

**COMPARATIVE STUDY OF GASTROINTESTINAL PARASITES
OF WILD RUMINANTS AND CHAURIS IN LANGTANG
NATIONAL PARK, RASUWA, NEPAL**



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

**Submitted to
Central Department of Zoology
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Tribhuvan University
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July, 2016**

DECLARATION

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

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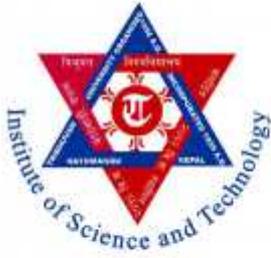
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This is to recommend that the thesis entitled "**Comparative study of gastrointestinal parasites of wild ruminants and chauris in Langtang National Park, Rasuwa, Nepal**" has been carried out by Bishnu Achhami for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under our supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LIST OF ABBREVIATIONS

CITES	-	Convention on International Trade in Endangered Species of Wild Fauna and Flora
cm	-	Centimetre
DNA	-	Deoxyribonucleic acid
DNPWC	-	Department of National Park and Wildlife Conservation
DoLS	-	Department of Livestock Services
ELISA	-	Enzyme linked immunosorbent assay
et al.	-	And his associates
FAO	-	Food and Agriculture Organization
GIT	-	Gastrointestinal tract
i.e.	-	That is
IUCN	-	International Union for Conservation of Nature
KDa	-	Kilodalton
kg	-	Kilogram
km ²	-	Kilometre square
m	-	Metre
MoAD	-	Ministry of Agricultural Development
NBS	-	National Bureau of Statistics
P value	-	Probability value
rpm	-	Revolutions per minute
rRNA	-	Ribosomal ribonucleic acid
sp.	-	Species
SPSS	-	Statistical Package for Social Science
UK	-	United Kingdom
USA	-	United State of America
WHO	-	World Health Organization
WWF	-	World Wildlife Found

ABSTRACT

The study was conducted from May to June 2014 in Langtang National Park to show the prevalence of gastrointestinal parasites in wild ruminants and chauris and compare the gastrointestinal parasites between wild ruminants and chauris. A total of 71 fecal samples including 16 from Himalayan Tahr, 31 from Barking Deer, 9 from Musk Deer and 15 from Chauri were collected. Fecal samples were preserved in a 50 ml vial with 10% ethyl alcohol and analyzed by concentration method in the laboratory of Central Development of Zoology, Tribhuvan University, Kirtipur, Kathmandu. Result revealed that the overall prevalence of gastrointestinal parasites was 85.92%. Seven species of parasites were identified with one protozoan (*Eimeria* sp.), four nematodes (*Ascaris* sp., strongyle, *Strongyloides* sp. and *Trichuris* sp.), one cestodes (*Moniezia* sp.) and one trematode (*Paramphistomum* sp.). Statistically it was found that the parasites of wild ruminants and chauri had no significance difference i.e. the parasites found in both ruminants were same. It was found that the parasites can transmit from wild ruminants to Chauri and vice versa. Contaminating the grazing land of wild ruminants by chauri was found to be one of the main reasons for presence of gastrointestinal parasites in wild ruminants. Besides these, presence of vectors or intermediate host may play the important role in transmitting the gastrointestinal parasites where moist and shady condition of Langtang National Park favoured the parasites viability. Controlling or restricting the grazing of domestic ruminants in the habitat of chauri is must to control the parasitic infection in wild ruminants and vice versa.

1. INTRODUCTION

1.1 Background

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 feces 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).

Attempt to control disease in wildlife populations or to avoid diseases transmission between wildlife and livestock have been based on setting barriers, habitat management and feeding bans, vector control, treatment and vaccination (Wobeser 2002, Gortazar *et al.* 2006).

1.2 General information on wild ruminants and chauri

The National Park and Wildlife Conservation Act, 1973 had provided various degree of protection to wildlife based on the national status of the species. Out of 203 mammal species recorded in Nepal, 23% are considered to be nationally threatened with extinction, 4% critically endangered, 12% endangered and 7% vulnerable (Jnawali et al 2011).

1.2.1 Musk Deer

Musk Deer (*Moschus chrysogaster* Hodgson, 1839) belongs to order Artiodactyla and family Moschidae. It is distributed in Afghanistan, Bhutan, China, India, Myanmar, Nepal and Pakistan (NBS 2002). In Nepal this species is distributed in Api Namppa Conservation Area, Khaptad National Park, Rara National Park, Shey Phoksundo National Park, Dhorpatan Hunting Reserve, Annapurna Conservation Area, Manaslu Conservation Area, Langtang National Park, Makalu Barun National Park and Kanchanjunga Conservation Area (Baral and Shah 2008, Aryal *et al.* 2010, Aryal and Subedi 2011, Jnawali *et al.* 2011). This species inhabits mostly in mixed forest, closely followed by Rhododendron forest whereas avoids Alpine scrub followed by Betula forest (Shrestha and Meng 2014). It is widely distributed along the Himalayas from 2,200m to 4,200m of elevation (Jnawali *et al.* 2011). The potential habitat of Musk Deer throughout the country is 30,177.19 km² but only 19.26% (5,815.08 km²) of potential habitat is inside the protected area (Aryal and Subedi 2011, Jnawali *et al.* 2011).

They are very shy and solitary animal found active during dawn and dusk and at night can be seen in the open areas of their habitat as they graze while during day remain in dense cover (Huffman 2004). It's colour coat is brown, bristly coated with a darker throat (Shrestha 2003, Jnawali *et al.* 2011) and attains a height of 53-80cm, head to body length of 86-100cm and weighs about 13-18 kg (Zhihotshenko 1988). This species mainly feed on grasses, shrubs, leaves, moss, lichens, shoots and twigs (Shrestha 2003). They are more primitive than cervids or true deer because they lack antlers and facial glands (Fox and Myers 2001). Males and females possess elongated upper canine teeth that project below the lower lip and male canine reaches 6-8cm which are used in fights between rivals (Zhihotshenko 1988). They breed seasonally from November to December and gestation period is of 178-198 days where the litter size ranges from 1 to 3 (Green and Kattel 1998).

Musk gland found in adult male is approximately walnut sized (4-6cm long and 3.5-4.5cm wide) and is situated in the preputial region between the abdomen and the genitals (Homes 1999). It produces musk from the age of 12-18 months onwards (Green 1989). Musk is produced in average of 25gm per animal per year (Homes 1999).

The population is decreasing (Wang and Harris 2008) due to poaching for trade of musk gland, habitat encroachment and disease transmission from livestock (Jnawali *et al.* 2011).

The Government of Nepal has protected Musk Deer as an endangered species under the National Park and Wildlife Conservation Act, 1973 and CITES listed it in appendix I and the IUCN Red List of threatened species listed it as endangered (Wang and Harris 2008).

1.2.2 Barking Deer

Barking Deer (*Muntiacus vaginalis* Boddaert, 1785) belongs to order Artiodactyla and family Cervidae. It is chestnut red coloured and brown black facial markings with small antlers (Jnawali *et al.* 2011). It is found up to the elevation of 3500m (Timmins *et al.* 2008). The height of an adult ranges from 50 to 75cm and weighs about 22 to 23 kg (Shrestha 2003). Antlers are small consisting of a short brow-tine and an unbranched beam (Dey 2007). Frontal glands on the forehead is believed to be scent glands which are activated during the mating period and serve as a means of attracting the hinds (female stag) (Shrestha 2003).

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

It is widely traded and hunted for wild meat and antlers (Timmins *et al.* 2008). Hunting, habitat loss and degradation due to human encroachment, clearing for agriculture and livestock grazing are the main threats (Jnawali *et al.* 2011). The population estimated in Nepal is greater than 10,000 individuals which has declined rapidly over the past 15 years (Timmins *et al.* 2008, Jnawali *et al.* 2011).

The Government of Nepal has protected Barking Deer as vulnerable species under the National Park and Wildlife Conservation Act, 1973, CITES listed it in appendix I and the IUCN Red List of threatened species listed it as least concern (Timmins *et al.* 2008).

1.2.3 Himalayan Tahr

Himalayan Tahr (*Hemitragus jemlahicus* Smith, 1826) belongs to order Artiodactyla, family Bovidae and is deep copper brown mountain goat, females and young are light brown, males are darken with large manes and both sexes lack beards (Jnawali *et al.* 2011). Adults' length is 90 to 100cm, attends a height of 40 to 100cm and weighs about 90kg (Shrestha 2003). Main diet consists of grasses, herbs, fruits, bamboo, leaves of rhododendron, lichens on rocks (Jnawali *et al.* 2011). The young one become sexually mature at 18 months and mating occur from October to January with female giving birth to one or two young in May or June after a gestation period of 180 to 242 days (Shrestha 2003, Jnawali *et al.* 2011). Life span is upto 22 years (Jnawali *et al.* 2011). They are diurnal with herd may range from 30-40 individuals following the guidance of an old male and social grooming with one licking the head and neck of another (Shrestha 2003). They are good climbers and have very strong hoof pads, slightly convex posteriorly and surrounded by a hard horny rim (Shrestha 2003).

It is found in the Himalayas including China (Southern Tibet), North India (Jammu and Kashmir to Sikkim) and Nepal (Bhatnagar and Lovari 2008). In Nepal this species distributed to lower parts of Kaski, Manang and Annapurna Conservation Area (Mustang), Kanchanjunga Conservation Area, Langtang National Park, Makalu Barun National Park, Manaslu Conservation Area, Sagarmatha National Park, Dolakha around Rolwaling and Sindhupalchowk (Jnawali *et al.* 2011). It occurs in temperate and sub alpine zone and in steep rocky mountain sides especially between 3,000-4,000m (Bhatnagar and Lovari 2008, Jnawali *et al.* 2011).

The estimated population of this species is more than 2,000 individuals in Nepal and actual population trend is decreasing (Bhatnagar and Lovari 2008, Jnawali *et al.* 2011). Habitat loss and anthropogenic activities such as poaching and livestock grazing are major threats for their survival (Jnawali *et al.* 2011). The Government of Nepal under the National Park and Wildlife Conservation Act, 1973 and IUCN Red List has placed it as near threatened species and CITES listed it in appendix I (Bhatnagar and Lovari 2008, Jnawali *et al.* 2011).

1.2.4 Chauri

The term Chauri, in this study involves the crosses of Yak (*Bos grunniens*) and local Hill Cow (*Bos indicus*) or Tibetan Yellow Cattle (*Bos taurus*) or local bull (Nak) and are confined in high hills and mountainous districts of Nepal mostly above 2,000m (Joshi 1982, Pradhan *et al.* 2000). This species belongs to order Artiodactyla and family Bovidae. Three species of the *Bos* genus are the parent stock of the cross breeds i.e. Tibetan Cattle (*Bos taurus*) which is small humpless cattle replenished from Tibet in the past, Zebu Cattle (*Bos indicus*) which is large hump cattle adapted to low to middle altitudes and Yak (*Bos grunniens*), the domesticated livestock at the higher altitude since long time ago (Dong *et al.* 2009). In Nepal, less than 124 different combinations of yak with different local types of cattles were conserved by the traditional community through their own breeding programme (Joshi 1982).

Chauri are found throughout Northern Nepal and are the prime component of the livestock production system for the substance of life by pastoralists (Das *et al.* 1998). Chauries are genetically superior and productive than either parent due to hybrid vigor and are better adapted than the parents to various ranges of altitudes (Joshi 1982). That's why the farmers prefer them as domestic animals.

Average first calving age is 3 year with lactation periods of 6 to 7 months (Chetri *et al.* 2011). The male offspring produced from back crossing either by female Chauri with Yak bull or Tibetan Cattle is generally considered as unproductive and killed or left to die without any rearing (Dong *et al.* 2009). The animals move towards high altitude pastures (upto 4,400m) in the monsoon season and to lower pastures (1,900m) fallow lands or community forest near settlement during the winter (Dong *et al.* 2009, Chetri *et al.* 2011).

Milk produced upto 4 litres and used for cheese, butter, ghee and churpi (hard cheese) production (Chetri *et al.* 2011) which is quite popular among the tourist. The major chauri products are churpi (hard cheese), ghee, soh-si (by product of the milk bucket and used for soap making), skin (mat), tail switch for religious purpose, Jopkyo for meat (Pande 2007) and are also beast of burden (Das *et al.* 1998).

In Nepal, Yak/Nak/Chauri population is estimated to be 70,165 (MoAD 2011/12). There are great opportunities to increase Yak and Chauri production through better breeding, feeding, herding and health care management of livestock and improved management of pasture land and fodder resources (Dong *et al.* 2009).

1.3 Parasitic interaction of wild and domestic ruminants

Wild animals were found to be infected with parasitic trematodes (Davis and Anderson 1971). The wild and domestic animals most commonly interact through direct competition for food, predation, pathogen exchange or hybridization (Foufopoulos *et al.* 2003). A stunning variety of pathogens can be transferred between domestic animals and wildlife posing great concern for pastoralists and ranchers and generating complicated problems for conservation biologist. Habitat overlap was found to have a significant effect on strongyle and coccidian abundance with abundance increasing with the number of bovid species in the habitat (Gulland 1992, Ezenwa 2002). In natural populations individuals may be infected with multiple distinct pathogens at a time (Davis and Anderson 1971, Thumbi *et al.* 2014). These pathogens may act independently or interact with each other and the host through various mechanisms with resultant varying outcomes on host health and survival (Thumbi *et al.* 2014). Physiological and behavioural elements of transmission should not be considered in isolation, as there is a two way interaction between host grazing behavior and parasitic burden. As the size of fecal deposits increases, the level of clustering of larvae increases, leading to rise in the severity of outbreaks of parasite burden (Fox *et al.* 2013).

For example, *Protostrongylus stillesi*, the sheep lungworm emerged in Musk ox (*Ovibos moschatus moschatus*) after reintroduction of this host into its historical range made it sympatric with Dall's sheep (*Ovis dalli*) (Kutz *et al.* 2004).

1.4 Rationale of the study

Ruminants are primary consumer in an ecosystem and play an important role in the ecosystem balance. The population trend of wild ruminants (Musk Deer, Barking Deer, Himalayan Tahr) are declining (Bhatnagar and Lovari 2008, Timmins *et al.* 2008, Wang and Harris 2010, Jnawali *et al.* 2011) due to poaching, habitat loss, human disturbance and parasitic diseases (Kusiluka and Kambarage 1996, Shrestha 2003, Jnawali *et al.* 2011). Sharing of common grazing land by domestic ruminants is one of the main threats in transmission of domestic ruminant's parasites to wild ruminants.

The lack of gastro-intestinal parasitic information on the wildlife and livestock in Nepal, motivate this study. This study will show the parasitic relationship between wild ruminants (Musk Deer, Barking Deer, Himalayan Tahr) and Chauri. If deadly and high prevalence of parasite find in Chauri then recommend authorized body and owner for the treatment of their livestock regularly and to avoid grazing in core wildlife habitat.

1.5 Objectives

1.5.1 General objective

The general objective of this study is to survey the gastrointestinal parasites of wild ruminants (Barking Deer, Musk Deer and Himalayan Tahr) and Chauris in Langtang National Park.

1.5.2 Specific objectives

- Characterize the gastro-intestinal parasites in Musk Deer (*Moschus chrysogaster*), Barking Deer (*Muntiacus vaginalis*), Himalayan Tahr (*Hemitragus jemlahicus*) and Chauri based on the morphology (shape, size and colour) of cysts/eggs under observation in light microscope.
- Determine the prevalence of gastro-intestinal parasites in both wild ruminants and chauris.
- Compare the intestinal parasites of wild ruminants and Chauris on the basis of morphological characters.

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, Boomker *et al.* 1989).

2.1 In global context

2.1.1 Parasites of 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 fecal 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 individuals in the groups and to individuals in different groups through dispersal or in areas of home range overlap.

Different researchers had done research in the parasites of Chauri and Yak especially from India and China. Goswami *et al.* (2013) found overall 24.13% prevalence with 26.31% and 20% prevalence in Yak of Arunachal Pradesh and Sikkim respectively. They were found infected with strongyle and *Eimeria* sp. Rahman *et al.* (2010) reported 20.68% prevalence of parasites in Yak of Sikkim. *Haemonchus* sp., *Nematodirus* sp., *Cooperia* sp. and *Dicrocoelium* sp. were observed in Yaks of Sikkim with 10.05% prevalence (Bandyopadhyay *et al.* 2010). Bam *et al.* (2012) reported strongyle, *Eimeria* sp., *Trichuris* sp., *Strongyloides* sp., *Dicrocoelium* sp., *Mammomonogamus laryngeus*, *Toxocara vitulorum* and *Fasciola gigantica* in Yak of Arunachal Pradesh, India. Highest

infection rate of nematode and coccidian was reported in Yaks of China (Yunfei *et al.* 2004) whereas Hogg (2004) recorded highest prevalence in strongyle followed by *Eimeria* sp.

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 *Cryptosporidium* sp., *Eimeria* sp., *Entamoeba* sp. (Maichomo *et al.* 2004, Regassa *et al.* 2006, Ayinmode and Fagbemi 2010, 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 Mbatl (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.

Asia being the developing country most of the people involved in animal husbandry. Most farmers use their own traditional practice in husbandry (Chetri *et al.* 2011). In Pakistan different researcher had done the research about gastrointestinal parasites of sheep, goat, cattle and buffalo. Asif *et al.* (2008) found overall prevalence of 65.7% in goats and sheep where they found higher parasites in sheep (72%) than in goats (63.7%). Similarly, Rahman *et al.* (2014) found 6 species of *Eimeria* in cattle where *E. bovis* had the highest prevalence. Although most cattle are exposed to coccidian and infected, most of the infections are self limiting and mild or asymptomatic. Lashari and Tasawar (2011) conducted research on various herds of Kacchi and Lohi breeds of sheep in Southern Punjab, Pakistan where overall parasitic prevalence of helminth parasites was 46.33%. In the cattle of Punjab overall prevalence of helminth was 51% (Muhammad *et al.* 2013). Age, sex, body weight and breed were found to be important factors which influence the prevalence of gastrointestinal parasites (Lashari and Tasawar 2011, Muhammad *et al.* 2013).

A total of 13 genera including 11 nematode: *Dictyocaulus* sp.(20.71%), *Haemonchus* sp.(9.7%), *Trichostrongylus* sp.(6.5%), *Strongylus* sp.(12.5%), *Neosaris* sp.(4.9%), *Nematodirus* sp.(4.5%), *Strongyloides* sp.(4.2%), one cestode, *Moniezia* sp.(6.7%) and one protozoan, *Eimeria* sp.(34.2%) were recorded from 810 fecal samples of goat and sheep of Palestine (Badran *et al.* 2012).

Cryptosporidium sp. is extensively recognized as pathogens of domesticated livestock, poultry animals and wildlife and is threat to public health. Out of 690 samples of calves in Myanmar, parasitic prevalence was 57.3% for *Cryptosporidium* sp., 34.1% for *Giardia* sp., 52.3% for *Eimeria* sp., 2.3% for *Toxocara vitulorum*, 7.4% for *Strongyloides papillosus* and 0.9% for *Tichuris* sp. (Khinlay 2007). A total of 504 fecal samples were collected from sheep and goat from Papua New Guinea. Samples were screened by nested PCR and genotyped at the 18S rRNA and at the 60KDa of glycoprotein (gp60) loci showed *Cryptosporidium* sp. prevalence of 2.2% for sheep and 4.4% for goats (Kionari *et*

al. 2014) but in the antigen ELISA of calves in Myanmar showed the prevalence of only 1.12% (Khinlay 2007).

Fecal samples of 241 goats from Korea showed that *Eimeria* sp. was significantly higher ($P < 0.05$) 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 gastro intestinal parasites of domestic ruminants. Biswas (2012) found 13 species of gastro intestinal 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 samples 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 gigantica*, *Paramphistomum* sp. and *Schistosoma indicum*) and 4 nematode (*Bunostomum* sp., *Trichuris* sp., *Strongyle* and *Strongyloides* sp.) species (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 of *Dictyocaulus* sp. (3.33%) in sheep.

A study was conducted to identify *Eimeria* sp. in lambs in Antakya Province of Turkey by Kaya (2004). Two hundred and forty eight samples were collected from 34 randomly selected lamb herds in 6 different towns. Ten different *Eimeria* sp. were identified. Another study was carried out in naturally infected cattle and sheep in Van Province in Eastern Turkey to assess prevalence of Paramphistomosis by Ozdal *et al.* 2010. Rumen and reticulum of slaughtered cattle and sheep were collected and examination showed 8.95% of cattle and 4.43% of sheep were positive for *Paramphistomum* infection. All sheep examined were infected with gastro intestinal nematodes of an abattoir in the Burdur region. Twenty two nematode species were identified and 38,639 nematodes were collected from infected sheep (Umur and Yukari 2005). Umur and Yakuri (2005) and

Ozdal *et al.* (2010) found that the highest infection was during the autumn followed by summer.

Four villages in two Provinces of West Cambodia were visited on monthly interval over a period of 11 months during which 2,391 cattle's fecal and blood samples for parasitological and haematological examination were taken. Overall proportion positive for gastrointestinal nematodes was 52% in 1-6 months calves, 44% in 6-24 months young and 37% in over 24 months adults. Six genera of strongyles (*Cooperia* sp., *Oesophagostomum* sp., *Haemonchus* sp., *Trichostrongylus* sp., *Mecistocirrus* sp. and *Bunostomum* sp.) were found (Dorny *et al.* 2011).

In cattle of Hemedan Province, Iran 9 species of *Eimeria* sp. with overall infection rate of 8.25% was found by Heidari *et al.* (2014) whereas Radfar *et al.* (2011) found 5 species of *Eimeria* with 89.27% of prevalence in Raeini goats. He also found *Trichuris* sp. in 44.75% fecal samples. In both research they found that *Eimeria* infection and the age or sex categories was not significant.

Jittapalapong *et al.* (2011) collected 1,599 fecal sample of dairy cows in Thailand and subjected to ethylacetate centrifugation and found overall prevalence was 46.6% with *Entamoeba* sp., rumen fluke, Coccidia, Strongyles, *Fasciola* sp., *Moniezia* sp., *Trichuris* sp., *Strongyloides* sp. and *Giardia* sp. as gastro intestinal parasites.

Kirkuk slaughter house, Iraq showed the highest rate of hydatid cysts and lung worm in cattle where as liver fluke was observed in buffaloes. Rate of liver flukes and lung worm in sheep and goats was highest in winter, while in cattle it was highest in autumn followed by winter (Kadir and Rasheed 2008).

Out of 3,300 fecal samples of sheep 76 were infected with adult cestodes in Riyadh city, Saudi Arabia. Cestodes were *Moniezia expansa* (96.3%) and *M. deucticulata* (3.7%). The highest infection rate was in autumn and lowest was in summer (Abdel and Qureishy 2008).

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 Kashmir 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).

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 samples 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 fecal samples of California bighorn sheep (*Ovis canadensis californiana*) in Northeastern Washington DC, USA. Identity of these dorsal larvae was established by single conformation polymorphism (SSCP) analyses of a partial fragment of the first international transcribed spacer of the ribosomal DNA and were identified as *Parelaphostrongylus odocoilei* (Chilton *et al.* 2006). Fecal samples 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). Fecal samples 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).

There are a range of internal parasites that cause production losses in the Australian sheep flock. In the winter high rainfall area, medium stomach worm (*Ostertagia* sp.) and black scour worm (*Trichostrongylus* sp.) are the roundworm parasites of major importance (McLeod 1995). *Haemonchus placei* is abomasal parasite of cattle primarily in tropical and subtropical areas of the world. In Australia this nematode can be extremely pathogenic in summer rainfall areas, particularly in the hot, sub tropical Kimberley region of Western Australia. Although *H. contortus* is found in cattle in the temperate Southern region of Western Australia, it appeared that *H. placei* also occurs in Southern West Australia (Jabbar *et al.* 2014).

In buffalo farms of Khadagzai district 6 species of nematode (*Trichostrongylus* sp., *Trichuris* sp., *Haemonchus* sp., *Strongyloides papillosus*, *Ostertagia* sp., *Toxocara vitulurum*) and one species of trematode (*Fasciola* sp.) were identified (Azam *et al.* 2002). Overall prevalence of 64.61% with helminthes 76.15% and protozoan 13.62% was found in cattle of Khyber Pakhtunkhwa (Rafiullah *et al.* 2011) whereas overall helminth prevalence of 78.1% (nematode infestation 37.5%, trematode 7.9%, cestode 2.6%) and protozoan 0.8% was found in sheep and goat flocks of Cholistan desert (Raza *et al.* 2014).

2.1.2 Parasites of wild ruminants

Wild animals are the most likely source of new emerging disease that put at risk the health of human being and livestock (Anonymous 2004). Digestive tract of Far Eastern Musk Deer (*Moschus moschiferus turovi*) of Primorsky Krai, Russia was analyzed by postmortem and *Spiculoptera spiculoptera*, *Nematodirus filicolis*, *Pygarginema skrjabini* were found in abomasum whereas in colon *Trichuris* sp. was found (Kuznetsov *et al.* 2014).

Three blackbuck antelope (*Antelope cervicapra*) in Texas were examined through postmortem and parasites identified were *Camelostromylus mentulatus*, *Haemonchus contortus* in abomasum, *Nematodirus spathiger*, *Trichostrongylus axei*, *T. colubriformis*, *T. probolurus* in small intestine and *Oesophagostomum* sp. and *Trichuris* sp. in large intestine (Thornton *et al.* 1973). Worley and Eustace (1972) collected 44 Mule Deer (*Odocoileus homionus*) from semiarid rangeland in Garfield and Rosebud countries, Montana, 43 animals were infected with one or more of 13 species of helminth parasites (*Trichostrongylus colubriformis*, *Nematodirus odocoilei*, *Skrjabinema parva*, *Cooperia oncophora*, *Protostrongylus macrotis*, *Trichuris* sp., *Ostertagia bisonis*, *Taenia hydatigena cysticerci*, *Thysanosoma actinoides*, *Pseudostertagia bullosa*, *Haemonchus contortus* and *Trichostrongylus longispicularis*). Identified helminths were similar to that of livestock. 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). Eve and Kellogg (1977) recorded *Skrjabinagia odocoilei*, *Ostertagia mossi* and *O. dikmansii* from medium stomach, *Haemonchus contortus* from large stomach and *Trichostrongylus 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 Virginia (Prestwood *et al.* 1976).

Similarly, Grey Brocket Deer (*Mazama gouazoubira*) of Brazilian Pantanal wetlands were found to be infected with *Haemonchus* sp. (LuxHoppe *et al.* 2010).

Survey of abomasal parasites in cervids from Central Spain was done by Duran *et al.* (2004) where 147 Red Deer (*Cervus elaphus*) and 17 Fallow Deer (*Dama dama*) were collected and necropsy examination was done. *Spiculoptera quadrispiculata* was

reported for the first time in Red Deer from Spain and *Trichostrongylus axei* and *Ostertagia drozdzi* were also recorded. Lungs of 102 Roe Deer (*Capreolus capreolus*), 136 Moose (*Alces alces*), 68 Fallow Deer and 6 Red Deer were examined during hunting season and worm were identified following PCR amplification of the internal transcribed spacer of ribosome DNA (ITS2) followed by hybridization with 4 species of ribosome oligonucleotides. All species from Roe Deer were identified as *Dictyocaulus capreolus* whereas those from Red Deer and Roe Deer were identified as *D. eckerti* (Divina *et al.* 2002). The alimentary canal of Fallow Deer hunted in Southern Poland showed *Ashworthius sidemi*, *Spiculoptera sp.*, *Nematodirus filicollis*, *Aonchotheca bovis*, *Oesophagostomum radiatum* as the main parasites (Kowal *et al.* 2012). Common Deer (*Cervus elaphus*), Fallow Deer, Rein Deer (*Rangifer tarandus*), Guanaco (*Lama guanacoe*) of Tomisoara zoo have found parasitized by *Eimeria* sp., *Nematodirus* sp. and *Trichostrongylus* sp. (Darabus *et al.* 2009).

Fecal samples of captive wild animals 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 Blue bulls (85.71%), Sambars (83.33%), Chausighas (80%), Spotted Deer (38%) and Blackbucks (35%) (Thawait *et al.* 2014). A mini zoo in Coimbatore, Tamil Nadu showed prevalence of 58% for helminth and 6% for protozoan of wild animals with strongyle, *Trichuris* sp., *Strongyloides* sp. and Coccidia as the main parasites (Varadharajan and Kandasamy 2000). Axis Deer of scrub forest of Borgaon Manju in Western Vidabha region of Maharashtra 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, Neelgai, Chital and Gaur around Jabalpur, India.

Coprological study was conducted by Gurley *et al.* (2010) to determine prevalence of helminth infections at Samsun zoo, Turkey. They found 36.4% prevalence with 1 cestode, 1 trematode and 12 nematode eggs or larvae among 184 animals including different mammals and birds.

Gastrointestinal parasites and their level of infestation in the Arabian oryx (*Oryx leucoryx*) were studied at King Khalid Wildlife Research Center in Thumamah, Riyadh Province, Saudi Arabia and fecal examination revealed *Eimeria saudiensis*, *Cryptosporidium* sp., *Nematodirus spathiger* and *Trichuris* sp. as the gastrointestinal parasites (Mohammed *et al.* 2012).

Radhy *et al.* 2013 in Baghdad showed that protozoal infection occurs almost in all species of captive wild animals and are risk of infection by various gastro intestinal protozoa like *Entamoeba coli*, *Giardia* sp., *Sarcocysts* sp and *Cryptosporidium* sp.

In Sanda Kyarimi Park, Maiduguru, Nigeria, Africa 36 captive wild ruminants representing 9 different species were examined where strongyle egg, *Trichostrongylus axei*, *Haemonchus contortus* and *Trichuris* sp. were common parasites present in the gastro intestinal tract (Ibrahim *et al.* 2012). Giraffe (*Giraffa camelopardalis angolensis*) were found infected with *Parabronema skrjabini*, *Skrjabinema* sp., *Haemonchus mitchelli*, *Echinococcus* sp. in Etosha National Park, Namibia (Krecek *et al.* 1990). Seventeen warthogs were harvested from their natural habitat and parasites were examined where *Telamodinium onyx*, *Megadinium aethiopicum* and *Teratodinium sphaereden* were identified (Booyse *et al.* 2002).

2.2 In the national context

Intestinal parasites occur in the 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). Cryptosporidiosis is a common protozoan disease in humans and animals in Nepal acquired by ingesting of oocysts excreted in the feces of infected individuals (Paudyal *et al.* 2013). Persistent shedding of oocysts by reservoir hosts like calves, deer etc. possess great threat to the transmission to general public. In 1983 A.D., with the realization of need of efficient veterinary service, veterinary hospitals were established in 75 districts (DoAH 2013/14).

2.2.1 Parasites of domestic ruminants

Fecal samples 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). 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 *Ornithobilharzia turkestanicum* were reported for the first time in Nepal from Buffaloes (Mukhia *et al.* 2007). More over *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 2000). *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. for the first time in Nepal from cattle.

2.2.2 Parasites of wild ruminants

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. Regarding the parasitic infection of Musk Deer, there was no literature found but there were few literature found on the gastrointestinal parasites of other wild ruminants. Thapa (2013) reported *Eimeria* sp., *Moniezia* sp., *Oxyuris* sp., *Strongyloides* sp., *Ascaris* sp., *Trichostrongylus* sp., *Dictyocaulus* sp., *Muelleuris* sp. and *Haemonchus* sp. from Himalayan Tahr and *Eimeria* sp., *Moniezia* sp., *Oxyuris* sp., *Ascaris* sp., *Trichuris* sp., *Dictyocaulus* sp. and *Haemonchus* sp. from Barking Deer of Rara National Park.

In Khairapur, Bardia Ban (2012) had reported strongyle, *Trichuris* sp., *Trichostrongylus* sp., *Paramphistomum* sp., *Fasciola* sp., coccidian, *Strongyloides* sp., *Moniezia* sp. and *Schistosoma* sp. as the main gastrointestinal parasites of Blackbuck (*Antelope cervicapra*). *Entamoeba* sp., *Eimeria* sp., *Paramphistomum* sp., *Fasciola* sp., *Moniezia* sp., *Trichostrongylus* sp., *Ascaris* sp., *Haemonchus* sp., *Strongyloides* sp., *Bunostomum* sp., *Trichuris* sp. and *Oxyuris* sp. were the parasites of Blackbuck in Blackbuck Conservation Area, Bardia and Shuklaphanta Wildlife Reserve, Kanchanpur (Chaudhary 2014).

3. MATERIALS AND METHODS

3.1 Study Area

Langtang National Park (28° 10' 25" N and 85° 33' 11" E) was first proposed by C. Caughley in 1969 and later endorsed by J. Blower in 1974 (Heinen and Kattel 1992). It was established and gazetted in 1976 and covers an area of 1,710 square kilometer (Shrestha 2003). In 1998 an additional 420 square kilometer was added to the park as a buffer zone (Heinen and Kattel 1992). It is located in the central Himalayas of Nepal and is 32 km north of Kathmandu (Shrestha 2001) and extends over parts of Nuwakot, Rasuwa and Sindhupalchowk districts in the southern mountainous terrain of the Nepal China border (LNP 2015).

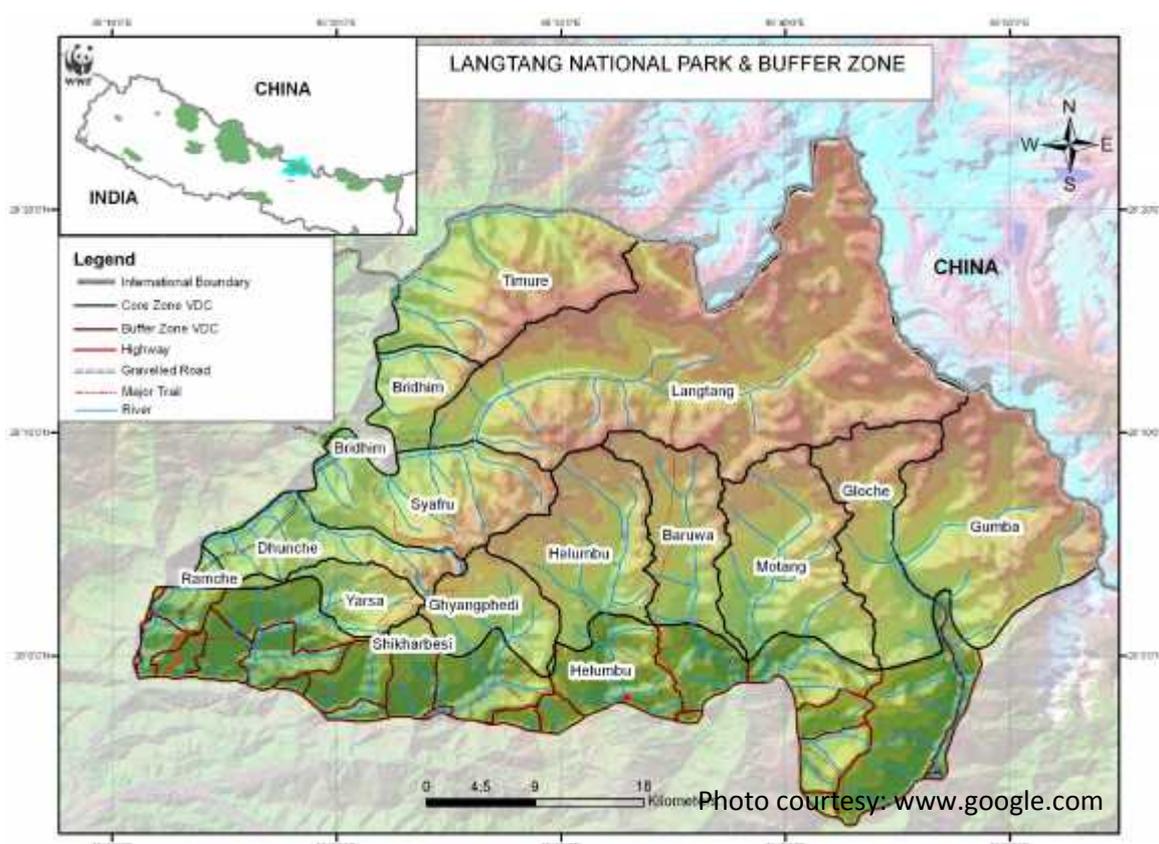


Figure 1: Map of Langtang National Park

It is Nepal's third largest protected area with variation of altitude from 720 m near Bhotekoshi to 7,245 m of Langtang Lirung (Shrestha 2001). Samples were collected from Cholangpati, Polangpati and Guppche.

Main flora are Pine (*Pinus wallichiana*), Birch (*Betula utilis*), Rhododendron (*Rhododendron arboreum*), Juniper (*Juniperus indica*), Spruce (*Picea smithina*), Oak

(*Quercus semecarpifolia*), Cypress (*Cupres sutorulosa*), Chestnut (*Aesculus indica*), Walnut (*Juglan sregia*), Bamboo (*Thamnocalamus* sp. and *Yushania/Chimnobambusa* sp) (LNP 2015). Similarly, Himalayan Black Bear (*Ursus thibetanus*), Common Leopard (*Panthera pardus*), Musk Deer (*Moschus chrysogaster*), Barking Deer (*Muntiacus vaginalis*), Goral (*Nemorhaedus goral*), Himalayan Tahr (*Hemitragus jemlahicus*), Red Panda (*Ailurus fulgens*), Wild Boar (*Sus scrofa*), Wild Dog (*Cuon alpinus*) are the fauna of the park (Shrestha 2003). Langtang expansive high meadows provide summer habitat for numerous ungulate species such as Musk Deer and Himalayan Tahr (LNP 2015).

3.2 Materials

During the research the materials used have been listed below:

3.2.1 Materials used

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

3.2.2 Chemicals used

- | | |
|------------------------------|---------------------|
| i. 10% ethyl alcohol | ii. Distilled water |
| iii. Saturated NaCl solution | iv. Methylene blue |
| v. Lugol's Iodine solution | |

3.3. Identification of pellet

The pellet were identified on the basis of following characteristics

3.3.1 Pellet of Barking Deer

- Slender in shape but sometime pointed at one end, one side is bulged and other side depressed
- Black in colour

3.3.2 Pellet of Himalayan Tahr

- Slender in shape, more or less blunt at the end and somewhat larger than that of Barking Deer
- Grey with blackish in colour

3.3.3 Pellet of Musk Deer

- Slender in shape with one end pointed and other end blunt
- Shiny black in colour

3.4 Sample collection method

A total of 71 fecal samples, 15 from Chauri, 9 from Musk Deer, 16 from Himalayan Tahr and 31 from Barking Deer, were collected between May and June 2014. The transect lines was made at an interval of 200 m. A total of 4 transect lines between 3,000 m – 3,600 m were used. Each sample comprised of 20 gm of feces taken from either a pellet group or a pile of dung. Chauri samples were collected immediately after they were defecated, and each sample was visually confirmed to be from different individuals. Wild ruminants were not directly sighted so, for their fecal samples the rule of a minimum distance (300 m – 400 m) was taken into account to reduce the chance of duplicated samples from the same individuals. To increase sample size, feces that were less than 300 m – 400 m apart were also collected, if the pellet sizes were visually different (different pellet sizes are likely from different individuals), and opportunistic samples were also collected while walking from one transect line to another provided that they met the minimum-distance/pellet-size criteria.

3.5 Preservation of samples

The collected fecal sample was immediately placed in a 50 ml sterile vile with 10% ethyl alcohol and transported back to the laboratory at the Central Department of Zoology, Tribhuvan University, Kathmandu. The samples were stored at 4°C for one week prior to further processing.

3.6 Lab process

The samples were processed for microscopic examination. The ova/oocysts/cyst and larvae of different parasites were identified according to the morphology and quantitative

estimation by using concentration method (flotation and sedimentation) and Stoll's counting technique to determine mix infection and intensity of parasites (Soulsby 1982).

3.6.1 Concentration method

Eggs/cysts are often low number in faeces that they are difficult to be detected in direct smears or mounts. Therefore, these procedures were performed which include flotation and sedimentation techniques.

3.6.1.1 Differential Floatation Technique

Nematode and cestode eggs present in feces is detected through this technique. This technique ensures the egg float in the floatation liquid, which helps to identify the eggs.

Approximately 3 gm of fecal sample was taken in a beaker and added 20 ml of water then the sample was grinded lightly with the help of mortar and pestle and filtered the solution by tea strainer. The filtrate solution was poured into a centrifuge tube of 15 ml and centrifuged at 2,000 rpm for 5 minutes. The tube's water was replaced with saturated sodium chloride solution and again centrifuged.

After centrifuge more saturated sodium chloride solution was added to develop convex surface at the top of the tube and one drop of methylene blue (to stain) where a cover slip can be placed for a few minutes and then cover slip was removed and placed on a slide and examined at 10X and 40X. Photographs of cyst and eggs were taken and identified based on egg's color, shape, and size.

3.6.1.2 Sedimentation technique

This technique is used for the detection of trematode eggs. It provides good results as the eggs of the trematode are bit heavier than the other, where sediments of centrifuged contents were taken for eggs detection.

Saturated salt solution was removed gently from the test tube after examined the flotation portion and poured the sediment content into the watch glass and stirred the content gently to mix it. One drop from the mixture was taken to prepare a second slide. The specimen was stained with iodine wet mounts solution.

In this way two slides were prepared from one sample (one from flotation and one from sedimentation) and examined under 10X and 40X magnification of microscope to detect eggs of helminthes and trophozoites or cyst of gastrointestinal protozoans.

3.6.2 Egg, cyst and larva size measurement

- using ocular and stage micrometer
- length and breadth measured by calibration

3.7 Identification of cysts, eggs and larvae of parasites

Cysts, eggs and larvae of parasites were identified on the basis of morphological characters (shape and size) by using books of Yamaguti (1961), Soulsby (1982), Zajac and Conboy (2012), other published and unpublished article and also from internet sources.

3.8 Data analysis

The presence and absence of parasites data were entered in MS Excel 2007, from where prevalence of parasites was calculated. To show the significance of parasites between wild ruminants and Chauri, the chi-square and P value was calculated from SPSS 21.

Glimpse of photograph during field and lab work



Photo 1: Chauri in their shed



Photo 2: Chauri grazing in the habitat of wild ruminants



Photo 3: Researchers at the field



Photo 5: Pellets of Himalayan Tahr in the field



Photo 5: Pellets of Himalavan Tahr



Photo 6: Pellets of Musk Deer



Photo 7: Pellets of Barking Deer



Photo 8: Transferring the filtered fecal sample to the centrifuge tube



Photo 9: Centrifuging the samples



Photo 10: Observing the slide on microscope

4. RESULTS

4.1 Gastrointestinal parasites of wild ruminants

A total of 56 fecal samples of wild ruminants including 31 from Barking Deer, 9 from Musk Deer and 16 from Himalayan Tahr were analyzed.

4.1.1 Parasites of Barking Deer

4.1.1.1 Parasitic prevalence of Barking Deer

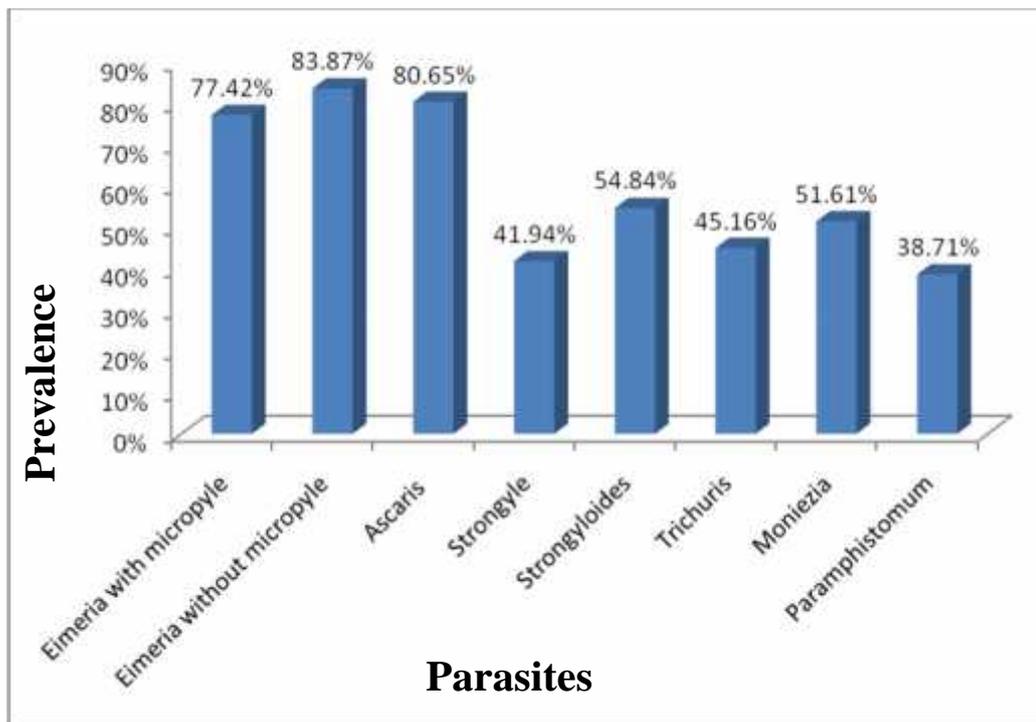


Figure 2: Parasitic prevalence of Barking Deer

Out of 31 analyzed fecal samples of Barking Deer, 26 (83.87%) were found positive with gastrointestinal parasites. Seven genus of parasite including protozoan (1), cestode (1), trematode (1) and nematode (4) were identified (Figure 2). *Eimeria* sp. with micropyle and without micropyle had the prevalence of 77.42% and 83.87% respectively. Among the identified nematode *Ascaris* sp. had the prevalence of 80.65%. *Moniezia* sp. had the prevalence of 51.61% and *Paramphistomum* sp. had 38.71% prevalence.

4.1.1.2 Mixed infections

Mixed parasitic infection was found in all positive fecal samples of Barking Deer (Table 1). *Eimeria* sp., *Ascaris* sp., *Moniezia* sp. and *Trichuris* sp. were found in 5 (16.13%) samples. Eight samples (25.81%) were found mixed infection with *Eimeria* sp., *Ascaris* sp.

and strongyle where as nine samples (29.03%) were found mixed infection with *Ascaris* sp., *Eimeria* sp. and *Paramphistomum* sp.

Table 1: Mixed infection of parasites in Barking Deer

Parasites	Number of samples	Prevalence
<i>Eimeria</i> sp., <i>Ascaris</i> sp., <i>Moniezia</i> sp., <i>Trichuris</i> sp.	5	16.13%
<i>Eimeria</i> sp., <i>Ascaris</i> sp., strongyle	8	25.81%
<i>Moniezia</i> sp., <i>Paramphistomum</i> sp.	5	16.13%
<i>Ascaris</i> , strongyle, <i>Strongyloides</i> sp.	4	12.90%
<i>Ascaris</i> sp., <i>Eimeria</i> sp., <i>Paramphistomum</i> sp.	9	29.03%

4.1.1.3 Intensity of parasites in Barking Deer

In this study, heavy parasitic infection was considered as those samples which has 6 or more eggs or cysts observed per field. Two samples of *Eimeria* without micropyle, one sample of *Ascaris* sp. and one sample of strongyle showed heavy infection. Two samples of *Eimeria* with micropyle, *Eimeria* without micropyle and *Ascaris* sp showed moderate infection. All positive samples showed light and moderate infection (Table 2).

Table 2: Intensity of parasites in Barking Deer

S.N.	Class	Parasites	Light	Mild	Moderate	Heavy
1.	Sporozoa	<i>Eimeria</i> with micropyle	15	7	2	-
2.		<i>Eimeria</i> without micropyle	13	9	2	2
3.	Nematode	<i>Ascaris</i> sp.	16	6	2	1
6.		<i>Trichuris</i> sp.	9	4	1	-
7.		Strongyle	9	2	1	1
9.		<i>Strongyloides</i> sp.	15	2	-	-
10.		Cestode	<i>Moniezia</i> sp.	14	2	-
12.	Trematode	<i>Paramphistomum</i> sp.	10	2	-	-

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 = eggs/cysts/ larva per field

4.1.2 Parasites of Musk Deer

4.1.2.1 Parasitic prevalence of Musk Deer

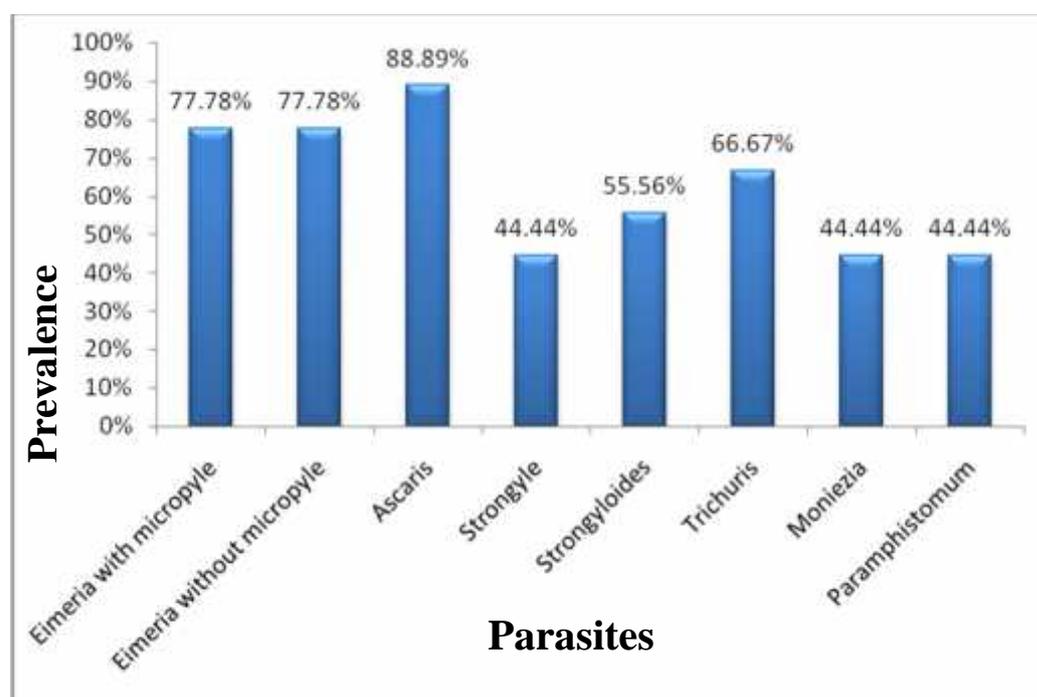


Figure 3: Parasitic prevalence of Musk Deer

Of the 9 analyzed fecal samples of Musk Deer, 8 (88.89%) samples were found positive with different gastrointestinal parasites. Seven genus of parasite including one protozoan, one cestode, one trematode and four nematode were identified (Figure 3). *Eimeria* sp. with and without micropyle had the prevalence of 77.78% whereas *Moniezia* sp., strongyle and *Strongyloides* sp. had the prevalence of 44.44%.

4.1.2.2 Mixed infection of parasites

Table 3: Mixed infection of parasites in Musk Deer

Parasites	Number of samples	Prevalence
<i>Eimeria</i> sp., <i>Ascaris</i> sp., <i>Strongyloides</i> sp.	5	55.56%
<i>Eimeria</i> sp., strongyle, <i>Paramphistomum</i> sp.	2	22.22%
<i>Trichuris</i> sp., <i>Moniezia</i> sp.	3	33.33%
Strongyle, <i>Strongyloides</i> sp., <i>Ascaris</i> sp.	1	11.11%

Mixed parasitic infection was found in all the positive samples of Musk Deer (Table 3). *Eimeria* sp., *Ascaris* sp. and *Strongyloides* sp. were found in 5 (55.56%) samples. Two samples (22.22%) were found mixed infected with *Eimeria* sp., strongyle and *Paramphistomum* sp. but only one sample (11.11%) were found infected with strongyle, *Strongyloides* sp. and *Ascaris* sp.

4.1.2.3 Intensity of parasites

Two samples of *Eimeria* without micropyle, one sample of *Eimeria* with micropyle, *Ascaris* sp. and *Strongyloides* sp. showed moderate infection (Table 4). All identified parasites showed light infection whereas no parasites showed heavy infection.

Table 4: Intensity of parasites in Musk Deer

S.N.	Class	Parasites	Light	Mild	Moderate	Heavy
1.	Sporozoa	<i>Eimeria</i> with micropyle	3	3	1	-
2.		<i>Eimeria</i> without micropyle	2	3	2	-
3.	Nematode	<i>Ascaris</i> sp.	5	2	1	-
6.		<i>Trichuris</i> sp.	5	1	-	-
7.		Strongyle	2	2	-	-
9.		<i>Strongyloides</i> sp.	3	1	1	-
10.	Cestode	<i>Moniezia</i> sp.	4	-	-	-
12.	Trematode	<i>Paramphistomum</i> sp.	2	2	-	-

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 = eggs/cysts/ larva per field

4.1.3 Parasites of Himalayan Tahr

4.1.3.1 Parasitic prevalence of Himalayan Tahr

Out of the 16 analyzed samples of Himalayan Tahr, 14 (87.50%) samples were found positive with seven genus (Figure 4). *Eimeria* with micropyle (81.25%), *Eimeria* without micropyle (75%), *Ascaris* sp. (87.50%), strongyle (37.50%), *Strongyloides* sp. (62.50%), *Trichuris* sp. (56.25%), *Moniezia* sp. (56.25%) and *Paramphistomum* sp. (12.50%) were the identified parasites.

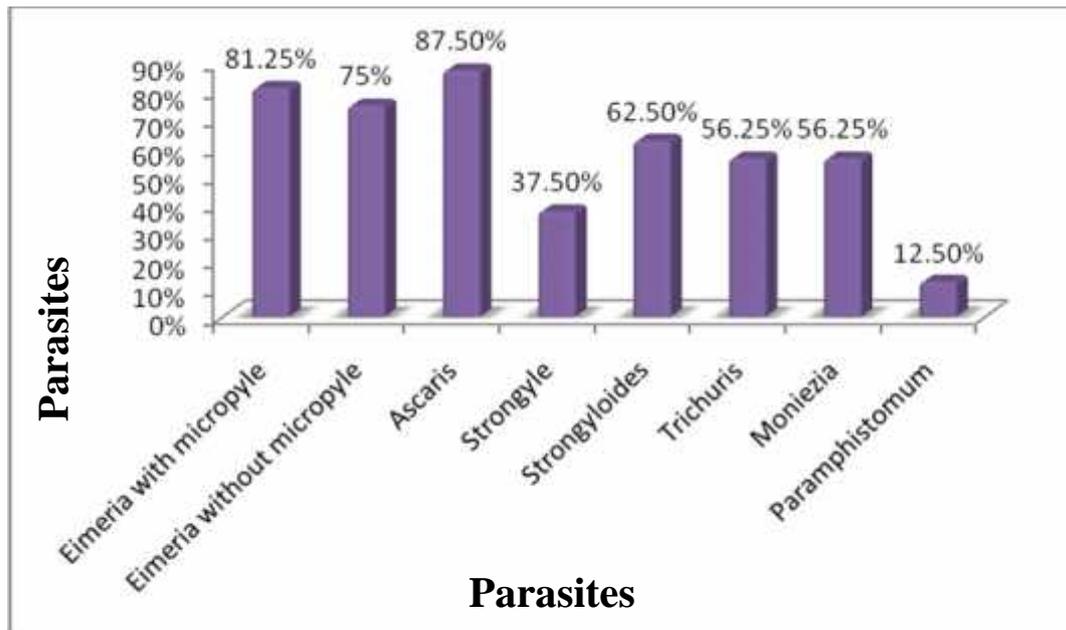


Figure 4: Parasitic prevalence of Himalayan Tahr

4.1.3.2 Mixed infection of parasites

Table 5: Mixed infection of parasites in Himalayan Tahr

Parasites	Number of samples	Prevalence
<i>Eimeria</i> sp., <i>Ascaris</i> sp., <i>Moniezia</i> sp.	6	37.50%
<i>Paramphistomum</i> sp., <i>Strongyloides</i> sp.	2	12.50%
<i>Ascaris</i> sp., <i>Trichuris</i> sp., strongyle	5	31.25%
<i>Eimeria</i> sp., strongyle, <i>Strongyloides</i> sp.	4	25.00%

All positive samples of Himalayan Tahr were found infected with mixed parasitic infection (Table 5). *Eimeria* sp., *Ascaris* sp. and *Moniezia* sp. were found in 6 (37.50%) samples, *Paramphistomum* sp. and *Strongyloides* sp. in 2 (12.50%) samples, *Ascaris* sp., *Trichuris* sp. and strongyle in 5 (31.25%) samples.

4.1.3.3 Intensity of parasites

Three samples of *Ascaris* sp., two samples of *Eimeria* with micropyle, one sample of *Eimeria* without micropyle and strongyle showed moderate infection. All identified

parasites showed light infection whereas expect *Paramphistomum* sp. all parasites showed mild infection (Table 6).

Table 6: Intensity of parasites in Himalayan Tahr

S.N.	Class	Parasites	Light	Mild	Moderate	Heavy
1.	Sporozoa	<i>Eimeria</i> with micropyle	7	4	2	-
2.		<i>Eimeria</i> without micropyle	6	5	1	-
3.	Nematode	<i>Ascaris</i> sp.	5	6	3	-
6.		<i>Trichuris</i> sp.	6	3	-	-
7.		Strongyle	3	2	1	-
9.		<i>Strongyloides</i> sp.	8	2	-	-
10.	Cestode	<i>Moniezia</i> sp.	8	1	-	-
12.	Trematode	<i>Paramphistomum</i> sp.	2	-	-	-

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 = eggs/cysts/ larva per field

4.2 Gastrointestinal parasites of Chauri

A total of 15 fecal samples of Chauri (female hybrids of Yak and local hill cow) were collected and examined for the gastrointestinal parasites. Out of 15, 13 samples were positive for the gastrointestinal parasites.

4.2.1 Parasitic prevalence of Chauri

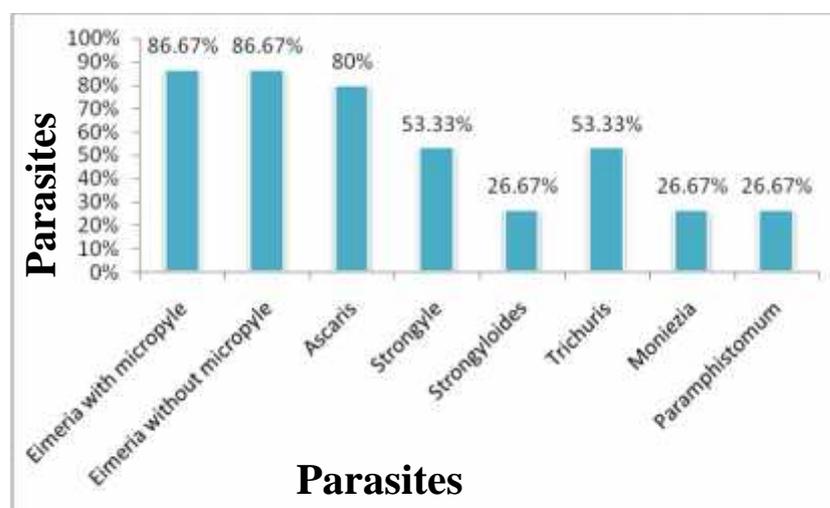


Figure 5: Parasitic prevalence of Chauri

Chauri was found to be infected with seven genus of parasites including one protozoan (*Eimeria* sp. with micropyle and micropyle), one cestode (*Moniezia* sp.), one trematode (*Paramphistomum* sp.) and four nematode (*Ascaris* sp., strongyle, *Strongyloides* sp. and *Trichuris* sp.) (Figure 5). *Eimeria* sp. had the highest prevalence (86.67%) followed by *Ascaris* sp. (80%). Strongyle and *Trichuris* sp. had the prevalence of 53.33% whereas *Strongyloides* sp., *Moniezia* sp. and *Paramphistomum* sp. had the prevalence of 26.67%.

4.2.2 Mixed infection of parasites

Table 7: Mixed infection of parasites in chauri

Parasites	Number of samples	Prevalence
<i>Eimeria</i> sp., <i>Ascaris</i> sp., <i>Moniezia</i> sp.	6	37.50%
<i>Paramphistomum</i> sp., <i>Strongyloides</i> sp.	2	12.50%
<i>Ascaris</i> sp., <i>Trichuris</i> sp., strongyle	5	31.25%
<i>Eimeria</i> sp., strongyle, <i>Strongyloides</i> sp.	4	25%

All positive samples of Chauri (13) were infected with different mixed parasites (Table 7). *Eimeria* sp., *Ascaris* sp. and *Moniezia* sp. were found in 6 (37.50%) samples whereas *Paramphistomum* sp. and *Strongyloides* sp. in 2 (12.50%) samples. *Ascaris* sp., *Trichuris* sp. and strongyle were found in 5 (31.25%) samples and *Eimeria* sp., strongyle and *Strongyloides* sp. in 4 (25%) samples.

4.2.3 Intensity of parasites

Two samples of *Eimeria* without micropyle, one sample of *Eimeria* with micropyle and *Ascaris* sp. showed heavy infection whereas two samples of *Ascaris* sp., one sample of *Eimeria* with micropyle, *Eimeria* without micropyle and strongyle showed moderate infection (Table 8). All identified parasites showed light and mild infection.

Table 8: Intensity of parasites in Chauri

S.N.	Class	Parasites	Light	Mild	Moderate	Heavy
1.	Sporozoa	<i>Eimeria</i> with micropyle	5	6	1	1
2.		<i>Eimeria</i> without micropyle	9	1	1	2
3.	Nematode	<i>Ascaris</i> sp.	6	3	2	1
6.		<i>Trichuris</i> sp.	6	2	-	-
7.		Strongyle	5	2	1	-
9.		<i>Strongyloides</i> sp.	3	1	-	-
10.	Cestode	<i>Moniezia</i> sp.	2	2	-	-
12.	Trematode	<i>Paramphistomum</i> sp.	2	2	-	-

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 = eggs/cysts/ larva per field

4.3 Comparison of parasites between Chauri and Musk Deer

4.3.1 Comparison of protozoan parasites

The only protozoan parasites found in Chauri and Musk Deer was *Eimeria* sp. In Chauri the both *Eimeria* had the prevalence of 86.67% whereas in the Musk Deer it was 77.78%. There was no significance difference in protozoan parasites between the Chauri and Musk Deer ($\chi^2 = 0.320$, $P = 0.572$, d.f. = 1).

4.3.2 Comparison of helminth parasites

Cestode, trematode and nematode were found in the Chauri as well as in Musk Deer. In Musk Deer highest prevalence of nematode was followed by *Ascaris* sp. (88.89%), *Trichuris* sp. (66.67%), *Strongyloides* sp. (55.56%) and strongyle (44.44%) but in Chauri it was followed by *Ascaris* sp. (80%), *Trichuris* sp., strongyle (53.33%) and *Strongyloides* sp. (26.67%). No significance difference was found in nematode parasites of Chauri and Musk Deer ($P > 0.05$).

Moniezia sp. was found in both Chauri and Musk Deer with the prevalence of 26.67% and 44.44% respectively. There was no significance difference between the cestode ($\chi^2 = 0.800$, $P = 0.412$, d.f. = 1) of both ruminants. The only trematode found in both ruminants was *Paramphistomum* sp. with the prevalence of 26.67% in Chauri and 44.44% in Musk Deer. There was no significance difference between the trematode parasite ($\chi^2 = 0.800$, $P = 0.412$, d.f. = 1).

Table 9: Comparison of parasites between the Chauri and Musk Deer

S.N.	Class	Parasites	Positive samples in Chauri (Prevalence)	Positive samples in Musk Deer (Prevalence)	² value	P Value
1	Sporozoa	<i>Eimeria</i> with micropyle	13 (86.67%)	7 (77.78%)	0.320	0.572
2		<i>Eimeria</i> without micropyle	13 (86.67%)	7 (77.78%)	0.320	0.572
3	Nematode	<i>Ascaris</i> sp.	12 (80%)	8 (88.89%)	0.320	0.572
6		Strongyle	8 (53.33%)	4 (44.44%)	0.178	1.0 [#]
8		<i>Strongyloides</i> sp.	4 (26.67%)	5 (55.56%)	2.003	0.212 [#]
9		<i>Trichuris</i> sp.	8 (53.33%)	6 (66.67%)	0.411	0.521
11	Cestode	<i>Moniezia</i> sp.	4 (26.67%)	4 (44.44%)	0.800	0.412 [#]
12	Trematode	<i>Paramphistomum</i> sp.	4 (26.67%)	4 (44.44%)	0.800	0.412 [#]

Fisher exact test accepted due to less than five expected value

4.4 Comparison of parasites between Chauri and Barking Deer

4.4.1 Comparison of protozoan parasites

Chauri and Barking Deer were found to have *Eimeria* sp. as protozoan parasite. In Chauri *Eimeria* with micropyle and *Eimeria* without micropyle had the equal prevalence (86.67%) but in Barking Deer *Eimeria* with micropyle and without micropyle had the prevalence of 77.42% and 83.87% respectively. There was no significance difference between the protozoan parasites of Chauri and Barking Deer ($P > 0.05$).

4.4.2 Comparison of Helminth parasites

Four species of nematode were found in both the Chauri and Barking Deer. In Chauri strongyle and *Trichuris* sp. had the prevalence of 53.33%, *Strongyloides* sp. with 26.67% and *Ascaris* sp. with 80%. In Barking Deer *Ascaris* sp., *Strongyloides* sp., *Trichuris* sp. and strongyle had the prevalence of 80.65%, 54.84%, 45.16% and 41.94% respectively. There was no significance difference between the nematode parasites of Chauri and Barking Deer ($P > 0.05$).

Moniezia sp. had the prevalence of 26.67% and 51.61% in Chauri and Barking Deer respectively. *Moniezia* sp. ($\chi^2 = 2.560$, $P = 0.128$ at d.f. 1) had no significance difference between Chauri and Barking Deer.

The prevalence of *Paramphistomum* sp. was 26.67% and 38.71% for Chauri and Barking Deer respectively. There was no significance difference of trematode between the Chauri and Barking Deer ($\chi^2 = 0.646$, $P = 0.520$ at d.f. 1).

Table 10: Comparison of parasites of Chauri and Barking Deer

S.N.	Class	Parasites	Positive samples in Chauri (Prevalence)	Positive samples in Barking Deer (Prevalence)	χ^2 value	P Value
1	Sporozoa	<i>Eimeria</i> with micropyle	13 (86.67%)	24 (77.42%)	0.549	0.459
2		<i>Eimeria</i> without micropyle	13 (86.67%)	26 (83.87%)	0.061	0.805
3	Nematode	<i>Ascaris</i> sp.	12 (80%)	25 (80.65%)	0.003	0.959
6		Strongyle	8 (53.33%)	13 (41.94%)	0.529	0.467
8		<i>Strongyloides</i> sp.	4 (26.67%)	17 (54.84%)	3.234	0.115 [#]
9		<i>Trichuris</i> sp.	8 (53.33%)	14 (45.16%)	0.271	0.603
11	Cestode	<i>Moniezia</i> sp.	4 (26.67%)	16 (51.61%)	2.560	0.128 [#]
12	Trematode	<i>Paramphistomum</i> sp.	4 (26.67%)	12 (38.71%)	0.646	0.520 [#]

Fisher exact test accepted due to less than five expected value

4.5 Comparison of parasites between Chauri and Himalayan Tahr

4.5.1 Comparison of protozoan parasites

The only protozoan parasites found in both ruminants was *Eimeria* sp. *Eimeria* with micropyle and *Eimeria* without micropyle had the prevalence of 86.67% in Chauri whereas in Himalayan Tahr *Eimeria* with micropyle and *Eimeria* without micropyle had the prevalence of 81.25% and 75% respectively. *Eimeria* sp. was not significantly different between the Chauri and Barking Deer ($P > 0.05$).

Table 11: Comparison of parasites of Chauri and Himalayan Tahr

S.N.	Class	Parasites	Positive samples in Chauri (Prevalence)	Positive samples in Himalayan Tahr (Prevalence)	χ^2 value	P Value
1	Sporozoa	<i>Eimeria</i> with micropyle	13 (86.67%)	13 (81.25%)	0.168	0.682
2		<i>Eimeria</i> without micropyle	13 (86.67%)	12 (75%)	0.675	0.411
3	Nematode	<i>Ascaris</i> sp.	12 (80%)	14 (87.50%)	0.322	0.570
6		Strongyle	8 (53.33%)	6 (37.50%)	0.784	0.376
8		<i>Strongyloides</i> sp.	4 (26.67%)	10 (62.50%)	4.014	0.073 [#]
9		<i>Trichuris</i> sp.	8 (53.33%)	9 (56.25%)	0.027	0.870
11	Cestode	<i>Moniezia</i> sp.	4 (26.67%)	9 (56.25%)	2.783	0.095
12	Trematode	<i>Paramphistomum</i> sp.	4 (26.67%)	2 (12.50%)	0.995	0.394 [#]

Fisher exact test accepted due to less than five expected value

4.5.2 Comparison of helminth parasites

Four species of nematodes were recorded from both ruminants and in both *Ascaris* sp. had the highest prevalence. There was no significance difference between the nematode of Chauri and Himalayan Tahr ($P > 0.05$).

Chauri and Himalayan Tahr had only one cestode, *Moniezia* sp. *Moniezia* sp. was not significantly difference ($\chi^2 = 2.783$, $P = 0.095$ at d.f. 1).

The only trematode, *Paramphistomum* sp. had the prevalence of 26.67% and 12.50% in Chauri and Himalayan Tahr respectively. There was no significance difference of trematode between the two ruminants ($\chi^2 = 0.995$, $P = 0.394$ at d.f. 1).

4.6 Diameter of eggs/cysts of different gastrointestinal parasites of wild ruminants and Chauri

In the present study, the diameter of eggs/cysts of different gastrointestinal parasites were measured which is given below;

***Eimeria* sp.** : Diameter of oocyst of *Eimeria* sp. was 23 ± 9 μm without micropyle while 28 ± 11 μm with micropyle. Eggs are small in size, pink in colour and contain morula which is located centrally or sub-centrally filled up. Micropyle occurs in one side.

***Ascaris* sp.** : Eggs are 31 ± 8 μm in size, nearly spherical, yellowish brown, granular contents and unsegmented, thick aleveolated albuminous shell.

***Strongyloides* sp.** : Eggs are small, measure $51 \pm 10 \times 52 \pm 7$ μm in size, oval with rounded edges or ellipsoidal, thin shelled and contain fully developed larvae that can be seen under low power.

***Trichuris* sp.** : Eggs are 75 μm size, contains unsegmented embryo, brown in colour, barrel shaped with a transparent plug at either pole.

***Moniezia* sp.** : Eggs are triangular or quadriangular in shaped, somewhat irregular having a circular or pear shaped (pyriform) apparatus at one end and measure 53 ± 9 μm in size.

Strongyle egg : Eggs are thin shelled, broad ellipse, barrel shaped side walls, blastomeres present, number vary and measures 80 ± 15 μm in size.

***Paramphistomum* sp.** : Its egg is $90 \pm 5 \times 52 \pm 7$ μm in size, operculum in one pole, pale grey or greenish in colour, contains five blastomeres surrounded by about 50 yolk cells, morula located centrally or somewhat subcentrally.

Photographs of identified gastrointestinal parasites



Photo 11: Cyst of *Eimeria* without micropyle (23 μ m)



Photo 12: Cyst of *Eimeria* with micropyle (28 μ m)

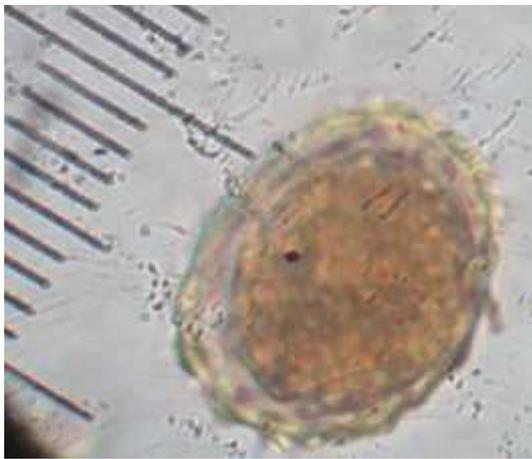


Photo 13: Corticated egg of *Ascaris* sp. (38 μ m)



Photo 14: Decorticated egg of *Ascaris* sp. (31 μ m)



Photo 15: Egg of strongyle (87 μ m)



Photo 16: Egg of strongyle (80 μ m)

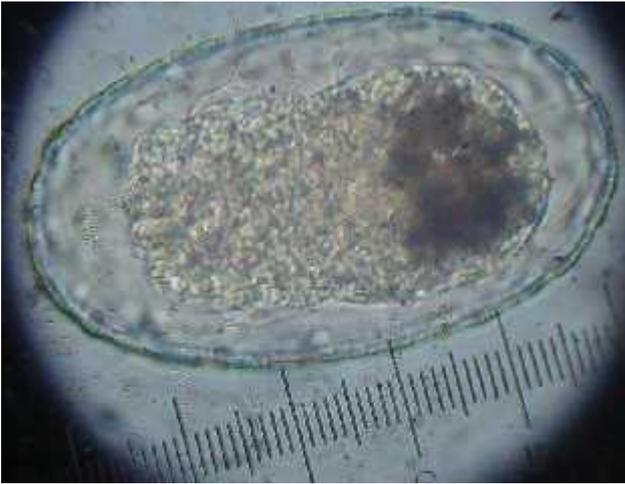


Photo 17: Egg of strongyle (85 μ m)



Photo 18: Egg of *Trichuris* sp. (75 μ m)



Photo 19: Larva of *Strongyloides* sp. (260 μ m)



Photo 20: Egg of *Strongyloides* sp. (51 μ m)

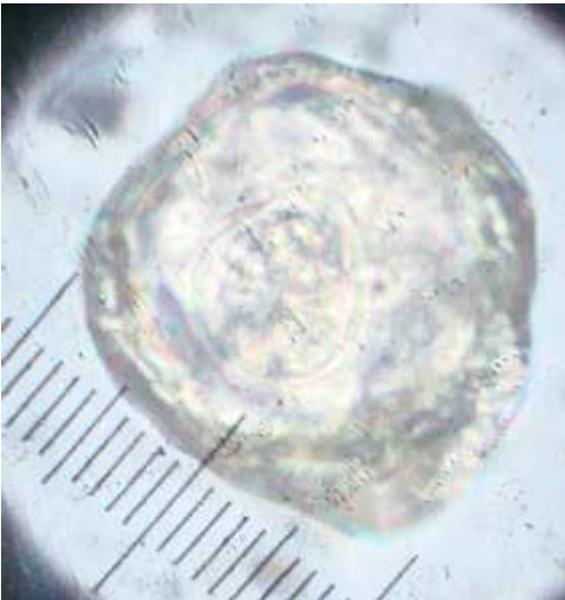


Photo 21: Egg of *Moniezia* sp. (59 μ m)

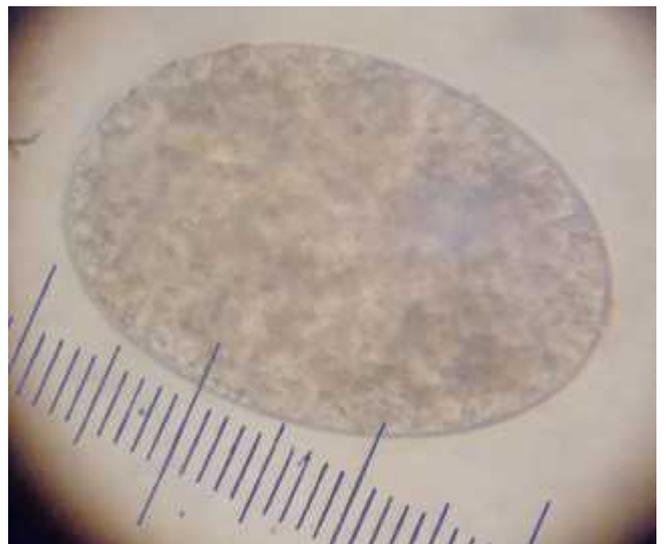


Photo 22: Egg of *Paramphistomum* sp. (89 μ m)

5. DISCUSSION

In the present study 31 fecal samples from Barking Deer, 9 from Musk Deer, 16 from Himalayan Tahr and 15 from Chauri were analyzed. Out of 71 samples, 61 (85.92%) samples were found positive for gastrointestinal parasites. Overall prevalence was almost similar with the findings in Mule Deer, Axis Deer, Yak and Chauri (Worley and Eustace 1972, Meshram *et al.* 2008, Byanju *et al.* 2011, Shrestha and Bindari 2013) and higher than the findings of Yak of Sikkim and Arunachal of India, different Deer species of Timisoara zoo, animals of Samsun zoo and herbivore of Dhaka zoo (Darabus *et al.* 2009, Gurley *et al.* 2010, Bandyopadhyay *et al.* 2010, Goswami *et al.* 2013, Rahman *et al.* 2014). The difference in the prevalence in different study was due to the different animal host, their age, the study area, condition of the study area and the resistance capacity of different animals with the parasitic infection.

The overall prevalence of parasites in chauri, Barking Deer, Musk Deer and Himalayan Tahr were 86.67%, 83.87%, 88.89% and 87.50% respectively. The prevalence in wild ruminants was found almost similar with the prevalence of Chauri because during the fecal sample collection it was found that the grazing land of wild ruminants was heavily over grazed and was contaminated by Chauri by their fecal matter. It was also found that the grazing land was shady with the high moisture that favours the survival of eggs or cysts of parasites.

Coccidiosis is one of the most common parasitic diseases of cattle in different parts of the world (Rahman *et al.* 2010). Wild ruminants and Chauri were found to be infected with *Eimeria* sp. as protozoan parasite. In this study *Eimeria* sp. was differentiated into two groups (*Eimeria* with micropyle and *Eimeria* without micropyle) on the basis of their morphological character (micropyle present or absent). *Eimeria* is a coccidian parasite which is a leading killer of small ruminants under 4 months of age and can cause significant economic losses in cattle (Harwood 2006). Himalayan Tahr has 81.25% and 75% and Barking Deer has 77.42% and 83.87% prevalence for *Eimeria* with micropyle and *Eimeria* without micropyle respectively. Musk Deer and Chauri has 77.78% and 86.67% of equal prevalence for both *Eimeria* with micropyle and *Eimeria* without micropyle respectively. *Eimeria* sp. was also recorded from Yak of Manaslu Conservation Area of Nepal (Byanju *et al.* 2011), Chauri of Ramechhap of Nepal

(Shrestha and Bindari 2013), Yak of Arunachal Pradesh and Sikkim of India (Bandyopadhyay *et al.* 2010, Goswami *et al.* 2013, Bam *et al.* 2012), Yak of Sichuan and Gansu Province of China (Hogg 2004, Yunfei *et al.* 2004), Himalayan Tahr and Barking Deer of Nepal (Thapa 2013), Blackbuck of Nepal (Ban 2012, Chaudhary 2014) and Sambar of India (Gupta *et al.* 2011).

Oocysts of *Eimeria* pass in the feces of Chauri 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 diarrhea, straining, loss of appetite, fever, debility (weakness) and even death (in severe cases) (Mass 2007). Occurrence of *Eimeria* sp. may be due to overcrowding of pastoral zones by other herbivore and presence of livestock and moist condition of pastoral zones because oocysts require moist condition to undergo sporulation.

Helminths occur in the wild as well as in domestic animals (Gupta 1997). In the present study, six species of helminth species belonging to nematode (4 species), cestode (1 species) and trematode (1 species) were recorded from wild ruminants and Chauri. The recorded parasites were *Ascaris* sp., strongyle, *Strongyloides* sp., *Trichuris* sp., *Moniezia* sp. and *Paramphistomum* sp. Many helminths of domestic ruminants are transmissible to wildlife and vice versa and transmitted by vectors or intermediate host and these may introduce an infection into areas previously free of that infection (Boomker *et al.* 1998).

In this study prevalence of *Ascaris* sp. was 80%, 80.65%, 88.89% and 87.50% in Chauri, Barking Deer, Musk Deer and Himalayan Tahr respectively. *Ascaris* sp. was also reported from Yak of Arunachal Pradesh and Darjeeling of India (RangoRao *et al.* 1994, Pradhan *et al.* 2011, Bam *et al.* 2012), Chauri of Ramechhap and Yak of Manaslu Conservation Area of Nepal (Byanju *et al.* 2011, Shrestha and Bindari 2013), Himalayan Tahr and Barking Deer of Rara National Park, Nepal (Thapa 2013) and Blackbuck of Bardia and Shuklaphanta, Nepal (Chaudhary 2014). In this study the prevalence of *Ascaris* sp. was found higher in wild ruminants than the Chauri. It indicates that *Ascaris* sp. may be transmitted from wild ruminants to Chauri or Chauri became resistant to *Ascaris* in some extent. It may be possible that transmission of *Ascaris* to wild ruminants was from the eggs which were shed by the Chauri or other animals long time ago. The

thick outer shell of *Ascaris* sp. insists them to be viable for longtime upto 15 years (Hagel and Giusti 2010) and cause diarrhea, malnutrition and obstruction of intestine.

Strongyloides sp. prevalence was 26.67%, 54.84%, 55.56% and 62.50% in Chauri, Barking Deer, Musk Deer and Himalayan Tahr respectively. In this case also prevalence of *Strongyloides* sp. was higher in wild ruminants than Chauri. This shows that the wild ruminants can be infected from other wild animals or from their own groups because of their gregarious behavior. Infection of *Strongyloides* sp. may be due to wet and muddy condition of pasturing land contaminated with fecal matter (Puthiyakunnon *et al.* 2014). Yak of Arunachal Pradesh, India (Bam *et al.* 2012), Himalayan Tahr of Rara National Park, Nepal (Thapa 2013), Blackbuck of Bardia and Shuklaphanta of Nepal (Chaudhary 2014), Blackbuck of Bardia, Nepal (Ban 2012), Axis Deer and Sambar of India (Meshram *et al.* 2008, Gupta *et al.* 2011) were also found infected with *Strongyloides* sp.

Chauri, Barking Deer, Musk Deer and Himalayan Tahr had the prevalence of *Trichuris* sp. as 53.33%, 45.16%, 66.67% and 56.25% respectively. Prevalence of *Trichuris* sp. was lower in Chauri than the most of the wild ruminants. This case also indicates that the transmission of parasites can be possible in either way i.e. from wild to domestic or from domestic to wild ruminants. It is also possible that the wild or domestic animals get infected with *Trichuris* sp. from the eggs which was shed by the infected animals a long time ago because eggs of *Trichuris* sp. can remain in the environment for upto 4 years (Peregrine *et al.* 2009). *Trichuris* sp. was recorded from Himalayan Tahr, Barking Deer, Blackbuck, Chauri and Yak of Nepal (Byanju *et al.* 2011, Ban 2012, Shrestha and Bindari 2013, Thapa 2013, Chaudhary 2014). Axis Deer of India and Hawaii (McKenzie and Davidson 1989, Meshram *et al.* 2008), Musk Deer of Russia (Kuznetsov *et al.* 2014, Maksimova *et al.* 2014), Yak and Sambar of India (Gupta *et al.* 2011, Bam *et al.* 2012) were also infected with *Trichuris* sp. Blackbuck, Axis Deer, Red Deer, White Tailed Deer, Fallow Deer, Mule Deer of different countries have *Trichuris* sp. in their gastrointestinal tract (Worley and Eustace 1972, Thornton *et al.* 1973, Sleeman 1983, Richardson and Demarais 1992, Farooq *et al.* 2012).

Stongyle nematodes representing 3 superfamilies: Ancylostomatidae, Strongyloidea and Trichostrongyloidea have been reported as parasites of the gastrointestinal system in wild bovids and cervids (Hoberg *et al.* 2001). Strongyle prevalence in Chauri, Barking Deer,

Musk Deer and Himalayan Tahr was found as 53.33%, 41.94%, 44.44% and 37.50% respectively. In this study the strongyle eggs were doubted as *Trichostrongylus* sp. and *Cooperia* sp. but they were not confirmed because most strongylid and trichostrongylid species are similar in appearance and overlapping in size. Therefore those eggs were considered as strongyle group. The present strongyle prevalence is almost similar with the findings of Hogg (2004) and Byanju *et al.* (2011) whereas lower than the findings of RangoRao *et al.* (1994) and Goswami *et al.* (2013). Strongyle was also recorded from Yak, Himalayan Tahr, Musk Deer, Blackbuck, Mule Deer, White Tailed Deer, Mountain goat, Red Deer, Fallow Deer, Roe Deer, Rein Deer, Sambar, Grey Brocket Deer and Axis Deer of different countries of the world (Worley and Eustace 1972, Thornton *et al.* 1973, Presteod *et al.* 1976, Sleeman 1983, McKenzie and Davison 1989, Hoberg *et al.* 2001, Divina *et al.* 2002, Duran *et al.* 2004, Irvine *et al.* 2006, Darabus *et al.* 2009, LuxHoppe *et al.* 2010, Gupta *et al.* 2011, Kowal *et al.* 2012, Goswami *et al.* 2013, Thapa 2013, Chaudhary 2014, Kuznetsov *et al.* 2014, Maksimova *et al.* 2014). *Trichostrongylus* sp., *Cooperia* sp., *Haemonchus* sp., *Nematodirus* sp., *Oesophagostomum* sp., *Oestertagia* sp. were recorded as a strongyle parasite by different researchers (Worley and Eustace 1972, Thornton *et al.* 1973, Richardson and Demarais 1992, Duran *et al.* 2004, Farooq *et al.* 2012, Kowal *et al.* 2012). Presence of strongyle may be due to over crowding and competition for food between wild ruminants and Chauri. Habitat overlap was found to have significant effect on strongyle abundance (Ezenwa 2002).

The cestode parasite identified in this study was *Moniezia* sp. Prevalence of *Moniezia* sp. was 26.67%, 51.61%, 44.44% and 56.25% in Chauri, Barking Deer, Musk Deer and Himalayan Tahr respectively. In this case also the prevalence of parasite was higher in wild ruminants compare to Chauri. Wild ruminants or other wild animals may harbouring the parasite and transmitting to other wild animals or even Chauri. May be Chauri are resistant to few parasite upto some level as they are the hybrid animals. RangoRao *et al.* (1994) and Chaudhary (2014) found lower prevalence of *Moniezia* sp. compare to this study. Barking Deer of Rara National Park and this study have almost similar prevalence of *Moniezia* sp. whereas Himalayan Tahr of Rara National Park has higher prevalence of *Moniezia* sp. than of this study (Thapa 2013). *Moniezia* infections are generally harmless and asymptomatic even when the tapeworms are present in large numbers (Elliott 1986). However, heavy infection may cause intestinal obstruction, diarrhea and weight loss. The

presence of *Moniezia* sp. in ruminants may be due to consumption of oribatid mite (with mature cysticercoids in it) while grazing (Slinitson 1931).

The prevalence of *Paramphistomum* sp. in Chauri, Barking Deer, Musk Deer and Himalayan Tahr was 26.67%, 38.71%, 44.44% and 12.50% respectively. *Paramphistomum* sp. being trematode have indirect life cycle and require intermediate host, fresh water snail. The prevalence of *Paramphistomum* sp. in each animal was lower 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). Yak and Sambar of India were also found infected with amphistome (RangoRao *et al.* 1994, Gupta *et al.* 2011). *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). 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).

In this study high prevalence of parasitic infection were observed in those parasites which have direct lifecycle, such as coccidian and gastrointestinal nematodes.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Wild ruminants and chauri of Langtang National Park were found infected with different gastrointestinal parasites. The overall prevalence of gastrointestinal parasite was 85.92% (61). The identified parasites were one protozoan (*Eimeria* sp.), one cestode (*Moniezia* sp.), one trematode (*Paramphistomum* sp.) and four nematode (*Ascaris* sp., strongyle, *Strongyloides* sp. and *Trichuris* sp.) based on the morphology of the eggs.

The parasites recorded in this study were similar in both wild ruminants and Chauri. Statistically it was found that there was no significance difference ($P > 0.05$) between the parasites of wild ruminants and Chauri. It was found that some parasites of wild ruminants had higher prevalence than Chauri and some parasites of wild ruminants had lower prevalence than Chauri. From this finding it can be assumed that the parasites can transmit in either way.

Transmission of parasites to wild ruminants was not only from the domestic animals, it may be transmitted from their own groups because of gregarious behavior or from other wild animals and from different vectors or intermediate host. In some extent the domestic animals including Chauri play one of the major role in transmitting the gastrointestinal parasites to wild ruminants because during the field work it was observed that the Chauri were grazing in the habitat or pasturing land of wild ruminants and contaminating their pasturing land (with food and water) by the fecal matter.

6.2 Recommendations

On the basis of the conclusion following recommendations are made to reduce the transmission risk of gastrointestinal parasites from domestic ruminants to the wild ruminants.

- Identification of parasites was done on the basis of their morphological character. To know the exact parasites upto species level molecular identification is necessary.
- Grazing of domestic animals in the habitat of wild animals must be controlled or

banned because gastrointestinal parasites can transmit from wild to domestic animals and vice versa.

- To minimize the risk of transmission of gastrointestinal parasites to wild animals also from chance domestic animals should be dewormed using anthelmintic in a regular basis.
- To know the status of gastrointestinal parasites of wild animals in a regular basis National park should establish veterinary laboratory and gastrointestinal parasites should not be neglected by the conservation biologist because they are one of the main threats for wild animals.
- Isolation and identification of parasites from wild ruminants should be done from postmortem of the dead animals.
- Seasonal study of parasitic prevalence of wild animals must be conducted by National Park or researcher to know the prevalence of parasites in a season wise pattern.

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