shows that among 10 sample of barking deer there is 100% prevalence. Different nematodes like *Oxyuris* sp., *Bunostomum* sp., *Trichostrongylus* sp., *Haemonchus* sp. and Trematodes like *Fasciola* sp. and *Paramphistomum* sp. founded.

2.2 Scenario of gastro-intestinal parasites in wild ruminants

Wild animals are the most likely source of new emerging disease that put at risk the health of human being and livestock (Anonymous, 2004). Besides, barking deer research had conducted on ruminants of different countries in worlds on wild as well as captive condition. In different continent there were different scenario regarding on prevalence of gastrointestinal helminth parasites on ruminants.

In Europe, research were conducted on different country like Poland (Kowal *et al.*, 2012; Kobak and Pilarczyk, 2011), Ukraine (Kuzmina *et al.*, 2010), Greenland (Steele *et al.*, 2011), Spain (Duran *et al.*, 2004) and Norway (Davidson *et al.*, 2014). The gastrointestinal tract parasites investigated by Kowal *et al.* (2012) of Fallow Deer hunted in Southern Poland recorded *Ashworthius sidemi*, *Spiculoptera sp.*, *Nematodirus filicollis*, *Aonchotheca bovis*, *Oesophagostomum radiatum* as the major parasites. In the Notecka Forest region in the Wielkopolska Province, Poland gastrointestinal nematodes and trematodes (*Fasciola hepatica* and *Paramphistomum cervi*) were found in water buffaloes (Kobak and Pilarczyk, 2011). In Ukraine, Kuzmina *et al.* (2010) found helminth fauna of roe deer (*Capreolus capreolus*) that prevalence of helminths was 92.4%. Where, *Paramphistomum cervi*, *Haemonchus contortus*, *Ashworthius sidemi*, *Marshallagia marshalli*, *Nematodirus oiratinus*, *Trichostrongylus axei*, *Moniezia expansa*, *Bunostomum phlebotomum* were found. *Trichostrongylus axei* was found on red deer (*Cervus elaphus*) in Norway and Central Spain (Davidson *et al.*, 2014; Duran *et al.*, 2004). But red deer of Norway were infected by more parasites like *Ostertagia leptospicularis*, *Spiculoptera spiculoptera*, *Capillaria bovis*, *Cooperia oncophora*, *Oesophagostomum venulosum*, *Trichuris globulosa* and tapeworm segments were encountered (Davidson *et al.*, 2014). *Spiculoptera quadrispiculata* was recorded first time from Red Deer in Spain by Duran *et al.* (2004). A survey in West Greenland caribou populations revealed

nematodirinae and anoplocephalidae, *Marshallagia* sp. eggs and *Eimeria* sp. oocyst (Steele *et al.*, 2011).

In Africa, some common helminth parasites like *Trichostrongylus* sp., *Haemonchus* sp. and *Strongyloides* sp. were recorded in wild impala antelope, Zambia (Nalubamba *et al.*, 2012), captive wild ruminants of Sanda Kyarimi Park, Nigeria (Ibrahim *et al.*, 2012). *Trichuris* sp. was found from the gastro intestinal tract in Sanda Kyarimi Park, Nigeria (Ibrahim *et al.*, 2012). Wahed (2004) reported gastrointestinal nematode eggs including *Fasciola hepatica*, *Dicrocoelium lanceolatum*, *Moniezia benedeni* and *Moniezia expansa*, *Coccidia*, *Sarcosporidio* sp. the larvae of *Trichostrongylus colubriformis*, *Ostertagia ostertagi* and *Oeseophagostomum* sp. from living Egyptian Deer (*Dorcas gazelles*) in Egypt. Similarly, Swai *et al.* (2013) revealed *Trichostrongylus* sp., *Oesophagostomum* sp., *Strongylus* sp., *Bunostomum* sp., *Ostertagia* sp., *Toxocara* sp., *Fasciola* sp., *Paramphistomum* sp. of parasites in free ranging African Buffaloes in wildlife protected areas of Tanzania. Giraffe (*Giraffa camelopardalis*) were found infected with *Parabronema skrjabini*, *Skrjabinema* sp., *Haemonchus mitchelli*, *Echinococcus* sp. by Krecek *et al.* (1990) in Etosha National Park, Namibia.

In American continent, some deer found to be infected by GI parasites. Worley and Eustace (1972) collected 44 Mule Deer (*Odocoileus hemionus*) from semiarid rangeland, Montana, USA and found helminth parasites like *Trichostrongylus colubriformis*, *Nematodirus odocoilei*, *Trichuris* sp., *Ostertagia bisonis*, *Taenia hydatigena cysticerci*, *Haemonchus contortus* and *Trichostrongylus longispicularis*. But white tailed deer of West Virginia were infected by *Sarcocystis* sp., *Cysticercus tenuicollis*, *Oesophagostomum venulosum*, *Cooperia punctata* and *Gongylonema pulchrum* (Prestwood *et al.* 1976). Likewise, *Eimeria* sp., *Haemonchus* sp., *Bunostomum* sp., *Cooperia* sp. and *Trichostrongylus* sp. had found in cattle and Mule Deer (*Odocoileus hemionus*) in Mexico Cossio-Bayugar *et al.* (2015). Grey Brocket Deer (*Mazama gouazaubira*) of Brazilian Pantanal wetlands were found to be infected with *Haemonchus* sp. (LuxHoppe *et al.*, 2010).

In Malaysia, same Intestinal parasites were found in various animals at a zoo conducted by (Lim *et al.*, 2008). Where, 45.7% of hoofed mammals (Bovidae, Cervidae) were infected with intestinal parasites among them hookworm had the highest prevalence (34.3%) followed by *Trichuris* spp. and *Cryptosporidium* spp. (5.7%). Gastrointestinal parasites and their level of infestation in the Arabian oryx (*Oryx leucoryx*) were studied at King Khalid Wildlife Research Center in Thumamah, Riyadh Province, Saudi Arabia and fecal examination revealed *Eimeria saudensis*, *Cryptosporidium* sp., *Nematodirus spathiger* and *Trichuris* sp. as the gastrointestinal parasites (Mohammed *et al.*, 2012).

In case of India, some survey were done in wild ruminants by Senger *et al.* (2017) for free-ranging wild herbivores and adjoining livestock of Panna Tiger Reserve, Madhya Pradesh, by Mir *et al.* (2016) in captive wild animals in Bir Moti Bagh mini zoo (Deer Park), Patiala, by Bandyopadhyaya *et al.* (2010) in yaks (*Bos poepagus*) in Gurudogmer Plateau, a cold desert area in North Sikkim, by Meshram *et al.* (2008) in free range deer in the scrub forest of Borgaon Manju in Western Vidarbha region, by Singh *et al.* (2006)

on Mahendra Choudhury Zoological Park, Punjab, by Yadav *et al.* (2005) on bovines at Jammu. Most of research resulted same helminth parasites in wild ruminants. Parasites like *Strongyle* sp., *Trichuris* sp., *Strongyloides* sp. (Gupta *et al.*, 2017; Senger *et al.*, 2017; Meshram *et al.*, Yadav *et al.*, 2005), *Moniezia expansa* (Gupta *et al.*, 2017; Senger *et al.*, 2017; Yadav *et al.*, 2005), *Ascaris* sp. (Mir *et al.*, 2016; Yadav *et al.*, 2005), *Amphistomes* sp. (Singh *et al.*, 2006; Yadav *et al.*, 2005) and *Bunostomum* sp. (Meshram *et al.*, 2008) were found in wild ruminants. *Capillaria* sp. was found in Bir Moti Bagh mini zoo (Deer Park), Punjab by Mir *et al.* (2016). Senger *et al.* (2017) found *Amphistome* (9.09%), *Balantidium* sp. (3.20%) in free-ranging wild herbivores and adjoining livestock of Panna Tiger Reserve, Madhya Pradesh. But Bandyopadhyaya *et al.* (2010) found *Nematodirus* sp., *Cooperia* sp. and *Dicrocoelium* sp. on study of faecal samples collected from yaks (*Bos poephegus*) in North Sikkim, India.

In Pakistan, gastrointestinal parasites like *Haemonchus* sp., *Trichostrongylus* sp., *Moniezia* sp., *Trichuris* sp., *Strongyloides* sp. (Farooq *et al.*, 2012; Rana *et al.*, 2015) were found on both domesticated and Wild Ruminants in Cholistan Desert of Pakistan (Farooq *et al.*, 2012) and captive hog deer, Pakistan (Rana *et al.*, 2015). Muhammad Arshad Rana *et al.* (2015) found seven different gastro-intestinal endo-parasite namely *Paramphistomum cervi*, *Moniezia expansa*, *Moniezia benedeni*, *Strongyloides papillosus*, *Trichuris globulosa*, *Trichostrongylus* sp. and *Haemonchus contortus* in captive hog deer, Pakistan.

In Bangladesh, there was also same type of result by investigation of gastrointestinal parasites of herbivores at Dhaka National Zoological Garden of Bangladesh (Rahman *et al.*, 2014) and Deer in Char Kukri Mukri upazilla of Bhola district of (Barmon *et al.*, 2014). Where some GI parasites like *Paramphistomum* sp., *Fasciola* sp., *Ascaris* sp., *Strongyloides* sp., *Balantidium coli* were recorded. Khan *et al.* (2014) identified protozoan (*Balantidium coli*, *Coccidius* sp.) and helminthes (Hook worm, *Trichuris* sp., sp., *Strongyles* sp.) in Siddhartha Garden Zoo animals.

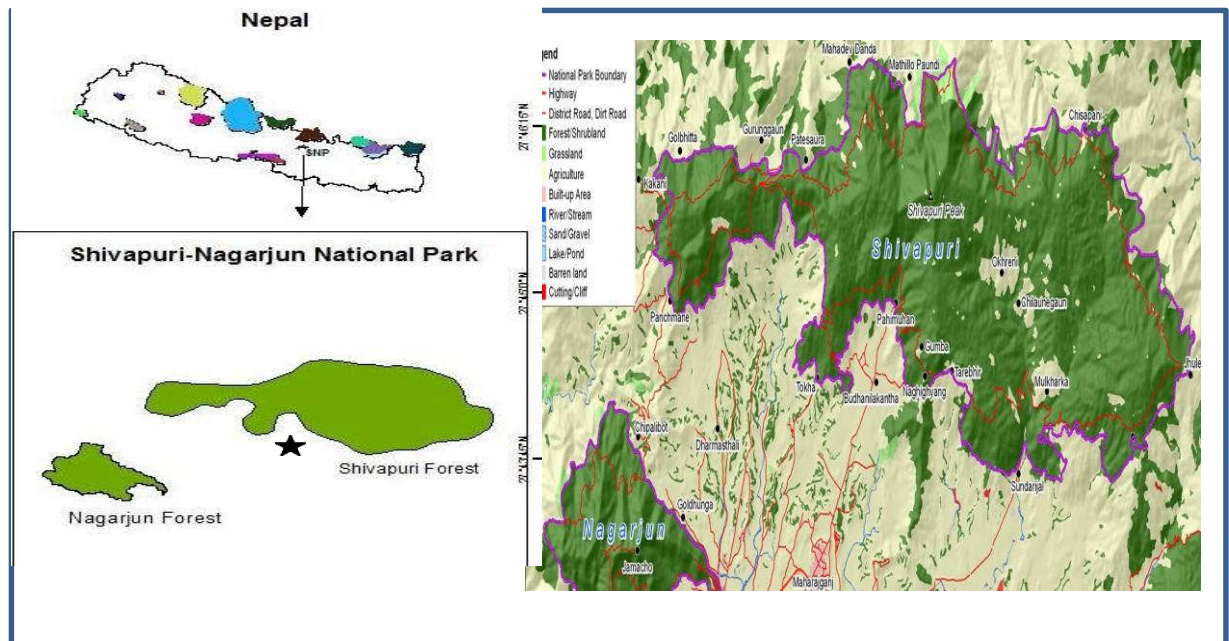
In Nepal, study showed that 168 species of helminth parasites has been compiled with 33 species belonging to trematodes, 67 to the nematodes and 36 to the cestodes (Gupta, 1997). Cryptosporidiosis is a common protozoan disease in humans and animals in Nepal acquired by ingesting of oocysts excreted in the feces of infected individuals (Paudyal *et al.*, 2013). Different numbers of parasites have been found on wild ruminants of Nepal (Oli, 2018; Airee, 2018; Pandey, 2017; Gupta, 2017; Achhami, 2016; Thapa, 2013).

Oli (2018) found *Trichuris* sp., *Ascaris* sp., *Strongyloides* sp. nematodes parasites on Spotted deer of Banke National Park which were found also in Swamp Deer of Shuklaphata National Park (Pandey, 2017), wild ruminants of Langtang National Park (Achhami, 2016), Blackbuck in Bardia (Chaudhary, 2014). Similarly, other nematodes like *Trichostrongylus* sp. (Airee, 2018; Gupta, 2017; Chaudhary, 2014; Thapa and Maharjan, 2015), *Haemonchus* sp. (Airee, 2018; Gupta, 2017; Chaudhary, 2014; Thapa and Maharjan, 2013), *Mullerius* sp. (Airee, 2018; Thapa and Maharjan, 2015), *Bunostomum* sp. (Airee, 2018; Chaudhary, 2014) were found from different wild ruminants of Nepal. Trematodes like *Fasciola* sp. (Airee, 2018; Pandey, 2017,

Chaudhary, 2014) and *Paramphistomum* sp. (Pandey, 2017; Achhami, 2016; Chaudhary, 2014) were also found. Besides these other nematodes were also noticed. In Koshi Tappu Wildlife Reserve, Gupta (2017) found *Toxocara* sp. Thapa and Maharjan (2013) found *Strongyloides* sp., *Dictyocaulus* sp. from Himalayan Tahr and Barking Deer of Rara National Park. Only cestodes found in Nepal was *Moniezia* sp. found by Pandey (2017) in Swamp Deer of Shuklaphata National Park, Achhami (2016) in Rara National Park and Chaudhary (2014) from Blackbuck Conservation Area, Bardia.

3. MATERIALS AND METHODS

3.1 Study area



Map showing location of study area (SNNP, 2019)

Shivapuri Nagarjun National Park (IUCN categories: II) is established in 2002 which is nearest national park from Kathmandu. Shivapuri Nagarjun National Park (SNNP) covers 159 square kilometers area and is located between two isolated islands forest Shivapuri and Nagarjun. Nagarjun lies adjacent to Kathmandu city while Shivapuri is of 4 km distance to North. Geographically lies between 27°45' to 27°52' latitude and 85°15' to 85°30' longitude (SNNP, 2019). Prior its declaration as national park, it was managed under the Shivapuri Watershed Development Board, and was later declared as Shivapuri Watershed). The Shivapuri Nagarjun is the true representation of the mid-hills in the protected area system of Nepal. Its altitude varies from 1350m to 2732m. The park lies in the transition zone between sub-tropical to temperate regions.

In Shivapuri Nagarjun National park, there are three locations according to entry transit point near from the Kathmandu valley.

- Nagarjun
- Panimuhan
- Sundarijal

Nagarjun side is one isolated part from Sivapuri section located under Latitude 27°43' to 27°46' N and Longitude 85°13' to 85°18' E. Its area is about 15 km² which is locating southern west part of national park (SNNP, 2019). Panimuhan and Sundarijal section are located on Shivapuri area which is main part of national park covers area about 144 km². It lies on northern side of Kathmandu valley which is main sources for water in Valley which is literally bioclimatic zone mid hills.

Climate

Shivapuri has subtropical to warm temperate climate. The 33 years (1985-2017 AD) climatic data of the weather station at Kakani (altitude 2066 m.) provided by Department of Hydrology and Meteorology shows the record of average maximum temperature of 19.90 C and that of average minimum temperature of 11.150 C. The mean annual precipitation was 236.5 mm mostly occurring during monsoon period. Its annual rainfall is 2727 mm (SNNP, 2019).

Flora and Fauna

The floral composition park has been categorized into four types viz. i) Lower mixed hardwood forests, ii) Chirpine forests, iii) Oak forests, and iv) Upper mixed hardwood forest. The major plant species found are *Schima wallichii*, *Castonopsis indica*, *Pinus roxburghii*, *Myrica esculenta*, *Pyrus pasia*, *Rhododendron arboreum*, *Juglans regia* and *Quercus* sp. (SNNP, 2019). Park record shows that it is supporting a number of animals of ecological significance. SNNP provides shelter to 33 species of mammals (excluding bats) such as Clouded leopard (*Neofelis nebulosa*), Common leopard (*Panthera pardus*), Leopard cat (*Felis bengalensis*), Jungle cat (*Felis chaus*), Pangolin (*Manis* sp), Rhesus monkey (*Macaca mulata*), Barking deer (*Muntiacus vaginalis*, Assamese monkey (*Macaca assamensis*), Himalayan black bear (*Ursus thibetanus*) and other prey species. The park is important bird areas (IBAs) with over 318 species of birds have been recorded where 117 species are migratory. There are more than 20 species of reptiles and 9 species of amphibians. This is good habitat of 102 species of butterflies (SNNP, 2019).

3.2 Materials

During the research the materials used have been listed below:

3.2.1 Materials for field:

- i. Sterile vials
- ii. GPS
- iii. Camera
- iv. Globes
- v. Potassium dichromate ($K_2Cr_2O_7$)

3.2.2 Materials for laboratory:

- | | | |
|--------------------------|--------------------------|---------------------------|
| i. Beaker | ix. Tea strainer | xvi. Cotton |
| ii. Droppers | x. Measuring cylinder | xvii. Electric microscope |
| iii. Glass rod | xi. Toothpicks | xviii. Gloves |
| iv. Globes | xii. Needle | xix. Stage |
| v. Slides | xiii. Centrifuge Machine | xx. Refrigerator |
| vi. Cover slips | xiv. Centrifuge tube | xxi. Ocular-micrometer |
| vii. Volumetric flask | xv. Mask | |
| viii. Mortars/
Pestle | | |

3.2.3 Chemicals:

- | | | | |
|------|--|-----|-------------------------|
| i. | Potassium dichromate
(K ₂ Cr ₂ O ₇) | iv. | Methylene blue |
| ii. | Distilled water (D/W) | v. | Lugol's iodine solution |
| iii. | Saturated NaCl solution | | |

3.3 Methods

3.3.1 Identification of pellet

Pellet identification keys were prepared by direct observation of pellet after defecation by respective animals. Pellet of Barking Deer (Ph.1, Ph.2) are characterize as:

- Black in colour.
- Slender in shape but sometime pointed at one end.
- Size: 8±1mm in length and 6±1mm in diameter.

3.3.2 Sample collection/preservation

Samples were collected between May 2018 and September 2018 respectively in three different locations i.e. Nagarjun, Panimuhan and Sundarijal. Sampling collection was done during day time without repetition in location within different time interval. Faecal samples were collected from purposive sampling method where barking deer habitat was finalized from key informant like national park officers and security personal of SNNP. Then sampling had done on different place with minimal repetition and also applied opportunistic sampling method in walking trail to get best number of sampling. Collected samples were labeled. There was collection of total 90 fecal samples. About 20-25gm fecal matter was placed in 20 ml sterile vial and 2.5% K₂Cr₂O₇ were added to cover the fecal sample completely. Then, vial was air tightly closed and put in cool box. The collected fecal samples were transported to laboratory at Central Department of Zoology, Tribhuvan University.

3.3.3 Examination of faecal samples

Faecal samples were processed for microscopic examination of eggs and were identified according to the morphology and quantitative estimation by using concentration method (flotation and sedimentation).

3.3.3.1 Concentration method

Eggs were often low number in feces that they are difficult to be detected in direct smears or mounts. Therefore, this procedures were performed which includes flotation and sedimentation techniques (Soulsby, 1986).

Differential Floatation Technique

Nematode and cestode eggs present in wild and domestic ruminant's faeces are detected through this method. This method ensures the egg float in the floatation liquid (conc. NaCl solution), which helps to identify the egg.

Approximately 3 gm of faecal sample was taken in a beaker and added 20 ml of water then the sample was grinded lightly with the help of mortars and pestle and filtered the solution by tea strainer. The filtrate solution was poured into a centrifuge tube of 15 ml and centrifuged at 1000 rpm for 5 minutes. The tube's water was replaced with saturated sodium chloride solution and again centrifuged.

After centrifuge more saturated sodium chloride solution was added to develop convex surface at the top of the tube and one drop of methylene blue was added where a cover slip was finely placed for a few minutes (15 min.) and then cover slip was removed and placed on a slide and examined at 10X and 40X. Photographs of eggs of parasites were taken and identified based on morphological characters (Dryden et al., 2005).

Sedimentation Technique

This technique is used for the detection of trematode eggs. It provides good results as the eggs of the trematode are bit heavier than the other, where sediments of centrifuged contents were taken for eggs detection. Saturated salt solution was removed gently from the test tube after examined the flotation portion and poured the sediment content into the watch glass and stirred the content gently to mix it. One drop from the mixture was taken to prepare a second slide. The specimen was stained with iodine wet mounts solution. In this way two slides were prepared from one sample (one from flotation and one from sedimentation) and examined under 10X and 40X magnification of microscope to detect eggs of helminthes (Dryden et al., 2005).

3.3.4 Egg and larva size measurement

- Using ocular and stage micrometer
- Length and breadth measured by calibration

3.3.5 Identification of eggs and larvae of parasites

Eggs and larvae of helminthes parasites were observed in microscope (Ph.3) and identified on the basis of morphological characters compared with published articles (Rahman et al., 2014; Thapa and Maharjan, 2015) and books by Soulsby (1986) and help of my supervisor.

3.3.6 Data Analysis

The presence and absence of parasites data were entered in Ms Excel 2010, from where prevalence were identified, and chi-square and P value were calculated by using Prop.test in R-software (r-3.4.1).

4. RESULTS

Various infectious diseases of the animals affects negatively on the health and wellbeing of animals. Among them parasitic diseases are neglected but emerging health problems of wild ruminants. Barking deer of Shivapuri Nagarjun National Park (SNNP) were also found to be infected with various helminthes parasites. Overall prevalence of gastrointestinal helminthes parasites revealed 77.78% in barking deer of SNNP.

Identification of gastro-intestinal parasites

Morphological characters of eggs and their references were used to identify gastrointestinal parasites.

Table 1: Identified eggs of GI-parasites

Name of parasites	Range of length and diameter of eggs and cysts (in μm)		Morphology characters	Reference values (Soulsby,1982 ; Foreyt, 2001)
	Length	Width		
<i>Haemonchus</i> sp.	65-90	40-50	Eggs are oval, thin-shelled, and grayish in color	70-90 \times 40-55 μm
<i>Strongyloides</i> sp.	65-70	25-30	Eggs are small, measure in size, oval with rounded edges or ellipsoidal, thin shelled and contain fully developed larvae that can be seen under low power.	51-65 \times 20-30 μm
<i>Trichostrongylus</i> sp.	90-110	35-40	Irregular ellipse dissimilar, kidney-shaped not very wide poles, one of which was more rounded than the other, dissimilar side-walls.	70-90 \times 35-50 μm
<i>Ascaris</i> sp.	45-60	35-40	Eggs are elongated covered with rough, blumpy outer surface.	45-75 \times 35-40

Prevalence of GI helminthes parasites

Barking deer of SNNP were found infected by two classes of helminthes i.e. nematodes and trematodes which include nine genus of parasites, one genus belongs to trematode and eight genus belongs to nematode. From the identified parasites only *Fasciola* sp. was observed belonging to trematode group. Among nematodes, *Strongyloides* sp. had maximum prevalence followed by *Haemonchus* sp., *Ascaris* sp., *Trichostrongylus* sp., *Muellerius* sp., *Capillaria* sp. and least number *Trichuris* sp. were observed (Table 2). *Fasciola* sp. mainly affect liver of ruminants while most nematodes like *Strongyloides* sp., *Trichostrongyloides* sp., *Oxyuris* sp., *Haemonchus* sp., *Ascaris* sp., *Capillaria* sp. affect small intestine. *Trichuris* sp. affects large intestine and *Haemonchus* sp. mainly affected on stomach (abomasum). *Muellerius* sp. is the only lung nematode parasites affecting respirating system of barking deer. Most of helminthes parasites were infected through fecal oral route except *Strongyloides* sp. which entered through skin penetration.

Table 2: Prevalence of helminth parasites of barking deer in SNNP.

Class	Parasites	Prevalence
Nematodes	<i>Strongyloides</i> sp.	41.1%
	<i>Haemonchus</i> sp.	25.6%
	<i>Ascaris</i> sp.	15.6%
	<i>Trichostrongylus</i> sp.	13.3%
	<i>Muellerius</i> sp.	10%
	<i>Capillaria</i> sp.	8.5%
	<i>Trichuris</i> sp.	5.6%
	<i>Oxyuris</i> sp.	3.33%
Trematodes	<i>Fasciola</i> sp.	7.78%

Fecal samples of barking deer were collected from three different locations i.e. Nagarjun, Panimuhan, Sundarijal site on the basis of entry point of national park area from Kathmandu valley. Higher number of parasitic infection was found in faecal samples collected from panimuhan as compared to other. While barking deer of Nagarjun and Sundarijal were found almost equally infected (Figure 1).

Statistically there was not any significant difference of helminths prevalence in three locations ($X^2 = 0.9482$, $df = 2$, $p\text{-value} = 0.6224$).

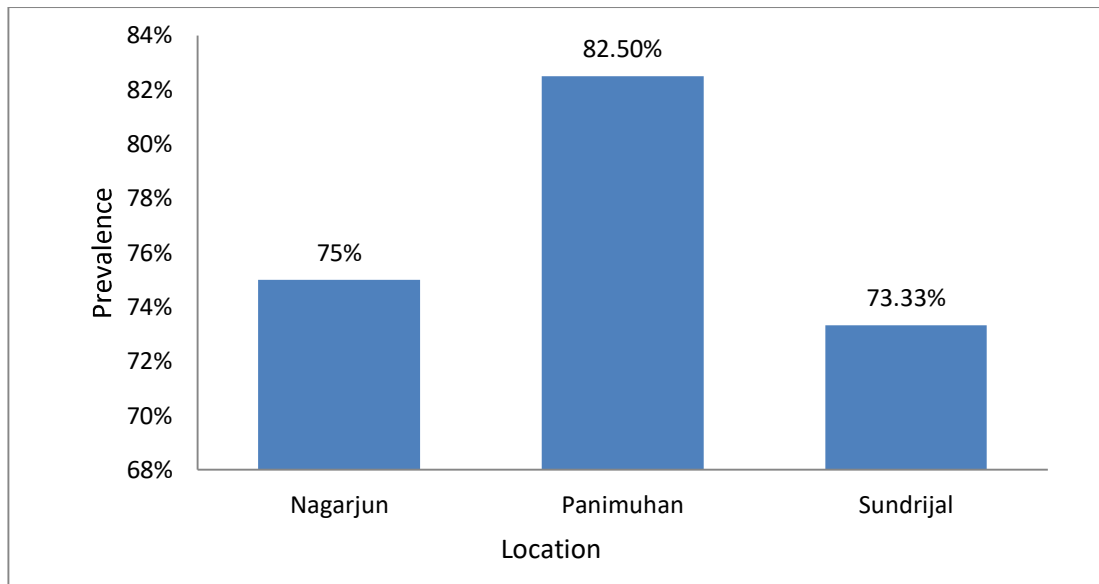


Figure 1: Prevalence of gastrointestinal helminth parasites of barking deer in different locations of SNNP.

Table 3: Prevalence and association of GI helminth parasites of barking deer in different locations of SNNP.

Parasites	Nagarjun (N=20)	Sundarijal (N=30)	Panimuhan (N=40)	X ² value	P value
Nematodes					
<i>Strongyloides</i> sp.	60%	30%	40%	10.521	0.0051
<i>Haemonchus</i> sp.	25%	10%	35%	5.8055	0.0548
<i>Ascaris</i> sp.	10%	23.3%	12.5%	2.1358	0.3437
<i>Trichostrongylus</i> sp.	10%	10%	17.5%	1.0817	0.5872
<i>Muellerius</i> sp.	5%	13.3%	10%	0.9259	0.6294
<i>Capillaria</i> sp.	0	20%	5%	7.2713	0.0263
<i>Trichuris</i> sp.	0	6.7%	7.5%	1.5353	0.4641
<i>Oxyuris</i> sp.	0	0	7.5%	3.8793	0.1438
Trematodes					
<i>Fasciola</i> sp.	5%	0	15%	5.654	0.0591

Comparison of helminthes parasitic prevalence in three different locations of Shivapuri Nagarjun National Park showed that most of the nematode parasites like *Strongyloides* sp., *Haemonchus* sp., *Ascaris* sp., *Trichostrongylus* sp., *Muellerius* sp. are common in all three places. But only trematodes i.e. *Fasciola* sp. was found in Nagarjun and Panimuhan. Likewise, *Trichuris* sp. and *Capillaria* sp. were found in Panimuhan and Sundarijal but absence in Nagarjun side. *Oxyuris* sp. was found in Panimuhan only. Prevalence of *Strongyloides* sp. was dominant in all locations followed by *Haemonchus* sp. (Table 3).

Statistical comparison of helminth parasites in three different sites also showed prevalence of *Strongyloides* sp. (X-squared = 10.521, df = 2, p-value = 0.0051) and *Capillaria* sp. (X-squared = 7.2713, df = 2, p-value = 0.0263) significant of different in three locations where as rest of the parasites were not (Table 3).

Barking deer were found to be infected with either single parasitic infection or multiple. Maximum of them were infected by single species of parasites followed by double, triple and quaternary infection. Among the single infection maximum of them were infected by *Strongyloides* sp. Statistically, there was significance difference in concurrency of parasitic infection(X-squared = 54.324, df = 3, p-value<0.05).

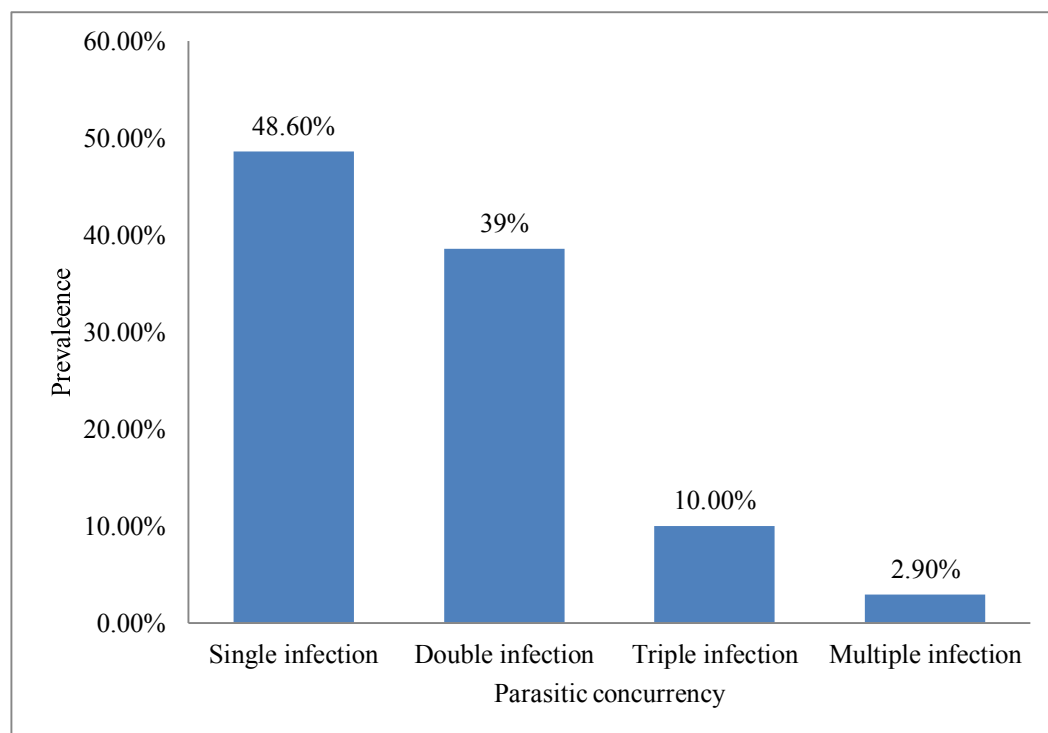


Figure 2: Concurrency of GI helminth parasites of barking deer in SNNP.

Table 4: Helminth parasite specific mix infection of barking deer in SNNP.

Mixed infection	Combination	Total
Single Infection		48.6%
Double infection	<i>Strongyloides</i> sp.+ <i>Muellerius</i> sp.	4.3%
	<i>Haemonchus</i> sp.+ <i>Strongyloides</i> sp.	5.7%
	<i>Haemonchus</i> sp.+ <i>Capillaria</i> sp.	1.4%
	<i>Haemonchus</i> sp.+ <i>Muellerius</i> sp.	2.9%
	<i>Strongyloides</i> sp.+ <i>Trichostrongylus</i> sp.	4.3%
	<i>Strongyloides</i> sp.+ <i>Capillaria</i> sp.	2.9%
	<i>Strongyloides</i> sp.+ <i>Ascaris</i> sp.	4.3%
	<i>Fasciola</i> sp.+ <i>Strongyloides</i> sp.	2.9%
	<i>Fasciola</i> sp.+ <i>Muellerius</i> sp.	2.9%
	<i>Trichostrongylus</i> sp.+ <i>Trichuris</i> sp.	1.4%
	<i>Trichostrongylus</i> sp.+ <i>Ascaris</i> sp.	1.4%
	<i>Trichostrongylus</i> sp.+ <i>Capillaria</i> sp.	1.4%
<i>Trichostrongylus</i> sp.+ <i>Oxyuris</i> sp.	2.9%	
Triple infection	<i>Fasciola</i> sp.+ <i>Strongyloides</i> sp.+ <i>Trichostrongylus</i> sp.	1.4%
	<i>Haemonchus</i> sp.+ <i>Strongyloides</i> sp.+ <i>Trichostrongylus</i> sp.	1.4%
	<i>Haemonchus</i> sp.+ <i>Strongyloides</i> sp.+ <i>Muellerius</i> sp.	1.4%
	<i>Haemonchus</i> sp.+ <i>Ascaris</i> sp.+ <i>Trichostrongylus</i> sp.	1.4%
	<i>Haemonchus</i> sp.+ <i>Ascaris</i> sp.+ <i>Oxyuris</i> sp.	1.4%
	<i>Strongyloides</i> sp.+ <i>Capillaria</i> sp.+ <i>Ascaris</i> sp.	1.4%
	<i>Strongyloides</i> sp.+ <i>Capillaria</i> sp.+ <i>Trichostrongylus</i> sp.	1.4%
Multiple infection	<i>Haemonchus</i> sp.+ <i>Trichostrongylus</i> sp.+ <i>Trichuris</i> sp.+ <i>Muellerius</i> sp.	1.4%
	<i>Fasciola</i> sp.+ <i>Haemonchus</i> sp.+ <i>Trichostrongylus</i> sp.+ <i>Ascaris</i> sp.	1.4%

Among the multiple infections there was different combination of specific mix infection. In double infection maximum 13 different types of parasitic combination of mix infection were observed. In triple infection seven different combinations and in multiple infection two different mix combinations were observed. *Strongyloides* sp. made maximum six different combination of mix infection in double infection and five different combinations in triple infection followed by *Trichostrongyloides* sp. which made five combinations in double and four combinations in triple infections (Table 3).

In comparative study of concurrency of helminth parasites on different locations showed different variation. Panimuhan got maximum single, double, triple infection compare to other two locations. Single infection was equal in Nagarjun and Sundarijal site. Quaternary infection were least among and absent in Nagarjun site. In comparison Panimuhan location showed high concurrency and Nagarjun showed least concurrency of parasites (Figure 3).

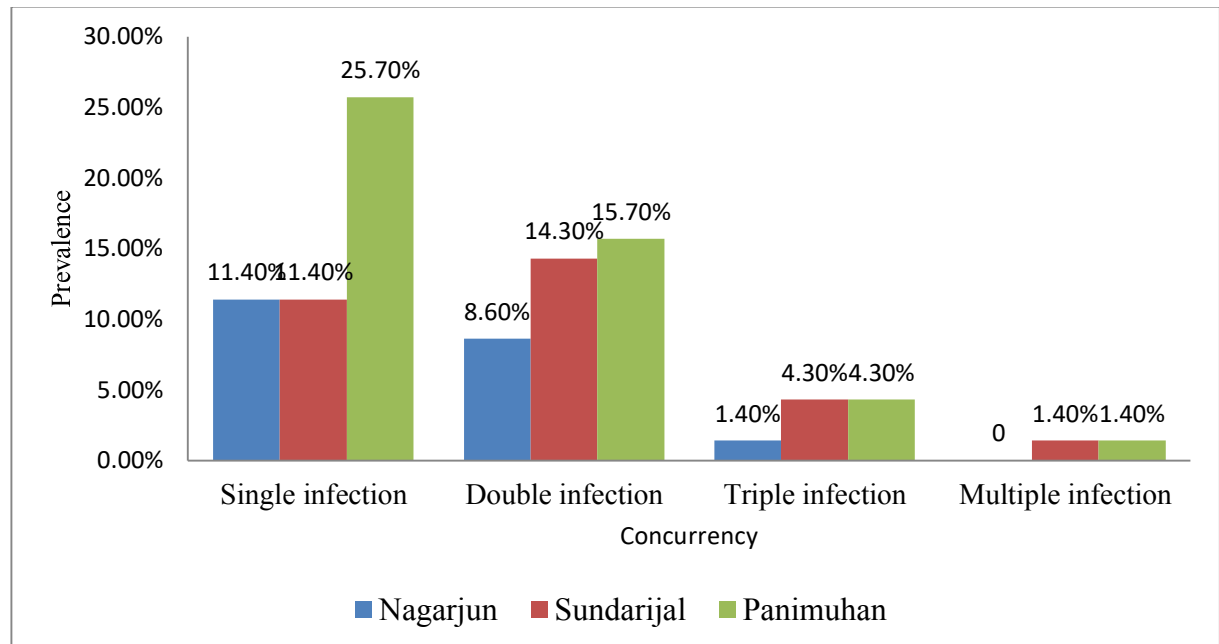
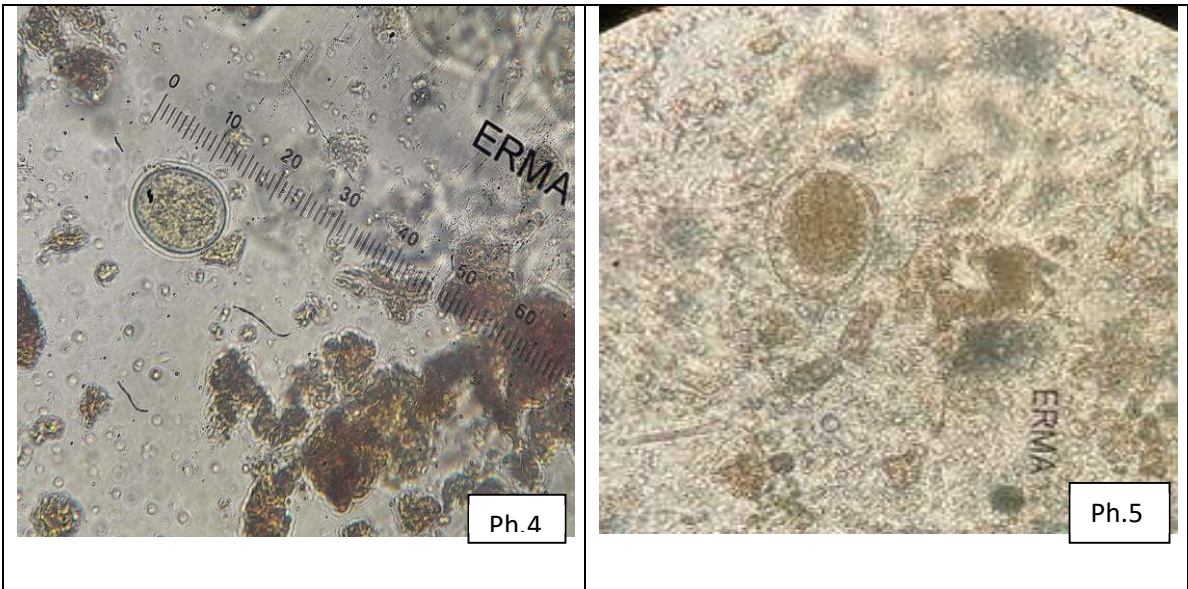
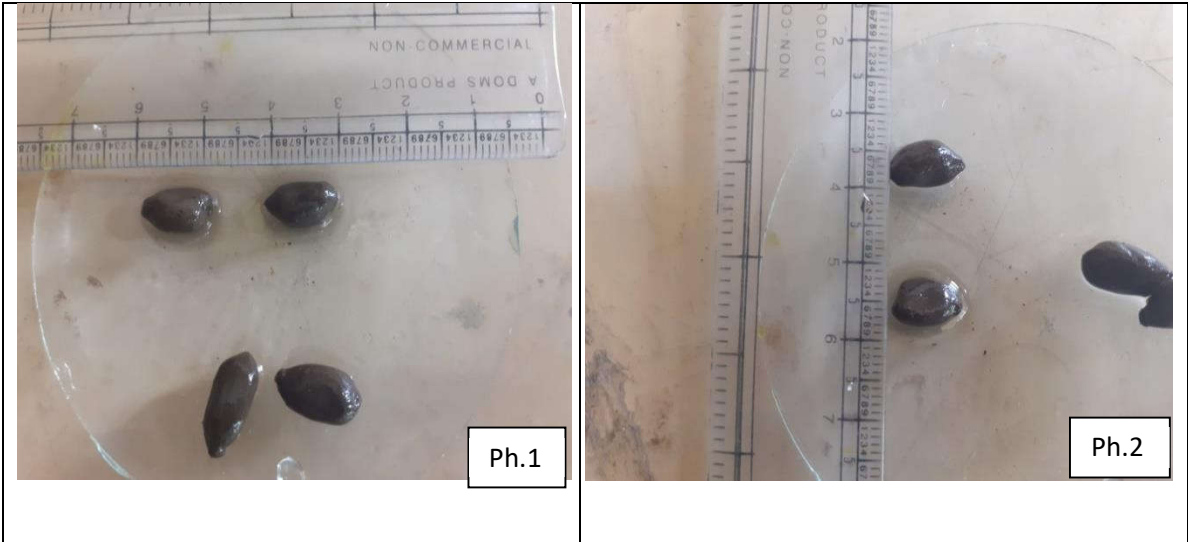
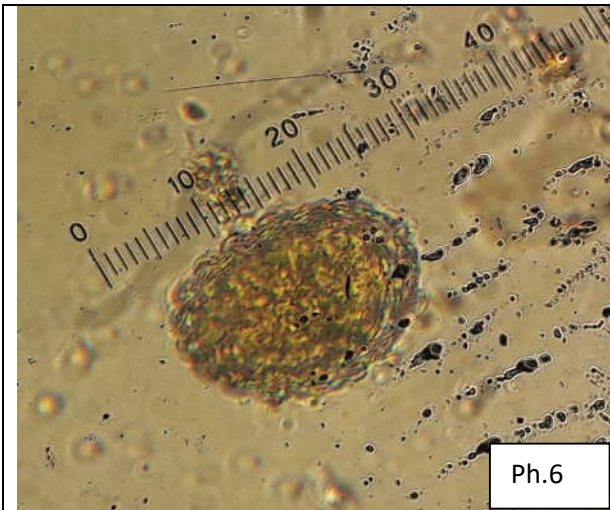


Figure 3: Concurrency of GI helminthes parasites of barking deer in different locations of SNNP.



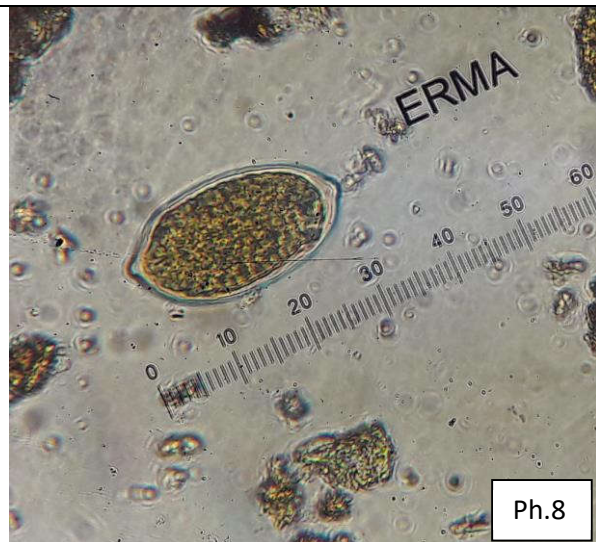
Ph.1: Length of pellet of barking deer, Ph.2: Width of pellet of barking deer, Ph.3: Observing in microscope, Ph.4: Egg of *Haemonchus* sp.(51/38 μ m) , Ph.5: Infertile egg of *Ascaris* sp.(50/32 μ m)



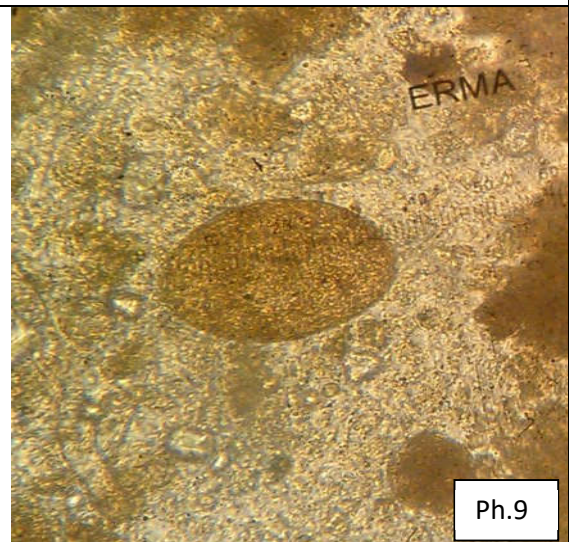
Ph.6



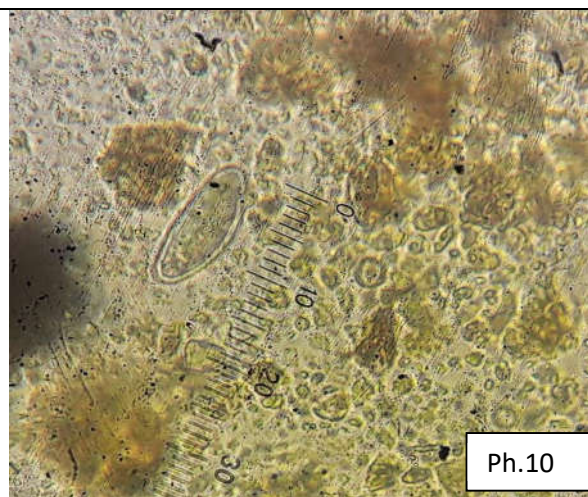
Ph 7



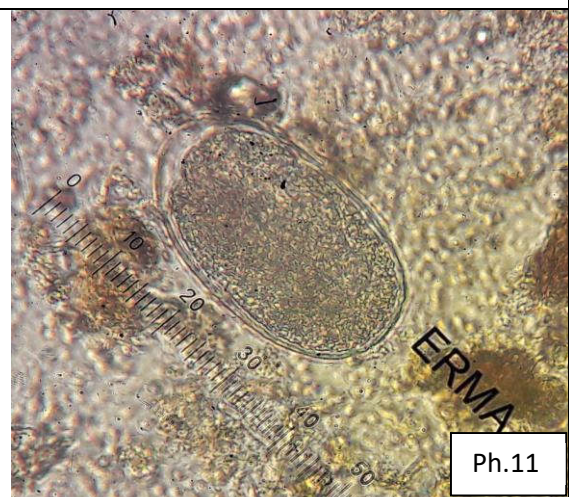
Ph.8



Ph.9

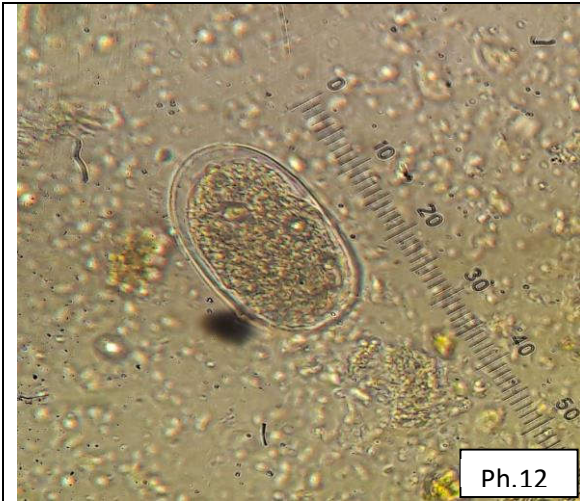


Ph.10

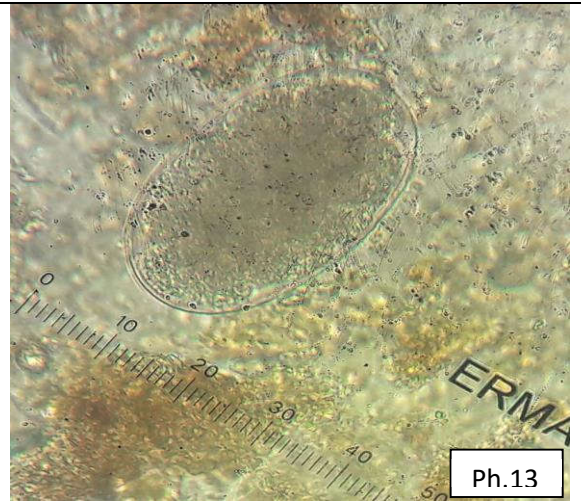


Ph.11

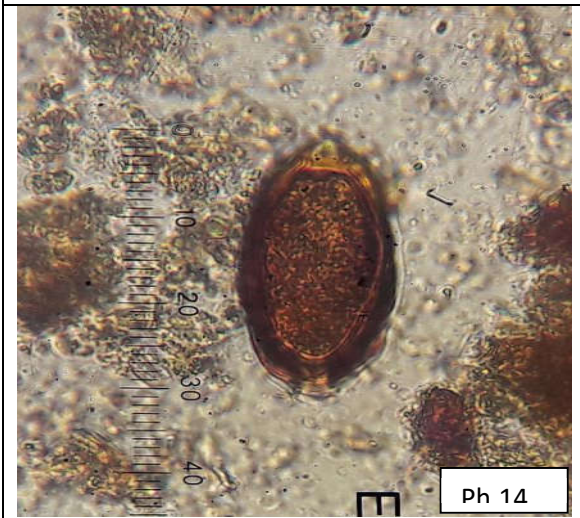
Ph.6: Infertile egg of *Ascaris* sp.(65/40 μ m), Ph.7: Fertile egg of *Ascaris* sp.(52/32 μ m), Ph.8: Egg of *Capillaria* sp.(77/32 μ m), Ph.9: Egg of *Fasciola* sp.(102/51 μ m), Ph.10: Egg of *Oxyuris* sp.(38/15 μ m), Ph.11: Egg of *Strongyloides* sp.(102/55 μ m)



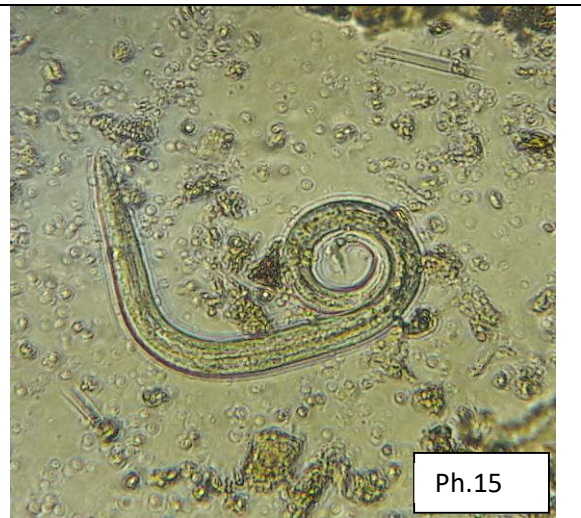
Ph.12



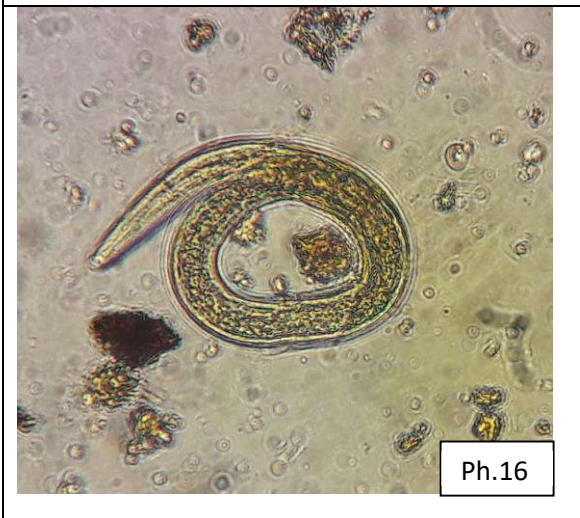
Ph.13



Ph 14



Ph.15



Ph.16

Ph.12: Egg of *Trichostrongylus* sp.(102/52 μ m), Ph. 13: Egg of *Trichostrongylus* sp.(80/50 μ m), Ph.14: Egg of *Trichuris* sp.(77/45 μ m), ph.15: Larvae of *Muellerius* sp., Ph.16: Larvae of *Strongyloides* sp.

4. DISSCUSSION

Among 20 protected areas Shivapuri Nagarjun National Park (IUCN categories: II) is one of the nearest national park from Kathmandu. The park is a home to many species of animal including barking deer. Barking Deer were reported from dense tropical and subtropical forests along with dense wooden hills of India, Bangladesh, Bhutan, Myanmar, Sri Lanka, Thailand, Cambodia, China, Hong Kong, Indonesia and Nepal (Jnawali *et al.*, 2011). Globally they are listed as least concern while national red list data categorized them as vulnerable status. Due to illegal hunting (Timmins *et al.*, 2008), habitat disturbance, conflict with human and disease their number is gradually decreasing in present condition (Jnawali *et al.*, 2011).

Infection and disease are important determinant of the health and well-being of animal population (Scott, 1988). Wild animals have been reported to be infected by various diseases such as bacterial, viral as well as parasitic (Hudson *et al.*, 2002; Gerber *et al.*, 2005). Gastrointestinal parasitic diseases directly as well as indirectly effect on the health status of wild animals especially in young, alter the condition, reduces body weight and brings about reproductive disorder (Fox, 2000). SNNP is the nearest National Park from human settlement and one of the main sites for travelling.

In present study, a total of 90 faecal samples of barking deer from three different locations were examined by direct smear and concentration methods and found 77.78% samples positive for gastrointestinal parasites. Previously, many studies have been conducted nationally and internationally on barking deer (Kanungo *et al.*, 2010; Rahman *et al.*, 2014; Thawait *et al.*, 2014; Aviruppola *et al.*, 2016, Thapa and Maharjan, 2015; Achhami, 2016; Pun, 2018 and Kandel, 2018). Researches were done on wild ruminants (Gupta *et al.*, 2017; Senger *et al.*, 2017; Rana *et al.*, 2015; Farooq *et al.*, 2012; Kowal *et al.*, 2012; Bandyopadhyaya *et al.*, 2010; Meshram *et al.*, 2008; Singh *et al.*, 2006; Yadav *et al.*, 2005) with captive condition such as zoo (Hossian, 2012; Mir *et al.*, 2016; Aviruppola *et al.*, 2016).

Furthermore, occurrence of GI parasitic infection in wild ruminants has been reported greater than 77% by different authors like (Kanungo *et al.*, 2010; Meshram *et al.*, 2008); Kuzmina *et al.*, 2010; Thapa and Maharjan, 2015; Achhami, 2016; Pun, 2018; Kandel, 2018; Chaudhary, 2014). However, some study like (Gupta *et al.*, 2017; Yadav *et al.*, 2005; Farooq *et al.*, 2012; Rahman *et al.*, 2012; Lim *et al.*, 2008; Oli, 2018) has shown lower prevalence of parasites than current study. But some results by Barmon *et al.* (2014), Kanungo *et al.* (2010) and Pandey (2017) have given almost similar results. Due to wide area and varying climatic condition, habitat might cause variation of prevalence in wild ruminants. Where helminth prevalence were most common which was lower than present result (Oli, 2018; Pandey, 2017, Rahaman *et al.*, 2014; Thapa and Maharjan, 2015).

Comparatively lower prevalence rate of GI parasites in captive ruminants was found than the present study such as University of Ilorin Zoological Garden (Kolapo *et al.*, 2017); Bir Moti Bagh Mini Zoo, India (Mir *et al.*, 2016); Dehiwala National Zoological Gardens,

Sri Lanka (Aviruppola *et al.*, 2016); NandanVan Zoo, India (Thawait *et al.*, 2014); Rangpur Recreational Garden and Zoo, Bangladesh (Khatun *et al.*, 2014); Federal University of Agriculture Zoological Park, Abeokuta (Egbetade *et al.*, 2014); Warsaw Zoological Garden, Poland (Maesano *et al.*, 2014); seven Zoological Gardens of Romania (Darabus *et al.*, 2014); AL-Zawraa Zoo, Baghdad (Radhy *et al.*, 2013). This lower prevalence rate of GI parasites in captive form might be due to sanitation and health care system of zoo.

Different study done in domestic animals like goat (Rizal, 2010; R. Purja, 2015; Dabasa *et al.*, 2017; Singh *et al.*, 2017), cows (Jittapalapong *et al.*, 2011; Samaddar *et al.*, 2015; Paul *et al.*, 2016) and buffaloes (Shreedevi and Hafeez, 2014; Alam *et al.*, 2016; Marskole *et al.*, 2016) show more infection of helminthes parasites among domestic animals than barking deer. Due to common grazing and accommodation, poor sanitation might cause higher prevalence of helminthes parasites in domestic animals.

Soil transmitted helminth (STH) are especially infection with particularly open soil enclosures, which couldn't be constituted a major problem to wild animals in fixed enclosure (Elena *et al.*, 2011). Nematode parasites are the most important helminth of Veterinary importance which had been incriminated as gastrointestinal tract (Singh *et al.*, 2006) causing serious disease condition in animals. They impact negatively on the conservation ecology and health of wildlife (Hotez *et al.*, 2008; Gillespie, 2006; Pedersen *et al.*, 2005). Other intestinal parasites (cestode and trematode) need an intermediate host and are less likely to gather in a fixed enclosed environment, because their intermediate host might not occur in easily accessible area.

Wild animals have large territory resulting in greater exposure to different climatic condition and habitat, which increases the probability of sharing parasites among different host, consequently found more (in terms of genera) parasitic infection as compare to captive form because of management as well as health care system. Although barking deer of SNNP were found to be infected with nine genera of gastro intestinal helminth parasites, which was higher number than spotted deer (Airee, 2018; Oli, 2018), swamp deer (Pandey, 2017), axis deer (Barmon *et al.*, 2014; Meshram *et al.*, 2008). Number of Helminth were lower than Kandel (2018) and equal with result of Thapa and Maharjan (2015). But, they were observed cestode free similar to the findings of Airee (2018), Aviruppola *et al.* (2016), Rahman *et al.* (2014), Barmon *et al.* (2014), Mir *et al.* (2016), Gupta *et al.* (2011), Meshram *et al.* (2008) but Kanungo *et al.* (2010), Chaudhary (2014), Thapa and Maharjan (2015), Achhami (2016), Pandey (2017) had reported cestode (*Moniezia* sp).

Although, *Strongyloides* sp. infection was found most prevalent (41.1%) among helminth. This belongs to family strongyloididae. The prevalence of *Strongyloides* sp. was higher than previous reports by Meshram *et al.* (2008), Kanungo *et al.* (2010), Barmon *et al.* (2014), Kandel (2018), Gupta (2017) but lower than Achhami (2016) and Gupta *et al.*, (2017) in India. On the other hand, 25.56% of barking deer were infected with *Haemonchus* sp. which was higher than previous results of Meshram *et al.* (2008), Thapa and Maharjan (2015), Kandel (2018). But some results were higher than present value

like Kanungo *et al.* (2010) in India and Rana *et al.* (2015) in Pakistan. They were nearly in equal result with Kandel (2018) in Buffer zone Chitwan National Park.

Here, 15.6% of studied barking deer were found to be infected with *Ascaris* sp. Which is higher than reported by Rahman *et al.* (2014), Lim *et al.* (2008), Oli (2018) in spotted deer and Thapa and Maharjan (2015) in barking deer. But it was lower than swamp deer in Suklaphata (Pandey, 2017) and Himalayan tahr by (Thapa and Maharjan, 2015) and blackbuck (Chaudhary, 2014) and Wild ruminants of Langtang (Achhami, 2016). Similarly, 13.3% infected with *Trichostrongylus* sp. which was differ from previous reports in India (Meshram *et al.*, 2008), Bangladesh (Kunungo *et al.*, 2010) and Nepal (Chaudhary, 2014) with comparatively higher prevalence rate than current study. However, it has previously been described from other wild ruminants (Davidson *et al.*, 2014; Rana *et al.*, 2015; Thapa and Maharjan, 2015) which were lower than current study. This indicates that *Trichostrongylus* sp. has wide range of host.

Trichuris sp. and *Capillaria* sp. have identical resemblance on egg having mucus plug. But *Trichuris* sp. egg have thick shelled and longer mucus plug than *Capillaria* sp. eggs. *Capillaria* sp. (8.9%) weren't reported much from world around but higher than Bangladesh (Kunungo *et al.*, 2010). But *Trichuris* sp. (5.6%) were found in most of the wild ruminants in India, Pakistan (Meshram *et al.*, 2008; Rana *et al.*, 2015; Gupta, 2017) and barking deer of Nepal (Thapa and Maharjan, 2015) which were comparatively higher than present study.

Only founded lung nematode i.e. *Muellerius* sp. infected 10% of barking deer in SNNP. This has not been reported from most of international publication. They were lower than himalayan tahr (Thapa and Maharjan, 2015) but comparatively higher than and (Airee, 2018) in Nepal. On the other hand *Oxyuris* sp. had been recorded in least amount 3.33% in barking deer which was much lower compared to previous results in Blackbuck (Chaudhary, 2014), wild ruminants of Baghmara buffer zone (Kandel, 2018) and himalayan tahr and barking deer of RNP (Thapa and Maharjan, 2015) but greater than report on spotted deer of Suklaphata (Airee, 2018). This variation in the prevalence rate could be related to the habitat as well as climatic aspect.

Trematodes need intermediate host to complete their life cycle. Barking deer of SNNP were found infected with only *Fasciola* sp. trematode parasites. Previously some reports had recorded *Fasciola* sp. from different wild ruminants, where Airee (2018) reported from spotted deer, Chaudhary (2014) from blackbuck and Pandey (2017) from swamp deer got higher prevalence of *Fasciola* sp. than present study. But Gupta *et al.* (2011) got similar prevalence on sambar, chital and nilgai whereas Oli (2018) got lower prevalence on spotted deer than present study.

Concurrency of parasites represents the different genus of parasites occurrence on single host. Out of infected wild barking deer, 48.60% found single infection more than Oli (2018) and Pandey (2017). Among them 51.5% revealed mixed infection, which was lower than the previous studies Himalayan tahr of RNP (Thapa and Maharjan, 2015), Kanchanpur (Chaudhary, 2014) and in LNP (Achhami, 2016). However, double infection

was found most prevalent in most of the previous result followed by triple and quaternary infections with significant difference.

5. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Overall result revealed that barking deer of Shivapuri Nagarjun National Park were found to be infected by helminth parasites. Overall prevalence of gastro-intestinal helminth parasites was 77.78% in National Park where Panimuhan got highest infection (82.50%) followed by Nagarjun (75%) and Sundarijal (73.33%). Barking deer of SNNP were found infected by two classes of helminthes i.e. nematodes and trematodes which include nine genus of parasites, one genus belongs to trematode and eight genus belongs to nematode. From the identified parasites only *Fasciola* sp. (7.78%) was observed belonging to trematode group. Among nematodes, *Strongyloides* sp. had maximum prevalence of 41.11% followed by *Haemonchus* sp. (25.56%), *Ascaris* sp. (15.56%), *Trichostrongylus* sp. (13.33%), *Muellerius* sp. (10%), *Capillaria* sp. (8.89%) and least number *Trichuris* sp. (3.33%) were observed. No cestodes were found during study period.

Barking deer were found to be infected with highest single parasitic infection (48.60%) followed by 39% of double, 10% of triple and 2.90% of quaternary infection. Maximum of them were infected by single species of parasites followed by double, triple and quaternary infection. Among the single infection maximum of them were infected by *Strongyloides* sp.

The present study confirmed that barking deer are susceptible and infected by various gastrointestinal helminth parasites. This study can be considered as baseline documentation on the study of gastro-intestinal parasites. It will be helpful while designing control strategies of gastro-intestinal parasites in barking deer and ruminants.

6.2 Recommendations

On the basis of conclusion following recommendations have been proposed.

- Further study recommended for characterization about shape of egg and larvae.
- Larvae culture should be done to better confirmation of result.
- Periodically extensive study on parasites of barking deer should be carried up to molecular level.

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