PREVALENCE OF GASTRO-INTESTINAL PARASITES OF SPOTTED DEER (*Axis axis* Erxleben, 1777) IN SHUKLAPHATA NATIONAL PARK, KANCHANPUR, NEPAL.

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Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

February, 2018

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 the sources of information have been successfully acknowledged by references to the author(s) or institutions(s).

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RECOMMENDATION

This is to recommend that the thesis entitled "**PREVALENCE OF GASTRO-INTESINAL PARASITES OF SPOTTED DEER *(Axis axis* Erxleben, 1777) IN SHUKLAPHATA NATIONAL PARK, KANCHANUR, NEPAL"** has been carried out by **Sabita Airee** for the partial fulfillment of **Master's Degree of Science in Zoology** with special paper **Parasitology.** This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been stimulated for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor **Associated Professor Dr. Mahendra Maharjan**, this thesis submitted by **Mrs. Sabita Airee** entitled " **Prevalence of Gastro-intestinal Parasites of Spotted Deer (*Axis axis* Erxleben, 1777) in Shuklaphata National Park ,Kanchanpur, 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

SNP : Shuklaphanta National Park

CITIES : Convention on International Trade in Endangered Species of Wild Fauna and Flora.

DNPWC: Department of National Park and Wildlife Conservation.

EPG : Egg per Grain.

GON : Government of Nepal

i.e. : That is

IUCN : International Union for Conservation of Nature

NaCL : Sodium Chloride

p value : Probability value

sp. : Species

T.U. : Tribhuvan University

ABSTRACT

Spotted deer (*Axis axis*) as a least concern species (International Union for Conservation of Nature) are conserved in Shuklaphata National park, Kanchanpur district. The aim of this study was to determine the prevalence of gastrointestinal parasites in spotted deer of Shuklaphata National park, Kanchanpur district. A total of 150 fresh faecal samples were collected from the grassland of SNP and microscopically examined using proper concentration methods (sedimentation and floatation techniques) in the laboratory of central department of zoology. The result revealed that the overall prevalence of gastrointestinal parasites was 98%. Out of them, helminthes infection (79.31%) was found comparatively more common than the protozoan infection in spotted deer. Among helminthes, high prevalence was found for nematode (51.99%) followed by trematode (27.32%) and protozoan (18.66%) however, no cestodes were recorded during the study. Spotted deer of SNP were found to be infected with eight different species of parasites which includes, *Eimeria* sp. (with micropyle 8% and without micropyle 10.66%) among protozoans; *Paramphistomum* sp. (10.66%) and *Fasciola* sp. (16.66%) among trematodes; *Trichostrongylus* sp. (10%), *Bunustomum* sp. (8%), *Haemonchus* sp. (10%), *Strongyloides* sp. (23.33%) and *Mullerius* sp. (0.66%) among nematodes. Among identified nematodes, *Strongyloides* sp. revealed highest prevalence in spotted deer. During the study, four types of infection were recorded, single (14.96%), double (34.01%), triple (30.61%) and multiple (20.4%). Out of them, double infection were found to be more prevalent. In single infection, it was observed that most of deer were infected with *Strongyloides* sp. while in mixed infection most of them were infected with *Eimeria* sp. and *Strongyloides* sp. In this study, intensity of parasites suggests light infection is prevalent than that of heavy infection. The study indicated that spotted deer of SNP were found to be highly infected with different types of parasites, which could be prevented by adopting the best preventive and control measures against the identified parasites in order to conserve spotted deer.

1. INTRODUTION

1.1 Background

Conservation is the planning and wildlife management of resources, so as to maintain and enhance their quality, value and diversity for the present and future use by the mankind. Wildlife conservation involves the protection, preservation, perpetuation and judicious control of the rare species in their natural habitats (Rastogi, 1996). In Nepal, 12 national park, one wildlife reserve, six conservation areas and one hunting reserve have been established for the protection of wildlife (DNPWC- 2017).

Shuklaphata national park lies in extreme southwest of the terai in Kanchanpur district of far-western Nepal. It is one of the Nepal's protected areas covering 305km² and altitude ranges from 150m to 600m. The name Shuklaphata comes from the largest of this grassland. Shuklaphata is two-third forest and one third grassland. This grassland is the largest in Nepal and occupied area 54sq km. It consists of different types of mammals, such as Tiger, Elephant, Rhinoceros, Hasped hare and many more. Among them spotted deer is also one of protected species. These are valuable wildlife helpful in maintaining ecological balance as well as regarding economic point of view too, and are the most ancient and noble group of mammals..

Spotted deer occur over 8-30°N in India, Nepal, Bhutan, Bangladesh and Srilanka. The past distribution of spotted deer ranged from the foothills of Himalayas and upper part of Assam to a few places in Indo- gigantic plain from the Sundarbans to upper Sind and locally through the area between the Ganges and Godavari (Blandford, 1888-1891). Chitals declined drastically throughout its ranges up until the first two-third of the 20th centeury. Karanth *et al*. (2010) documented an estimated average extinction rate of 45% over the last 50 years in the Indian sub continent. It is however locally abundant in well-protected parts of its range in mixes deciduous habitats. Chital thrive in a variety of habitats, but avoid extreme, such as dense moist (evergreen) forest and open semi-desert or desert.

The spotted deer (*Axis axis*) listed as least concern species (IUCN), locally known as Chital which is a common animal in Shuklaphata National park. The spotted deer is the most widely distributed and abundant cervid species in the Indian protected areas (Arora, 1982). Deer is one of the ruminant wildlife having economic value worldwide. Males are larger and heavier than females. The bright reddish-brown coat of both sexes is marked with scattered white spots. Males alone carry antlers. Spotted deer are more active in the morning and late afternoon. Mostly spotted deer are found in 25-100 numbers in a group. Chital eats a wide variety of plants. It is predominantly a grazer but consumes more fallen leaves, flowers and fruits in winter and dry season (Sankar, 1994). According to the data of spotted deer, counting and monitoring programme of Shuklaphata 2013, there are about 2301, spotted deer in this grassland.

Endoparasites play an important role in the health status of the wild animals. In nature, practically no animal is free from parasites. The animals suffer from a variety of infectious and non infectious diseases, particularly that of parasitic origin (Iqbal *et al.,* 2000). These parasites may be transmitted to their host through ingestion, skin penetration, by the vectors, direct contact and others. Parasites are cosmopolitian and play a significant role in the morbidity and mortality of human as well as animals in many parts of the world.

Ruminants are affected with different kinds of parasites (Coop and Kyriazakis 1999). In some cases, may be fatal due to the type of parasites or the load of parasites (Zhang *et al.,* 2008, Maublanc *et al.,* 2009). Parasites can affect host survival and reproduction directly through pathological effect (blood loss, tissue damage, spontaneous abortion, cogenital malformation and death) and indirectly by reducing the hosts immunity and affecting the physical condition (Thawait *et al*., 2014). Domestic ruminants may transmit the intestinal parasite to the wild ruminants by sharing same pasture and vice-versa. Different types of parasites had been reported in spotted deer such as, *Capillaria bovis, Elephostrongylus cervi, Haemonchus contortus,* and *Trichuris ovis*. Infections with helminthes are a major health issue in captive and wild deer (Goossens *et al.,* 2005). Although outbreaks of parasitic diseases in deer are not so deadly but it is most important to keep the deer free from parasites. In captive deer, parasites such as gastrointestinal nematodes, *Dictyocaulus viviparous* and *Elaphostrongylus cervi* are common (Fletcher, 1982 and Mason, 1994) and often cause mortality and morbidity in deer (Fletcher, 1982). Captivity of wildlife creates an unnatural system and distrupts the balance between parasites and host, and it makes a stressful environment and become animals diseased or can even die from parasites loads, that they would have survived under natural condition (Van Wyk and Boomker, 2011).

In most of the cases, wild and domestic animals share the common grazing land (Walker, 1995). So, an individual host harbouring an infectious agent to the environment through fecal matter and infects other animals in close proximity or that come in contact with contaminated soil, food items or other substance (Mawdsley *et al.,* 1995 and Nunn *et al.,* 2011). Wildlife can be exposed to domestic animal diseases resulting in severe consequences on their population (Gulland 1992, Daszak *et al.,* 2000). The frequent occurrence of diseases has been one of the major factors associated with the decline in numbers of some species of wild and domesticated mammals (Shrestha 2003, Wolfe *et al.,* 2005, and Morgan *et al.,* 2006). Transmission of parasites to spotted deer was not only from domestic animals, it may be transmitted from their own groups because of their gregarious behavior or from wild animals and from different vectors or intermediate host through contaminating pasturing land.

Reports on the mortality of spotted deer in Shuklaphata national park due to the endoparasites have not been studied yet. The decline of spotted deer from this area may be due to diseases, predation as well as illegal poaching and hunting. Although the outbreaks of the parasitic diseases in deer are not so deadly, but it is almost important to keep free from parasites. Recent researches have done to determine the prevalence of parasites in captive deer (Opara *et al*., 2010), but no extensive work has been done in wild condition. So this research focused on studying, the prevalence of the gastrointestinal parasites of spotted deer in SNP.

A regular programme of gastrointestinal parasite surveillance and control measures based on correct diagonosis, effective treatment and proper prophylaxis would be helpful to improve and progress in the health of animals. Application of existing knowledge of disease control and prevention would significantly reduce gastrointestinal helminthesis (Barmon, 2014).

1.2 OBJECTIVES

1.2.1 General objective

- To determine the prevalence of intestinal parasites of spotted deer of Suklaphata National park, Kanchanpur, Nepal.

1.2.2 Specific objectives

- To determine the distribution of intestinal parasites of spotted deer of Shuklaphata National Park, Kanchanpur, Nepal.
- To find out the intensity of the intestinal parasites of spotted deer of Shuklaphata National park, Kanchanpur, Nepal.

1.3 Significance of study

Spotted deer is one of the important wildlife of Nepal. Intestinal parasites are the major problems of various wildlife. Some researches has done to determine the prevalence of captive deer, but no detail work has been done in deer at wild condition. Therefore, this present study attempts to identify the gastrointestinal parasites and to determine the prevalence and intensity of parasitic infection in deer at wild condition, and helps to recommend future deer conservation plan at Shuklaphata National park

2. LITERATURE REVIEW

Ruminants are the primary consumer and the secondary producers. They play a vital role in the ecosystem by providing the food or being food for the prey. They get infected with different parasitic diseases and spread to their surrounding animals (Hutchinson, 2009). Domestic ruminant may transmit the intestinal parasites to the wild ruminants by sharing same pasture and vice-versa. Most of the studies have been carried out on parasites of domestic ruminants. However, only few reports are available on wild ruminants worldwide. This is the first study on parasites of spotted deer of Shuklaphata National Park.

2.1 In Global Context

Some major research regarding intestinal parasites of wild ruminant have been carried out from different national parks, zoo and zoological gardens of different countries. Mckenzie and Davidson (1989) reported *Trichostrongylus axei, Haemonchus contortus* and *Capillaris bovis* in spoted deer in island of Molakai, Hawaii. However, blackbuck antelope (*Antilope cervicapra*) in Texas were examined through postmortem and parasites identified were *Camelostrongylus mentulatus*, and *Haemonchus contortus* in abomasum, *Nematodirus spathiger, Trichostrongylus axei, T. colubriformis,* and *T. probolurus* in small intestine and *Trichuris* sp. in large intestine (Thornton *et al.,* 1973). Worley and Eustace (1972) reported 13 species of helminth parasites in Mule deer (*Odocoileus homionus*) from semiarid rangeland in Garfield and Rosebud countries, Montana. Whereas among white tailed deer (*Odocoileus virginianus*) of the Southeastern United states *Sarcocystis* sp. were found in 51% by light microscopic examination of muscle (Crum and Prestwood, 1982).While another study was carried out by Eve and Kellogg (1977), which recorded *Skrjabinagio odocoilei, Ostertagia mossi* and *O. dikmansi* from medium stomach, *Haemonchus contortus* from large stomach and *Trichostrongylous askivali, T. axei* and *T. dosteri* from small stomach. *Sarcocystis* sp., *Cysticercus tenuicollis, Oesophagostomum venulosum, Cooperia punctata* and *Gongylonema pulchrum* were the parasites found in white tailed deer of West Verginia (Prestwood *et al.,* 1976). Similarly, Grey brocket deer (*Mazama gouazaubira*) of Brazilian Pantanal wetlands were found to be infected with *Haemonchus* sp. (Lux Hoppe *et al.,* 2010). Another study in Mongstad oil refinery, Norway, single species infection was observed in red deer (*Cervius elaphus*) by (Davidson *et al.,* 2014).

Endoparasites of wild ruminants consist of both protozoan and helminthes parasites. Maia *et al.,* (1999) investigated the parasitic fauna of red Deer and fellow deer in various national park in Portugal and has reported *Fasciola hepatica* and *Haemonchus contortus* for the first time in Portugal. In Slovenia 100% *Fasciola* sp. was recorded (Vengust, 2003). Whereas, Roe deer of Pomerania has been found to be infected with

100% nematode parasites along with *Eimeria* sp. (Pilarczyk *et al.,* 2005). However, helminthic burden were higher in fallow deer than in sympatric deer in western Spain (Satin-Duran *et al.,* 2004). While another study was carried out in NW Spain over a period of last 12 years, Coprological analysis revealed *Eimeria sp., Strongyloids sp.* and *Trichirus sp.* (Vazquez *et al.,* 2010). The overall prevalence rate of fallow deer, red deer and roe deer were 92.0%, 96.5% and 73.5% respectively in western pomerania (Cisek *et al.,* 2004). *Strongyloids* sp. were the most commonly found parasites of Red deer and Fallow deer in Lativia (Medne *et al.,* 2009). The another study reported 92.9% prevalence of helminths infection in roe deer of Ukraine (Kuzmina *et al.,* 2010). Similarly, overall prevalence of helminths infection was 36.9% among 23 ruminants of Turkey (Gurler *et al.,* 2010). While in another research the examination revealed the prevalence of *Trichuris* sp., *Trichostrongyloides* sp., *Strongyloides* sp. among wild animals including spotted deer of Macedonia (Atanaskova *et al.*, 2011). Pacon (1994) revealed the prevalence of gastrointestinal nematodiasis 50% to 81% from May 1998 to 1992 in roe deer of Silesa forest, Europe. However the examination revealed very low species count was observed in *Trichuris* sp., *Capillaries* sp. and *Strongyloides* sp. in red deer of Tapada national de Mafre (Maia *et al.,* 2001). Similarly the overall prevalence of parasites of six herbivores, including spotted deer was 67.8% in Timisoara Zoo (Darbus *et al*., 2009). Wherease, both intensity and prevalence of abomasal parasitism were recorded higher in older animals in wild red deer of central Spain (Satin-Dural *et al*., 2008). However, Medne *et al.,* (2009) recorded *Strongyloides* sp. were the most commonly found parasites in deer of Lativia. Valcarcel *et al.,* (2002) examined 196 faecal samples in *Cervus elaphus* in central Spain, 90.3% of deer were infected (mean intensity was 348.3 worms per animals) where males were more frequently and heavily infected than females. Ferte *et al*.,(2000) conducted a study of helminth fauna of roe deer and red deer in France between 1985 and 1998 which revealed the presence of nematodes belonging to two genera (*Haemonchus* and *Ashworthius*) in the sub family Haemonchinae.

Many parasites infecting wild ruminants have been recorded in Egypt, such as *Moniezia expansa, M. benedani,* and *Fasciola hepatica* by Wahed (2004). Similarly, from wild animals *Entameoba* sp. and *Schistosoma* sp. has been reported from South East Nigeria (Opara *et al.,* 2010). Also the nematodes *Strongyle* sp. were recorded in the faecal sample of wild animals in Ethopia (Bogale *et al.,* 2014). While another study was carried out in same country Ethopia, *Dictyocaulus filarial* and *Trichostrongylus columbriformis* having high prevalence has been observed in Sheep (Bekele *et al.,* 1992). Some reports have indicated the presence of *Giardia* species in wild ruminant in southeast Nigeria (Hunter and Thomson, 2005).

Lim *et al.,* (2008) in Malaysia reported the overal prevalence of primates and hoofed mammals were 54.5% and 45.75% respectively. Whereas three genera of intestinal parasites, *Eimeria* sp, *Dirocoelium chinesis, Trichuris ovis* were reported in sika deer (*Cervus nippon*) of Japan (Shibashi *et al.,* 2003). The comparative study was carried

out in hog deer *(Axis porcinus*) of Pakistan, the overal prevalence was 86.05% (Rana *et al*., 2015). As in the same country, study of endoparasites of samber and ghoral was conducted, prevalence of helminth infection was 77.5% with *Trichostrongylus* sp. in large number were reported (Rana *et al.,* 2015). The parasitic infection was studied in fallow deer, examination revealed 57.32% average prevalence and intensity of 529 EPG, where *Trichostrongylus* were found more frequently (Pilarczyk *et al.,* 2015).

Some major research regarding intestinal parasites of wild ruminant have been carried out from different national parks, zoo and zoological garden of different countries. Barmon *et al.,* (2014) reported the prevalence of gastro-intestinal parasite of deer were 69.29% with higher prevalence of helminthes parasites in Char Khukri, Mukri Upazilla of Bhola district of Bangladesh. Similarly, another study was carried out in Dhaka National Zoological Garden of Bangladesh where overall prevalence of gastrointestinal parasite of deer was 76.5% of which 61.5% for helminthes and 55.8% for protozoan (Rahman *et al.,* 2014). The overall incidence of *Fasciola* sp. infection was 82.5% that has been reported from domesticated deer of Chittagong, Bangladesh (Masuduzzaman *et al.,* 2005). In the same place, *Paramphistomum* sp. were recorded 100% in spotted deer *(*Islam *et al.,* 2003) The coprological study of gastrointestinal parasites of spotted deer was conducted at Rajpur recreational garden and Zoo in Bangladesh, mixed infection of *Strongyloides* sp. and Coccidian (*Eimeria* sp.) was observed (Khatun *et al.,* 2014).

Meshram *et al.,* (2008) revealed 89.05% of parasitic prevalence in spotted deer of Borgaon Manju in Maharastra, India. While, in the same country infection with lungworm (*Mullerius capillaries*) was reported among the deer of national park (Ramaswamy *et al.,* 1991). The parasitic prevelance 38.19% was reported in spotted deer of Mahendra Choudhury zoological park, Punjab (Singh *et al.,* 2009). Parasites such as *Ascaris* sp., *Trichurus* sp., *Strongyloides* sp. and *Fasciola* sp. were reported from the zoo animals of Bihar (Modi *et al.,* 1997). While, another study was carried out in six different location of Nagpur, examination revealed 96.13% of parasitic prevalence with 100% helminthic infection in spotted deer (Kashid *et al.,* 2002). Again in Nagpur, overall prevalence of helminthic infection 90.02% was observed in spotted deer (Hussain *et al.,* 2002). Also from Maharajbag zoo, Nagpur, out of 66 samples of spotted deer 30 were positive for helminthic eggs and larva (Borghare *et al.,* 2009). Whereas maximum infection of *Strongyle* spp 41.7% were reported in free ranging chital of Mudumalai wildlife sanctuary, Tamilnadu (Mandal *et al.,* 2002). While the parasites like *Trichuris* sp. *Strongyle* sp. and coccidia sp. were reported from spotted deer of typical zoo, Coimbatore, Tamilnadu (Varadharajan *et al.,* 2000). Infections with helminthes are the major health issues in captive and wild deer (Goossen *et al.,* 2005) .

Bante *et al.,* (2013) reported 42.90% prevalence for helminth parasite in wild animals kept at Kamala Prani Sangrahalaya, Indore. The investigation on seasonal variation in captive spotted deer of Nagpur, the parasites *Strongyloides* sp. infection was found predominant in the both season (Jadhav *et al.,* 2010). However, another study was

carried out in Jabalpur, the prevalence of *Strongyloides* sp. was found highest followed by *Trichuris* sp.and *Fasciola* sp. in spotted deer (Gupta *et al.,* 2011). Similarly 74% of animal infected with helminthes and 23% infected with protozoans were reported in wild animals in zoological garden of Kerela (Varadharajan *et al.,* 1999). In the same way, *Trichostrongylus* sp. and coccidia were reported out of two samples of spotted deer (Parsani *et al.,* 2001). While another study was carried out in Bir Moti Bagh Zoo, Patiala, Punjab, where mixed infection were observed in 48% of captive wild animals and no cestodes were reported during study (Mir *et al.,* 2016). In the same way the research revealed 17.7% of spotted deer were positive for either single or mixed parasitic infection (Banerjee *et al.,* 2005).

2.2 In National context

Most of the researches have been carried out regarding to the domestic ruminant however few research have been done to the parasite of wild ruminant in context of Nepal. Protozoan parasites like coccidia, *Eimeria sp., Cryptosporidium* sp. and *Isopora* sp. (Khanal, 2006) and (Ban, 2012) have been reported in blackbuck from Khairapur, Bardia. Similarly, Thapa (2013) has reported two types of *Eimeria* sp. in the faecal samples of himalayan Thar and barking deer from Rara National Park such as *Eimeria* with micropyle and *Eimeria* without micropyle that seems to be non pathogenic. Whereas, the parasites like *Entamoeba* sp., *Eimeria* sp., *Paramphiostomum* sp., *Fasciola* sp., *Strongylous* sp., *Trichostrongylous* sp. and *Oxyuris* sp. were reported in blackbuck of Blackbuck Conservation Area, Bardia and Shuklaphata Wildlife National park, Kanchanpur (Chaudhary, 2014). While, in spotted deer no work has been reported from Nepal.

The *Haemonchus* sp. (wire worm) is a blood feeding parasites that causing anemia, edema, weakness and others in bovids has been identified in deer, similarly *Trichostrongylous* sp. *and Trichuris* sp. has also been identified in different ruminants including deer at Mrigusthali forest, Pashupati (Rimal, 2011). While, another study was carried out in Langtang national park, to show the prevalence of gastrointestinal parasites in wild ruminants and Chauris, examination revealed that the overall prevalence of gastrointestinal parasites was 85.9%. Seven species of parasites were identified with one protozoan *(Eimeria* sp.), four nematodes, one cestode and one trematode (Achhami, 2016).

There are numerous parasites found in the intestine of wild as well domestic animal that have been reviewed by different researchers. Overall prevalence of helminth was 81.53% in goat of Kalanki khasibazar (Parajuli, 2007) where 46% were found positive for helminth in winter and 90.3% in summer (Karki *et al.,* 2012) whereas 79.70% of prevalence with trematode 5.94%, cestode 4.45% and nematode 69.30% was found in the goat of Baghbazar, khasibazar (Pathak, 2011). In addition trematode

Dicrocoelium lanceatum and *Ornothobilharzia turkestanicum* were reported for the first time in Nepal from Buffaloes (Mukhia *et al.*, 2007). Moreover, *Paramphistomum* sp. and *Fasciola* sp. were found as common parasites of cattle in Kathmandu valley (Thakuri and Mahato 1990, Shrestha, 1996 and Sapkota *et al.*, 2006). In the case of buffaloes of slaughter house, Kirtipur *Fasciola hepatica* (59.67%) was found slightly higher than *Fasciola gigantica* (52.63%) (Shrestha, 2010). While in spotted deer no work has been done from Nepal.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Shuklaphata National park, which is a protected area in the Terai of the Far-Western region, kanchanpur, Nepal. It covers 305sq. km of open grassland, forests, riverbeds and tropical wetlands at an altitude of 174 to 1,386 meters. Area, 117.8 m². It is situated in Kanchanpur district which lies between 28°35'0" North and 80°25'0" south. It extends north of the east west highway to create a corridor for seasonal migration of wildlife into the sivalik hills. SNP was gazetted in 1976 as Royal Shuklaphanta National Park. The reserve was upgraded to National park status on 3rd March 2017, by government of Nepal.

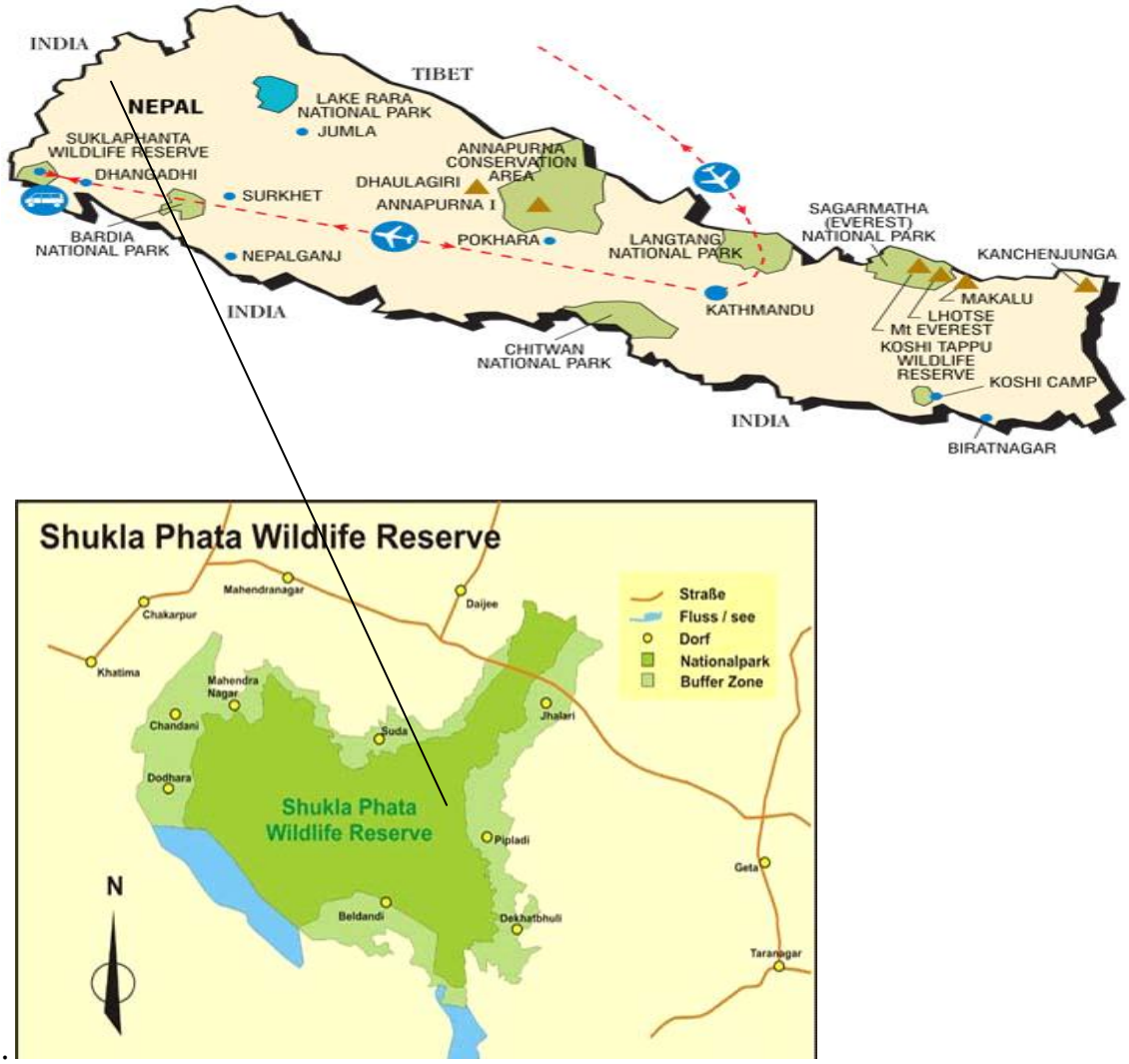


Figure 1- Map showing study area in Shuklaphata National Park

Shuklaphata National park is important both nationally and internationally for its extensive grassland or phantas that constitute almost half of national park vegetation. There are other smaller phantas in the reserve which are also important for wild animals especially for birds. These are Singhpur phanta, Karaiya phanta, Barkaula phata, Hirapur phanta, Arjuni phanta and some smaller phantas near Jhilmila and Radhapur. The main grass species include Siru (*Imperata cylindrica*) and Kans (*Heteropogon contortus)*. Besides these tree species like *Bombax ceiba* and *Butea monospermae* growing along the pheriphery of the phantas and provide ideal habitat for different animals. These grasses are extensively used by the local people for thatching.

SNP has a hot and dry mansoon climate. The climate is predominantly tropical to monsoon with more than 90% of annual precipitation. The average daily temperature during winter ranges from 10-20°C and reaches as high as 32-35°C in summer. The maximum temperature reaches upto 42°C in summer.

3.2 Materials Required

The materials used during research work have been listed below

3.2.1 Apparatus

i. Weighing machine
ii. Slide
iii. Beaker
iv. .Coverslip
v. Dropper
vi. Pipette
vii. Forceps
viii. Needle
ix. Wooden box
x. Electric microscope
xi. Oculometer
xii. Test-tube stand
xiii. Vials
xiiv. Centrifuge machine
xv. Toothpick
xvi. Gloves and mask
xvii. Petridis
xviii. Conical flask
xix. Binocular microscope
xx. Centrifuge
xxi. stage micrometer
xxii. cotton

3.2.2 Chemicals

- 2.5% Potassium dichromate solution
- Sodium chloride solution (NaCl)
- Distilled water
- Lugol's iodine solution
- Methylene blue

3.2.3 Preparation of 2.5% potassium dichromate

2.5 gram of potassium dichromate was weighed and dissolved in 100ml of distilled water. This solution was used for the preservation of parasites which contained in the faecal matters (Zajac and conboy, 2012).

3.2.4 Preparation of Normal saline

Normal saline was prepared by dissolving 8.5 gm of sodium chloride in 1000ml of distilled water which was used in unstained preparation (Zajac and Conboy, 2012).

3.3 Study Design

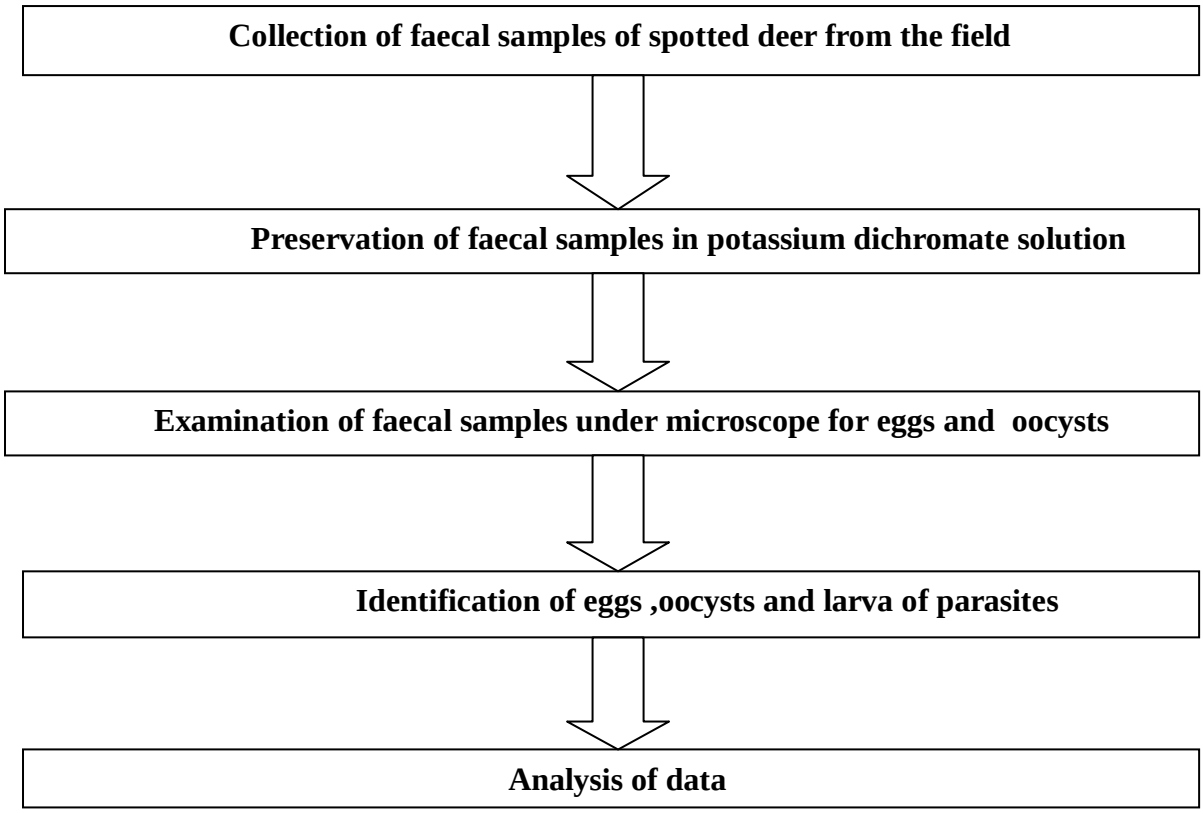


Figure 2: Research flow chart

3.3.1 Sample size

A total of 150 faecal samples of spotted deer were collected from different grassland areas of Shuklaphata National park, near Ranital, Khairbadi ,and Majgau from November 2016.

3.3.2 Collection of stool sample

Stool sample of spotted deer was collected from November 2016 to Febrauray 2017. Fresh faecal samples were collected systematically from dawn to dusk. About 5gm of fresh sample were collected in 20ml sterile vial and 2.5% potassium dichromate was added to cover the sample completely. Then vial was tightly closed and put in cool box. The collected faecal sample was transported to lab at central department of zoology and was kept in refrigerator at 4°c for further processing.

3.3.3 Examination of stool sample

After collection, all the faeces were examined macroscopically for presence of blood or mucus, adult or larval helminthes. Samples were transported to the laboratory of Central Department of Zoology (CDZ), TU, Kirtipur, Kathmandu. In the laboratory the sample were microscopically examined using both direct smear and concentration method (Floatation and sedimentation techniques).

3.4 Methods

3.4.1 Saline wet mount

Small quantity (about 2gm) of faeces was mixed in a drop of saline placed on a clean slide. Any grass fiber or particles were removed and covered with coverslip. The smear was examined under microscope at 10X and 40X (Soulsby, 1982).

3.4.2 Iodine wet mount

It was done by emulsifing the faecal samples in a drop of Lugol's iodine solution on a slide covered with a clean coverslip and examined under microscope as above.

3.4.3 Floatation method

The floatation technique is widely used for the detection of nematode and cestode eggs which are relatively small and light. This technique ensures the eggs to float in the floatation liquid. Approximately 3gm of faecal sample were taken in a beaker and 20 ml of water were added. Then the samples were mixed or grinded with the help of mortar or pestle and filtered the solution by tea strainer. The filtrate solution was poured into a centrifuge tube of 15ml and centrifuge at 2000 rpm for 5 minutes. The tube water was replaced with saturated sodium chloride solution and again centrifuge. After centrifuge more saturated sodium chloride solution were added to develop convex surface at the top of tube and one drop of methylene blue (to stain) where a cover slip could be placed at top for a few minutes and then cover slip were removed and placed on a slide and examined at 10X and 40X. Photographs of egg/ cyst/ oocyst/larva were taken and identified based on egg/larvaes color, shape and size and with the help of different keys.

3.4.4 Sedimentation method

This technique is used for the detection of the trematode eggs. It provides good result as the egg of trematode are bit heavier then the other eggs and deposited at the bottom (Veterinary Lab, techniques 2003). Saturated salt solution were removed gently from the test tube after examination of floated portion and poured the sediment content into watch glass and stirred the content gently to mix it. One drop from the mixture was taken to prepare a second slide. The specimen were stained with iodine wet mounts solution. In this way two slides were prepared from one sample and examined under 10X and 40X magnification of microscope to detect eggs of helminthes and cyst of gastrointestinal protozoans.

3.5 Measurement of Diameter of Eggs/Oocysts/Cysts

The eggs/ ocysts/cysts and larva were identified in faecal samples of spotted deer, on the basis of their shape and size, using standared keys (Soulsby, 1982). The length and width of eggs/ocysts/cysts were measured by using a micrometer (Cable, 1965).

3.6 Data Analysis

Since the study was mainly focused on identification of different gastrointestinal parasites, the presence and absence of parasites data were entered in MS Excel 2007 and to show the significance of parasites the chi-square and p-value was calculated in SPSS 21 version.

Glimpse of photographs during field and lab work

Pellets of spotted deer in the field

Spotted deer in SNP

spotted deer in the field

Chemical used during field

Working in laboratory

Observing the slide on microscope

4. RESULTS

Spotted deer is a wild herbivores, widely found in Shuklaphata National park, Kanchanpur. The study was conducted to identify the gastrointestinal parasites in spotted deer of SNP through faecal examination, by using floatation, sedimentation and direct smear technique.

4.1 General prevalence of gastrointestinal parasites of spotted deer.

During the study period, a total of 150 fresh faecal samples of spotted deer were collected from different grassland area of SNP. Microscopic examination of the samples revealed overall parasitic prevalence of 98%. Maximum spotted deer were infected by either protozans or helminth parasites as well as both type of parasites.

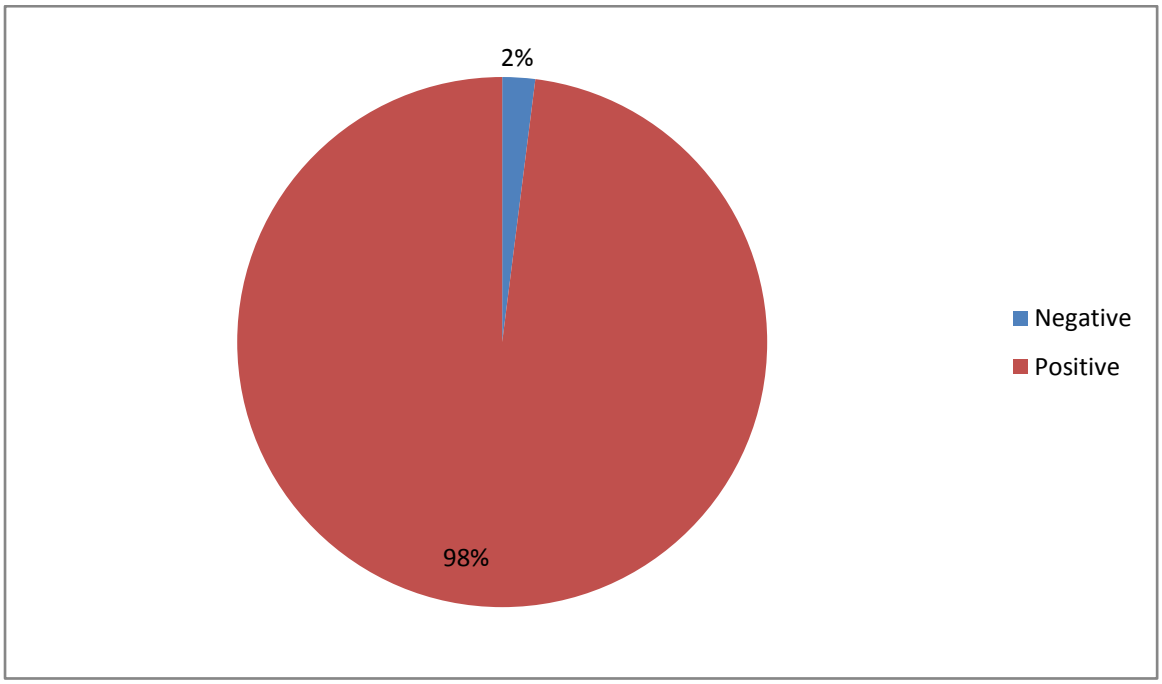


Fig: 1 General prevalence of spotted deer

4.2 Prevalence with parasitic group

At the time of study, a total of three parasitic groups were observed from spotted deer, protozoan, trematode and nematode. Among them high prevalence was found for nematode parasities (51.99%) followed by trematode (27.32%) and protozoans (18.66%). However no cestodes were recorded in this investigation. The result indicated that the nematode infection were more common then protozoan in spotted deer (fig 2).

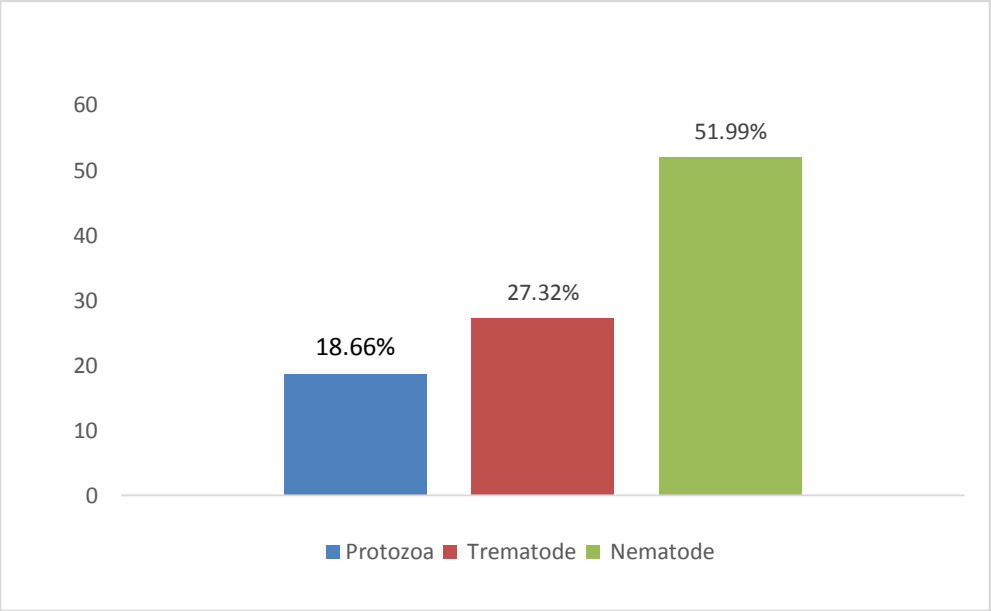


Fig : 2 prevalence of gastrointestinal parasites with parasitic group

4.3 Prevalence of intestinal parasites of spotted deer

During study, ten different species of gastrointestinal parasites were found in the spotted deer of SNP. Among protozoans only one genus *Eimeria* sp. was observed belonging to the class coccidian parasites. *Eimeria* sp. (coccidia parasites) couldn't be identified upto species level, hence broadly they were catogorised in two groups, *Eimeria* with micropyle and without micropyle. The result showed spotted deers were found to be infected with both type of *Eimeria* sp. almost equally with statistically insignificant association(χ^2= 0.64, P=0.42).

Table:1 prevalence of intestinal parasites of spotted deer

S.N.	Class	Identified intestinal parasites	Prevalence	χ^2 value	P-value
1	Protozoa	*Eimeria* with micropyle	8%	0.64	0.42
		Eimeria . without micropyle	10.66%		
2	Trematode	*Fasciola* sp.	16.66%	3.12	0.08
		Paramphistomum sp.	10.66%		
3	Nematode	*Trichostrongylus* sp.	10%	48.33	$8.04e^{1°}$
		Bunostomum sp.	8%		
		Haemonchus sp.	10%		
		Strongyloides sp.	23.33%		
		Mullerius sp.	0.66%		

Among helminth parasites, only nematode and trematode parasites were recorded. The result showed that they were infected with two species of trematodes parasites i.e, *Fasciola* sp. (in liver site) and *Paramphiostomum* sp. (in the small intestine) were identified without significant association. (χ^2= 3.12, P=0.08). While incase of nematode, five types of parasitic species were reported with high prevalence of *Strongyloides* sp. (23.33%) and low prevalence of *Mullerius* sp. (0.66%). The overall research, showed that the infection of nematodes were very high with statistically significant association ((χ^2= 48.33, P=$8.04e^{1°}$).(Table 1).

4.4 Mixed Infection in spotted deer of SNP

In the present study, the rate of mixed infection was also observed. Mixed parasitic infection was found in all the positive samples of spotted deer of SNP . Four types of infection (single, double, triple and multiple) were categorized. The occurrence of double infection was highest (34.01%) followed by single, triple and multiple infection. While studying mostly, *Strongyloides* sp. or *Paramphistomum* sp. were observed in single infection *Eimeria* sp. and *Strongyloides* sp. were observed in double infection, *Strongyloides* sp., *Eimeria* sp. and *Fasciola* sp. observed in triple infection. Similarly, *Eimeria* sp., *Strongyloides* sp., *Fasciola* sp., and *Haemonchous* sp. were observed in multiple infection.

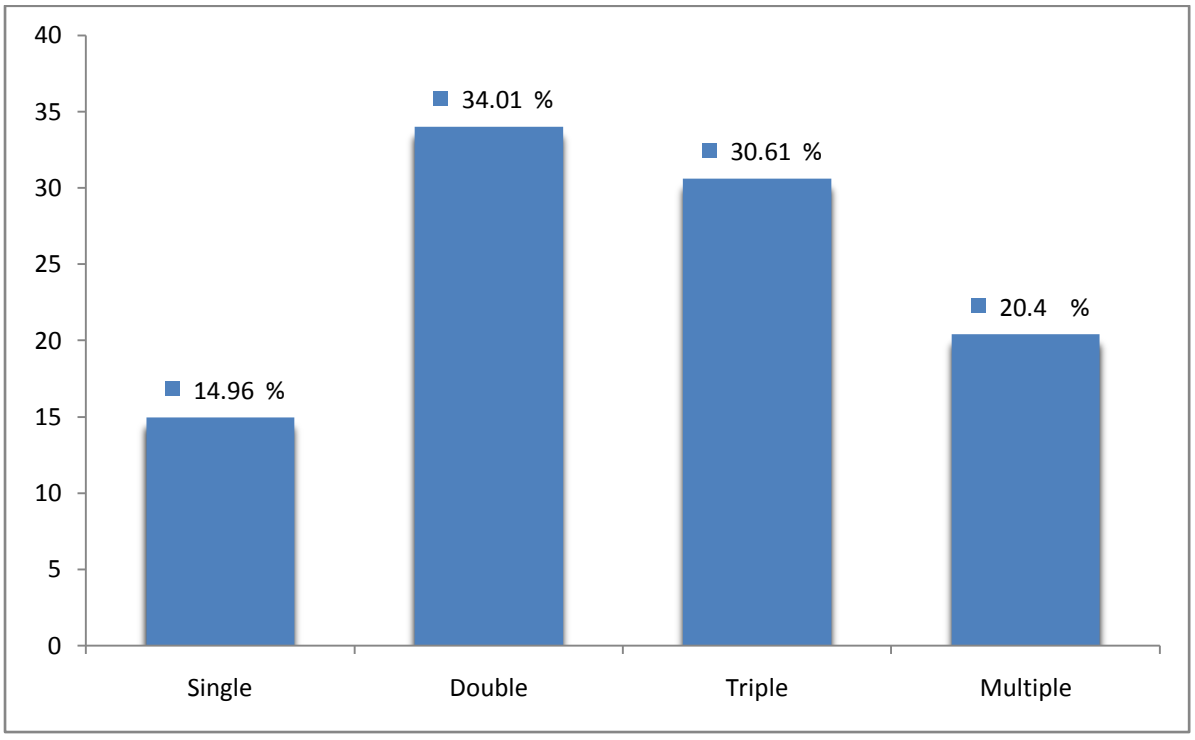


Fig: 3 Mixed infection in spotted deer.

4.5 Intensity of parasitic infection in spotted deer

Intensity of parasitic infection has been assessed based upon the number of egg/oocyst and larva found per field. In SNP heavy parasitic infection was considered in those samples which has 6 or more ova or oocyst observed per field. Among the total positive samples, *Eimeria* sp. (0.6%) belong to protozoa, *Strongyloide*s sp. (2.66%) belongs to nematode revealed heavy infection. While maximum number of spotted deer were infected with light infection which was considered < 2 ova/oocyst observed per field, and remaining all positive samples showed mild and moderate infection (Table 2).

Table 2: Intensity of parasitic infection

Class	Parasites	Light (+)	Mild (++)	Moderate(+++)	Heavy(++++)
Protozoa	*Eimeria with micropyle*	5 (3.33%)	3 (2%)	2 (1.33%)	1 (0.6%)
	Eimeria without micropyle	4 (2.66%)	9 (6%)	3 (2%)	-
Trematode	*Fasciola* sp.	15 (10%)	7 (4.66%)	3 (2%)	-
	Paramphistomum sp.	10 (6.66%)	6 (4%)	-	-
Nematode	*Trichostrongylus* sp.	8 (5.33%)	6 (4%)	1 (0.6%)	-
	Bunostomum sp.	9 (6%)	3 (2%)	-	-
	Haemonchus sp.	8 (5.33%)	4 (2.66%)	3 (2%)	-
	Strongyloides sp.	9(6%)	16 (10.66%)	6 (4%)	4 (2.66%)
	Mullerius sp.	1 (0.66%)	-	-	-

Note: Light infection = < 2 eggs/cysts/ larva per field (+)

Mild infection = 2-4 eggs/cysts/ larva per field (++)

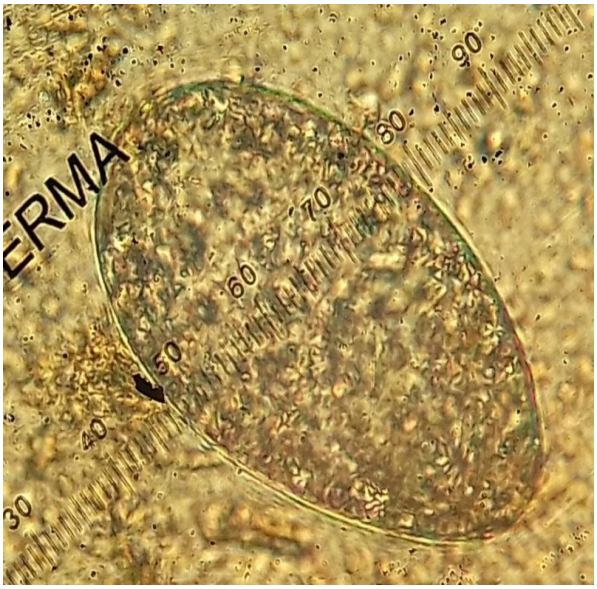
Moderate infection = 4-6 eggs/cysts/ larva per field (+++)

Heavy infection = ≥ 6 eggs/cysts/ larva per field (++++).

Photographs of identified gastrointestinal parasites in 40X.

Photograph 1*: Paramphistomum* **sp.(137µm)**

Photograph 2: ***Haemonchus*** **sp.(78µm)**



Photograph 3: ***Fasciola*** **sp.(130µm)**

Photographs 4: ***Mullerius*** **sp.**

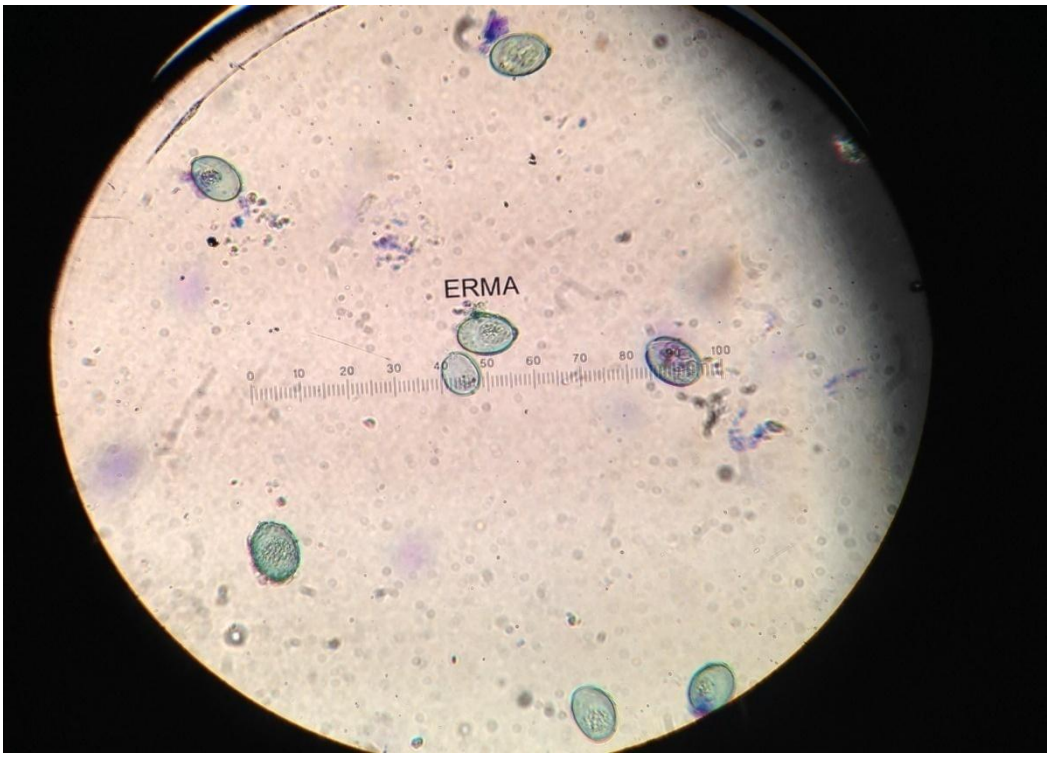
Photograph 5*: Strongyloides* sp.(91µm)

Photograph 6: *Trichostrongylus* sp.(104µm)

Photograph 7 :*Bunustomum* sp.(90µm)

Photograph 8*: Eimeria* with micropyle (31µm)

Photograph 9: *Eimeria* without micropyle (26µm)



Photograph 10: *Eimeria* sp. in group

Table 3 : Identified eggs and cysts of parasites found in spotted deer

Name of Parasites	Range of diameter of eggs and oocysts (in µm)		Morphological character	Refrence values (Soulsby,1982)
	Length	width		
Eimeria sp.	31	-	Cysts are small, rounded or spherical with four nucleus.	15-55µm
Paramphistomum sp.	137	78	Operculated get stained in methylene blue.	114-176µm × 73-100µm
Fasciola sp.	130	70	Thin shells, operculated, brown.	130-150µm × 63-90µm
Strongyloides sp.	91	33	Blunt ends , thin shells, contain fully developed embryo or larva.	40-60µm × 20-40µm
Haemonchus sp.	78	46	Embryo divided into 16-32 cells when passed in faeces.	70-85µm × 41-48µm
Trichostrongylus sp.	104	39	.Irregular, elipse dissimilar, kidney shaped not very wide poles,one of which was more rounded than the other,dissimilar side walls.	70-108µm × 30-40µm
Bunustomum sp.	90	49	Bluntly rounded, embryonic cells darkly granulated	79-97µm × 47-50µm

5. DISCUSSION

Ruminants are the primary consumer and play important role in ecosystem. Spotted deer *(Axis axis*) is one of the protected species of SNP. In the present research, the study of gastrointestinal parasites of spotted deer has been carried out in SNP for the first time in Nepal. The research so far has not been adequate enough, especially in context to our country so, the prevalence figure of the present work are compared with the work done in other ruminants including deer species and wild mammals. The present study explore parasitic prevalence, multiple infection and intensity in spotted deer. The prevalence of gastrointestinal parasites in spotted deer of SNP has been mostly influenced by various environmental factors such as season, relative temperature, climate and others. An attempt was made to control as many confounding factor as possible in spite of several limitation.

Different reserchers has reported the prevalence of parasitic infection which is similar to the present study. Cisek *et al*., (2004) who reported overall prevalence rate in fallow deer, red deer and roe deer as 92.0%, 96.5% and 73.5% respectively in western Pomernia. The result is more or less similar to the present study which revealed 98% of overall prevalence in spotted deer of SNP. Rana *et al*., (2015) and Khasid *et al*., (2002) also reported the similar result to the present study, that reported 86.05% and 96.13% as overall prevalence in hog deer of Pakistan and spotted deer of India. The prevalence in spotted deer was found high, because during the faecal sample collection it was found that the grazing land for wild ruminant was heavily over grazed by other animals and contaminated by their faecal matter or contamination from their own groups because of their gregarious nature . It was also found the grazing land was shady with high moisture that favors the survival of egg or cysts of parasites.

The present study shows that spotted deer were mostly infected with nematode parasites while protozoan, trematodes and cestodes were comparatively less predominant to them. Varadharjan *et al*., (1999), Pilarcyzk *et al*., (2005), Satin- Duran *et al*., (2008) and Thawait *et al*., (2014) reported that deer was highly susceptible to gastrointestinal nematodiasis which support the present study which revealed 51.99% of nematodes infections . Nematode infection were found to be highest is spotted deer in the present study. Nematodes have direct life cycle and are mostly present in soil. Grasses grown in such contaminated soil also may contain eggs and L3 larva of nematodes. This might be the reason behind heavy infection of nematodes in spotted deer. During the present study no cestodes parasites were recorded in spotted deer of SNP which is similar to the findings of Borghare *et al*., (2009) who reported the absence of cestode infection in captive deer at Maharajbag zoo, Nagpur. Spotted deer doesnot feed on intermediate host such as fish and crustaceans. Or, the intermediate host required for the cestodes to complete its life cycle might not be present in the study area, are might be the reason behind the absence of cestode eggs in spotted deer.

Several protozoans and helminthes parasites can infect the GI tract of ruminant. Helminthes are major health issue in captive and wild deer (Goossens *et al*., 2005). Varadharajan *et al*., (1999) reported 74% of animals were infected with gastrointestinal helminthes at Kerela, India, which is more or less similar with the present study which revealed 79.3% of helminth infection and 18.66% of Protozoan infection. The present study also confirm the report of Varadharajan and Kandasamy (2000), who recorded that 58% of animals were positive for helminths infection and only 6% were positive for protozoan infection in a wild animal in a mini Zoo Coimbatore. Khatun *et al*., (2014) reported 55.6% of prevalence of helminth infection which was slightly lower that of present study while 24% prevalence of protozoan infection which is slightly higher than present study. Whereas, Kashid et al., (2002) reported 100% helminthes infection in sported deer in Nagpur, India. Such variation between the prevalence of gastrointestinal helminthes and protozoan infection might be that helminths eggs are resistant to moisture and temperature whereas protozoan parasites are very sensitive to moisture, temperature and other environmental condition and also due to geographical condition or method of sample collection.

Infection Regarding with Coccidian species, Diez *et al*., (2010) reported *Eimeria* species from Galicia in roe deer, Mohammad *et al.*, (2012) reported *Eimeria saudiensis* from Saudi Arabia and Tovassoli *et al*., (2011) reported one or more *Eimeria* sp. from NW Spain which is similar to the present study which encounter only genus *Eimeria* sp. in spotted deer of SNP. According to the previous report, *Eimeria* sp. can not be differentiated into species level in present study because it needs culture to identify the species of this parasite. But it can be differentiated into two types (i.e. *Eimeria* with micropyle and without micropyle) on the basis of morphological structure. Corden *et al*., (2008) obtained 17.3% prevalence of *Eimeria* sp. in Blackbuck which is more or less similar to the present study which revealed 18.66% of prevalence in *Eimeria* sp. in spotted deer. Oocyst of *Eimeria* sp. passess in the faeces of spotted deer and are resistant to disinfectants and can remain in the environment (moist and shady area) for long period of time and maintain their infectivity (Kennedy, 2001) which can cause diarrohea, loss of appetite, weakeness and even death in several cases.

During the study, the *Fasciola* sp. was recorded 16.66% which was less then reported by Kanungo *et al*.,(2010), who reported strong infection of *Fasciola* sp. 20% in spotted deer and 36.36% in para deer in Dhako zoo and in Safari park, India. It was evident from these result that deer was susceptible to *Fasciola* sp. While, Gupta *et al*., (2011) and Barmon *et al* ., (2014) reported the prevalence of *Fasciola* sp. 6.7% and 8.66% in deer around Jabalpur and at Char Khukri Mukri in Bhola district, Bangladesh which was less than the present study. The spotted deer of SNP was found to be mostly infected by *Fasciola* sp. This might be due to the location of animals area, availability of intermediate host such as snails, fishes and other animals to complete their lifecycle. The probable cause of trematode infection was, strongyle

connected with mud snail which lives in water and located in the conservation area, as well as by eating short grasses which may contaminated with metacercaria.

Various species of nematode were isolated from the faeces of wild and captive ruminants. The present study also explore the nematode species such as, *Mullerius* sp., *Trichostrongylus* sp., *Haemonchus* sp., *Bunostomum* sp. and *Strongyloides* sp. Ramaswamy *et al.,* (1991) investigated the prevalence of parasitic infection among the spotted deer in three national parks in India, and revealed infection with the lungworm *Mullerius capillaries* which is similar to the present study. Cross transmission of *Mullerius capillaries* occur between wild and domesticated animals. *Haemonchus contortus* (wire worm) is one of the important pathogens for blackbuck (Thornton et al, 1973), producing a disease known as Haemonchiasis, which can cause anemia, growth loss, edema and even death (Roberts and Janovy , 2005) which was recorded by Meshram e*t al* ., (2008) and Thapa (2013) as 13.80% and 2.94% in spotted deer and in barking deer which was higher and comparatively lower than the present study, which revealed 10%. The parasites such as *Trichostrongylus* sp. (11.50%) and *Bunostomum* sp. (4.0%) were also recorded by Meshram *et al* ., (2008) in spotted deer which is more or less similar to the present study. High prevalence infection was observed in those parasites which have direct lifecycle such as coccidian and gastrointestinal nematodes.

In the present study, the mixed parasitic infections were found to be more common in spotted deer due to high contamination of pasture by grazing of domestic animals. Single infection was observed 14.96%, double (34.01), triple (30.61%), and multiple (20.4%) in spotted deer of SNP. The present result was similar to the finding of Kanungo *et al.,* (2010) who documented that higher rate of mixed infection was noted in majority of deer and Thapa (2013) who described the mixed infection was most common in himalayan thar and barking deer. Mixed infection rate was observed as 15% single, 20% ,double, 15% triple 30% quadriple and 5% quinruple (Seong-jun *et al.,* 2011) which are higher than single infection, lower than double infection, higher than triple infection of present study. While, quadruple and quinruple infection were not observed in present study.

During the study, mixed infection of *Eimeria* sp. and *Strongyloides* sp. was found more common in spotted deer which was in conformity with the finding of Rahman *et al.,* (2014) at Dhaka National Zoological Garden of Bangladesh. Mixed infection of *Eimeria* sp. and *Strongyloides* sp. was found higher in spotted deer in comparision to other parasites. The reason might be, the size of *strongyloides* sp. is comparatively larger and thus offers less space for other parasites having similar size. However, *Eimeria* sp. being smaller in size can cause mixed infection along with strongyle sp.

Present study encountered that single infection of *Strongyloides* sp. were common in spotted deer which in supported by record of *Strongyle* ova in spotted deer at Mahendra Choudhary Zoological Park, Punjab and Siddhartha Garden, Maharastra by Singh *et al.,* (2009) and Khan *et al.,* (2014) respectively. Meshram *et al.,* (2008) and

Singh *et al.,* (2009) reported presence of *Strongyle* eggs in free- ranging spotted deer at Van Vihar National Park, Bihar. Similarly, Mandal *et al.,* (2002) reported maximum infection of *Strongyloides sp.* in free ranging chital (*Axis axis*) of Tamilnadu which is similar to the present study. Infection by *Strongyloides* sp. was found to be highest among GI parasites. *Strongyloides* sp. can enter inside the host through faecal-oral route as well as by direct penetration and also have high reproduction rate, might be the reason of highest prevalence of *Strongyloides* sp. Moreover, *Strongyloides* sp. being larger in size and act as a dominant parasitic species, leaving very little space for other parasites.

The intensity of different parasites in spotted deer of SNP was observed in this study. According to result, maximum numbers of spotted deer were found to be infected with light infection which is asymptomatic conditions and cannot cause serious diseases in animals while less number of spotted deer were infected with heavy infections revealed by *Eimeria* sp. and *Strongyloides* sp. The heavy infection is symptomatic and cause serious diseases or death in animals. The result of present study was similar to the report of Thapa (2013) due to same measurement techniques. Intensity is the number of adult worm infecting a host and many factors contribute to the number eggs expelled in host faeces. Though in present study, the eggs of all parasitic species recovered were counted and analysed as their intensity relating that parasitic eggs or oocyst load could be same indication of intensity.

The spread of infection doesn't necessarily present a major risk for wild ruminants. Many parasites have co-evolved with their host to have low levels of virulence (Anderson and May, 1978). *Strongyloides* sp. has been found significantly higher in spotted deer of SNP.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study was carried out to determine the prevelance of gastrointestinal parasites of spotted deer in Shuklaphata National park, Kanchanpur district. The spotted deer were found infected with different gastrointestinal parasites. From the present study, it was clear that gastrointestinal parasites are highly prevalant (98%) in spotted deer of SNP. Finding of this study showed that gastrointestinal helminthes infection in spotted deer were comparatively more common than the protozoan infection in spotted deer. Among helminthes, nematodes were more prevalent followed by trematode and protozoan while no cestodes were recorded during the study. This prevalence rate showed that spotted deer are highly susceptible to endoparasites. Eight different parasitic genera were identified in spotted deer of SNP, such as *Eimeria* sp. among protozoan, *Paramphistomum* sp. and *Fasciola* sp. among trematode, *Trichostrongylus* sp., *Bunustomum* sp., *Haemonchus* sp., *Strongyloides* sp. and *Mullerius* sp. among nematode. Out of all these identified gastrointestinal parasites, *Strongyloides* sp. (23.33%) showed the highest prevalence in spotted deer. Multiple infection in spotted deer was found to be 85.02%. Out of them, double infection (34.01%) was more prevalent in spotted deer followed by single (14.96%) triple (30.61) and multiple (20.4%) infections. In the present study, maximum number of spotted deer were found to be infected with light infection which is asymptomatic condition while less number of spotted deer were infected with heavy infection revealed by *Eimeria* sp. and *Strongyloides* sp. The heavy infection is symptomatic condition and cause sever diseases in animals.

Based on the outcome of present study, following recommendations have been made to reduce the risk of gastrointestinal threat in the conservation of spotted deer.

6.2 Recommendation

- The veterinary laboratory should be established in the conservation areas for the regular diagonosis of parasitic diseases and treatment.

- Molecular techniques should be applied for accurate identification of parasites up to species level.

- Conservation areas and wildlife reserves should be strictly prohibited from domestic animals since they are the major source of infection for spotted deer.

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