GASTROINTESTINAL PARASITES OF SWAMP DEER (*Rucervus duvauceli duvauceli* Cuvier, 1892) IN SHUKLAPTHATA NATIONAL PARK, KANCHANPUR, NEPAL"

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DECLARATION

I hereby declare that the work presented in this thesis entitled "Prevalance of gastro-intestinal parasites of Swamp deer (*Rucervus duvauceli duvauceli* Cuvier, 1892) in Suklaphanta National Park" has been done by myself, and has not been submitted anywhere for the award of any degree. All the sources of the information have been specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATIONS

This is to recommend that the thesis entitled "Prevalance of gastro-intestinal parasites of Swamp deer (*Rucervus duvauceli duvauceli* Cuvier, 1892) in Suklaphanta National Park" has been carried out by Miss Binita Pangeni for the partial fulfillment of the requirements for the Degree of Master of Science in Zoology with special paper 'Parasitology'. This is her original work and has been carried out under our supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institutions. I recommend that the thesis be accepted for the Degree of Master of Science in Zoology (parasitology), Tribhuvan University, Kirtipur, and Kathmandu, Nepal.

Date: 9th Feb, 2021

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LETTER OF APPROVAL

On the recommendation of supervisor Professor Mahendra Maharjan (Ph D), this thesis submitted by Miss Binita Pangeni entitled "Prevalance of gastro-intestinal parasites of Swamp deer (*Rucervus duvauceli duvauceli* Cuvier, 1892) in Suklaphanta National Park" is approved for the examination and ~~submitted to the Tribhuvan University~~ in partial fulfillment of the requirements for the Degree of Master of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF APPROVAL

This thesis submitted by Miss Binita Pangeni entitled "Prevalance of gastro-intestinal parasites of Swamp deer (*Rucervus duvauceli duvauceli* Cuvier, 1892) in Suklaphanta National Park" has been approved as a partial fulfillment of the requirements for the Degree of Master of Science in Zoology (Parasitology).

EVALUATION COMMITTEE

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviation
Approx.	Approximately
df	Degrees of Freedom
IUCN	International Union for Conservation of Nature
Ktm	Kathmandu
SNP	Suklaphanta National Park
VU	Vulnerable
DNPWC	Department of national park and wildlife conservation.
RMP	Revolution per minute
D/W	Distilled water
CITIES	Convention on International Trade in Endangered Species

ABSTRACT

The swamp deer is one of the attractive hoofed ungulates found in Nepal. It is found in the low laying protected area in the grassland and floodplain located in the southwestern part of Nepal. To determine the distribution of intestinal parasites of Swamp deer, 100 faecal samples were collected from Suklaphanta National Park in three different grasslands i. e Suklaphanta grassland (main grassland), Barkaulaphanta and Singpurphanta in April, 2019. All the samples were macroscopically as well as microscopically examined by faecal floatation and sedimentation techniques. Out of 100 faecal samples examined 80 samples is found to be positive. Total of (80%) were found to be positive for intestinal parasites. Altogether, ten different intestinal parasites were found to be prevalent in Swamp deer. Swamp deer of SNP were found infected by protozoa and helminthes i. e. nematodes and trematodes. Swamp deer were found to be infected with coccidian parasite i. e *Eimeria* sp. with micropyle and without micropyle 11. 25% and 26. 25% respectively. The deer were also found infected with two species of trematode parasite i. e. *Fasciola* sp. (80%) and *Paramphistomum* sp. (60%). Among nematodes, *Strongyloides* sp. had maximum prevalence of 15% followed by *Strongyle* sp. (10%), *Muellerius* sp. (10%), *Trichostrongylus*sp. (2.5%), and least number *Ascaris* sp. (1.25%), Haemonchus sp. (1. 25%) and *Dictylocalus* sp. (1.25%) were observed. The deer were not found to be infected with cestode parasites. The study of intensity of parasitic load reported that maximum number of samples revealed the light intensity followed by mild, moderate and heavy intensity. Single infection was found more common than mixed infection. It was observed that the swamp deer residing in main grassland of suklaphanta were infected by parasites with significantly high rate compared to other two study area. To know the exact parasites upto the level and to know the better confirmation of result molecular identification and larvae culture should be done respectively.

1. INTRODUCTION

1. 1 BACKGROUND

Protected areas are widely considered to be among the most effective means of conserving biological diversity in situ. Protected area management received a real thrust in the 1970s in Nepal. Not only protected areas came to be added but also action on both their protection and conservation was intensified. The first organized approach to managing Swamp deer in Nepal dates back to the year 1976. Suklaphanta wildlife reserve was established in 1976. It is the only protected area which has been set up exclusively for swamp deer. The presence of world's largest herd of Swamp deer in Suklaphanta makes SWR a globally important site for conservation standpoint (Poudel, 2007).

Suklaphanta wildlife reserve has been changed to Suklaphata National Park in 2017. It is one of the famous national parks in Nepal after Chitwan and Bardia National park. It occupies a total area of 305 sq. km and located in the southern Terai of Far-Western Nepal in Kanchanpur District. The reserve lies between 80° 25' east longitude and 28° 35' north latitude. It also has a large lake and the Bahini River flows through the park. The park also has over 300 species of birds. Reptiles include gharial and mugger crocodiles, Indian python, cobras, kraits, rat snakes and monitor lizards (Resource Profile, 2004). One third of the total area of the reserve is grassland and two third are covered by forest (Resource Profile, 2004). The major tree species include *Terminalia balerica*, *Adina Cardifolia* and *Bombax Ceiba* are found along the rivers. The grasslands of the reserve is the prime habitat of the swamp deer (Resource Profile, 2004). This reserve is also famous for wild elephants, Tigers, Spotted deer, Barking deer, Swamp deer, Blue bull and Wild boar. Wetlands such as Rani Tal, Solagaudi Tal, Kalikich Lake, Tara Tal and Lalpani Tal provide suitable habitats for waterfowl and reptiles (DNPWC 2019).

Grazing animals of the grasslands include five species of deer Barasingha, Samber, Chital, Hog deer and Muntjac. The largest herd of swamp deer in the world is found in Nepal’s largest grassland, Suklaphata grassland. According to the data of Barasinga counting and monitoring programme of Suklaphanta 2013, there were about 2301 Swamp deer in the grassland. Out of which 777 are male, 1390 are female and 134 are fawn (Sukhlaphanta Barsik Pratibedan, 2072/2073). Swamp deer (*Rucervus duvaucelii duvaucelii*) also known as the Barasingha lives in the swampy grasslands and floodplains (Tiwari et al., 2013).

Swamp deer was placed on the 1st appendix of CITIES list as an endangered species, but nowadays due to their habitation and conservation initiatives the number is increasing. Thus from 1996 to 2004, it was included in IUCN Red list with vulnerable status.

1. 2 Parasitic infections in Swamp deer

Parasites and infectious diseases have become a major concern in conservation of endangered as well as other species as they can lead to mortality, dramatic population declines, and even contribute to local extinction events (Mir et al., 2016). The gastrointestinal parasites are important in Zoo and free ranging wild animals (Isaza et al., 1990). Gastrointestinal helminths can have a detrimental effect on the fitness of wild ungulates (Aleuy et al., 2018). Nutrition plays an important role in a host's ability to cope with the negative effects of gastrointestinal parasites and a host's ability to regulate parasite establishment, growth, and fecundity.

Intestinal parasites are parasites that can infect the gastro-intestinal tract of humans and other animals (Loukopoulos et al. 2007). They can live throughout the body, but prefer the intestinal wall (Coop and Holmes 1996, Coop and Kyriazakis 1999). Protozoa can be directly infectious when they are passed in the faeces into the environment, but helminthes required period of maturation in the soil to become infectious, other require the involvement of an intermediate host (Arcari et al., 2000, Fabrizio 2014). The most favourable sites for intestinal parasites are the duodenum, ileum, cecum and large intestine (Cuomo et al., 2000). To survive or reproduce in the gastrointestinal tract the parasites have to adapt to continuous physiological changes relative to the feeding habitats of the host (Lyons et al., 1914, Leonard 1987, Cuomo et al., 2000). Ruminants are affected with different kinds of parasites (Coop and Kyriazakis 1999), in some case may be fatal due to the type of parasites or the load of parasites (Zhang et al., 2005, Maublanc et al., 2009). Nematode parasites of domestic ruminants are the main disease problem in grazing livestock system (Waller and Thamsborg 2004). For example, *Haemonchus contortus* is regarded as serious problem causing blood loss in cattle (Prestwood and Kellogg 1971). In most of the cases, wild and domestic animals share the common grazing land (Walker 1995). So, an individual host harbouring a gastrointestinal parasite shed 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 (Bryan 1977, Mawdsley et al. 1995, 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, Morgan et al., 2006).

The wild and domestic animals most commonly interact through direct competition for food, predation, pathogen exchange or hybridization (Foufopoulos et al., 2003. Deer are host to range of endoparasite, such as helminthes, insects larvae and certain protozoan's (Rehbein et al., 2001), cestodes (Chapman and Chapman, 1997) and other endoparasites. 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 and Cray fish. 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).

Nematode parasites are commonly known as roundworms 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 faeces 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).

The increased mortality of Barasingha in Suklaphanta could be due to some endoparasites. The decline of Barasingha from this area may be due to disease and predation, as well as illegal poaching and hunting (Pandey, 2017). Although the outbreaks of the parasitic disease in deer are not so deadly, but it is utmost important to keep free from parasite. This study is also focused on studying the prevalence of gastrointestinal parasites in the Swamp deer in the Suklaphanta National Park.

1. 3 Obectives

1. 3. 1 General Objective

Gastrointestinal parasites of Swamp deer (*Rucervus duvaucelii duvaucelii*) in Suklaphanta National park, Nepal.

1. 3. 2 Specific Obectives

- To determine the prevalence of gastro-intestinal parasites in Swamp deer of SNP.
- To assess the intensity of gastro-intestinal parasites in Swamp deer.

1. 4 Significance of the Study

Globally, many researches have been carried out regarding gastro-intestinal parasites of wild ruminanats. In case of Nepal, some researches have been done regarding GI-parasites of other wild ruminants but, very little research has been carried out regarding GI-parasites of Swamp deer (*Rucervus duvaucelii duvaucelii*). This study is to assess the prevalence of GI-parasites of Swamp deer in SNP Nepal. Wild animals are regulated by some complex abiotic and biotic factors. One of the biotic factors is parasite. As we know that, parasites are present in individual host, but if number of population becomes infected with parasites which have a many harmful effect, then the dynamics of the host population may be partly or greatly regulated by the parasites (Anderson, 1979). Research on parasitic fauna can actually add a new knowledge to the understanding of ecological interaction, pattern of host distribution and to the complex history of regions and habitats. So, parasites and their impact on wild population should be highly concerned. Therefore, it is important to find out exact data on diversity and abundance of parasite in order to know the role of infectious agents in decline and extinction of wildlife (Smith et al., 2009; Thompson et al., 2010). Parasitic diseases constitute one of the major problems causing morbidity and even mortality in wild animals. The population of Swamp deer has been decreasing throughout Nepal (Pandey, 2017) and need of urgent attention is required though it is not listed in protected species of Nepal. Due to feeding habits and behavioral factors Swamp deer might be infected with several gastrointestinal parasites. But there is lack of study regarding parasitic infection in Swamp deer in case of Nepal. Thus, this study will help to fulfill this need. Hence, parasitic impact on Swamp deer is higher concerning topic for research. This study will provide reference for future researchers.

2. LITERATURE REVIEW

The gastrointestinal parasitic infection is a key indicator of health status of wild animals including swamp deer. Most of the parasitic diseases cause significant effect on morbidity and mortality of wildlife. Besides bacterial, fungal and viral infections, the wild ruminants are also susceptible to various disease like coccidiosis, fascioliasis, fasciolopsiasis, schistosomiasis, nematodiasis and respiratory disease. Although coccidiosis doesnot affect wild ruminants but it can cause diarrohea, weight loss, weakness, growth and retardation. As all other domestic herbivoures, wild ruminants are also suffered from different endoparasites by sharing same pasture. Considering this lot of research works have been carried out on endoparasites of domestic as well as wild and captive ruminants. But in Nepal, only a few researches have been reported among wild ruminants. In case of swamp deer, very little research work has been carried out regarding parasitic infection. Wild ruminants can be infected by different parasites including protozoans, trematodes, cestodes and nematodes. In this section some important published work related with the present work has been reviewed.

2. 1 Global scenario of gastro-intestinal parasites of deer

Swamp deer is a large yellowish brown deer (*Rucervus duvaucelli duvaucelli*) having six points on each antler and sometime spotted with white. Swamp deer is the wild ruminant and host of different types of intestinal parasites like *Eimeria* sp., *Moniezia* sp., *Fasciola* sp., *Paramphistomum* sp., Ascaris sp., *Trichuris* sp., *Haemonchus* sp., *Strongyloides* sp., *Tricostrongylus* sp. And *Mullerius* sp. Microscopic examination of faecal sample of swamp deer in india was carried by (Talukdar et al., 2010) and reported swamp deer were infected by gastro-intestinal parasite by *Strongyle* sp., *Trichostrongylus* sp., *Fasciola* sp., *Moniezia* sp. and *Paramphistomum* sp. Beside swamp deer there are some research which are done on deer sp. in many countries.

In American continent, some deer found to be infected by GI parasites. Worley and Eustace (1972) collected the faecal sample of 44 Mule deer (*Odocoileus homionus*) 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 Verginia were infected by *Sarcocystis* sp., *Cysticercus tenuicollis*, *Oesophagostomum venulosum*, *Cooperia punctata and Gongylonema pulchrum*

(Prestwood et al., 1976). Likewise, *Eimeria* sp., *Haemonchus* sp., *Bunostonum* sp., *Cooperia* sp. *and Trichostrongylus* sp. had found in 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 *Haemonchussp* (LuxHoppe et al., 2010).

In Europe, research were conducted on different country like Poland (Kowal et al., 2012), 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, Spiculopteragia* sp., *Nematodirus filicollis, Aonchotheca bovis, and Oesophagostomum radiatum* as the major parasites. In Ukrain, (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*, *Spiculopteragia spiculoptera*, *Capillaria bovis*, *Cooperia oncophora*, *Oesophagostomum venulosum*, *Trichuris globulosa* and tapeworm segments were encountered (Davidson et al., 2014). *Spiculopteragia 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, *Marshallagi*a sp. eggs and *Eimeria* sp. ocyst (Steele et al., 2011).

In Bangladesh, there was also same type of result by investigation of gastrointestinal parasites of captive deer at Dhaka National Zoological Garden of Bangladesh and Dulahajara sufari park (Kanungo et al., 2010) 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. and *Strongyles* sp.) in Siddhartha Garden Zoo animals.

In case of India, some survey were done in free range deer in the scrub forest of Borgaon Manju in Western Vidarbha region, by Singh et al.,(2006) on Mahendra Choudhury Zoolodical Park, Punjab, byYadav et al., (2005) on bovines at Jammu. Most of research resulted same helminth parasites in wild deer. 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. Fecal sample of captive deer of Nandan Van zoo, Chhattisgarh, India were screened and revealed 46.2% prevalence where Barking deer showed highest prevalence (100%) of gastro intestinal parasites followed by Sambars (83.33%), Spotted deer (38%) and Blackbucks (35%) (Thawait et al., 2014). Axis deer of scrub forest of Borgaon Manju in Western Vidabha region of Maharastra showed 89.05% of prevalence for parasites representing Strongyloides sp., *strongyle*, *Haemonchus sp*., *Trichostrongyloides sp*., *Trichuris sp*. and *Bunostomum sp*. (Meshram et al., 2008). Gupta et al. (2011) found coccidian, *strongyle*, Strongyloides sp., *Trichuris sp*., *Toxocara sp*., *Moniezia sp*., *amphistome* and *Fasciola* sp. as common parasites in Sambar around Jabalpur, India.

2. 1. 1 Scenario of gastro-intestinal parasites in wild ruminants

In America, gastro-intestinal parasites recorded from Bos gaurus of Nilgiri Hills were treatodes (13.3%) include *Fasciola* sp. (3.3%), *Amphistome* sp. (6.7%) and *Schistoma* sp. (3. 3%), cestodes (20. 0%) include Moniezia sp. (20. 0%), nematodes (43.3%) include *Toxocara* sp. (6. 7%), Strongyles (23.3%), *Oesophagostomum* sp. (3.3%), Trichuris sp. (6. 7%) and *Mecistocirrus* sp. (3.3%) and unsporulated coccidian oocysts (13.3%) (Allwin et al., 2016). A survey carried out to detect the gastrointestinal parasites in Bison herds at Manitoba and Saskatchewan, *Trichostrongyle sp*., *Eimeria sp*., *Moniezia* sp., *Capillaria sp*., *Nematodirus sp. and Trichuris sp*. were detected from fecal sample of adult Bison (Murray et al., 2014). Cestode like *Echinococcus granulosus* (Fuller, 1959; Wade, 1979) was reported from Bison of Wood Buffalo National Park. Similarly, *Capillaria bovis* (Worley, 1980), *Nimatodirus filicollis* and *Skrjabinagia bisonis* (Yamaguchi, 1958) reported from American Bison.

In Europe, study conducted on prevalence of GI parasites on Wild boar, a total of 47 fecal sample were collected from Wild boars of eastern Spain and reported the parasites as followes: *Ascarops strongylina* (87%), *Ascaris suum* (21%), *Macracanthorhynchus hirudinaceus* (21%), *Taenia hydatigena* cysticercus (19%) and *Physocephalus sexulatus*

(6%) (Fiere et al., 2001). Nilgai, Wild boar and Asian elephant having the common parasites i. e *Amphistomes*, *Strongyles* and Coccidia, while *Fasciola* sp. were detected only in Nilgai, in the survey of parasitic infections in wild animals (Banerjee et al., 2005).

In India, study carried out to assess the gastrointestinal parasitic prevalence in wild herbivores kept at Mahendra Choudhury Zoological Park, Chhatbir in Punjab, India. The overall prevalence of GI- parasites based on 389 faecal samples examined for helminthes eggs and cysts/oocysts of protozoa was found to be 25.7% (Singh et al., 2006). An investigation carried out for the determination of prevalence and management of parasitic infections of zoo animals and birds, conducted in Mahendra Mohan Choudhury Zoological Park, Chhatbir, Punjab a total of 909 animals and 549 bird's faecal sample were collected. Result revealed that 232 (25.52%) animals and 206 (37.52%) bird faecal sample were found positive (Moudgil, 2015). Study on prevalence of GI parasites in captive wild animals of Nandan Van Zoo, Raipur, Chhatisgarh, a total of 120 fecal sample were collected from 14 different captive wild animals including, Chausingha, Blue bull, Rhesus monkey, Lion, Leopard, Tiger, Bear, Hyaena, Jackal and Ratel from various enclosures of the zoo. Out of 210 faecal sample examined, 97 (46. 2%) were found positive for different helminth parasites. Among different captive wild animals, the prevalence of GI parasites was 45.6% in herbivores, 45.2% in carnivores and 60% in primates indicating higher prevalence of GI parasites in primates than carnivores and herbivores in zoo. Among herbivores, Blue bulls showed highest prevalence of (85.71% (Thawait et al., 2014).

2. 1. 2 Scenario of gastro-intestinal parasites in domestic ruminants

Domestic animals are one of the main sources of income for the peasant in developing country (Thornton et al., 1973). Mostly farmers kept goat, sheep, cow and buffalo for milk, meat and manure. They are the wealth for them. If they suffer from different kinds of disease causing morbidity or mortality it will be a great loss for them. Globally parasitic diseases continue to be a major constraint for poor developing countries. They are rarely associated with high mortality and effects are usually characterized by low outputs of animal products, by products and manure (FAO, 2002).

Many gastrointestinal parasites spreads through faeco-oral transmission routes which involve fecal contamination of the soil, food items or other substrates and subsequent consumption of infectious stages of the parasites by other hosts (Davis and Anderson 1971, Nunn et al., 2011). This contact may occur when individual from different groups overlap at food or water resources (Nunn et al., 2011). Newly infected individual then spread the infection to other individual in the groups and to individual in different groups through dispersal or in areas of home range overlap.

A survey was carried to determine and describe the prevalence and intensity of gastrointestinal parasite infections and *Dictyocaulus viviparous* (lungworm) in a dairy and a beef cattle farm of two different ecological zones in Costa Rica, Central America. Coprological techniques were used to detect helminth, protozoan and *D. viviparous*. Blood sample were analyzed for antibodies to *D. viviparous* by ELISA. Gastro intestinal parasites detected on both farms were *Eimeria* sp., *Strongylidae*, *Buxtonelia sulcata*, *Strongyloides papillosus*, *Moniezia benedeni*, *Trichuris* sp., *Toxocara vitulorum*, *Entamoeba bovis*, *Haemonchus* sp., *Cooperia* sp. and *Dictyocaulus viviparous* (Jimenez et al., 2007). Larval nematodes with a dorsal spine on the tail were recovered from faecal sample of California bighorn sheep (*Ovis canadensis californiana*) in north eastern Washington DC, USA. Identity of these dorsal larvae was established by single conformation polymorphism (SSCP) analyses of a partial fragment of the first internatonal transcribed spacer of the ribosomal DNA and were identified as *Parelaphostrongylus odocoilei* (Chilton et al., 2006). Fecal sample were collected from 819 calves (6-18 months of age) from 49 operations in the USA where prevalence of *Giardia* sp. was 33. 5% (Santin et al., 2012). *Cooperia punctata* has a deleterious effect on both appetite and nutrients uptake or utilization of cattle (Stromberg et al., 2012). Cattles were recognized as hosts for two species *Cryptosporidium parvum* and *C. muris* infecting the intestine and the abomasum and represented by small and large oocysts (Fayer et al., 2009). Faecal sample of goats of Western Santa Catarina, Brazil showed prevalence of 88. 9% with *Haemonchus* sp., *Trichostrongylus* sp., *Teladorsagia* sp., *Cooperia* sp., *Oesophagostomum* sp., *Thysanosoma* sp., *Trichuris* sp., *Moniezia* sp., *Neoascaris* sp., *Eimeria* sp., *Cryptosporidium* sp., *Giardia* sp. and *Entamoeba* sp. as main parasites (Radavelli et al., 2014). Bovid's parasites of UK were found infected with *Strongyloides sp*., *Trichuris* sp. , *Capillaria* sp. , *Moniezia* sp. , *Thysaniezia ovilla* and coccidia. Habitat overlap was found to have a significant effect on *strongyle* and coccidia abundance (Ezenwa, 2003). Study carried out on 400 sheep and 180 goats on Poland showed 80. 6% prevalence in goats with higher infection in goats than in sheep (Gorski et al., 2004). *Teladorsagia circumcincta*, *Trichostrongylus* sp., *Nematodirus* sp., *Cooperia* sp., *Oesophagostomum* sp., *Chabertia* *ovina*, *Bunostomum sp*. and *Moniezia sp*. were the common parasites of sheep in Poland and Iceland (Richter 2002, Gorski et al., 2004).

Parasitic investigations of domestic ruminants like sheep, goat, cow and buffalo are conducted globally. For examples, In African countries *Haemonchus* sp., *Trichostrongylus* sp., *Trichuris* sp., *Strongyloides* sp., *Fasciola* sp., *Moniezia* sp., *Bunostomum* sp. and *Oesophagostomum* sp. are the common helminth parasite of the domestic ruminants (Kusiluka and Kambarage 1996, Belem et al., 2001, Mekonnen 2007, Mhoma et al., 2011, Amadi et al., 2012, Edosomwan and Shoyeni 2012, Kingsely et al., 2013, Blackie 2014) whereas *Crytosporidium* sp., *Eimeria* sp., *Entamoeba* sp. (Maichomo et al., 2004, Regassa et al., 2006, Mhoma et al., 2011) are the common parasitic protozoan. Cryptosporidium sp. is one of the common intestinal parasites of domestic ruminants in Nigeria, especially in asymptomatic cattle that could serve as reservoirs for the zoonotic infection in humans (Ayinmode and Fagbeni 2010, Pam et al., 2013). In Ethiopia, season and age were shown to have association with prevalence of parasites with the highest worm burden occurred during the rainy season (Regassa et al., 2006, Mekonnen 2007). Rainfall or moisture is the most important factor which influences the survival, development, dissemination and availability of free living stages of helminthes (Kusiluka and Kambarage 1996, Belem et al. 2001). Most common species of trematodes associated with gastrointestinal parasite in small ruminants of Sub Saharan countries are *Haemonchus contortus*, *Oesophagostomum columbianum*, *Trichostrongylus colubriformis*, *T. axei*, *Bunostomum trigonocephalum*, *Cooperia curticei*, *Trichuris ovis*, *T. globulosus*, *Strongyloides papillosus*, *Gaigeria pachyscelis and Chabertia ovina* (Kusiluka and Kambarage 1996). Belem et al. (2001) in Burkinafaso identified the 9 different helminthes i. e. *Cooperia punctata*, *C. pectinata*, *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Bunostomum phlebotomum*, *Moniezia expansa*, *Avitellina* sp., *Oesophagostomum radiatum and Trichuris* sp. in cattle. Overall prevalence of nematodes in the calves, sheep and goat was found to be 69.2%, 80% and 82% respectively by Maichomo et al., (2004) in Kenya whereas Kanyari et al., 2010 found that strongyle were the most common nematodes especially among under one year old cattle. Tsotetsi and Mbati (2013) in Northern free state, Abouzeid et al., (2010) in Egypt, Lamrioui et al., (2013) in Morocco, Blackie (2014) in Ghana found that Haemonchus sp. was the dominant nematode genera in domestic ruminants whereas Phiri et al., (2006) in Zambia and Mhoma et al., (2011) in Tanzania found that *Paramphistomum* sp. was the common parasite of domestic ruminants.

Faecal sample of 241 goats from Korea showed that *Eimeria* sp. was significantly higher than that of other gastrointestinal parasites where 22. 4% were nematodes and 2. 1% cestodes (Gebeyehu et al., 2013). Bangladesh is another developing country of Asia. Animal farming is one of its sources of livelihood (Talukder et al., 2013, Uddin et al., 2006 2013). Many researches had done in the gastrointestinal parasites of domestic ruminants. Biswas (2012) found 13 species of gastrointestinal parasites including 4 trematodes, 6 nematodes, 1 cestode and 2 protozoans in fecal examination of buffaloes in Bhola district. Similarly, Saha et al. (2013) found 5 types of helminth in Barisal district among fecal sample of buffaloes. In both researches *Fasciola* sp., *Amphistomes*, *Schistosoma* sp. were common. Out of 144 gastrointestinal tracts of Black Bengal goat slaughtered at different slaughter house in Mymensigh district 105 (72.92%) individuals were found infected with a single or multiple species of *Amphistomes* where 3 species of *amphistomes (Paramphistomum cervi*, *Cotylophoron cotylophorum* and *Gastrothylax crumenifer)* were identified (Uddin et al., 2006). One hundred fifty four sheep in Tangal district were found infected with 7 helminth i. e. 3 trematode (*Fasciola gigantic*, *Paramphistomum* sp. and *Schistosoma indicum*) and 4 nematode (*Bunostomum* sp., *Trichuris* sp., *Strongyle* and *Strongyloides* sp.), (Sangma et al., 2012). Moreover, Yeasmin et al., (2014) found 12 species of helminth parasites with highest prevalence of *Strongyloides* sp. (71.67%) and lowest *Dictyocaulus* sp. (3.33%) in sheep.

In India many people of village choose farming animals as their main occupation for livelihood. Visceral examinations of 284 sheep and 318 goats in Kashmir showed the higher helminthic infection in goats than in sheep (Lone et al., 2012). Necroscopic examination revealed 72.88% of helminthic infection in goats of subtropical Jammu region of Jammu and Kashmit state (Mir et al., 2012). Two hundred and forty two (39.4%) ruminants were positive for nematode infection in Nagpur where infection rate in buffalo, cattle and goat was 41.63%, 32.18% and 51.94% respectively. They were infected with *Haemonchus* sp., *Toxocara* sp., *Trichuris* sp. and *Strongyloides* sp. (Chauhan et al., 2008). Goats and sheep of Mathura showed the overall prevalence of 68. 75% (Singh et al., 2013) whereas goats of Maharashtra showed prevalence of 62.75% (Sutar et al., 2010). In Tamil Nadu prevalence of gastrointestinal parasites was higher in sheep (66.33%) than in goats (57.67%) where *Haemonchus* sp. was found to be predominant in both sheep and goat (Varadharajan and Vijayalakshmi, 2015). Helminth parasites of buffaloes brought to Ahmedabas slaughter house, Gujarat was trematodes 34%, nematodes 26% and cestodes

10% with overall prevalence of 64. 67%. Prevalence of helminth was maximum (46.39%) in young age group followed by adult (27.83%) and old animals (25.77%) (Patel et al., 2015). *Strongyle* (35.41%), *Strongyloides* sp. (0.49%), Toxocara sp. (0.099%), Fasciola sp. (4.44%), *amphistome* (11.06%), coccidia (1.19%), *Moniezia expansa* (0.64%) and *M. benedeni* (0.35%) were the parasites identified in cow and buffalo of Rajasthan (Swarnakar et al., 2015).

2. 2. National scenario of gastro-intestinal parasites in ruminants

Intestinal parasites occur in wild as well as within domestic animals. In Nepal a checklist of 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). Many works have conducted in the threats of wild ruminants but in the disease transmission or parasitic disease there are only few work have done. In case of the swamp deer the work related to the parasitic infection was carried out in Suklaphata National Park (Pandey, 2017). Where out of 150 faecal samples, 108 samples were found to be positive. The intestinal parasites like *Eimeria* sp., *Moniezia* sp., *Fasciola* sp., *Paramphistomum* sp., *Ascaris* sp., *Trichuris* sp., *Haemonchus* sp., *Strongyloides* sp., *Tricostrongylus* sp., *and Mullerius* sp. were found in the study of SNP (Pandey, 2017). There is some literature found on the gastrointestinal parasires of wild ruminants of Nepal (Oli, 2018; Airee, 2018; Gupta, 2017; Achhami, 2016; Thapa, 2013).

2. 2. 1 Wild ruminants

Oli (2018) found *Trichuris* sp., *Ascaris* sp., *Strongyloides* sp. nematode parasites on Spotted deer of Banke National Park which were found also 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, 2013), *Haemonchus* sp. (Airee, 2018; Gupta, 2017; Chaudhary, 2014; Thapa and Maharjan, 2013), *Mullerius* sp. (Airee, 2018; Thapa and Maharjan, 2013), *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.

Fecal sample of Yak of Manaslu Conservation Area showed the overall prevalence of 81. 82% (Byanju et al., 2011) whereas Chauri of Gumdel VDC of Ramechhap district showed overall prevalence of 90.38% (Shrestha and Bindari 2013). *Strongyle*, *Eimeria* sp., *Ascaris sp*., *Trichuris* sp. and *Amphistomum* sp. were the common parasites of yak and chauri (Byanju et al., 2011, Shrestha and Bindari 2013). Livestock contribute 31% of Nepal's GDP and small ruminants 12% (Sani et al., 2004).

2.2.2 Domestic ruminants

Different domestic ruminants brought for slaughtering purpose are found to infect with different parasites. In Nepal, small ruminant producers were estimated to experience largest economic loss from roundworms (Sani et al., 2004). Majority of calf mortality in Sankhuwasawa was due to ascariasis, diarrhea, dysentery and poisoning (Dhakal et al., 1996). 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, Sapkota et al. 2006). Kohar (2008) reported that prevalence of *Fasciola* sp. infection in buffalo was maximum in September (88.09%) followed by August (26.98%), October (19%) and July (15. 87%) whereas Pandey (2001) found that prevalence of *Fasciola* sp. gradually increased from the month of June (0.64%) to July (1.83%) and again August (1.49%) to November (5.35%) and decreased from December (2.64%) to February (0.47%). In the case of buffaloes of slaughter house Kirtipur *Fasciola hepatica* (59.67%) was found slightly higher than *Fasciola gigantica* (52.63%) (Shrestha, 2010). Mixed infection of *Haemonchus contortus*, *Ostertagia* sp. and *Trichostrongylus* sp. were recorded below 2,000m, only Ostertagia sp. was recorded above 3,500m altitude in migratory sheep and goats of Nepal (Joshi, 1999). Among the three Nepalese sheep breeds (Kage, Baruwal and Lamphuchhre), Kage breed indicated the relative superior resistance against *Haemonchus contortus* (Joshi, 1999).

Strongyloides sp., *Haemonchus* sp., *Moniezia* sp., *Trichostrongylus* sp., *Trichuris* sp., *Fasciola* sp., *Nematodirus* sp., *Paramphistomum* sp., *Ostertagia* sp., *Oesophagostomum* sp., *Chabertia* sp., *Cooperia* sp. and *Toxocara* sp. were reported from the Mule of Banke (Rani ,2000). Dhakal (2011) reported cestode *Anoplocephala* sp. from cattle.

3. MATERIALS AND METHODS

3. 1 Study area

3. 1. 1 Location

The study was conducted in grassland of Suklaphata National Park. Suklaphata National Park lies in the extreme south western part of terai in Kanchanpur district. SNP was managed as a hunting reserve at the beginning in 1969. It has been gazette as a wildlife Reserve in 1976 and as National park currently in2017. National park covers 305 sq. km (118sq. mi) of open grassland, forest, riverbed and tropical wetlands and altitude of 174 to 1386 meters (571 to 4547ft). The reseve was extentended eastwards with north latitude 28. 84° and east longitude 80. 228° to create more habitats and a corrider from the terai into the churia hills for seasonal migration of wildlife. SNP is important both nationally and internationally for its extensive grasslands or phatas that largest protected patch of continuous grassland in Nepal. It covers 54. 4 km^2 in area. In the eastern half of the main phanta, the grassland is wet or damp with large areas of marshes and pools. There are other smaller phatas in the reserve which are also important for wild animals especially for birds. These are Singhpur phata, Karaiya phata, Dubhiya phata, Barkaula phata, Hirapur phata, Arjuni phata, Sundari phata, Tarapur phata and other smaller phantas.

3. 1. 2 Flora and Fauna

Besides swamp deer, it provides habitat for wide range of vertebrates which include endangered species such as wild Elephant(*Elephas maximus*, Bengal Tiger (*Panthera tigris*),Spotted deer (*Axis axis*), Blue bull (*Boselephus tragocamelus*), Barking deer(*Muntiacus muntjak*) Hog deer(*Hyelapus porcinus*) wild boar (*Sus scrota*), Leopard (*Panthera pardus*), Jackal (*Canis aureus*),Langur (*Semnopithecus schistaceus*), Flycatcher (*Eumyias thalassinus*,) Crocodile(*Crocodylus porosus*), Cobra (*Naja naja*), Python (*Python molurus*), Bengal Florican (*Houbaropsis bengasensis*) and so on along with more than 424 species of birds and insects(Baral, 1997).

The main grass species include Siru (*Imperata cylindric*) and Kans (*Heteropogoncontortus*). Beside these Dhaddi (*Saccharam sp*), Narkat (*Phragmites karka*), Beldande are the other major species of grass with tree species like *Bombax ceiba* and *Butea* monospermae growing along the periphery of the phantas and provide ideal habitat for different animals. Sal is the dominant forest type in the reserve with khair (*Acaciacatechu*) and Sisso (*Dalbergia sissoo*) alongside rivers. There are many small lakes with associated marshes where dense grasses of phragmites karka and saccharum spontaneum are predominante.

3. 1. 3 Climates

The climate is predominantly tropical to monsoon with more than 90% of the annual precipitation. The average daily temperature during winter ranges from (10-20)°C and rises to(22-25) °C in spring and reaches as high as 32-35°C in summer. The maximum temperature reaches up to 42°C in summer.

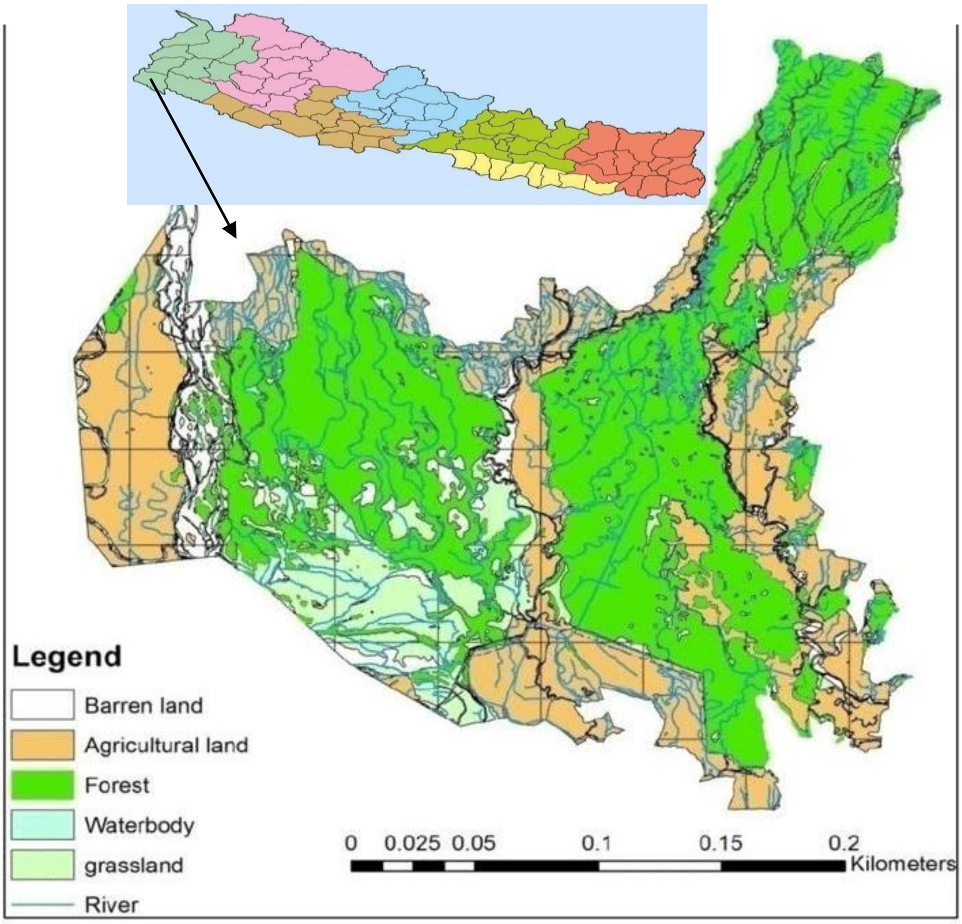


Fig 1: Map of Suklaphata National Park (DNPWC, 2019).

3. 2 Materials Required

3. 2. 1 Materials for field:

i. Sterile vials
ii. GPS
iii. Camera
iv. Globes
v. Potassium dichromate

3. 2. 2 Materials for laboratory:

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

3. 2. 3 Chemicals:

i. Potassium dichromate
ii. Distilled water (D/W)
iii. Saturated NaCl solution
iv. Methylene blue
v. Lugol's iodine 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 (Soulsby, 2012). Pellet of Swamp deer are characterize as:

- Black in colour.
- Slender in shape but sometime pointed at one end.
- Size: 8±1mm (n=15) in length and 6±1mm (n=15) in diameter.

3. 4 Study Design

The present study was designed to determine the parasitic prevalence of Swamp deer in SNP by applying faecal sample collection, preservation, examination and processing by direct smear and centrifugation method (Soulsby, 2012).

Study area

↓

Fecal sample collection

↓

Preservation in 2. 5% Potassium dichromate and transportation

↓

Processing by direct smear and centrifugation method

↓

Identification

↓

Data analysis

↓

Report dissemination

Fig. 2: Flowchart showing study design

3. 5 Sample collection

Sample was collected during the month of March 2019 in three different grassland i. e Suklaphata (main grassland), Barkaulaphata and Singpurphata. Sampling collection was done during day time without repetition in location within different time interval. Fresh sample were collected from the ground. Proper care was taken when collecting the faecal palletes from the ground to prevent contamination.

3. 6 Preservation and Transportation

Collected fecal sample of Swamp deer were preserved immediately in sterile airtight vials containing 2.5% of potassium dichromate at 4ºC temperature by using ice box and preserved sample were safely transported to the laboratory of Central Department of Zoology.

3. 7 Laboratory Examination

After preservation and transportation of fecal sample, examination of all sample were processed at the laboratory of Central Department of Zoology, T.U, Kirtipur, and Kathmandu. The fecal sample were examined under microscope for trophozoite, cysts, oocysts, eggs and larvae of gastrointestinal parasites by stained smear preparation and concentration method viz. floatation and sedimentation techniques (Soulsby, 2012; Zajac and Conboy, 2012). The Stoll's count technique was used to determine mix infection and intensity of parasites (Soulsby, 2012).

3. 7. 1 Stained Smear Preparation

Preparation of smear is required for many laboratory procedures. The purpose of making smear is to fix the parasitic cysts/ova/eggs onto the slide. It is useful to study the nuclear character and identification of protozoan cysts. A small portion of fecal sample was picked up with a clean bamboo toothpick and emulsified with Lugol's iodine solution on a clean glass slide and covered with a cover slip. The smear was examined under electric microscope at 10X and 40X (Soulsby, 2012).

3. 7. 2 Concentration Methods

The concentration procedures include floatation and sedimentation techniques for the detection of eggs/cysts/trophozoites/larva of parasites (Soulsby, 2012; Zajac and Conboy, 2012). In case of heavy infection, parasites can be easily seen in smears but in case of light infection it is difficult to detect the parasitic form in smears or mounts. Hence, in the study, concentration method (Floatation and Sedimentation) were carried out.

a. Floatation Techniques

This technique is based on the principle that lighter eggs of helminthes and protozoans float on the medium having greater density (Maplestone 1940) (Soulsby, 2012).About 1 gm of feces was taken in a glass pestle and a little quantity of water was added and mixed well. Suspension was strained to remove the 18 debris. The suspension was centrifuged at 1000 revolution per minute (rpm) for 5 minutes. The tube's water was replaced with super saturated NaCl solution and again centrifuged. After centrifused, more saturated NaCl solution was added to develop convex meniscus at the top of the tube and one drop of Methylene blue was also added. The eggs and cysts float to the top and were collected by placing a cover slip on the surface of the meniscus at the top of the tube. The surface layer was examined under low power microscope. The presence of eggs was identified through their morphological characteristics (Bowman, 1999).

b. Sedimentation Technique

This method is used for trematode eggs but not suitable for protozoan cysts. About 1gm of feces was taken in a glass pestle and a little quantity of water was added to it and mixed well. Suspension was strained to remove the debris and poured into a centrifuge tube up to an inch below the brim. Centrifuged at 1000 revolutions per minute (rpm) for 5 minutes. The supernatant was discarded and form the sediment. Iodine wet mount was prepared for each sample by mixing 1-2 drops of the sediments with Lugol's iodine solution in a glass slide and examined under low power objective (10X) by covering with a cover slip. The presence of eggs was identified through their morphological characteristics (Bowman, 1999). Prepared slides were examined under microscope at 10X and 40X respectively. Size of eggs/oocysts/cysts was measured using stage and ocular micrometer. The eggs, cysts and larva were identified by comparing the structure, color and size of eggs, cysts and larva of published articles, journals and books (Soulsby, 2012; Gardiner et al. , 1988; Taylor et al., 2007; Hussam, 2015).

3. 9 Intensity

Calculation of GI Parasitic Intensity of gastro-intestinal parasites was calculated depending on the number of eggs/oocysts and larvae found per microscopic field (Soulsby, 2012).

3. 10 Data Analysis

Since, the study was focused on identification of different intestinal parasites, the data were analyzed by using MS-Excel 2007 and statistical analysis was performed using "R", version

3.5.2 software package with chi-squared test. In all cases 95% confidence interval (CI) and $p<0.05$ was considered for statistically significant association.

4. Results

A total of 100 fecal sample of swamp deer were collected from the study area i. e Suklaphata National Park, Nepal. Swamp deer were found to be infected with various helminthes parasites. Microscopic examination of the sample revealed overall parasitic prevalence of 80%. Maximum swamp deer were infected by either protozoans or helminth parasite as well as both type of parasite.

4. 1 parasitic prevalence in swamp deer

Swamp deer were found to be infected with three groups of parasites i. e Protozoa, Trematoda and Nematoda. Among them high prevalence was found for trematode parasites (75%) followed by nematode (37.5%) and protozoans (23.75%). However no cestodes were recorded in this investigation. The result indicated that the swamp deer were highly susceptible for helminth parasites compared to protozoans. The difference in the prevalence of protozoa, trematode and nematode in swamp deer were found statistically significant (χ^2=45. 425, df=2, p<0. 05)(Figure:-1).

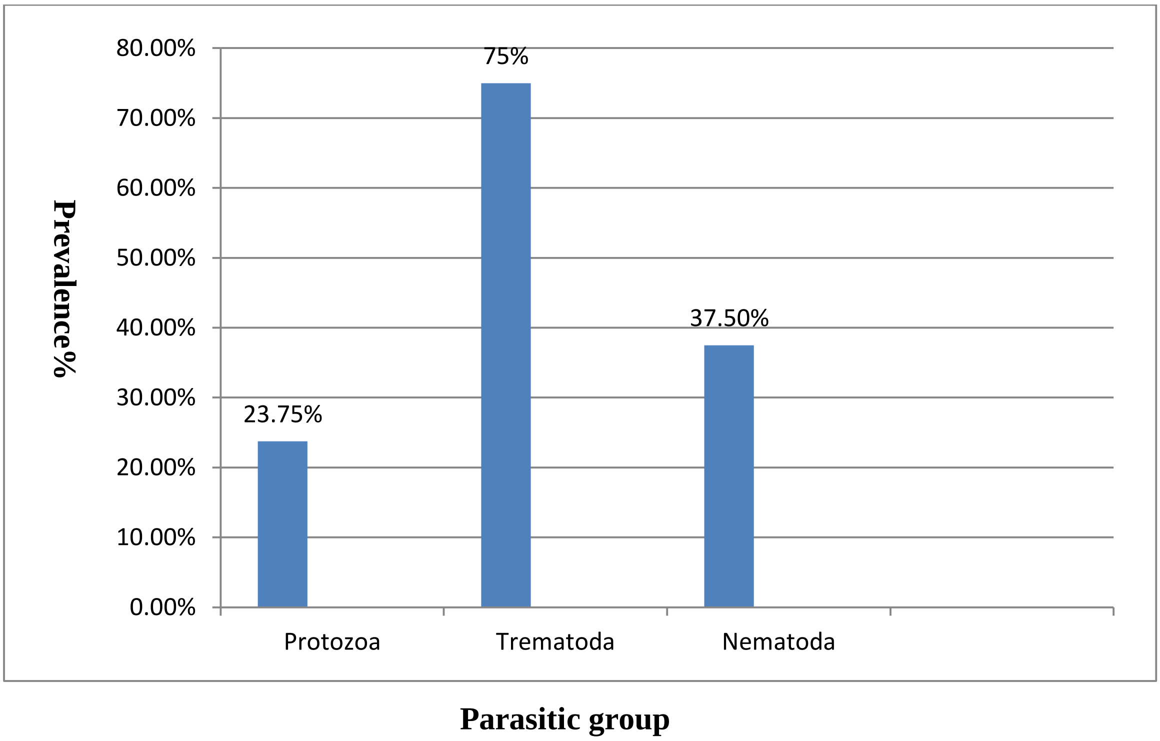
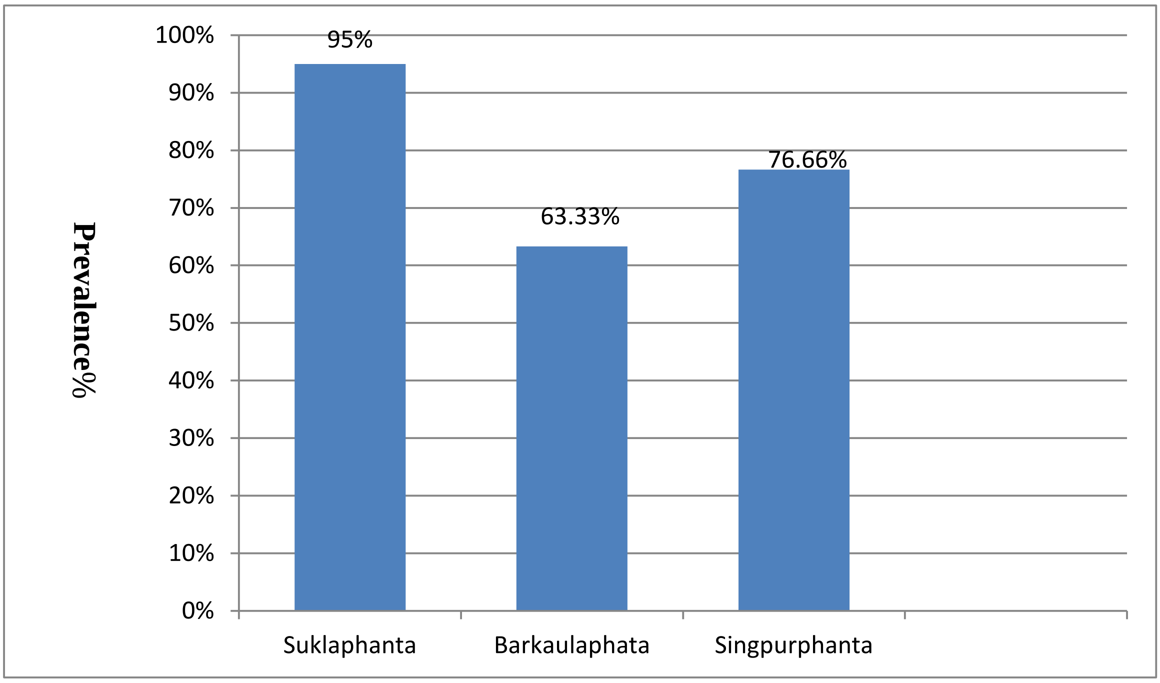


Figure 3:- Parasitic prevalence in swamp deer

The sample of swamp deer were collected from the different grassland of SNP i. e Suklaphata grassland (main grassland), Barkaulaphata and Singpurphata grassland.

Maximum prevalence of parasite was found in the sample of main farm land. Statistically there was significant difference of parasitic prevalence in three locations. (χ^2=11.042, df=2, p<0. 05) (Figure:-2).



Locations

Figure 4:-Location wise prevalence of GI parasite of SNP

4. 2 Overall prevalence of GI parasite of swamp deer in SNP

Among protozoan parasites only one species of protozoa i. e *Eimeria* were reported. Swamp deer were found infected with two morphologically distinct *Eimeria* species i. e with and without micropyle. In between the *Eimeria* species *Eimeria* without micropyle were found significantly different in three study site. (χ^2=8. 6354,df=2,p=0. 013333). Swamp deer of SNP were found infected with seven different species of nematode parasite. Among them *Strongyloide* sp. were found among swamp deer of all three sites with high prevalence i. e 36%.

Maximum prevalence of almost all parasitic infection was found in the sample of Suklaphata grassland (main grassland). *Ascaris, Haemonchus* and *Dictylocalus* were found in the sample of Suklaphata grassland i. e main farmland but absent in Barkaulaphata and Singpurphata. Least prevalence of parasitic infection was found in singpurphata. *Fasciola* and *Paramphistomum* were common in all three locations. (Table:-1).

Table 1 :- Overall prevalance of GI parasite of swamp deer in SNP

Parasite		Suklaphata N=40	Barkhaulaphata N=30	Singpurphata N=30	χ^2	P – value
Protozoa	*Eimeria* with micropyle	5%	10%	3. 33%	1. 3002	0. 522
	Eimeria without mycropyle	25%	6. 66%	3. 33%	8. 6354	0. 01333
Nematode	*Trichostrongylus*	2. 5%	3. 33%	0%	0. 93537	0. 6264
	Strongyloides	12. 5%	20%	3. 33%	3. 9615	0. 138
	Ascarid	2. 5%	0	0	1. 5152	0. 4688
	Dictylocalus	2. 5%	0	0	1. 5152	0. 4688
	Haemonchus	2. 5%	0	0	1. 5152	0. 4688
	Mullerius	7. 5%	10%	0	2. 9255	0. 2316
	Strongyle	12. 5%	6. 66%	0	4. 1219	0. 1273
Trematode	*Fasciola sp.*	37. 5%	30%	33. 33%	0. 43821	0. 8032
	Paramphistomum	22%	26. 66%	30%	0. 51109	0. 7745

4. 3 Overall mixed infection

Swamp deer were found to be infected with either single parasite infection or multiple. Maximum of them were infected by single species of parasites followed by double, triple and quartenary infection. Among the single infection maximum of them were infected by *Fasciola* sp. The result concluded that, concurrent parasitic infection in swamp deer was statistically significant. (χ^2=116. 13,df=2,p <0.05). (Table:-2)

Table 2 :-Overall mixed infection

SN	Infection	Percentage
1.	Single	67. 5%
2.	Double	25%
3.	Triple	6. 25%
4.	Quadriple	1. 25%

4. 5 Helminth parasite specific mix infection of swamp deer in SNP.

Swamp deer were found to be infected with maximum single infection i. e 67.5%. 12 different types of parasitic combination were found in double infection while in triple infection 5 different combination and in quadriple infection only one mixed infection were oveserved. The parasitic association between protozoa and nematode in double infection were found to be maximum where *Eimeria* sp. from protozoa was associated with *Dictylocalus*, *Ascaris*, *Mullerius*, *Strongyloides* and *Strongyle* sp. of nematodes while parasitic association between the same parasitic groups were found to be least. (Table:-3)

Table 3:- Helminth parasite specific mix infection of swamp deer in SNP.

Mixed infection	Combination	Total
Single Infection		67.5%
Double infection	*Fasciola* sp. + *Paramphistomum* sp.	8.75%
	Strongyloides sp. +*Eimeria* sp. without micropyle	2. 5%
	Strongyloides sp. + *Muellerius* sp.	2. 5%
	Eimeria sp. with micropyle+ *Dictylocalus* sp.	1.25%
	Fasciola sp. + *Eimeria* sp. without micropyle	1.25%
	Strongyloides sp. +*Strongyle* sp.	1. 25%
	Eimeria sp. without micropyle+ *Ascaris* sp.	1. 25%
	Fasciola sp. + *Trichostrongylus* sp.	1. 25%
	Fasciola sp. + *Muellerius* sp.	1. 25%
	Eimeria sp. without micropyle+ *Muellerius* sp.	1. 25%
	Eimeria sp. with micropyle. + *paramphistomum* sp.	1. 25%
	Eimeria sp. with micropyle+ *Strongyloides* sp.	1. 25%
Triple infection	*Fasciola* sp. + S*trongyloides* sp. +*Strongyle* sp.	1. 25%
	Fasciola sp. + *Paramphistomum* sp. +*Strongyle* sp.	1. 25%
	Fasciola sp. + *Paramphistomum* sp. + *Muellerius* sp.	1. 25%
	Fasciola sp. + *Paramphistomum* sp+ *Eimeria* sp. with micropyle	1. 25%
	Fasciola sp. + *Paramphistomum* sp+ *Eimeria* sp. without micropyle	1. 25%
Quaternary infection	*Fasciola* sp. + *Paramphistomum* sp. + *Muellerius* sp. + *Strongyle* sp.	1. 25%

4. 6 Intensity of GI parasite in swamp deer

Heavy parasitic infection may cause the serious problem in swamp deer. Heavy parasitic infection was considered in that sample which has six or more then six egg or oocyst observed per field. Among the total parasitic sample there was no any heavy infection. *Fasciola*, *Paramphistomum* and *Strongyloides* showed the light infection in most of the sample which may not cause any serious issue to the swamp deer. *Eimeria* sp from protozoa revealed moderate infection which may show some symptoms in swamp deer (. Table:-4).

Table 4:-Intensity of GI parasite in swamp deer

Class	Name of parasite	Light(+)	Mild(++)	Moderate (+++)	Heavy (++++)
Protozoa(Sporozoa)	*Eimeria* sp. with micropyle	4(5%)	1(1. 25%)	1(1. 25%)	
	Eimeria without micropyle		5(6. 25%)		
Trematode	*Fasciola* sp.	17(21. 25%)	9(11. 25%)	5(6. 25%)	
	Paramphistomum	12(15%)	15(18. 75%)	1(1. 25%)	
Nematode	*Trichostrongylus*	2(2. 5%)			
	Strongyloides	13(16. 25%)			
	Ascarid	1(1. 25%)			
	Dictylocalus	1(1. 25%)			
	Haemonchus	1(1. 25%)			
	Mullerius	7(8. 75%)	2(2. 5%)		
	Strongyle	4(5%)	2(2. 5%)		

Light infection = < 2 eggs/cysts/ larva per field

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

Moderate infection = 5-6 eggs/cysts/ larva per field

Heavy infection = >6 eggs/cysts/ larva per field

5. DISCUSSION

Suklaphata National park is one of the protected areas of Nepal. More than 53 species of mammals including protected species such as swamp deer, Bengal tiger, Sloth bear, Elephant, Indian Leopard, Hispid Hare and Greak one Horned Rhinoceros are found in SNP. (Jnawali et al., 2011) . Around 30% area is covered by the grassland which is suitable habitat of swamp deer. Total population of swamp deer was 2301(Suklaphata Barsik Partibedan, 2072/2073). Large number of the infections of the gastrointestinal tract infections is caused by the parasites that are cosmopolitan in distribution. All the animals' i. e domestic animals and wild animals bear different kind of parasites. (Hodsun et al., 1998; Garber et al., 2005). Faecal sample of swamp deer were collected and examined for this study which was accomplished to find out the prevalence of GI parasite of swamp deer in three different site of the SNP. This study revealed the prevalence of 80% infection among the swamp deer and also showed the presence of both helminth and Protozoan parasites.

Previously, different researchers have worked on GI parasite of deer as well as other wild ruminants from Nepal, India, Pakistan and Italy. Comparatively less research has been directed towards understanding the origins of diseases, particularly on swamp deer, Parasitic prevalence on swamp deer was reported in SNP of Nepal (Pandey, 2017). Similarly many other research has been carried out in deer species of Nepal (Thapa, 2019, Thapa and Maharjan, 2015; Achhami, 2016; Pun, 2018 and Kandel, 2018). Likewise from other countries many study has been reported on the prevalence of GI parasites of deer (Talukdar et al., 2019 ,Barmon et al., 2001;Kanungo et al., 2010; Rahman et al., 2014; Thawaitet al., 2014; Aviruppola et al.). Some of the study was conducted to know the prevalence of GI parasites of wild ruminants(Gupta et al., 2017; Senger et al., 2017; Rana et al., 2015; Farooq et al., 2012; Kowal et al., 2012; Bandyopadhya et al.,2010; Meshram et al., 2008; Singh et al., 2006; Yadav et al., 2005) and some researchers were done on captive condition such as zoo (Hossian, 2012; Mir et al., 2016; Aviruppola et al., 2016;Sengar et al., 2007).

The higher parasitic prevalence had been reported from wild ruminants of various parts of Nepal e.g Pandey (2017) on swamp deer of Suklaphta National Park, Chaudhary (2014) on Blackbuck of Blackbuck conservation area of Bardiya National Park, Kandel (2018) on wild and domestic ruminants in Bagmara Buffer zone of Chitwan National Park, Achhami et al., (2016) on musk deer of Langtang National Park. Similarly from India the higher parasitic prevalence has been reported by the various researchers in wild ruminants (Banerjee et al., 2008, Singh et al., 2006, Sengar et al., 2007, kumar et al., 2009 and from Bangladesh (Khatun et al., (2014) on captive animals , Rahman et al., (2014) on wild herbivores. But some of the researchers have reported comparatively less parasitic prevalence i. e <80% in wild ruminants of Nepal Thapa(2019), Adhikari(2019) on deer of shivapuri Nagarjun National park and Mrigasthali of Kathmandu respectively, Pun(2010) on ruminants of central zoo Kathmandu , Bishnu et al., (2014) in wild ruminants of Langtang National Park and from India less parasitic prevalence has been carried out in wild ruminants, Gupta et al., (2011) around Jabalpur, Thawait et al., (2014) in captive wild animals. Similarly less prevalence has also been reported from other countries such as deer of Bangladesh, Barmon et al., (2014) and cervids of Spain, Santin et al., (2004). Nearly similar results was reported on Barking deer of Nepal (Thapa, 2019) and small ruminants of India (Singh et al., 2016).

Many protozoans and helminth parasites can infect the gastro-intestinal tract of ruminants and other animals which has been repoted by various studies. In the present study, swamp deer found to be infected with three groups of parasites i.e protozoa (23.75%), Trematode (75%) and Nematode (37.5%). The result revealed that helminth infection is more common than protozoan infection in swamp deer. This investigation was also supported by the this study carried out by Pandey (2017), who reported higher prevalence of helminth infection than protozoan infection of swamp deer along with cestode parasite which was not detected in this study. Similarly, different study done in domestic animals like goat (Rizal, 2010; 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) also showed more infection of helminthes parasites among domestic animals than swamp deer. Cestodes were not recorded in this study which resembles the findings in the study carried out in Nepal Airee (2018) in spotted deer at Suklaphata National Park and Oli (2018) at Bardiya National Park, Adhikari (2019) at Mrigasthali Kathmandu. Absence of cestodes in present study is also supported by the study of ruminants in Bhubaneshwor, India Nayaka (2016) . The findings of Borghare et al.,(2009) also resembles the present study who reported the absence of cestode infection in captive deer at Maharjbag zoo, Nagpur. The difference in the prevalence of the protozoan and helminth parasite was due to the different animal host,their study area, and due to the common grazing, poor sanitation. Absence of cestoda might have been due to their indirect life cycle or the intermediate host required for the cestode to complete its life cycle might not be present in the study area.

The prevalence rate of parasite was found to be very high in suklaphata grassland i. e main farmland among the three study area. This finding was supported by the study carried out by Pandey (2017) in swamp deer of SNP.

Parasites can affects host survival and reproduces directly through pathological effects indirectly by reducing the host`s immunity and affecting the physical condition, Thawaitet al., (2014). The swamp deer of SNP were found to be infected with 10 groups of GI parasites. Among protozoan parasites swamp deer of SNP were found to be infected only with the coccidian parasite i.e *Eimeria*. In the present study coccidian parasites were identified upto genus level and differentiated into two groups on the basis of presence and absence of micropyle. In the current study the prevalence of *Eimeria* was 53%, *Eimeria* oocyst have been reported in wild ruminants, Pandey (2017), Singh et al., *(*2009), Saud et al., (2012). Maesano et al., (2014), Darabus et al., (2014). This prevalence was higher than that of Tiwari et al., (2009), Barmon et al.,(2014) and lesser then that of Achhami (2016), singh et al., (2009). Higher prevalence of *Eimeria* sp. may be due to overcrowding of other animals and habitat of swamp deer and moist condition of the grassland because oocyst requires moist condition to undergo sporulation.

Gastrointestinal nematodes rank highest on global index (Perry et al., 2002). In current study nematode shows the prevalence (37.5%). High prevalence of nematode might be due to the direct lifecycle and wide range of host. In this study, seven groups of nematode has been reported they are *Trichostrongulus*, *Strongyloides*, *Ascarid*, *Dictylocalus*, *Haemonchus*,

Mullerius and Strongyle. The above study resembles the study of Arghali (2013) on the study of Himalayan tahr and Barking deer of Rara National Park. Among Nematodes *Strongyloides* sp. infection was found most prevalent (36%). 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) but lower than (Achhami, 2016), (Gupta et al., 2017) in India. On the other hand, 19.16% of swamp deer were infected with *Strongyle* sp. which was higher than previous results of (Meshram et al., 2008), (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.

Ascaris were found to be very least prevalent in the current study i. e 2.5% but the previous study by (Pandey, 2017) showed high prevalence of *Ascaris* in SNP. Which is lower than reported by (Rahman et al., 2014), (Lim et al., 2008), (Oli, 2018) in spotted deer. Similarly, 5. 8% 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. *Muellerius* sp. infected 17. 5% of swamp deer in SNP. 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 *Haemonchus* sp. had been recorded in least amount 2. 5% in swamp deer which was much lower compaired to previous results in Himalayan thar and Barking deer (Thapa and Maharjan, 2015), (Kanungo et al., 2007) Captive deer of Bangladesh. Similarly *Dictylocalus* sp was also reported from the current study which was less prevalent then the study carried out by (Thapa and Maharjan, 2015) in wild ruminant.

Among trematode, the prevalence of *Paramphistomum* sp. was found to be high than the prevalence of *Fasciola* sp. *Paramphistomum* sp. being trematode have indirect life cycle and require intermediate host, fresh water snail. The prevalence of *Paramphistomum* sp. in each animal was higher than the other identified parasites because the factors determining the availability, development and survival of intermediate host in the environment influence the level and severity of trematode infections (Kusikula and Kambarage 1996). High prevalence of *Paramphistomum* sp. was also reported from Blackbuck of Bardia and Shuklaphanta, Chauri of Ramechhap and Yak of Manaslu Conservation Area of Nepal (Bjanju et al., 2011, Ban 2012, Shrestha and Bindari 2013, Chaudhary 2014). Yak and Sambar of India were also found infected with *Paramphistomum* (Rangorao et al., 1994, Gupta et al., 2011). Infections of *Paramphistomum* sp. was may be due to ingestion of metacercariae while grazing in contaminated pastures which generally occurs in rumen and cause intestinal wall erosions, haemorrhage, oedema and necrosis of ruminal papillae (Love and Hutchinson 2003).

Concurrency of parasites represent the different genus of parasitic occurrence on single host. Out of infected swamp deer 67. 5% were found single infection which is more then the single infection found from Nepal in Barking deer of Rara National park (Thapa and Maharjan, 2014), Thapa (2019) in spotted deer of Shivapuri Nagarjun National park. similarly others reasearchers from the other countries by (Rahman et al., 2014; Barmon et al., 2014 and Nayak, 2014) at Dhaka National Zoological Garden of Bangladesh,Char Kukri Mukri in Bhola District of Bangladesh and at Bhubaneshwor in India respectively. Among them 32.5% revealed mixed infection which was lower then the previous studies by Chaudhary (2014) and Achhami (2016) in Langtang National Park. Result obtained from mixed infection is in accordance with previous individual results (Gupta et al., 2011, Tiwari et al., 2009, Barmon et al., 2014). The huge diversity and densities of pathogen species represent huge diversities of life cycle, transmission routes and pathogenicity that causes great harm to animals and affecting wildlife can be threat to conservation (Woolhouse, 2002). So, that single infection was not highly harmful as comparative to double, triple and multiple infections.

The intensity of different gastro intestinal parasites in swamp deer of SNP was calculated in present study. According to the result, the maximum number of swamp deer was found to be infected with light infection. This study resembles with the study of many researchers from Nepal i. e (Thapa and Maharjan, 2014) on Barking deer of Rara National Park, (Achhami, 2016) on wild ruminants of Langtang National Park. No any parasitic load has been reported among the Swamp deer of SNP which suggest that they are less susceptible to the diseases.

6. CONCLUSIONS AND RECOMMENDATIONS

6. 1 Conclusions

A study on gastrointestinal parasite in swamp deer was undertaken in march 2019. The prevalence of gastro-intestinal parasites was observed on the basis of saline wet mount and concentration (floatation and sedimentation) techniques. The present study demonstrates that the swamp deer of SNP were found infected with different gastrointestinal parasites. The overall prevalence of gastro-intestinal parasites was 80% in National Park where Suklaphanta grassland (main farmland) got highest infection (95%) followed by Singpurphanta (76.66%) and Barkaulaphanta (63.33%). The study showed that gastro-intestinal helminthes infection was comparatively more common than the protozoan infection in swamp deer. Among helminthes, nematodes were more prevalent followed by protozoan and trematode while cestode were lacking in the present study. Swamp deer were found to be infected with coccidian parasite i. e *Eimeria* sp. with micropyle and without micropyle. Swamp deer of SNP were found infected by two classes of helminthes i. e. nematodes and trematodes which include nine genuses of parasites, two genus belongs to trematode and seven genus belongs to nematode. From the identified helminthparasites Fasciola sp. were found to be predominant sp. having the prevalent rate of 80%. Among nematodes, *Strongyloides* sp. had maximum prevalence of 15% followed by *Strongyle* sp. (10%), *Muellerius* sp. (10%) and *Trichostrongylus* sp. (2.5%), where *Ascaris* sp., *Haemonchus* sp. and *Dictylocalus* sp. showed the similar prevalence rate of 1.25%. Swamp deer were found to be infected with highest single parasitic infection (67.5%). 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 *Fasciola* sp. Similarly, in intensity of parasitic load, maximum number of samples revealed the light intensity followed by mild, moderate and heavy intensity.

6. 2 Recommendations

On the basis of the conclusion, following recommendations are made.

- ❖ To know the exact parasites upto species level molecular identification is necessary.
- ❖ Larvae culture should be done to better confirmation of result.

The veterinary laboratory should be established in the conservation areas and wildlife resereve for the regular diagnosis of parasitic disease and treatment.

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Appendix 1

Identification of gastro-intestinal parasites

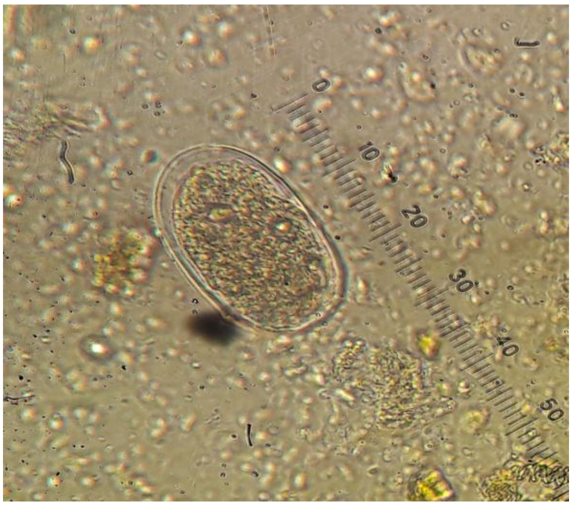
Morphological characters of eggs and their references were used to identify gastrointestinal parasites.

Name of parasites	Range of length and diameter of eggs and cysts in µm.		Morphology characters	Reference values (Soulsby, 1982; Foreyt, 2001)
	Length	Width		
Haemonchus sp.	65 (n=1)	40(n=1)	Eggs are oval, thin-shelled, and grayish in color	70-90×40-55 µm
Strongyloides sp.	65-70(n=6)	25-30(n=6)	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.	51-65×20-30 µm
Trichostrongylus sp.	90-110(n=3)	35-40(n=3)	Irregular ellipse dissimilar, kidney-shaped not very wide poles, one of which was more rounded than the other, dissimilar side-walls.	70-90×35-50 µm
Ascaris sp.	45mm (n=1)	35mm (n=1)	Eggs are elongated covered with rough, blumpy outer surface.	
Eimeria with micropyle	30-40(n=6)	20-25(n=6)	Ovoid or ellipsoidal shaped, contained polar cap (micropyle)	23-38x 16-24µm

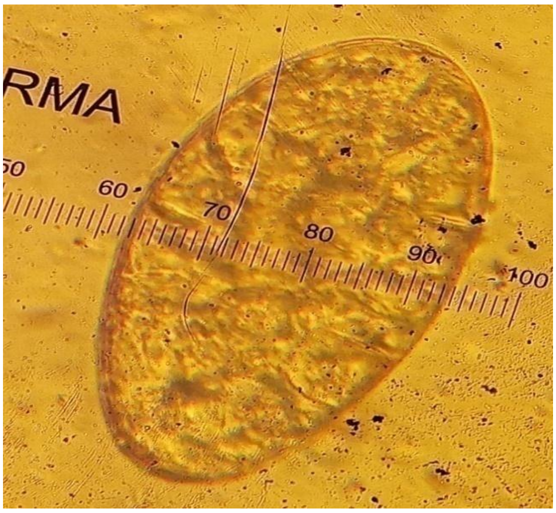
Eimeria without micropyle	25-30(n=6)	15-20(n=6)	Ovoid and spherical shaped, without polar cap (micropyle).	20-29µm x14-22µm.
Fasciola sp.	120-140(n=6)	40-50(n=6)	Oval, nonembryonated, thin egg shell, operculated and immature	125-130µm x 40-65µm
Paramphistomum sp.	140-150(n=6)	60-70(n=6)	Barrel-shaped, clear shell and operculum at one end.	125-130µm x40-65µm
Dictyocaulus sp.	0. 5mm (n=1)	0. 3mm (n=1)	First stage larva passed in the feces, presence of small cuticular knob at the anterior extremity and numerous brownish food granules in the intestinal cells	0. 55-0. 58mm
Strongyles	75-80(n=3)	40-45(n=3)	Oval, thin-shelled, segmenting when laid.	70-85µm x 40-47µm

Appendix 2

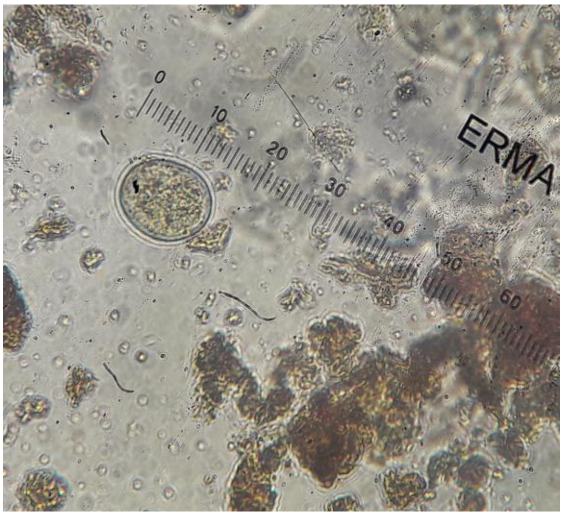
Photograph No. 1 & 2: Lab work at Central department of zoology



Photograph 3: Egg of *Haemonchus* at x100, at Luooglo’s iodine



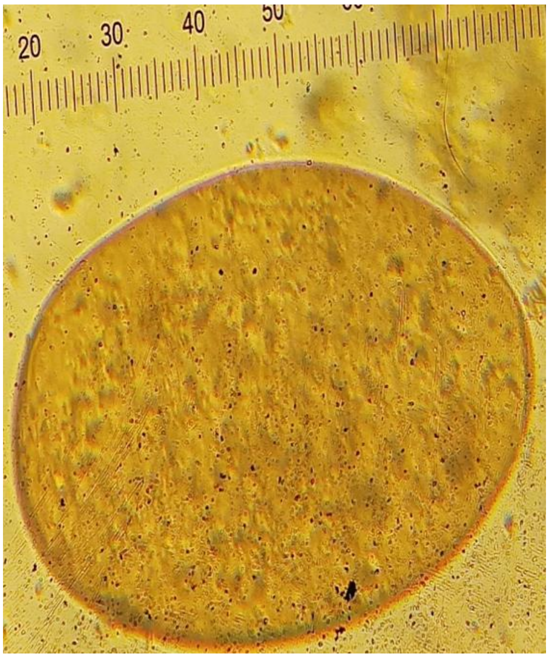
Photograph 4: Egg of *Fasciola* at x100, at Luooglo’s iodine



Photograph 5: Egg of *Ascaris* at x100, at Direct smear method

Photograph 6: Larva of *Muellerius* at x100, at Luooglo’s iodine staining.

Photograph 7: Egg of *Strongyle* at x400, at Luooglo's iodine staining.



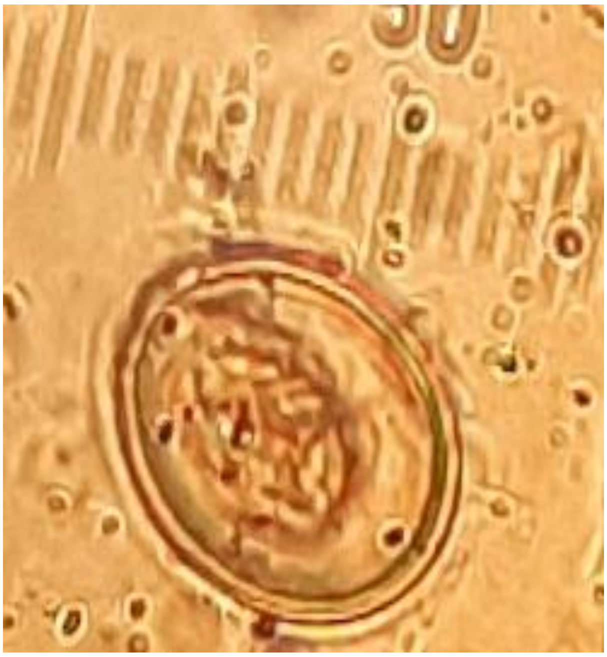
Photograph 8:Egg of *Paramphistomum* at x400, from direct

Photograph 9: Larva of *Muellerius* at x100, at Luooglo's iodine staining.

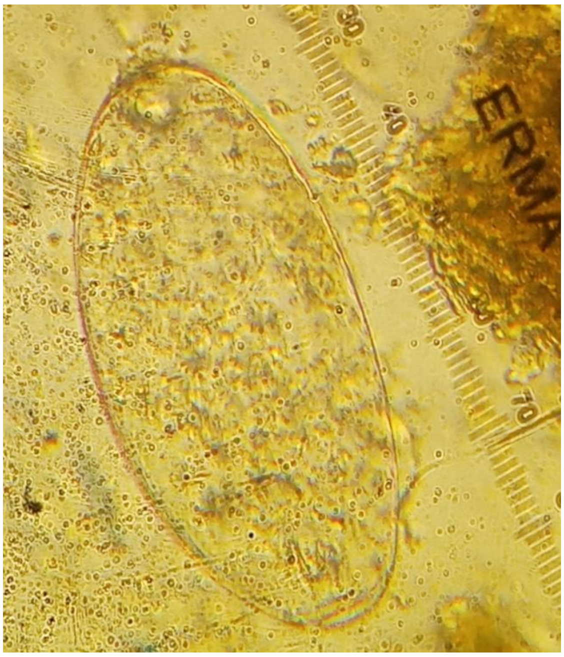
Photograph 10: Oocyst of *Eimeria* with micropyle at x100, at Luooglo's iodine staining.

Photograph 11: Larva of *Dictylocalus* at x100, at Luooglo's iodine staining.

Photograph 12: Egg of Strongyle at x400, at Luooglo's iodine staining.



Photograph 13: Oocyst of *Eimeria* without micropyle at x100, at Luooglo's iodine staining.



Photograph 14: Egg of *Fasciola* at x100, at Luooglo's iodine staining.