PREVALENCE OF GASTRO-INTESTINAL PARASITES IN RUMINANTS AT CENTRAL ZOO, KATHMANDU, NEPAL

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in
Zoology with special paper Parasitology.

Submitted to
Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
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Nepal
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DECLARATION

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

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RECOMMENDATION AND LETTER OF APPROVAL

This is to recommend and approve that the thesis entitled **"Prevalence of gastro-intestinal parasites in ruminants at Central Zoo, Kathmandu, Nepal"** has been carried out by **Tejab Pun** for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Tejab Pun** entitled **"Prevalence of gastro-intestinal parasites in ruminants at Central Zoo, Kathmandu, Nepal"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DoAH	Directorate of Animal Health
GI-parasite	Gastro-intestinal parasite
IUCN	International Union for Conservation of Nature
$K_2Cr_2O_7$	Potassium dichromate
MoAD	Ministry of Agriculture Development
NaCL	Sodium chloride
NPWC	National Park and Wildlife Conservation Act, Nepal
NTNC-Czoo	National Trust for Nature Conservation Central Zoo

ABSTRACT

The present study was done in Central Zoo of Nepal to find out the prevalence of gastro-intestinal parasites and basic management practice in present ruminant species. Zoo is the special place where wild animals are kept for exhibition which plays crucial role in recreation, relaxation, education, research and so on. Ruminants are herbivores with four chambered stomach. They are also get infected frequently by parasites. So, present study was conducted to find the parasitic infections in ruminants. A total of 104 fecal samples were collected from ruminants available at zoo in two different seasons' viz. winter (56) and summer (48) with the help of zookeeper. Fecal samples were preserved in 2.5% Potassium dichromate ($K_2Cr_2O_7$) in 25ml sterile vial and transported safely to laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal for microscopic observation. Concentration method (flotation and sedimentation) were employed to recover helminth eggs whereas iodine smear method was employed to recover protozoan cysts. Gastro-intestinal parasites were identified by microscopic examination. Results revealed overall prevalence of 59% parasitic infection whereas seasonal were 55% and 62% in winter and summer respectively. Only one protozoan, *Eimeria* sp. (*Eimeria* without micropyle (26%) and *Eimeria* with micropyle (15%)) was found and three helminth, nematode eggs were recorded with highly prevalent *Trichostrongylus* sp. (39%) followed by *Haemonchus* sp. (4%) and *Strongyloides* sp. (2%). No cestode and trematode were found during study period. Blue Sheep (*Pseudois nayaur),* Himalayan Goral (*Naemorhedus goral*) and Sambar Deer (*Rusa unicolor*) were found to be highly infected with intestinal parasites whereas Barking Deer (*Muntiacus muntjak)* showed lowest. Through interview and observation, management practice was found to be quite systematic but still care and deworming practice should be more effective.

1. INTRODUCTION

1.1Background

"Zoo" or "Zoological gardens" are those centers where various wild animals from different sources are kept for exhibition to public. Those animals play crucial role in aesthetic, education, entertainment, relaxation, research, cultural transportation, labor and preservation of endangered species. Although, zoo are sophisticated and well managed for wildlife as their natural habitat but they are not sufficient. So, captive animals are more susceptible to many diseases which cause morbidity and even mortality. One of the major causes for morbidity is parasitic infection, which can pose serious threat to endangered species (Muoria *et al.*, 2005). Fixed boundaries of zoo enclosures make captive animals more prone to different parasitic infections hence proper attention to feeding, water and maintenance of hygiene in captivity is required (Kashid *et al*., 2002).

1.1.1 Ruminants

Ruminants are the herbivores of suborder Rumantia having special four chambered stomach in which fermentation occurs by microbes for digestion of food. Among four compartments, rumen is the largest compartment in which partially chewed grass is stored and broken down into balls of cud. There are about 150 species of both wild and domestic ruminants such as buffalo, cattle, goat, sheep, tahr, deer, giraffe, goral, antelope etc. Here, present study includes ruminants of order Artiodactyla with four sub-family of two Families Bovidae and Cervidae.

1.1.1.2 Family: Cervidae; Sub-family: Cervinae (Deer)

The Sambar Deer (*Rusa unicolor*) is a large deer of Indian subcontinent, commonly known as Jarayo in Nepal. They are highly varied in mass and body length from west to east across distribution in decreasing order (Geist, 1998; Pocock, 1942). Males are larger in mass than females (Sankar and Acharya, 2004) with antlers on short pedicles (Brander 1923; Lydekker, 1915, 1898; Blanford, 1888; Jerdon, 1874). It occurs from sea level at various places in southeastern Asia to about 3,000 m in the Indian Himalayas (Green, 1985). Due to excessive hunting and habitat loss (Timmins *et al*., 2008), they are now rare in Bangladesh (Basbar *et al.*, 2001), Thailand (Ngampongsai, 1987), Laos (Timmins and Evans, 1996), and Vietnam (Khun and Kan, 1991). It is listed as vulnerable on the IUCN Red List since 2008.

The Swamp Deer (*Rucervus duvaucelii)* are commonly known as Barhasingha, the second largest deer found in the swampy grasslands and floodplains of Indian subcontinent. Swamp deer is placed on the appendix I on CITIES list and as a vulnerable species in IUCN Red List and also protected by the government of Nepal under National Parks and Wildlife Conservation Act 1973 (NPWC Act 1973). They are confined to swamp habitats of northern India and southern low land of Nepal (Ghimire, 1996; Groves, 1982; Schaaf, 1978).

The Spotted Deer (*Axis axis*) are the attractive, third lagrest deer with white spots on the skin and huge antlers. They are commonly known as Chital or Cheetal, species of south Asia, occurring in Nepal, India, Sri lanka and Bangladesh (Schaller, 1967; Pratar, 1934). It is listed as least concern on the IUCN Red List since 2008.

The Barking Deer (*Muntiacus vaginalis*) are distributed throughout south and south-east Asia, commonly known as Ratuwa Mirga. It is listed as least concerned species on the IUCN Red List. They are widely distributed from lowland to the high mountains and use a variety of habitats from dense forest (Pokharel and Chalise, 2010; Brodie and Brockelman, 2009; Barrete, 1977) to scrub grassland and thorny shrub land (Teng *et al*., 2004). Populations of these species are decreasing mainly because of habitat loss and fragmentation, and illegal hunting (Jnawali *et al.*, 2011; Timmins *et al.,* 2008; Mallon and Kingswood, 2001).

1.1.1.2 Family: Bovidae; Sub-family: Antilopinae

The Four-horned Antelope (*Tetracerus quadricornis*) are commonly known as Chousingha or Chauka, are small antelope found in India and Nepal. It has four horns, one pair of horns is located between the ears, and the other on the forehead and posterior horns are always longer than the anterior horns, which distinguish it from most other bovids. The four-horned antelope is diurnal and feeds on grasses, herbs, shrubs, foliage, flowers and fruits. It is listed as vulnerable on the IUCN Red List since 2008, placed in appendix III of CITES list and also protected by NPWC Act 1973.

The Black Buck (*Antilope cervicapra*) is commonly known as Krishnasar, gazelle like beautiful, medium sized animal native to Indian Subcontinent. The male have dark brown to black colour of the dorsal (upper) part of the coat which made it attractive. It is listed as near threatened on the IUCN Red List since 2008, placed in appendix III of CITES list and also protected by NPWC Act 1973. Their populations are isolated in Black Buck Conservation Area, Khairaphanta, Bardiya and Suklaphanta National Park, Kanchanpur in Nepal.

1.1.1.3 Family: Bovidae; Sub-family: Caprinae (Blue Sheep and Goral)

The Himalayan Goral (*Naemorhedus goral)* are goat like wild animals adapted for climbing on mountains. It is listed as near threatened on the IUCN Red List and place in appendix I of CITES. It is diurnal animal, most active in the early morning and late evening. They feed on grassy ridges and steep rocky slopes in grasses, leaves, twigs, fruits, and nuts. They are widely spread along the southern foothills of Himalayan Mountains in India, Pakistan and Nepal, through Sikkim, Bhutan, and Assam and into northernmost Burma and Thailand (Schaller, 1967; Hayman, 1961).

The Blue Sheep (*Pseudois nayaur*) are medium sized ungulates, commonly known as Naur or Na or Bharal, found in the high Himalayas of India, Nepal, Bhutan, Tibet, and Pakistan. Their diet consists of *Stippa* sp., *Ulmus pumila* and *Poa* sp. (Liu *et al.,* 2007). The bharal is a major prey of the endangered Snow leopard. It is listed as least concern on the IUCN Red List.

1.1.1.4 Family: Bovidae; Sub-family: Bovinae (Blue Bull and Wild Water Buffalo)

The Wild Water Buffalo (*Bubalus arnee*) are commonly known as Arna, native of Indian Subcontinent and Southeast Asia, found in only Koshi Tappu of Nepal. It is listed as endangered on the IUCN Red List since 1986, placed in appendix III of CITES and protected by NPWC Act 1973 by government of Nepal. It is mainly a grazer, consuming grass and leafy aquatic vegetation and feeds mainly in the morning and evenings, and in the midday heat it retreats to the shade or wallows in water or muddy pools, in which it is sometimes almost completely submerged with only its nostrils showing.

The Blue Bull (*Boselaphus tragocamelus*) are commonly known as Nilgai, largest antelope of Nepal, endemic to the Indian subcontinent. They prefer areas with short bushes, scattered trees in scrub forests and grassy plains. They are common in around agricultural lands rather than deep forest.

1.1.2 Gastro-intestinal Parasites

Gastro-intestinal parasites are the major problem in captive animals causing severe infection even up to mortality (Singh *et al*., 2006; Isaza *et al*., 1990). Nematode helminths are negatively important to conservation and health of wildlife (Hotez *et al*., 2008; Gillespie *et al*., 2006; Pedersen *et al*., 2005). Probably it is possible for helminths to coexist with or without any pathology (Ryan *et al*., 2012; Krief *et al*., 2010) however in some cases consequent harm may be more severe (Muller *et al*., 2010; Empike *et al*., 2007; Empike *et al*., 2002). Occurrence of gastro-intestinal parasites in animals housed in zoo might vary according to the type of husbandry practices, disease prophylaxis and treatment administrated. Intensive husbandry of animals produces conditions which facilitates to the spread of parasites. In general, captive animals do not show any signs and symptoms of parasitism as regular deworming practices are carried out in zoo (Parsani *et al*., 2001). Although, the frequent use of deworming with anti-helminthic drug often cause resistant strains to evolve. Mostly the nutritional status of zoological animals can also enhance or diminish their resistance to disease (Geraghty *et al*., 1982).

1.1.2.1 Protozoan Parasites

Protozoans are ubiquitous single celled organisms, mostly free living and harmless, but few are parasitic that can affect the body at a cellular level, causing problems especially in the circulatory, endocrine and gastrointestinal system. They get transmitted through ingestion of oocyst with contaminated food or water. Protozoan parasites that commonly found in intestinal tract of ruminants are *Giardia* sp., *Blastocystis* sp., *Balantidium* sp., *Entamoeba* sp. (Aviruppola *et al.*, 2016), *Eimeria* sp., (Pandey, 2017; Achhami, 2016; Chaudhary, 2014) etc.

1.1.2.2 Helminth Parasites

Helminths are multicellular organisms mostly endoparasites causing the intestinal infection (Morariu *et al.*, 2012).

1.1.2.2.1 Trematode parasites:

Trematodes are commonly known as a flukes which reside in the bile duct and intestine causing minor to severe damage to host. These species play a vital role in the degradation of health of domestic as well as wild animals. Intermediate host plays important role in completion of their life cycle such as snail, Cray fish etc. *Paramphostomum* sp. (Achhami, 2016), *Fasciola* sp. (Pandey, 2017; Chaudhary, 2014) etc. are common trematode parasites of ruminants.

1.1.2.2.2 Cestode parasites:

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

1.1.2.2.3 Nematode parasites:

Nematode parasites are commonly known as round worms which are most prevalent parasites among other. Important nematode parasites of the ruminants are strongyles (*Trichostrongyylus* sp., *Strongyliodes* sp. and *Strongylus* sp.), ascarids (*Ascaris* sp.), *Oesophagostomum* sp., *Nematodirus* sp. and hook worm (*Haemonchus* sp.) have the highest prevalence (Foreyt, 2001). *Trichostrongyylus* sp. and *Strongyliodes* sp. resides on small intestine feeding on mucus and sucking blood where as *Haemonchus* sp. on rumen. Normally nematodes are transmitted through contaminated food and water with feces containing eggs and larvae of parasite where as some species such as *Strongyliodes* sp. are transmitted by direct penetration by infective larvae or through oral route.

1.2 Objectives

1.2.1 General objective

To determine the prevalence of gastro-intestinal parasites in ruminants at Central Zoo, Kathmandu, Nepal

1.2.2 Specific objectives

1. To identify the gastro-intestinal parasites in ruminant species by morphological characters of eggs.
2. To compare the prevalence of gastro-intestinal parasites in ruminants.
3. To compare the concurrent infections of gastro-intestinal parasites in ruminants in winter and summer season.
4. To assess the basic management practices for ruminants at Central Zoo.

1.3 Rationale of the study

Unfortunately, there are only a few detailed and comprehensive studies on the prevalence of intestinal parasites that are carried out in zoological gardens. Few unpublished reports were found from Central Zoo of Nepal so it can be considered as baseline study of gastro-intestinal parasites and basic management practices in ruminants. This would be useful for zoo, researchers and concerned authorities for references, making plan and policies.

2. LITERATURE REVIEW

2.1 Global context

2.1.1 In Zoo or Zoological Gardens

A survey conducted by Mir *et al*. (2016) showed nematode parasites (strongyle, *Strongyloides* sp., *Trichuris* sp., ascarid and *Capillaria* sp.) where trematode and cestode were not detected in captive wild animals at Bir Moti Bagh Mini Zoo (Deer Park), Patiala, Punjab. In same year, Aviruppola *et al.* (2016) found *Trichuris* sp., *Strongyloides* sp., *Toxocara* sp., *Spirometra* sp., *Moniezia* sp., *Nematodirus* sp., *Giardia* sp., *Blastocystis* sp., *Balantidium* sp., *Entamoeba* sp., strongyle type eggs, hookworm, and coccidian oocyts in a cross sectional, coprological survey on gastrointestinal parasites of captive mammals in the Dehiwala National Zoological Gardens of Sri Lanka. Similar to these studies Rana *et al.* (2015) recorded *Paramphistomum cervi*, *Moniezia expansa*, *Moniezia benedeni*, *Strongyloides papillosus*, *Trichuris globulosa*, *Trichostrongylus* sp. and *Haemonchus contortus* in Hog Deer (*Axis porcinus*) at Jallo Wildlife Park, Lahore Safari Park and Lahore Zoo, Pakistan. In the newly established Federal University of Agriculture, Abeokuta Zoological garden (Adegbulu *et al.*, 2015), a cross-sectional study revealed seven species of gastrointestinal parasites which includes hookworm, *Trichuris* sp., *Ascaris* sp., *Enterobius* sp., *Trichostrongylus* sp., *Entamoeba* sp., and *Strongyloides* sp.

Thawait *et al*. (2014) showed the highest prevalence (100%) of GI parasites in Barking Deer followed by Blue Bull (85.71%), Sambar Deer (83.33%), Chausingha (80%), Spotted Deer (38%) and Black Buck (35%) which had single infection of *Ascaris* sp. and *Toxocara* sp. But an investigation carried in Rangpur Recreational Garden and Zoo at Rangpur in northern Bangladesh showed *Fasciola sp., Moniezia benedeni, Strongyloides sp., Dictyocaulus sp., stomach worm, Coccidia sp. and Balantidium coli* infection *in herbivores as* (Khatun *et al.*, 2014). Similar study carried by Egbetade *et al*. (2014) found *Trichuris* sp., strogyle sp., *Capallaria* sp., ascaridae, *Echinococcus* sp., *Isopspora* sp. and *Eimeria* sp. in different animals kept in Federal University of Agriculture Zoological Park, Abeokuta. In the same year Rahman *et al*. (2014) investigate the gastrointestinal parasites from 21 herbivores at Dhaka zoo which revealed *Balantidium coli, Paramphistomum* sp., *Fasciola* sp., stomach worm, *Ascaris* sp. and *Strongyloides* sp. as major parasites from Dhaka Zoo. Khan *et al*. (2014) identified protozoan (*Balantidium coli*, *Coccidius* sp.) and helminthes (Hook worm, *Trichuris* sp., *Spirometra* sp., *Strongeles* sp.) in Siddhartha Garden Zoo animals.

Maesano *et al*. (2014) reported gastrointestinal strongyles as a most prevalent parasite in ruminants where Coccidia (*Eimeria* sp.), *Trichuris* sp. and *Nematodirus* sp. were also recorded in the Warsaw Zoological Garden (Poland). In herbivores, protozoa (*Eimeria* sp.), trematode (*Dicrocelium lanceolatum*), and digestive and pulmonary nematodes were identified which showed prevalence of 54.2%. Furthermore necropsy of the Deer revealed

the presence of *Dictyocaulus filaria* in the lungs, *Ostertagia ostertagi* in the abomasum, and *Trichuris capreoli* in the caecum from seven Zoological Gardens located in Romania (Darabus *et al*., 2014). Radhy *et al.* (2013) investigated gastro-intestinal protozoa in feces of the animals of AL-Zawraa Zoo in Baghdad showed protozoan infection in all species of captive wild animals which contains *Entamoeba coli*, *Giardia* sp., *Sarcocysts* sp. and *Cryptosporidium* sp.

Nalubamba *et al.* (2012) recovered eggs of *Trichostrongylus* sp., *Haemonchus* sp., *Strongyloides* sp. and *Monezia* sp. by coproculture where larvae of the *Trichostrongylus* sp. were most predominant from captive wild Impala (*Aepyceros melampus melampus*) in a game facility south of Lusaka, Zambia. An investigation carried in Bangabandhu Sheikh Mujib Safari Park, Dulahazra, Cox's Bazar revealed *Fasciola* sp. and *Balantidium coli* parasites in Spotted Deer and *Balantidium coli* and stomach worm parasites in Sambar Deer (Hossian, 2012). Gastrointestinal parasites and their level of infection in the Arabian Oryx (*Oryx leucoryx*) were studied at King Khalid Wildlife Research Center in Thumamah, Riyadh Province, Saudi Arabia by Mohammed *et al.* (2012) which revealed *Eimeria saudiensis, Cryptosporidium* sp., *Nematodirus spathiger* and *Trichuris* sp. as the gastrointestinal parasites.

Ibrahim *et al.* (2012) in Sanda Kyarimi Park, Maiduguru, Nigeria, Africa found strongyle, *Trichostrongylus axei, Haemonchus contortus* and *Trichuris* sp. as common parasites from 36 samples of captive wild ruminants representing nine different species. An investigation was carried out in Bangladesh to study the prevalence of gastrointestinal helminthiasis in captive Deer which revealed 76.2% prevalence where *Paramphistomum* sp. & *Haemonchus* sp. were most prevalent parasites and Para or Hog Deer was most susceptible Deer species for helminthiasis followed by Barking Deer, Spotted Deer and Sambar Deer (Kanungo *et al.*, 2010). Overall prevalence of 36.4% was found with one cestode, one trematode and 12 nematode eggs or larvae among 184 animals including different mammals and birds at Samsun Zoo, Turkey (Gurley *et al.*, 2010). In the same year Opara *et al.* (2010) observed overall prevalence of 76.6% with nematodes (*Ascaris* sp., *Cooperia* sp., strongyles, *Trichuris* sp. and *Enterobius vermicularis*), trematodes (*Fasciola* sp., *Fasciolopsis buski, Schistosoma* sp.) and cestodes (*Taenia* sp.), while *Entamoeba* sp. and *Giardia lamblia* were the protozoa encountered at the zoological garden in Nekede, Owerri Southeast Nigeria. Also Fagiolini *et al.* (2010) examined fecal samples from mammals housed in two of the main Italian Zoological Gardens revealed 61.5% of overall prevalence among which 57.1% (*Trichuris* sp., *Toxocara vitulorum*, gastrointestinal strongyles and Paramphistomidae) of artiodactyls were infected with protozoa and helminthes parasites.

Gastrointestinal *Strongiloides* were the most common parasites in Red Deer, Fallow Deer and Moufflon with uneven *Paramphistomum* sp., *Trichostrongylus* sp., *Protostrongylus* sp.,

Dictyocaulus sp. *and Coccidia* sp. of parasites (Ruta *et al.*, 2009). Out of 60 samples of Deer from Maharajbag Zoo, Nagpur examined by Borghare *et al.* (2009) 30 were positive for eggs and larvae of helminthic parasites such as *Haemonchus* sp., *Dicrocoelium* sp., *Paramphistomum* sp., *Oesophagostomum* sp. *and Bunostomum* sp. A survey was undertaken to investigate the prevalence of intestinal parasites from different groups of mammals housed in a Zoological Garden in Malaysia by Lim *et al.* (2008) which discovered highest prevalence of hookworm followed by *Trichuris* sp. and *Cryptosporidium* sp. from hoofed animals. Corden *et al.* (2008) showed most frequent pathogenic endoparasites as *Eimeria* sp., *Trichuris* sp., *Strongyloides* sp., *Cyclospora* sp., *Cryptosporidium* sp. and *Isospora* sp. with most frequent cases of multiple parasitisms were *Eimeria* sp. and *Blastocystis* sp.

In the Mahendrda Choudhury Zoological Park, Chhatbir, Punjab Singh *et al.* (2006) recorded strongyle sp., *Trichuris* sp. and *Eimeria* sp. of gastrointestinal in wild herbivores. Goossens *et al.* (2005) recovered three genera and three species of nematodes at necropsy of one red deer and three Nelson's elk such as *Spiculopteragia spiculoptera, Trichostrongylus* sp., *Nematodirus filicollis, Capillaria* sp., *Oesophagostomum radiatum* and *Trichuris* sp. from two zoo ‘s of the Antwerp Zoo and the Animal Park Planckendael in Belgium. Kashid *et al.* (2003) analyzed faecal samples of various species of wild and zoo animals recovered amphistomes, strongyles, *Trichuris* sp., *Moniezia* sp., *Ascaridia galli, T. leonina, Raillietina tetragona, Paragonimus westermanii, Filaroides osleri, F. hirthi, Taenia taeniaeformis* and *Subulura* sp. of gastrointestinal helminths from six different locations of India. *Fasciola gigantica* was reported (Chakraborty, 2001) infection in 28 animals of different species, including Spotted Deer (*Axis axis*), Gayals (*Bos frontalis*) and Wild Water Buffalo (*Bubalus bubalis*) died at Assam State Zoo, Guwahati, India. In Rajkot Municipal Corporation zoo, Parasani *et al.* (2001) reported *Trichostrongylus* sp., *Strongyloid* sp. *Coccidia* sp. and *Balantadium coli* from herbivores among the infected mammals. Varadharajan and Kandasamy (2000) showed 58% of prevalence for helminth and 6% for protozoan of wild animals with strongyle, *Trichuris* sp., *Strongyloides* sp. and Coccidia as the major parasites in a Mini Zoo in Coimbatore, Tamil Nadu, India.

2.1.2 In Wild

Cossío-Bayugar *et al.* (2015) identified parasite genera *Eimeria, Haemonchus, Bunostonum, Cooperia*, and *Trichostrongylus* in cattle and Mule Deer (*Odocoileus hemionus*) and cattle (*Bos taurus*) in the Mapim Biosphere Reserve, Durango, Mexico where Fecal parasite counts increased during periods of higher precipitation and temperature. In Russia digestive tract of Far Eastern Musk Deer (*Moschus moschiferus turovi*) was analyzed by postmortem and found *Spiculopteragia spiculoptera, Nematodirus filicolis, Pygarginema skrjabini* in abomasum whereas *Trichuris* sp. in colon (Kuznetsov *et al.*, 2014). In the same year study carried on gastrointestinal parasites of Deer in Char Kukri Mukri upazilla of Bhola district of Bangladesh by Barmon *et al.* (2014) observed 69.29% of overall prevalence with *Fasciola*

sp. (8.66%), *Paramphistomum* sp. (20.47%), stomach worm (18.11%), hook worm (20.47%), *Strongyloides* sp. (1.57%), *Oesophagostomum* sp. (1.57%), *Eimeria* sp. (6.30%) and *Balantidium coli* (1.57%). In Red Deer (*Cervus elaphus*) wild isolated population in Nigeria, Davidson *et al.* (2014) found *Ostertagia leptospicularis*, *Spiculopteragia spiculoptera*, and *Trichostrongylus axei* in the abomasa examined and other were *Capillaria bovis*, *Cooperia oncophora*, *Oesophagostomum venulosum*, *Trichuris globulosa* and tapeworm fragments.

Swai *et al.* (2013) revealed *Trichostrongylus* sp., *Oesophagostomum* sp., *Strongylus* sp., *Bunostomum* sp., *Ostertegia* sp., *Toxocara* sp., *Fasciola* sp., *Paramphistomum* sp., *Gastrothylax* sp., *Ornithobilharzia* sp., *Fischoederius* sp., and Coccidia sp. of parasites in free ranging African Buffaloes in wildlife protected areas of Tanzania.

The study carried out to assess the prevalence of gastrointestinal helminths infections among wild and domestic ruminants in Cholistan desert of Pakistan revealed *Haemonchus* and *Trichostrongylus* as the most frequently genera followed by *Chabertia*, *Oesophagostomum*, *Schistosoma*, *Moniezia*, *Cooperia*, *Bunnostomum*, *Toxocara*, *Ostertagia*, *Nematodirus*, *Trichuris*, *Strongyliodes*, *Avitellina*, *Fasciola*, *Thelazia*, *Syngamus*, *Gaigeria*, *Skrjabinema*, *Cotylophoron*, *Metastrongylus* and *Gongylonema* as mixed or single species infections in different species of animals (Farooq *et al.*, 2012). From the study it was concluded that wild and domesticated ruminants of the Cholistan desert of Pakistan suffer with heavy infections of a variety of helminths including those of high economic significance.

The gastrointestinal tract investigated by Kowal *et al.* (2012) of Fallow Deer hunted in Southern Poland recorded *Ashworthius sidemi*, *Spiculopteragia* sp., *Nematodirus filicollis*, *Aonchotheca bovis*, *Oesophagostomum radiatum* as the major parasites. In the Notecka Forest region in the Wielkopolska Province, Poland gastrointestinal nematodes and trematodes (*Fasciola hepatica* and *Paramphistomum cervi*) were found in water buffaloes (Kobak and Pilarczyk, 2011). A survey in West Greenland caribou populations revealed 'strongyle-type', nematodirinae and anoplocephalidae, *Marshallagia* sp. eggs and *Eimeria* sp. ocyst (Steele *et al.*, 2011). Similarly Gupta *et al.* (2011) around Jabalpur, India showed higher prevalence in Sambar followed by Neelgai, Chital and Gaur where coccidian, strongyle, *Strongyloides* sp., *Trichuris* sp., *Toxocara* sp., *Moniezia* sp., *Amphistoma* sp. and *Fasciola* sp. found as common parasites. *Haemonchus* sp. was found in Grey Brocket Deer (*Mazama gouazaubira*) of Brazilian Pantanal wetlands (Lux Hoppe *et al.*, 2010).

Singh *et al.* (2009) studied gastrointestinal parasitism and body condition in free-ranging herbivores where Sambar was found highly infected followed by Chital and Nilgai with highest prevalence of Strogyles followed by *Strongyloides* sp., Cocidia sp., *Fasciola* sp., amphistomes and *Trichuris* sp. Axis Deer of scrub forest of Borgaon Manju in Western Vidabha region of Maharastra were positive for *Strongyloides* sp., strongyle, *Haemonchus*

sp., *Trichostrongylus* sp., *Trichuris* sp. and *Bunostomum* sp. with 89.05% of prevalence for parasites (Meshram *et al.,* 2008). Microscopic examination carried out on samples collected from forests of Tarai region in South- Uttaranchal reported 17.7%, 41.6%, 73.6% and 63.6% samples of Spotted Deer, Nilgai**,** Wild Pigs and Elephants, respectively by Banerjee *et al.* (2005) as positive for either single or mixed parasitic infections. Investigation of gastrointestinal parasites of Saiga Antelope (*Saiga tatarica*) grazing on livestock pasture by Morgan *et al.* (2005) found three species of cestode and 12 species of nematodes where most abundant species were *Marshallagia marshalli, Marshallagia mongolica, Nematodirus gazellae* and *Skrjabinema ovis* which were potentially enabling transmission of a wide range of parasitic helminthes between Saigas and domestic ruminants. A study was conducted on community land in southern Samburu by Muoria *et al*. (2005) in wild Grevy's zebra population which showed prevalence of *Trichostrongylus* sp., *Oesophagostomum* sp., *Haemonchus contortus*, *Parascaris* sp., *Nematodirus* sp., *Entamoemba coli*, *Balantidium coli*, *Entamoemba histolytica*, *Isospora belli* and *Dictyocanlus* sp. of parasites.

Wahed (2004) reported gastrointestinal nematode eggs including *Fasciola hepatica, Dicrocoelium lanceolatum, Moniezia benedeni* and *Moniezia expansa*, Coccidia, *Sarcosporidio* sp. the larvae of *Trichostrongylus colubriformis, Ostertagia ostertatgi* and *Oeseophagostomum* sp. *and Dictyocaulus* sp. from living Egyptian Deer (*Dorcas gazelles*) in Matrouh Governorate. In the same year Duran *et al.* (2004) examined abomasal parasites in cervids from Central Spain consists of 147 Red Deer (*Cervus elaphus*) and 17 Fallow Deer (*Dama dama*) which reported *Spiculopteragia quadrispiculata* first time from Red Deer whereas *Trichostrongylus axei* and *Ostertagia drozdzi* were also recorded. In wild herbivores highest prevalence was observed in bison followed by Chital Deer having common parasite Strongyles in Pench National Park, Maharashtra (Nighot *et al.,* 2004).

At Nagpur in axis deer, *Strongyloides* sp. was found most prevalent helminths parasites followed by mixed infection of strongyles and *Trichuris* sp., *Nematodirus* sp., *Haemonchus* sp. and *Oesophagostomum* sp. (Hussain *et al.,* 2002). *Dictyocaulus capreolus* was a species only identified from all the samples of lungs of Roe Deer whereas *D. eckerti* from Red Deer and Roe Deer were identified (Divina *et al.,* 2002). In the same year Booyse *et al.* (2002) identified *Telamodinium onyx, Megadinium aethiopicum* and *Teratodinium sphaereden* parasites from seventeen warthogs harvested from their natural habitat. Mandal *et al.* (2002) reported maximum infection of strongyles followed by amphistomes, *Fasciola* sp., *Strongyloides* sp. and *Ascaris* sp. in wild herbivores at Mudumalai Wildlife Sanctuary, Tamilnadu. Jayathangaraj *et al.* (2000) recorded the presence of *Bunostomum trigonocephalum* in Black Bucks having history of inappetance, weakness, persistent diarrhoea and anemia and having a rough coat in Chennai, Tamilnadu, India. Giraffe (*Giraffa camelopardalis angolensis*) were found infected with *Parabronema skrjabini*, *Skrjabinema*

sp., *Haemonchus mitchelli, Echinococcus* sp. by Krecek *et al.* (1990) in Etosha National Park, Namibia.

2.2 In 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).

Many works have been conducted in the threats of wild ruminants but in the disease transmission or parasitic disease there are only few work have been done. There were only few literature found on the gastrointestinal parasites of wild ruminants.

Pandey (2017) showed *Eimeria* sp., *Ascaris* sp. *Strongyle, Strongyloides* sp., *Trichuris* sp., *Moniezia* sp., *Paramphistomum* sp. and *Fasciola* sp. of parasites in Swamp Deer of Shuklaphata National Park, Nepal. In the same year in Koshi Tappu Wildlife Reserve, intestinal parasites of Wild Buffalo were investigated by Gupta (2017) where *Eimeria* sp., *Paramphistomum* sp., *Trichostrongylus* sp., *Haemonchus* sp., *Toxocara* sp., *Strongyloides* sp. and *Oxyuris* sp. were identified. Seven species of gastrointestinal parasites were identified by Achhami (2016) with one protozoan (*Eimeria* sp.), four nematodes (*Ascaris* sp., strongyle, *Strongyloides* sp. and *Trichuris* sp.), one cestode (*Moniezia* sp.) and one trematode (*Paramphistomum* sp.) where there were no significant difference in those parasites in wild ruminants (Musk Deer, Barking Deer and Tahr) and Chauris of Langtang National Park.

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 discovered parasites of Blackbuck by Chaudhary (2014) from Blackbuck Conservation Area, Bardia and Shuklaphanta Wildlife Reserve, Kanchanpur. Thapa (2013) conducted investigation in Rara National Park and found *Strongyloides* sp., *Trichostrongylus* sp., *Dictyocaulus* sp. and *Muelleuris* sp. from Himalayan Tahr and *Trichuris s*p. and *Dictyocaulus* sp. from Barking Deer whereas *Eimeria* sp., *Moniezia* sp., *Oxyuris* sp., *Ascaris* sp. and *Haemonchus* sp. were common parasites in both. Ban (2012) in Khairapur, Bardia reported strongyle, *Trichuris* sp., *Trichostrongylus* sp., *Paramphistomum* sp., *Fasciola* sp., coccidian, *Strongyloides* sp., *Moniezia* sp. and *Schistosoma* sp. as the major gastrointestinal parasites of Black Buck (*Antelope cervicapra*).

3. MATERIALS AND METHODS

3.1 Study area

Central Zoo (27˚40′ 23″ N 85˚18′ 39″ E / 27.6731849˚N 85.3109334˚E) is the only zoo of Nepal which is located at Jawalakhel, Lalitpur with an area of six hectares.

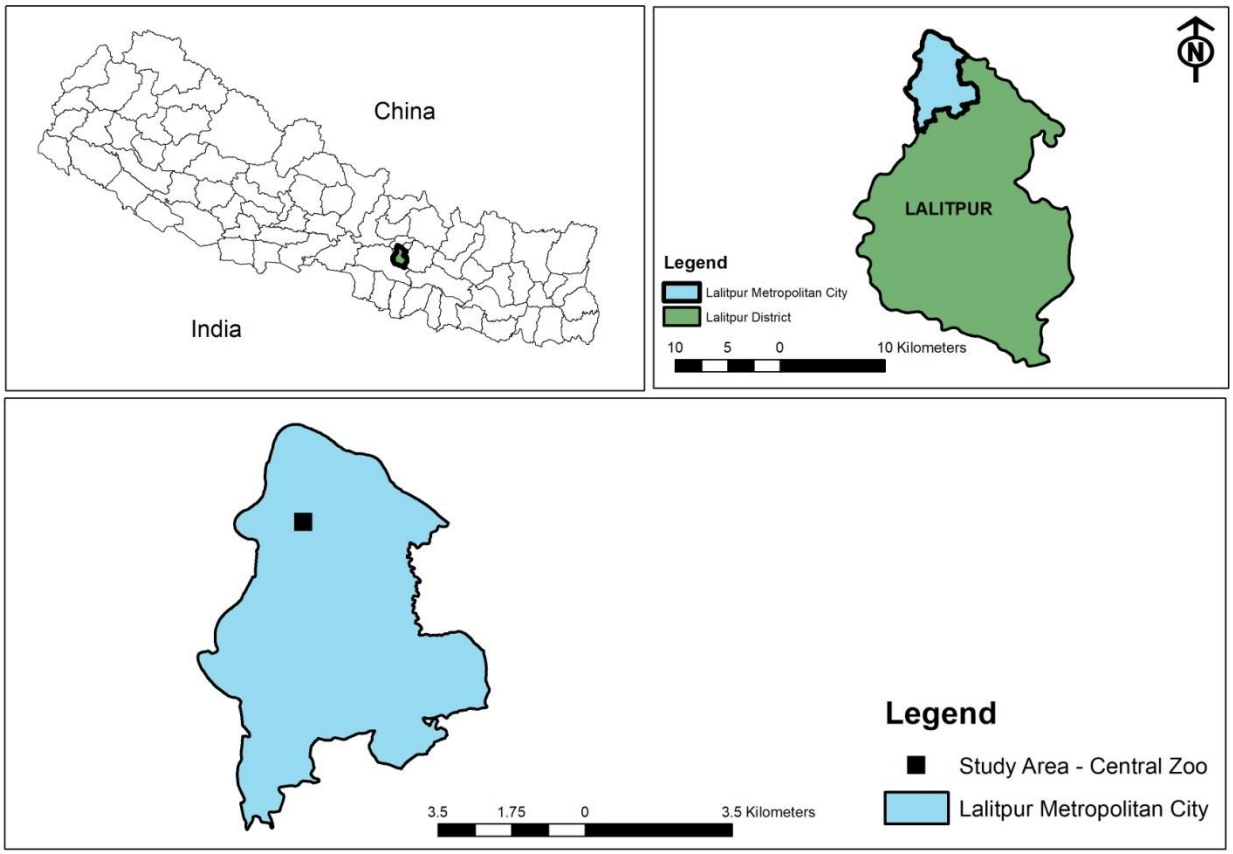

Figure 1: Map of Nepal showing location of Central Zoo, Nepal.

Zoo contains a total of 830 animals of 103 species including 10 species of ruminants including Wild Water Buffalo, Swamp Deer, Sambar Deer etc. (NTNC-Czoo, 2016). Ruminants are placed at left side of entrance gate. Almost each species are separately placed in different cells with complete information. Spotted Deer, Black Buck and Barking Deer are placed at first cell as they live together in herd in wild and have similar characteristics whereas Sambar Deer and Barking Deer are also placed in the same cell. Except these all species have separate compartments. Wild Water Buffalo are placed back of Assamese monkey and side of Simang cell.

3.2 Materials used

Gloves, Bamboo sticks, Sterile vials, Cool box, Refrigerator, Beaker, Glass rod, Mortar/pest, Measuring cylinder, Tea strainer, Centrifuge tube, Centrifuge Machine,

Dropper, Slides, Cover slips, Needle, Cotton, Stage micrometer, Ocular micrometer, Electric microscope etc. were used.

Chemicals used

2.5% of $K_2Cr_2O_7$, Distilled water, Saturated NaCl Sol^n, Methylene blue, Lugol's Iodine Sol^n etc. were used.

3.3 Methods

3.3.1 Sample collection/preservation

Samples were collected during December 2016 and March/April 2017 for winter and summer season respectively. With the help of zookeeper of the zoo, fecal samples were collected from each cell of different ruminant species and labeled. There were total 104 (56 + 48) fecal samples from 10 species of ruminants. About 20-25 gm fecal matter was placed in 20 ml sterile vial and 2.5% $K_2Cr_2O_7$ were added to cover the fecal sample completely. Then, vial was air tightly closed and put in cool box. The collected fecal samples were transported to laboratory at Central Department of Zoology, Tribhuvan University and kept in refrigerator at 4^Oc for further processing.

3.3.2 Lab process

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

3.3.3Flotation concentration and sedimentation concentration method (Dryden *et al.*, 2005)

3.3.3.1Flotation concentration method

Eggs of nematodes and cestodes are generally detected by this method as their eggs are comparatively lighter than trematodes. On saturated solution of sodium chloride they float. Approximately 3 gm of fecal sample was taken in a beaker and 20 ml of water was added. Then the sample was mixed or grinded with the help of mortar and filtered 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 was placed at top for a few minutes and then cover slip was removed and placed on a slide and examined at 10X and 40X of objective lens. Microphotographs of eggs were taken and identified based on eggs color, shape and size using identifying different keys.

3.3.3.2 Sedimentation concentration method

Saturated salt solution was removed gently from the test tube after examination of floated portion and sediment content was poured into watch glass and stirred the content gently to mix it. One drop from the mixture was taken to prepare a second slide. The specimen was stained with iodine wet mounts solution. Generally eggs of trematodes are by this method detected due to heavy and large size.

In this way two slides was prepared from one sample and examined under 10X and 40X of objective magnification of microscope to detect eggs of gastro-intestinal parasites.

3.3.3.3 Iodine smear method (Dryden *et al.*, 2005)

Protozoan eggs/cysts are generally detected by this method as Iodine makes it visible. A tooth pick was used to stir the fecal sample, a drop of Lugol's Iodine solution on a clean glass slide with emulsified a tooth pick head sample and then covered with a clean cover-slip. The smear was examined carefully under electric microscope at objective lens of 10X and 40X.

3.4 Egg size measurement

Size was measured with the help of calibrated micrometer in objective lens of 10X and 40X respectively.

3.5 Identification of eggs/cysts

By using books of Soulsby (1982), Foreyt (1989), and other published, unpublished articles and also from internet sources eggs were identified on the basis of morphological characters (shape and size).

3.6 Interview

Structured questions (Annex I) were asked to doctor, head zookeeper and animal caretakers for management practices of zoo.

3.7 Data analysis

Data was statistically analyzed with the help of statistical software R version 3.3.1. Prop-test was used to test significance and to compare.

4. RESULTS

Coprological investigation was carried out during Nov/Dec 2016 and Apr/May 2017 of ruminants at Central Zoo, Kathmandu, Nepal. A total of 104 fecal samples (Annex II) of all ruminant species (Annex III) were collected during the study period in which 59% were found to be infected with gastro-intestinal parasites. *Eimeria* sp. was the single protozoan parasite and *Haemonchus* sp., *Strongyloides* sp. and *Trichostrongylus* sp. were helminth parasites.

4.1 Identification of gastro-intestinal parasites

Morphological characters of eggs and cysts and their references were used to identify gastro-intestinal parasites (Annex IV).

Table 1: Identified eggs and cysts of GI-parasites

Name of parasites	**Range of length and diameter of eggs and cysts (in µm)**		**Morphology characters**	**Reference values (Soulsby, 1982; Foreyt, 2001)**
	length	**width**		
Eimeria sp.	15-35	-	Eggs were elongated oval shaped, having prominent cyst wall with micropyle and protoplasmic mass was spherical in centrally or sub-centrally.	13-44 µm
Haemonchus sp.	65-90	40-50	Eggs are oval, thin-shelled, and grayish in color.	70-90×40-55 µm,
Strongyloides sp.	65-70	25-30	Eggs are small, measure in size, oval with rounded edges or ellipsoidal, thin shelled and contain fully developed larvae that can be seen under low power.	51-65×20-30 µm
Trichostrongylus sp.	90-110	35-40	Irregular ellipse dissimilar, kidney-shaped not very wide poles, one of which was more rounded than the other, dissimilar side-walls.	70-90×35-50 µm

4.2 Comparison of overall prevalence of GI-parasites in ruminants

Out of total samples collected during winter and summer season, 59% samples were found to be infected. Statistically there was no significant difference (χ^2= 2.89, df = 1, p-value >0.05) in the prevalence of GI-parasites in different season.

During winter and summer season fecal samples were collected from different ruminant species. Out of which highest prevalence (62%) was found to be in summer season followed by winter season (55%). But statistically there was no significant difference between the prevalence of parasites in two season (t = -0.19642, df = 7.932, p-value >0.05).

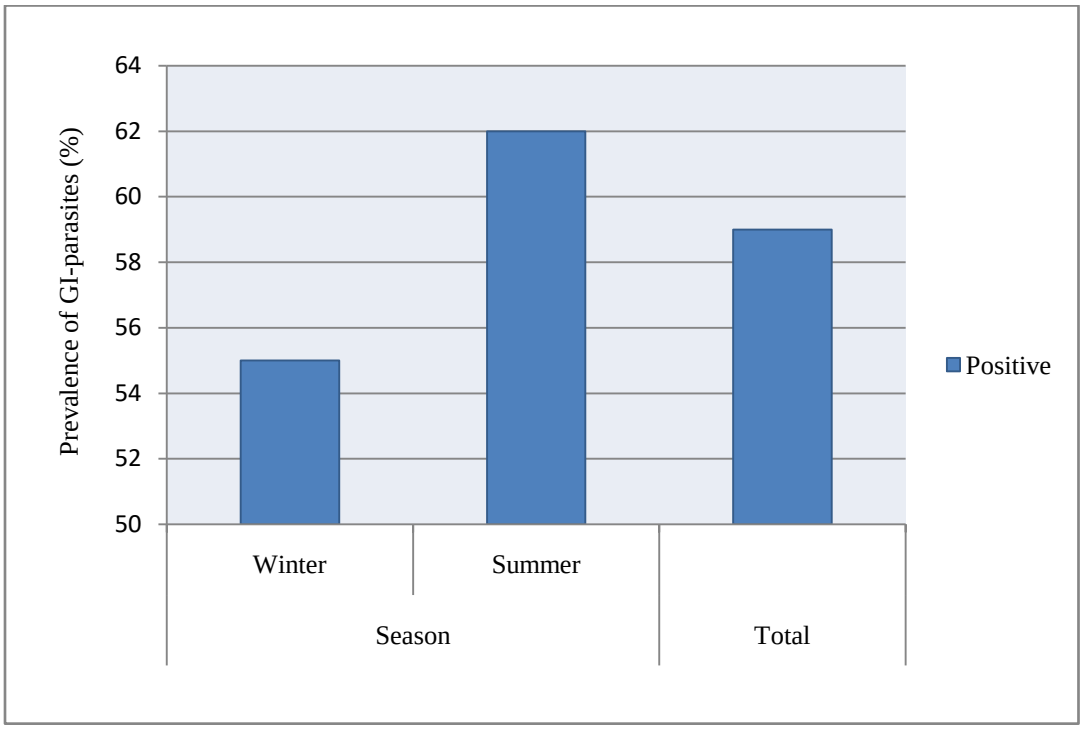

Figure 2: Comparison of overall prevalence of GI-parasites.

4.3 Comparison of ruminant-wise prevalence of GI-parasite in winter and summer season

Altogether 10 species of ruminants present in Central Zoo were sampled. They were Barking Deer (*M. vaginalis)*, Black Buck (*A. cervicapra)*, Blue Bull (*B. tragacamelus)*, Blue Sheep (*P. nayaur)*, Four-horned Antelope (*T. quadricornis)*, Himalayan Goral (*N. goral)*, Sambar Deer (*R. unicolor)*, Spotted Deer (*A. axis)*, Swamp Deer (*C. duvauceli)* and Wild Water Buffalo (*B. arnee)*. Among them Blue Sheep (*P. nayaur)*, Himalayan Goral (*N. goral)* and Sambar Deer (*R. unicolor)* showed the highest prevalence whereas Barking Deer (*M. vaginalis*) showed the lowest prevalence of GI-parasites.

Statistically there was no significant difference between the prevalence of GI-parasites in the different ruminant species in Central Zoo (t = -0.35385, df = 17.993, p-value >0.05).

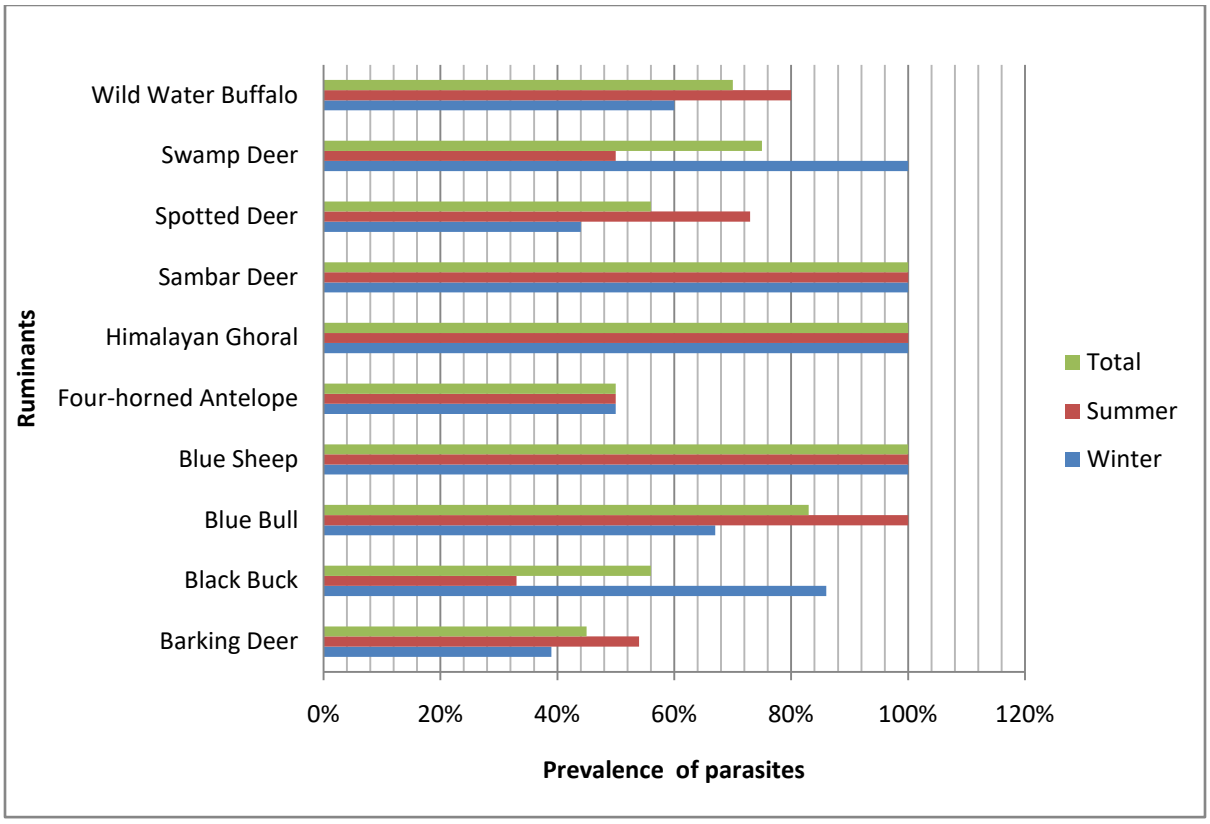

Figure 3: Comparison of ruminant-wise prevalence of GI parasites.

4.4 Comparison of parasites in winter and summer season

Altogether four genera of parasites were found. Helminths parasites (3 species) were found to be most prevalent than protozoan (1 species). No cestode and trematode parasites were found.

Table 2: Season-wise prevalence of parasites

SN.	GI-parasites	Prevalence (%)		
		Winter	Summer	Total
1.	*Eimeria* sp. without micropyle	25	27	26
2.	*Eimearia* sp. with micropyle	14	17	15
3.	*Haemonchus* sp.	4	4	4
4.	*Trichostrongylus* sp.	32	46	39
5.	*Strongyloides* sp.	-	4	2

Only single species of protozoa, *Eimeria* sp. was found with two different forms. Statistically there was no significant difference (χ^2= 0.068206, df = 1, p-value >0.05) in prevalence of protozoan parasite in two different season.

Among all the helminths parasites *Trichostrongylus* sp. had highest prevalence. Although summer season favored for helminth parasites in comparison but statistically there was no significant difference in two seasons (t = -0.22809, df = 3.9049, p-value >0.05).

4.5 Concurrency of GI-parasites in ruminant in winter and summer seasons

There was only single and double parasitic infections among the ruminant species studied. Statistically there was no significance difference in concurrency of parasitic infection (t = 0.16641, df = 1.7423, p-value >0.05).

Table 3: Showing multiple infections in winter and summer season

SN.	Hosts		Winter (%)		Summer (%)	
	Common names	**Scientific names**	**Single**	**Double**	**Single**	**Double**
1.	Barking Deer	*Muntiacus vaginalis* Boddaert, 1785	27	11.11	30.7	23.1
2.	Black Buck	*Antilope cervicapra* Linnaeus, 1758	57	28.6	22.22	11.11
3.	Blue Bull	*Boselaphus tragocamelus* Pallas, 1766	-	66.7	100	-
4.	Blue Sheep	*Pseudois nayaur* Hodgson, 1833	-	100	-	100
5.	Four-horned Antelope	*Tetracerus quadricornis* Blainville, 1816	50	-	50	-
6.	Himalayan Goral	*Naemorhedus goral* Hardwicke, 1825	50	-	-	100
7.	Sambar Deer	*Rusa unicolor* Kerr, 1792	50	-	100	-
8.	Spotted Deer	*Axis axis* Erxleben, 1777	43.7	-	54.5	-
9.	Swamp Deer	*Rucervus duvaucelii* Cuvier, 1823	100	-	-	50
10.	Wild Water Buffalo	*Bubalus arnee* Kerr, 1792	40	20	40	-

4.6 Results of the interview for basic management practices in Central Zoo

An interview with a doctor, head zookeepers and zookeepers revealed the following results of basic management practice in Central Zoo. There was regular monitoring of sanitation, health and half yearly deworming practice. Furthermore, in case of acute infection urgent

check up and medication was done in Zoo hospital within quarantine. Zookeepers were all professional as they have spent more than 20 years working in zoos. They were all trained, aware and capable about the proper management.

Table 4: Results of the Interview in Central Zoo, Jawalakhel, Kathmandu, Nepal.

SN.	Activities performed
A	**Health monitoring**
1.	Daily observation of health (signs and symptoms) of ruminants
2.	Rarely illness observed once a month that mainly occurs in rainy season
3.	If acute or chronic illness observed, treated in zoo hospital
B	**Deworming practice**
4.	Half yearly deworming practice
5.	Albendazole, Fenbendazole, Ivermectin, Tetracycline etc. were drugs used for deworming
C	**Feeding practice**
6.	Grams, beans, pulses, bran, cabbage, radish (for all ruminants) and straw (additional) for larger ruminants (Blue Bull and Wild Water Buffalo) grass were provided according to availability
7.	Fed according to animal requirements (twice or thrice)
D	**Hygiene and sanitation**
8.	Cleaning of ruminants cells (8-10 AM) everyday, using brooms and Kohrsolin as chemical disinfectant
9.	Drainage system was proper and pellet/dung and solid wastes were disposed to Municipal garbage collector
E	**Additional information**
10.	Postmortem of dead animals were done before burial
11.	Frequent trainings and awareness programs conducted for zookeepers

Observation by the researcher:

1. Daily visit of doctor and head zookeeper, treatment at zoo hospital, cleaning of cells, proper feeding etc. were observed as of interview.
2. Despite of all efforts for proper management, some drawbacks are needed to be findout.
3. Additionally, feral birds like Common Pigeon, House Crow, Common Myna, Oriental Magpie Robin, House Sparrow etc. and rodents were foraging inside the cell of ruminants.
4. Snails and slugs (intermediate hosts of cestode and trematode) were not observed inside the zoo periphery.
5. Sometimes visitors were seen feeding and teasing animals.

5. DISCUSSION

The prevalence of GI parasites has been reported from various zoos of Sri Lanka (Aviruppola *et al.*, 2016), India (Mir *et al*., 2016), Abeokuta (Adegbulu *et al.,* 2015), Poland (Maesano *et al*., 2014), Bangladesh (Rahman *et al*., 2014), Romania (Darabus *et al*., 2014), India (Thawait *et al*., 2014), Nigeria (Ibrahim *et al.,* 2012) etc. The present study reports baseline study of GI-parasites from Central zoo of Nepal. The overall prevalence of gastrointestinal parasites in the ruminants at Central zoo, showed 59% of parasitic infection. This is because of wild animals in captivity; increases chances of contamination which as a result becomes susceptible to infection. Also contiguity to humans (Wenz *et al*., 2009; Woodford *et al.*, 2002) may also endorse parasites in captivity to wildlife's. The present finding in respect to overall prevalence of parasites agreed with University of Ilorin Zoological Garden (62.9%) (Kolapo *et al.,* 2017); Bir Moti Bagh Mini Zoo, India (68%) (Mir *et al*., 2016); Dehiwala National Zoological Gardens, Sri Lanka (62.9%) (Aviruppola *et al.,* 2016); NandanVan Zoo, India (46.2%) (Thawait *et al*., 2014); Rangpur Recreational Garden and Zoo, Bangladesh (60%) (Khatun *et al.,* 2014); Federal University of Agriculture Zoological Park, Abeokuta (Egbetade *et al*., 2014); Warsaw Zoological Garden, Poland (48%) (Maesano *et al*., 2014); seven Zoological Gardens of Romania (54.2%) (Darabus *et al*., 2014); AL-Zawraa Zoo, Baghdad (63%) (Radhy *et al.,* 2013), but disagreed with Siddhartha Zoological Garden, Maharastra, India (15.84%) (Khan *et al.,* 2014) having lowest prevalence and higher prevalence in Jallo Wildlife Park and Lahore Zoo respectively, Pakistan (88% & 77.5% respectively) (Rana *et al.,* 2015); Dhaka Zoo, Bangladesh (76.9%) (Rahman *et al*., 2014); Shiddartha Municipal Zoo, Aurangabad (77.78%) (Ghoke *et al*., 2012); Dhaka Zoo, Chittagong Zoo and Dulahazar Safari Park, Bangladesh (76.2%) (Kanungo *et al.,* 2010); Zoological Garden, Owerri Southeast Nigeria (76.6%) (Opara *et al.,* 2010); Italian Zoological Gardens (77.1%) (Fagiolini *et al.,* 2010) respectively.

Results indicated that helminth infections were more common than protozoan infection in ruminants although there is only slight difference. In this study the prevalence of helminth infection (44.23%) was found higher than protozoan infection (41.34%). Aviruppola *et al.* (2016) reported much higher helminth infection (81.8%) than protozoan infection (47.7%). Khatun *et al*. (2014), Rahman *et al*. (2014), Hossain (2012) and Opara *et al*. (2010) also had similar results in Rangpur Recreational Garden and Zoo, Bangladesh, Dhaka National Zoological Garden of Bangladesh, Bangabandhu Sheikh Mujib Safari Park, Bangladesh and Zoological Garden, Nigeria. Fagiolini *et al*. (2010) reported similar fact where helminth had higher prevalence than protozoan from Italian zoo. This was more or less similar with the report of Parasani *et al.* (2001) who revealed that 50% animal were positive for helminth infections and 18.8% with protozoa in Rajkot Municipal Corporation zoo. Lim *et al.* (2008) reported 34.5% positive with helminth and 21.8% positive with protozoan infections which were much lower than the present study. Varadharajan and Kandasamy (2000) and

Varadharajan and Pythal (1999) showed 58% and 97.5% helminth infection whereas 6% and 3% protozoan infection respectively which further supports present finding. However, Cordon *et al.* (2008), Levecke *et al*. (2007) & Gomez *et al*. (1996) reported higher protozoan infection compared to helminth infection which is against several findings and present result. This may be happened due to local climatic conditions, method of sample collection and use of anthelmintic in captive animal.

Helminthiasis, soil transmitted helminths (STH) are especially infection with soil in which ova gathered in the surrounding environment, particularly in open soil enclosures, which couldn't be completely disinfected constitute a major and huge problem to wild animals in fixed enclosure (Elena *et al*., 2011). Nematode parasites are the most important helminth of Veterinary importance which had been incriminated as gastrointestinal tract (Singh *et al.,* 2006) causing serious disease condition in animals. They impart negatively on the conservation ecology and health of wildlife (Hotez *et al*., 2008; Gillespie, 2006; Pedersen *et al.,* 2005). Although coexistence of helminths is also possible with wildlife to exist with obvious pathology (Krief *et al.,* 2010; Ryan *et al.,* 2012) however in some instances, it may be more severe (Emikpe *et al.,* 2002; Emikpe *et al.,* 2007; Muller *et al.,* 2010). Other intestinal parasites (cestode and trematode) need an intermediate host and are less likely to gather in a fixed enclosed environment, because their intermediate host might not occur in the captivity (Lalošević, 2007). No cestode and trematode was found in present study period but nematode with higher prevalence i.e. 44.23% indeed. Three species of nematode were recorded from ruminant species viz. *Haemonchus* sp. (4%), *Strongyloides* sp. (2%) and *Trichostrongylus* sp. (39%).

Haemonchus sp. was previously recorded by Rana *et al*. (2015), Pencheva (2013), Borkovoka *et al*. (2013), Kanungo *et al.* (2010), Borghare *et al.* (2009) etc. from different part of zoo or zoological gardens/parks of the globe. *H. contortus* was found in large numbers in Goral (*N. goral*) whereas Sambar Deer (*R. unicolor*) in fewer number (Rana *et al*., 2015). *Haemonchus* sp. was common in Barking Deer (*M. vaginalis)*, Spotted Deer (*A. axis*) and Sambar Deer (*R. unicolor*) in zoos of Bangladesh (Kanungo *et al.,* 2010; Borghare et al., 2009) and highly prevalent in Barking Deer (*M. vaginalis)*.

Strongyloides sp. was identified in Ilorin Zoological Garden, Nigeria (Kolapo *et al.,* 2017), Bir Moti Bagh mini zoo, Punjab, India (Mir *et al.,* 2016), Dehiwala National Zoological Gardens, Sri Lanka (Aviruppola *et al.,* 2016), Federal University of Agriculture Abeokuta Zoological Park, Ogun State, Nigeria (Adegbulu *et al.,* 2015), seven Zoological Gardens In Romania (Darabus *et al.,* 2014), Dhaka National Zoological Garden of Bangladesh (Rahman *et al.,* 2014), Rangpur Recreational Garden and Zoo, Bangladesh (Khatun *et al.,* 2014), Zoological Garden in Skopje, Macedonia (Atanaskova *et al.,* 2011), two Italian Zoological Gardens (Fagiolini *et al.,* 2010), various Zoos of Bangladesh (Kanungo *et al.,* 2010), V.O.C

Park and Mini Zoo, Coimbatore (Varadharajan and Kandasamy, 2000) and The Zoological Garden, Thiruvanthanpuram, Kerala (Varadharajan and Pythal, 1999). *Strongyloides* sp. was common in Barking Deer (*M. vaginalis),* Spotted Deer (*A. axis*) and Sambar Deer (*R. unicolor*) in zoos of Bangladesh (Kanungo *et al.,* 2010).

Trichostrongylus sp. is one of the most important intestinal parasites of ruminant which are recorded time and again from Federal University of Agriculture Abeokuta Zoological Park, Ogun State, Nigeria (Adegbulu *et al.,* 2015), Jallo Wildlife Park and Lahore Zoo, Punjab, Pakistan (Rana *et al.,* 2015), seven Zoological Gardens of Romania (Darabus *et al*., 2014), Zoological Garden in Skopje, Macedonia **(**Atanaskova *et al.,* 2011), various Zoos of Bangladesh of Bangladesh (Kanungo *et al.,* 2010) and many more other Zoos and free ranging ruminants. *Trichostrongylus* sp. was highly prevalent in Sambar (*R. uniclor*) than Goral (*N. goral*) (Rana *et al.,* 2015). In this present study it is highly prevalent among ruminant.

Ruminants were found to be infected with *Eimeria* sp. (41.34%) as a protozoan parasite. In this present study *Eimeria* sp. was differentiated into two groups (*Eimeria* with micropyle (15%) and *Eimeria* without micropyle (26%)) on the basis of their morphological character (presence or absence of micropyle). Higher prevalence of *Eimeria* sp. (protozoan parasite) may be due to direct lifecycle pattern, humid climatic condition and open soil enclosure. Darabus *et al.* (2014) also reported *Eimeria* sp. from seven Zoological Gardens in Romania. Similar findings were reported earlier from the Zoological Garden in Skopje, Macedonia **(**Atanaskova *et al.,* 2011), captive wild animals of Kerala (Ravindran *et al.,* 2011), two Italian Zoological Gardens (Fagiolini *et al.,* 2010) and Mahendra Choudhary Zoological Park Chhatibhir, Punjab (Singh *et al.,* 2006).

Concurrency of infection with different intestinal parasites was only recorded in single and double occurrence. Blue Sheep (*P. nayaur)* (100%) had higher concurrency followed by Himalayan Goral (*N. goral*) (50%), Blue bull (*B. tragacamelus)* (33.3%), Swamp Deer (*C. duvauceli)* (25%), Black Buck (*A. cervicapra)* (19.85%), Barking Deer (*M. vaginalis)* (17.1%) and Wild Water Buffalo (*B. arnee)* (10%). Singly, hosts were infected with *Eimeria* sp. and *Trichostrongylus* sp. whereas double with *Eimeria* sp.+ *Trichostrongylus* sp., *Eimeria* sp.+ *Strongyloides* sp. and *Eimeria* sp.+ *Haemonchus* sp. Mir *et al.* (2016), Aviruppola *et al.* (2016), Rahman *et al*. (2014) and Varadharajan and Pythal (1999) found 48%, 25%, 42.3%.and 34% mixed infection respectively from various zoological gardens. Mixed infection was noted in majority of the deer (Kanungo *et al.,* 2010) and herbivore (Singh *et al*., 2006). The highest prevalence of mixed infection was observed in Nilgai (75%) (Mir *et al.,* 2016). Darabus *et al*. (2014) showed multiple infections with *Eimeria* sp. and *Trichostrongylus* sp. other intestinal parasites in deer from seven Zoological Gardens of Romania. These reports of mixed infections might be due to presence of host animals of all

age groups in the same shell, the feeding management and improper disposal of feces. So on similar to this study.

Blue Sheep (*P. nayaur)*, Himalayan Goral (*N. goral*) and Sambar Deer (*R. unicolor*) were found to be highly infected with intestinal parasites among 10 ruminants of Central Zoo whereas Barking Deer (*M. vaginalis)* showed lowest. Rana *et al.* (2015) identified *Trichostrongylus* spp. *and Gaigeria pachyscelis* in large numbers in Sambar (*R. unicolor*) where *G. pachyscelis*, *H. contortus* and *B. trigonocephalum* were recorded in Goral (*N. goral).* However in present study only *Trichostrongylus* sp. were recorded from Blue Sheep (*P. nayaur)*, Blue Bull (*B. tragacamelus)*, Swamp Deer (*C. duvauceli)*, Sambar Deer (*R. unicolor*), Spotted Deer (*A. axis)*, Black Buck (*A. cervicapra)*, Barking Deer (*M. vaginalis)*, Four-horned Antelope (*T. quadricornis),* and Wild Water Buffalo (*B. arnee)* and *Haemonchus* sp. from Himalayan Goral (*N. goral*). Borghare *et al.* (2009) reported *Haemonchus* sp. in deer from Nagpur and was further more in Barking Deer (*M. vaginalis)* in Bangladesh (Kanungo *et al.,* 2010). Kanungo *et al.* (2010) recorded multiple infections of *Haemonchus* sp., *Strongyloides* sp. and *Trichostrongylus* sp. from Spotted Deer (*A. axis)*, Barking Deer (*M. vaginalis)*, Sambar Deer (*R. unicolor*) and Para Deer. *Strongyloides* sp. in Sambar Deer (*R. unicolor*) (Aviruppola *et al.*, 2016), Black Buck (*A. cervicapra)* and Barking Deer (*M. vaginalis)* (Mir *et al.*, 2016), Spotted Deer (Khatun *et al.*, 2014) were recorded. Ravindran *et al.* (2011) recorded *Eimeria* sp. in Sambar Deer (*R. unicolor*) from Kerala.

Rana *et al.* (2015) and Gurley *et al*. (2010) reported higher prevalence of parasitic infection in winter rather than summer but highly prevalent in rainy season. The study period was only confined to winter and summer season which was against the finding of Rana *et al.* (2015) and Gurley *et al*. (2010) but there was no statistically significant difference between two seasons. This might be due to average temperature of zoo all around the year and humid environment (Maharjan and Regmi, 2014).

It has been observed that fixed enclosure of wild animals in zoo makes them more prone to different parasitic infections despite proper attention to feeding, water, and maintenance of hygiene which was supported by Kashid *et al.* (2002). Direct life cycle of nematodes and some coccidian parasites made easy for transmission (without intermediate host) by auto and feco-oral route through contaminated feed, water, and soil and can accumulate in captive environment (Thawait *et al.,* 2014). Contaminated water or fodder, and even carelessness of zoo workers have key role in transmission by acting as transmitter and transmitting parasites through their shoes, clothes, hands, food, or with working tools (Adetunji, 2014; Otegbade and Morenikeji, 2014; Dakshinkar *et al.,* 1983). Foraging wild birds, mouse and cats inside the captive fixed enclosure might be reason for transmission of intestinal parasites. In addition there is possibility that zoo keepers cleaning the cages and enclosures could act as a

vehicle (fomite) for the transmission of parasites as the pathogens might be present in the zoo environs. Visitors were not allowed to feed any animals at any circumstance but unseemly animals were feed and teased which in result increases stress and susceptibility to parasites increased.

Although regular deworming was carried out in every six months of year, satisfied results was not obtained. This study highlighted the importance of coprological analysis before administering deworming. Possibility of re-infection might occur through contaminated environment of the animals and feeding habits of the animals.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Ruminant species at Central Zoo of Nepal were found to be infected with both protozoan and helminth parasites. Overall prevalence of gastro-intesinal parasites was found to be 59% whereas seasonal were 55% and 62% in winter and summer respectively. Only one protozoan, *Eimeria* sp. (*Eimeria* without micropyle (26%) and *Eimeria* with micropyle (15%)) was found and three species of nematode eggs were recorded with highly prevalent *Trichostrongylus* sp. (39%) followed by *Haemonchus* sp. (4%) and *Strongyloides* sp. (2%). No cestode and trematode were found during study period. This might be due to isolation to intermediate host required to complete lifecycle for cestode and trematode.

Blue Sheep (*P. nayaur)*, Himalayan Goral (*N. goral*) and Sambar Deer (*R. unicolor*) were found to be highly infected with intestinal parasites whereas Barking Deer (*M. vaginalis)* showed the lowest. Blue Sheep (*P. nayaur)* (100%) has the higher concurrency followed by Himalayan Goral (*N. goral*) (50%), Blue bull (*B. tragacamelus)* (33.3%), Swamp Deer (*C. duvauceli)* (25%), Black buck (*A. cervicapra)* (19.85%), Barking Deer (*M. vaginalis)* (17.1%) and Wild Water Buffalo (*B. arnee)* (10%).

The present study confirmed that ruminants are susceptible and infected by various GI parasites. The management practices of the Central Zoo, Kathmandu, Nepal are good but still needs improvements because the ruminants were found infected by GI parasites. This study can be considered as baseline documentation on the gastro-intestinal parasites. It will be helpful while designing control strategies of gastro-intestinal parasites in ruminants and also be useful to other animals kept at zoo.

6.2 Recommendations

Based upon the above conclusion, following are recommendations suggested:

- Deworming practice needs to be improved to minimize the gastro-intestinal parasites of ruminants.
- Foraging birds and rodents need to be prevented to enter the cell of ruminants as they help in transmission of the parasitic infection in the animals at zoo as well as outside the zoo.

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ANNEXES

ANNEX I

Interview for prevalence of gastro-intestinal parasites of ruminants at Central zoo, Kathmandu, Nepal

1. Name:…….. Age:…… Sex:……
2. Designation:……. Service year:…….
3. Educational status:……
4. Is regular health monitoring of ruminants done?
   (A) Yes (B) No (C) Others……
5. How frequent is illness seen in ruminants?
   (A) Weekly (B) Monthly (C) Others……..
6. Are the ruminants dewormed regularly? If yes at what interval of time?
   (A) Yes (B) No ……
7. Which drug do you use to deworm them?
   ……..
8. What are the feeds of ruminants?
   ……..
9. Are the feed species specific?
   (A) Yes (B) No (C) Do not know (D) Others…….
10. What about natural fodder?
    (A) Yes (B) No (C) Do not know
11. How many times per day they are fed up? If what interval?
    (A) Once (B) Twice (C) Thrice …….
12. Are the ruminants cells cleaned every day? If at what time?
    (A) Yes (B) No …….
13. Did they use chemical disinfectant during cleaning?
    (A) Yes (B) No
14. Where did pellet and other waste are disposed?
    …….
15. Is there proper management of drainage?
    (A) Yes (B) No
16. What is done to ruminants if died?
    (A) Buried (B) Postmortem (C) Others…….
17. What about the training and awareness programs?
    ……..……

ANNEX II

1. Observation of pellets

Pellets were identified based on direct observation of defecation and following morphological characters.

1. Pellet of Barking Deer
    - Slender in shape but sometime pointed at one end; one side is bulged and other side depressed
    - Black in color
    - Size 7-9mm in length, 3-4mm in diameter
2. Pellet of Black Buck (Jayson, 1987)
    -Pointed at end, bulges at middle, sometimes both end are pointed
    -grey or dark grey/greenish in color
    -Size 8-10mm in length, 6-7mm in diameter
3. Pellet of Blue Bull
    -Direct observation of defecation
    -Slender, elongated, slightly pointed at one end and tapered at another end
    -Light green or dark green/brown/grey in color
    - Size 15-17mm in length, 8-10mm in diameter
4. Pellet of Blue Sheep
    -Direct observation of defecation
    -Slender, sharply pointed at a end and blunt at another end
    -Dark grey/brown in color
    - Size 10-12mm in length, 5-6mm in diameter
5. Pellet of Four-horned Antelope
    -Direct observation of defecation
    -Slender, somewhat pointed at end and another end round
    -Size 8-10mm in length, 3-5mm in diameter
6. Pellet of Himalayan Goral
    -Direct observation of defecation
    -More or less slender, pointed at a end and tapered at another end
    -Dark brown or grayish in color
    -Size 8-10mm in length, 4-5mm in diameter
7. Pellet of Sambar Deer (Jayson, 1987)
    -Nearly oval to spherical in shape, sometimes with a little pointed one end and sometimes may be slender with a little pointed at a end
    -Black in colour
    -Size 15-17mm in length, 12-13mm in diameter
8. Pellet of Spotted Deer (Jayson, 1987)
    -Slender in shape with one end somewhat pointed and other side depressed
    -Black in color, sometime greenish and when feed with cereals no balls

-Size 16mm in length, 8.5-10mm in diameter

9. Pellet of Swamp Deer
    -Direct observation of defecation
    -Nearly oval to spherical in shape, sometimes with a little pointed one end and bulge at another end
    -Black/ dark grey/green in color
    -Size 15-17mm in length, 11-13mm in diameter
10. Dung of Wild Water Buffalo
    -Spherical or round or semicircle
    -large quantity at a time depends up on age of animal
    -Greenish or dark green or dark brown color depending up on fodder

2. Pellets of ruminant species

Photo 1: Barking Deer

Photo 2: Black Buck

Photo 3: Blue Bull

Photo 4: Blue Sheep

Photo 5: Four-horned Antelope

Photo 6: Himalayan Goral

Photo 7: Spotted Deer

Photo 8: Swamp Deer

Photo 9: Sambar Deer

Photo10: Wild Water Buffalo

ANNEX III

Some photographs of ruminants and thesis work

Photo 11: Diagrammatic Zoo

Photo 12: Barking Deer (Male)

Photo 13: Black Buck (Male)

Photo 14: Blue Bull

Photo 15: Blue Sheep

Photo 16: Four-horned Antelope

Photo 17: Himalayan Goral

Photo 18: Sambar Deer

Photo 19: Spotted Deer

Photo 20: Swamp Deer

Photo 21: Wild Water Buffalo

Photo 22: Interviewing with zoo staff

Photo 23: Zookeeper collecting pellet

Photo 24: Group photos with zoo family

Photo 25: Microscopic examination

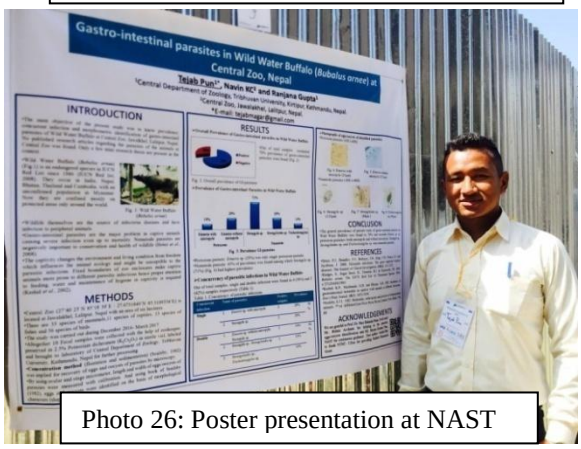

Photo 26: Poster presentation at NAST

ANNEX IV

Identified oocyst and eggs of GI parasites

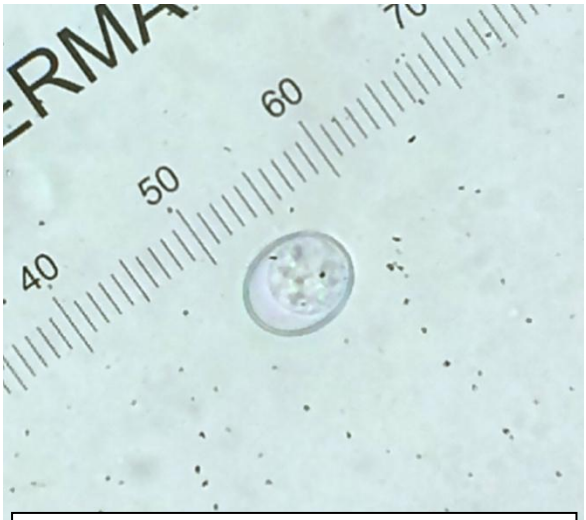

Photo 27: *Eimeria* sp. without micropyle (40X)

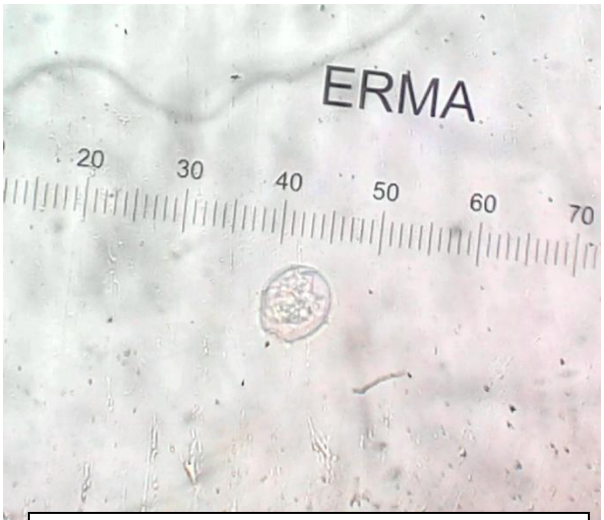

Photo 28: *Eimeria* sp. with micrpyle (40X)

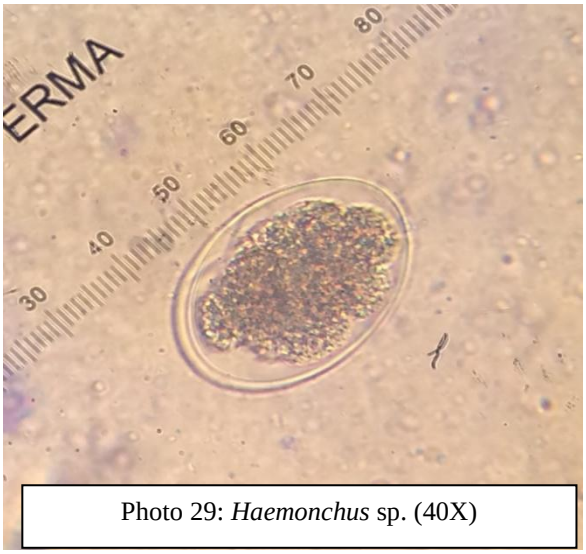

Photo 29: *Haemonchus* sp. (40X)

Photo 30: *Strongyloides* sp. (40X)

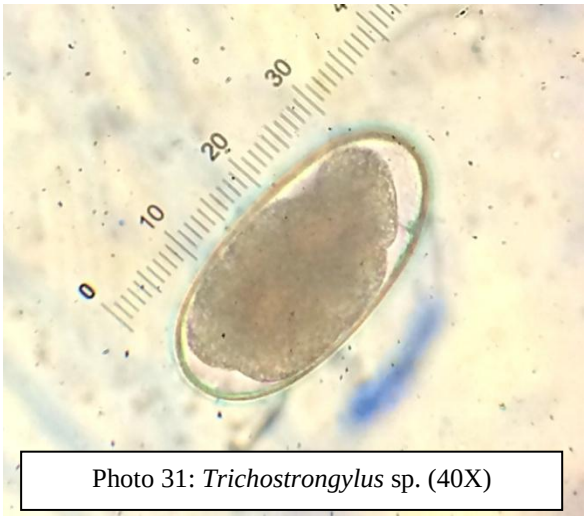

Photo 31: *Trichostrongylus* sp. (40X)