GASTROINTESTINAL PARASITES IN KRISHNASAR (*Antilope cervicapra*) OF BLACKBUCK CONSERVATION AREA, BARDIYA AND SHUKLAPHANTA WILDLIFE RESERVE, KANCHANPUR

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Submitted to

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Kirtipur, Kathmandu

Nepal

October, 2014

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 authors or institutions.

Date: **20 October 2014** ……..…

Ram Bahadur Chaudhary

RECOMMENDATION

This is to recommend that the thesis entitled **"Gastrointestinal Parasites in Krishnasar (*Antilope cervicapra*) of Blackbuck Conservation Area, Bardiya and Shuklaphanta Wildlife Reserve, Kanchanpur"** has been carried out by **Ram Bahadur Chaudhary** for the partial fulfilment 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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On the recommendation of supervisor **"Dr. Mahendra Maharjan"** this thesis submitted by **Ram Bahadur Chaudhary** entitled **"Gastrointestinal Parasites in Krishnasar (*Antilope cervicapra*) of Blackbuck Conservation Area, Bardiya and Shuklaphanta Wildlife Reserve, Kanchanpur"** is approved for the examination and submitted to the Tribhuvan University in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper **Parasitology**.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Ram Bahadur Chaudhary** entitled **"Gastrointestinal Parasites in Krishnasar (*Antilope cervicapra*) of Blackbuck Conservation Area, Bardiya and Shuklaphanta Wildlife Reserve, Kanchanpur"** has been accepted as a partial fulfilment 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
µm	Micrometer
BCA	Blackbuck Conservation Area
CPG	Cysts per gram
DNPWC	Department of National Park and Wildlife Conservation
EPG	Egg per gram
et al.	And his associates
ft	Feet
GI	Gastrointestinal
gm	Gram
IUCN	International Union for Conservation of Nature
km	Kilometer
mi	Mile
ml	Milliliter
NPWCA	National Park and Wildlife Conservation Act
NTNC	National Trust for Nature Conservation
OPG	Oocyst per gram/Ova per gram
rpm	Revolution per minute
SWR	Shuklaphanta Wildlife Reserve
WWF	World Wildlife Fund

ABSTRACT

Blackbuck (*Antilope cervicapra*) is near threatened species which are conserved in Blackbuck Conservation Area (BCA), Khairapur, Bardiya and Shuklaphanta Wildlife Reserve (SWR), Kanchanpur district. The present study was conducted to determine the prevalence of GI parasites of blackbuck in BCA and SWR. A total of 150 fresh faecal samples were collected from BCA and 70 faecal samples from SWR and examined by floatation, sedimentation and Stoll's counting technique by using Lugol's Iodine mount and saline mount. The GI parasites were found to be 90.00% in BCA and SWR. Protozoan parasites were found to be most prevalent in SWR (55.71%) compared to BCA (55.33%). While with regard to helminths, blackbucks of BCA were found to be more infected (90%) than SWR (88.57%). Helminths infection was comparatively more common than the protozoan infection in blackbuck of both BCA and SWR. Gastrointestinal parasites were more prevalent in female as compared to male and fawn. blackbuck of BCA were found to be infected with 12 different species of parasites which includes *Entamoeba* (20%) and *Eimeria* (45.33%) among protozoa; *Paramphistomum* (25.33%) and *Fasciola* (17.33%) among trematodes; *Moniezia* (14%) among cestode; *Trichostrongylus* (75.33%), *Ascaris* (57.33%), *Haemonchus* (18%), *Strongyloides* (16%), *Bunostomum* (12.67%), *Trichuris* (6%) and *Oxyuris* (4.67%) among nematodes while blackbuck of SWR were found to be infected with 10 different parasitic genera i.e. *Entamoeba* (8.57%) and *Eimeria* (51.43%) among protozoa; *Paramphistomum* (38.57%), *Fasciola* (21.43%) and *Schistosoma* (7.14%) among trematodes; *Trichostrongylus* (55.71%), *Ascaris* (38.57%), *Haemonchus* (14.28%), *Strongyloides* (12.86%) and *Bunostomum* (2.86%) among nematodes. Among protozoans identified, *Entamoeba* was most prevalent in BCA compared to SWR while *Eimeria* was comparatively higher in SWR than BCA. Three trematodes i.e. *Paramphistomum, Fasciola* and *Schistosoma* were more prevalent in SWR than BCA while *Schistosoma* was only reported from SWR. *Moniezia* was only observed in blackbuck of BCA. All of seven different nematodes were comparatively higher in BCA than SWR while *Trichuris* and *Oxyuris* were reported from BCA only.

1. INTRODUCTION

1.1 Background

Conservation is the planning and management of resources, so as to maintain and enhance their quality, value and diversity for the present and future use by the mankind. Wildlife conservation involves the protection, preservation, perpetuation and judicious control of rare species in their natural habitats (Rastogi 1996).

A conservation area represents human's sublime idea of giving space and resource for the other species to live in an un-interfered manner (DNPWC 2011). In Nepal, 10 national parks, three wildlife reserves, six conservation areas and one hunting reserve have been established for the protection of wildlife (DNPWC 2012). These protected areas cover 34,185.62 sq. km (23.23%) of the total geographical area of the country (DNPWC 2012). A total of 181 mammal species have been recorded to occur in Nepal (DNPWC 2012). Of them 23% are considered to be nationally threatened with extinction, 4 % of species considered as critically endangered, 12% endangered and 7% vulnerable. A further 3% are considered near threatened, 35% of Nepal's mammals are considered least concern and 38% are considered data deficient (Jnawali et al. 2011). Small isolated populations of wild animals are vulnerable to extinction through demographic, environmental and genetic stochasticity and catastrophes such as disease epidemics (Shaffer 1981).

1.2 Blackbuck (*Antilope cervicapra* Linneaus 1758)

Blackbuck (*Antilope cervicapra*), locally known as Krishnasar, is an elegant, gazelle-like animal regarded as the most handsome member of the Bovidae family. It belongs to the subfamily 'Antilopinae' and order 'Artiodactyla'. The scientific name "*Antilope*" is restricted to blackbuck only. Corbett and Hill (1977) and Mungall (1978) described four species of *Antilope cervicapra* according to the coat, color, length and the shape of the horn with distribution. They are; *Antilope cervicapra cervicapra* (South India), *A. cervicapra centralis* (Central India), *A. cervicapra rupicapra* (North India and Nepal) and *A. cervicapra rajputane* (North-West India and Pakistan).

This animal is built like a gazelle. There is great difference in coloration of the sexes (Grzimek 1972). The male blackbuck has striking black and white pelage and bears long spiral horns (Shrestha 2003). The brown areas in the males gradually darken with age

finally becoming black (Prater 1965). The female blackbuck, also known as doe has yellowish on the head and back. The male blackbuck stands 70-80 cm at shoulder height. Its weight varies between 34-45 kg, that of adults females between 31-39 kg (Ranjitsinh 1989). In both sexes, the under parts, insides of the legs and an area encircling the eyes are white. In males, the rape is rusty (Shrestha 2003). A light colored streak runs laterally along the upper flank of the body. They have pronounced pre-orbital glands which secrete a pungent sticky secretion which the animal uses to mark territories (Mungall 1978). The horns are marked with rings and make horns to force spirals in adults (Scaller 1967). These horns are non-deciduous horns that grow from an ossicone or nosecone on the forehead and are hollow giving rise to the designation bovids as hollow horned ruminants (Grzimek 1972).

The blackbuck is near threatened belongs to IUCN Red List of Threatened Species (IUCN 2008). In Nepal, blackbuck is one of the 27 protected mammals under National Park and Wildlife Conservation Act 1973.

Blackbuck is typically distributed in India, Pakistan and Nepal (Lydekker 1924). Ranjitsinh (1989) was estimated a total of 45,000 animals in India. The blackbuck was extinct in Bangladesh and also became extinct in Pakistan in 1970s but 10 animals were re-introduced from Texas, USA in Lal Sunhara National Park of Sindh Provience of Pakistan (Burton and Burton 1987). About 43,600 blackbucks were introduced to Argentina and the USA (Mallon and Kingswood 2001). In Nepal, before the malaria eradication programme, blackbuck was commonly found in eastern and western Terai; but later the population of blackbuck declined gradually. Scattered population of blackbuck occurred in Kanchanpur, Bardiya and Banke districts in western Nepal as late as 1960s (Pradhan et al. 1999). Blackbucks are restricted to Khairapur, Bardiya; few are in captive in central zoo, Mahendra Park, Nepalgunj mini zoo and semi-captive enclosures at Mrigasthali forest, Kathmandu (Khanal 2006). Now, blackbucks are also restricted to Hirapur phanta of SWR, Kanchanpur (DNPWC 2012). Twenty eight blackbucks were reintroduced to Hirapur phanta of SWR, Kanchanpur (DNPWC 2012). In 2012, the population of blackbuck was estimated at 287 in Blackbuck Conservation Area, Khairapur, Bardiya (Shah 2012).

The blackbucks inhabit in open field with short grazing grasses and tend to avoid environment dominated by tall grasses or forested areas (Scaller 1967). They are

gregarious and gather in herds. Blackbucks are true grazers, but sometimes they browse over leaves of trees and take some herbs and shrubs. While grazing and browsing, they keep themselves alert for danger and raise their heads to look around. Daily requirement of food by the blackbuck accounts for nearly 6 percent of its body weight (Kafle 1998). Blackbuck takes the rate of three chews per two seconds at the time of cud chewing (Nair 1975). Mating is throughout the year with two peak rutting seasons (July-August and February-March). The female antelope gives birth to one fawn at a time at the age of two year and gestation period lasts for a period of six months. Blackbucks maintain nearly constant breeding size consisting of 10-20 individuals, occasionally they range up to 40. The maximum life span recorded is 16 years and average is 12 years (http://www.en.wikipedia.org/wiki/blackbuck).

Blackbuck has important ecological roles in grassland ecosystem. It has genetic, medicinal (horns and skin to cure liver and heart diseases), scientific, aesthetic and re-creational value. In Hinduism and Buddhism, the horns and skin of blackbuck are used in rituals like 'Bratabandha' and in medication by Saints: respectively. In Australia, blackbuck has good meat value.

The main threats to blackbuck are poaching, predation, habitat destruction, overgrazing, diseases, inbreeding, visitors and natural disorder like flood, etc.

1.3 Parasitic Diseases of Blackbuck

The animals suffer from a variety of infectious and non-infectious diseases, particularly that of parasitic origin (Iqbal et al. 2000, Akhter and Arshad 2006). The major concern with wild ruminants like blackbuck is a decrease in animal health and even death. In nature, wild animals live on large areas and have consequently a low genetic resistance against parasitic infections because of low exposure (Muoria et al. 2005) and practically animal is not free from parasitic infection (Hossain 2012). Blackbucks are susceptible to various kinds of gastrointestinal parasitic infections like coccidiosis, paramphistomiasis, fascioliasis, schistosomiasis, taeniasis, nematodiasis and respiratory disorder, bacterial, viral and fungal infection. Mortality and health problem diagnosed mainly in young adult male and pregnant blackbucks include food shortage and malnutrition, overcrowding and parasitism in Texas, US (Thornton et al 197). Mortality also diagnosed in blackbuck of BCA, Khairapur, Nepal due to different viral diseases like diphtheria, foot and mouth

disease, etc (DNPWC 2009). Generally blackbuck can be infected with different types of endoparasites. The major gastrointestinal parasites reported in blackbuck are *Coccidia* (Khanal 2006 and Ban 2012), *Taenia hydatigena* (Thornton et al. 1973), *Camelostrongylus* sp. (Thornton et al. 1973, Flach 1986, Flach and Sewell 1987), *Haemonchus* sp. (Thornton et al. 1973, Flach 1986, Farooq 2009, Rimal 2011 and Farooq et al. 2012), *Trichostrongylus* sp. (Thornton et al. 1973, Farooq 2009, Rimal 2011, Farooq et al. 2012 and Ban 2012), *Nematodirus* sp. (Thornton et al. 1973, Flach 1986, Flach and Sewell 1987), *Oesophagostomum* sp. (Thornton et al. 1973, Rimal 2011 and Farooq et al. 2012), *Trichuris* sp. (Thornton et al. 1973, Farooq 2009, Rimal 2011, Farooq et al. 2012 and Ban 2012), *Fasciola* sp. (Farooq et al. 2012 and Ban 2012), *Paramphistomum* sp. (Khanal 2006, Rimal 2011 and Ban 2012), *Schistosoma* sp., *Moniezia* sp. and *Strongyloides* sp. (Farooq 2009, Farooq et al. 2012 and Ban 2012), *Strongyles* and *Ascaris* sp. (Khanal 2006 and Ban 2012), *Chabertia, Cooperia, Bunostomum* (Farooq 2009 and Farooq et al. 2012). Out of all these reported GI parasites, *Camelostrongylus mentulatus* was most dreadful nematode to blackbuck and also associated with chronic emaciation and death of dominant adult male animals.

1.4 Objectives of the Study

1.4.1 General objective

- To identify the diversity and intensity of GI parasites of blackbuck in BCA, Bardiya and SWR, Kanchanpur

1.4.2 Specific objectives

- To determine the prevalence of GI parasites of blackbuck in BCA, Bardiya
- To determine the prevalence of GI parasites of blackbuck in SWR, Kanchanpur
- To compare the diversity and intensity of GI parasites of blackbuck in between BCA and SWR

1.5 Rationale of the Study

Blackbuck is near threatened species (IUCN 2008) which are valuable and also important to maintain the ecological and biodiversity balance but this conception has not been established yet in our country. In the past, very little research work had been done on the parasites of blackbuck in BCA, Khairapur, Bardiya but till date parasitic studies has not

been carried out in SWR. Parasites and infectious diseases of wildlife are a major threat to conservation of threatened species (Lyles and Dobson 1993). Thus, there is a need for studying and documenting the prevalence of parasites among threatened species. Transmission of pathogens at the wildlife-livestock interface can occur in both directions and may therefore pose a threat to conservation (Bengis et al. 2002). Parasitism has been shown to affect impaired reproductive performance which can lead to a reduction in population size or the extinction of the host (Hoberg et al. 2001). From a conservation point of view, parasitological studies are important to understand mode of infection and the potential transmission of parasites between species, both native and introduced (Begon et al. 1999). In order to assess and manage the effect of parasites on population dynamics, it is also essential to evaluate the parasitic distribution and then extent of pathogenesity (Morner 2002). The present findings provided some baseline information on the parasitic burden in blackbuck and help to formulate appropriate strategies to mitigate the endoparasitic problem of blackbuck in SWR and BCA.

2. LITERATURE REVIEW

Besides bacterial, fungal and viral infections, the wild ruminants are also susceptible to various diseases like coccidiosis, fascioliasis, fasciolopsiasis, schistosomiasis, nematodiasis, respiratory diseases, etc. Although, coccidiosis does not directly affect wild ruminants but it can cause diarrohea, weight loss, weakness, growth retardation, etc. As all other domestic herbivores, wild ruminants are also suffered from different endoparasites by sharing same pasture. The gastrointestinal parasites are important in zoo animal (Isaza et al. 1990). Considering this lot of research works have been carried out on endoparsites of domestic as well wild and captive ruminants. But in Nepal, only a few researches have been reported among wild ruminants. In case of blackbuck, very little research works has been carried out regarding parasitic infection. Wild ruminants can be infected by different parasites including protozoans, trematodes, cestodes and nematodes. In this section some important published work related with the present work has been reviewed.

2.1 In Global Context

2.1.1 Endoparasites of wild ruminants

Endoparasites are those organisms living within their hosts, in the gut, body cavity, liver, lungs, gall bladder and blood or within the internal cavities, tissue or cell of the host. They completely depend upon their host causing infection to them. Parasites of the digestive system like small intestine and large intestine as well as those of respiratory and circulatory system infest grazing animals (Taylor et al. 2007). Especially, the protozoan and helminthes parasites have been reported in wild ruminants.

a. ***Protozoan parasites***

The protozoan parasites were reported from India by Parasani et al. (2001) and Iraq by Radhy et al. (2013). Different types of intestinal protozoan parasites such as *Cryptosporidium* (Corden et al. 2008, Lim et al. 2008, Fagiolini et al. 2010, Mohammad et al. 2012, Radhy et al. 2013), *Entamoeba* (Opara et al., 2010), *Isospora* (Corden et al., 2008), *Giardia* (Opara et al. 2010, Radhy et al. 2013), *Blantidium coli* (Varadharajan and Kandasamy 2000, Hossain 2012, Roki B Ur Raja 2012, Rahman and Mustafizur 2012), *Coccidia* (Varadharajan and Kandasamy 2000), *Eimeria* (Tovassoli et al. 2010, Fagiolini

et al. 2010, Mohammad et al. 2012), *Isospora* (Corden et al. 2008), *Cyclospora* (Corden et al. 2008) and *Blastocystis* (Corden et al. 2008) have been identified from different wild ruminants.

Many coccidian parasites infecting wild ruminant animals have been recorded in Egypt (Wahed et al. 2004), state of India (Varadharajan and Kandasamy 2000, Singh et al. 2009), Spain (Vazquez et al. 2009), Latvia (Ruta et al. 2009). The *Coccidia* include various species such as *Eimeria, Cryptosporidium, Isospora, Neospora,* etc. At least seven *Eimeria* species infect the wild ruminant animals have been reported from Galicia (Diez et al. 2010). They are *E. capreoli, E. cutebrina, E. superba, E. panda, E. rotunda, E. pondarosa* and *E. patavina. Eimeria* have also been reported in the faeces of ruminants in the state of India (Singh et al. 2009), Turkey (Arslan and Sari 2010), Italy (Fagiolini et al. 2010), Chungbok (Seong-Jun et al. 2011), Iraq (Saud et al. 2012), Poland (Maesano et al. 2014) and Romania (Darabus et al. 2014). *Eimeria saudiensis* have been reported by Mohammad et al. (2012) from Saudi Arabia. According to Tovassoli et al. (2011), the wild ruminants infected with one or more *Eimeria* species have been documented in North-West Iran.

At least 13 *Cryptosporidium* species occurimg in mammals, birds and fish but only two species are important in livestock animals (Taylor et al. 2007, Radostitis et al. 2008). They are *Cryptosporidium parvum* and *Cryptosporidium andersoni* (Taylor et al. 2007, Radostitis et al. 2008). *Cryptosporidium* complete their biological cycle on the surface of epithelial cells of digestive and respiratory system of birds, mammals and reptiles (Ponce Gordo et al. 2002). *Cryptosporidia* have been reported in the faeces of wild animals in Ireland (Skerrett and Holland 2001), Spain (Corden et al. 2008), Malaysia (Lim et al. 2008), Italy (Fagiolini et al. 2010), South Africa (Abu Samra et al. 2011), Galicia (Castro-Hermida et al. 2011), Saudi Arabia (Mohammad et al. 2012) and Iraq (Radhy et al. 2013).

Some reports have indicated the presence of *Giardia* species in wild ruminants. There is only one species described in this group of wild animals i.e. *Giardia intestinalis* or *G. duodenalis* or *G. lamlia* which is found in the small intestine of the host. Infection of this parasite occurs in a wide range of domesticated and wild animals and also humans. It is a potential pathogen in livestock causing diarrhoea, weight loss, poor condition and lethargy (Hunter and Thompson 2005). Olson et al. (2004) reported a high level of *Giardia* infection in young livestock, especially calves. *Giardia* sp. has also been reported

in Southeast Nigeria (Opara et al. 2010) and Galicia (Castro-Hermida et al. 2011). Few reports have indicated the presence of *Isospora, Cyclospora* and *Blastocystis* in wild ruminants in Spain (Corden et al. 2008).

Entamoeba cysts forming groups include non-pathogenic species, some of them described from wild animals. *E. coli* like group inhabits in the small intestine of the host. *Entamoeba* sp. has been reported from Southeast Nigeria (Opara et al. 2010). *Blantidium coli* are ciliate and normal inhabitants of intestine of wild ruminants, probably capable of becoming somewhat pathogenic under favorable conditions. It has been identified by Varadharajan and Kandasamy (2000) from India and Hossain (2012), Roki B Ur Raja (2012), Rahman and Mustafizur (2012) from Bangladesh.

b. Helminths

i. Trematodes

Trematode parasites include *Paramphistomum, Fasciola*, *Schistosoma, Dicrocoelium,* etc. They are found in the liver, bile duct and sometimes lungs of the ruminant's host. *Fasciola,* also known as fluke that cause fascioliasis, commonly called as namle, mate, lew, etc. *Fasciola* sp. has been identified in India (Mandal et al. 2000, Singh et al. 2009), Southeast Nigeria (Opara et al. 2010), Pakistan (Farooq et al. 2012) and Bangladesh (Rahman and Mustafizur 2012, Roki B Ur Raja 2012, Hossain 2012). *Fasciola hepatica* has also been recovered in Egypt by Wahed et al. (2004) and Pakistan by Farooq (2009). *Paramphistomum* sp. was found in India (Mandal et al. 2000, Singh et al. 2009 and Borghare et al. 2009), Latvia (Ruta et al. 2009), Bangladesh (Kanungo et al. 2010, Rahman and Mustafizur 2012, Roki B Ur Raja 2012) and Italy (Fagiolini et al. 2010). *Paramphistomum cervi* was reported by Kuzmina et al. (2010) from Ukraine and Aukstikalniene et al. (2013) from Lithuania. *Schistosoma* sp. have been encountered in Pakistan (Farooq et al. 2012), Southeast Nigeria (Opara et al. 2010). Likewise, *Dicrocoelium lanceolatum* was recorded in Egypt (Wahed et al. 2004), Romania (Darabus et al. 2014) while *Dicrocoelium* sp. in India (Borghare et al. 2009) and Turkey (Gurler et al. 2010). Another species, *Dicrocoelium dendriticum* has been identified by Aukstikalniene et al. (2013) in Lithuania.

ii. Cestodes

Different types of tapeworm belongs to the class cestoda such as *Moniezia* sp., *Taenia* sp., etc. that inhabit in the small intestine of the both wild and domestic ruminant animals i.e. goats, sheep, blackbuck, cattle, etc. *Moniezia* is a large tapeworm, also known as sheep tapeworm or double pored tapeworm that causes poor growth, intestinal obstruction, weight loss, diarrhoea during heavy infections. Cysticerci of *Taenia hydatigena* was recorded in wild ruminants from US (Thornton et al. 1973), Ukraine (Kuzmina et al. 2010) and Saudi Arabia (Mohammad et al. 2012). Similarly, *Taenia* sp. was found in Southeast Nigeria (Opara et al. 2010) and Iraq (Saud et al. 2012). *Moniezia* sp. has been reported in wild ruminants from India (Varadharajan and Kandasamy 2000), Central Spain (Garcia-Romero et al. 2000), Spain (Vazquez et al. 2009), Turkey (Gurler et al. 2010), and Ukraine (Kuzmina et al. 2010), Ethiopia (Bogale et al. 2014). Two species such as *Moniezia expansa* and *M. benedeni* have been identified by Wahed et al. (2004) from Egypt and Farooq (2009) and Farooq et al. (2012) from Pakistan.

iii. Nematodes

There are numerous nematode parasites found in the intestine of wild as well as domestic animal that have been reviewed by different researchers as given below.

The *Haemonchus contortus* (wire worm) is one of the most important pathogen for blackbuck in US (Thornton et al. 1973), producing a disease known as haemonchiasis which can cause anaemia, growth loss, edema, emaciation, etc and even death (Roberts and Janovy Jr. 2005). It was recovered in Poland (Cicek et al. 2003), India (Meshram et al. 2007, Borghare et al. 2009), Pakistan (Farooq 2009, Farooq et al. 2012), Ukraine (Kuzmina et al. 2010), Bangladesh (Kanungo et al. 2010) and Iraq (Saud et al. 2012). Mayo et al. 2013 was reported *Haemonchus* in Barbary sheep from Spain.

Three species of *Trichostrongylus* have been encountered in blackbuck of US such as *Trichostrongylus axei, T. colubriformis, T. probolurus* (Thornton et al. 1973) and like other wild ruminants in Poland (Cicek et al. 2003), Ukraine (Kuzmina et al. 2010), Saudi Arabia (Mohammad et al. 2012), *T. retortaeformis* in Belgium (Goossens et al. 2005), *T. vitrinus* and *T. capricola* in Spain (Mayo et al. 2013). *Trichostrongylus* sp. has also been reported in Central Spain (Garcia- Romero et al. 2000), India (Meshram et al. 2007), Pakistan (Farooq 2009, Farooq et al. 2012), North-Western Spain (Vazquez et al. 2009),

Latvia (Ruta et al. 2009), Bangladesh (Rahman and Mustafizur 2012, Roki B Ur Raja 2012, Hossain 2012) and Iraq (Saud et al. 2012). The larva of *T. colubriformis* was also recorded in Egypt by Wahed et al. (2004).

Camelostrogylus mentulatus was most prevalent pathogens for blackbuck and observed after post mortem from abdomen and small intestine of male blackbuck in US (Thornton et al. 1973), Endinburgh (Flach 1986, Flach and Sewell 1987). It was associated with chronic emaciation and death of dominant adult male blackbuck in Endinburgh (Flach 1986, Flach and Sewell 1987). *Camelostrogylus mentulatus* has also been reported abomasum in Belgium (Goossens et al. 2005), Saudi Arabia (Mohammad et al. 2012) and Spain (Mayo et al. 2013).

Nematodirus spathiger have been identified in wild ruminants from US (Thornton et al. 1973), Endinburgh (Flach and Sewell 1987), Saudi Arabia (Mohammad et al. 2012) and Spain (Mayo et al. 2013). *Nematodirus* sp. was also observed in the faeces of wild ruminants in Poland (Cicek et al. 2003, Maesano et al. 2014), Northwestern Spain (Vazquez et al. 2009), Chungbok Province (Seong-Jun et al. 2011), Pakistan (Farooq et al. 2012), Iraq (Saud et al. 2012), Lithuania (Aukstikalniene et al. 2013) and Ethiopia (Bogale et al. 2014). Another species has also been reported *Nematodirus fillicollis* in Belgium (Goossens et al. 2005), *Nematodirus oiratinus* in Ukraine (Kuzmina et al. 2010), *N. abnormalis* and *N. helvetianus* in Spain (Mayo et al. 2013).

Oesophagostomum sp. is a strongylid nematode of the small intestine of blackbuck in US (Thornton et al. 1973). It was also found in other wild ruminants reported from Central Spain (Garcia- Romero et al. 2000), India (Borghare et al. 2009) and Pakistan (Farooq et al. 2012). One species *Oesophagostomum venulosum* has also identified in Central Spain (Valcarcel et al. 2002), Poland (Cicek et al. 2003), Egypt (Wahed et al. 2004), Ukraine (Kuzmina et al. 2010) and North-West Iran (Tovassoli et al. 2010). Another species has also been reported *Oesophagostomum radiatum* in Belgium (Goossens et al. 2005), *Oesophagostomum dentatum* in Ukraine (Kuzmina et al. 2010).

Trichuris sp. is commonly known as whip worm usually found in the caecum of the host. It was observed in blackbuck of US (Thronton et al .1973). This species has also been identified in India (Varadharajan and Kandasamy 2000, Meshram et al. 2007, Singh et al. 2009), Central Spain (Garcia- Romero et al. 2000), Belgium (Goossens et al. 2005), Spain (Corden et al. 2008, Mayo et al. 2013, Maesano et al. 2014), Malaysia (Lim et al. 2008),

North-Western Spain (Vazquez et al. 2009), Turkey (Gurler et al. 2010), Southeast Nigeria (Opara et al. 2010), Italy (Fagiolini et al. 2010), Northwest Iran (Tovassoli et al. 2010), Chungbok Province (Seong-Jun et al. 2011) and Ethiopia (Bogale et al. 2014). *Trichuris globulosa* has identified in both wild ruminants from Pakistan (Farooq 2009, Farooq et al. 2012), *Trichuris cervicaprae* in Arabian oryx from Saudi Arabia (Mohammad et al. 2012) and *Trichuris ovis* from Lithuania (Aukstikalniene et al. 2013).

Ascaris sp has been reported in India (Mandal et al. 2000, Varadharajan and Kandasamy 2000), Southeast Nigeria (Opara et al. 2010), Bangladesh (Rahman and Mustafizur 2012, Roki B Ur Raja 2012) and Ethiopia (Bogale et al. 2014). *Bunostomum* sp. is a hookworm like nematodes (Lim et al. 2008) and their reports have been reviewed in India (Meshram et al. 2007, Borghare et al. 2009) and Pakistan (Farooq et al. 2012). Kuzmina et al. 2010 has been documented *Bunostomum phlebostomum* in roe deer from Ukraine.

Strongyloides sp belong to the family strongyloididae was identified from India (Mandal et al. 2000, Varadharajan and Kandasamy 2000, Meshram et al. 2007, Singh et al. 2009), Spain (Corden et al. 2008), Latvia (Ruta et al. 2009), Chungbok Province (Seong-Jun et al. 2011), Pakistan (Farooq et al. 2012), Bangladesh (Rahman and Mustafizur 2012, Roki B Ur Raja 2012, Hossain 2012), Iraq (Saud et al. 2012). *Strongyloides papilosus* was identified in the faecal samples of cervids from Lithuania (Aukstikalniene et al. 2013), Walia ibex from Ethiopia (Bogale et al. 2014).

Capillaria sp. is more or less similar to that of *Trichuris* sp. but in comparison to *Trichuris* eggs, the shell is almost colourless, barrel shaped having sides nearly parallel. It was observed in Central Spain (Garcia-Romero et al. 2000), Belgium (Goossens et al. 2005), Turkey (Gurler et al. 2010), Chungbok Province (Seong-Jun et al. 2011) and Lithuania (Aukstikalniene et al. 2013). Only one species *Capillaria bovis* (Cicek et al. 2003) has been documented in North-Western Poland. *Cooperia* sp. usually plays a secondary role in the pathogenesis of parasitic gastroenteritis in ruminants due to the presence of large number of *Trichostrongulus* sp. It has been reported in Central Spain (Garcia-Romero et al. 2000), Southeast Nigeria (Opara et al. 2010) and Pakistan (Farooq et al. 2012).

Chabertia sp, commonly known as large mouthed bowel worm is present usually in low number that found attached to the mucosa of colon of host. *Chabertia ovina* has been

reported in North-Western Poland (Cicek et al. 2003), Pakistan (Farooq 2009, Farooq et al. 2012), Ukraine (Kuzmina et al. 2010) and Iraq (Saud et al. 2012).

Few reports have been indicated the presence of *Ostertagia, Spiculopteragia, Dictyocaulus, Muellerius,* etc. in wild ruminants. *Ostertagia* sp. have been reported in Central Spain (Garcia-Romero et al. 2000) while *Ostertagia ostertagi* in Egypt (Wahed et al. 2004), *O. circumcincta* in Pakistan (Farooq 2009, Farooq et al. 2012), *O. leptospicularis* and *O. lyrata* in Spain (Mayo et al. 2013), *O. kolchida* in Poland (Cicek et al. 2003). Eggs of *Toxocara* sp. reported in feces of wild ruminants in Italy by Fagiolini et al. (2010) and Pakistan by Farooq et al. (2012). *Spiculoptergia* sp. in Central Spain (Garcia-Romero et al. 2000), *S. boehmi* in Poland (Cicek et al. 2003), *Dictyolaulus* sp. in Central Spain (Garcia-Romero et al. 2000), Egypt (Wahed et al. 2004), Latvia (Ruta et al. 2009) whereas *Dictyocaulus capreolus* in North-Western Spain (Vazquez et al. 2009), *Dictyocaulus filarial* and *Muellerius* in Turkey (Gurler et al. 2010), *Protostrongylus* in Ethiopia (Bogale et al. 2014), Latvia (Ruta et al. 2011) have been encountered in the faecal samples of different wild ruminants.

Similarly, the nematodes *Strongyle* type recorded in the faecal samples of wild ruminants in India (Varadharajan and Kandasamy 2000, Meshram et al. 2007, Singh et al. 2009), Turkey (Gurler et al. 2010), Southeast Nigeria (Opara et al. 2010), Italy (Fagiolini et al. 2010), Chungbok Province (Seong-Jun et al. 2011), Saudi Arabia (Mohammad et al. 2012), Poland (Maesano et al. 2014) and Ethiopia (Bogale et al. 2014). Two types of *Strongyles* i.e. pulmonary and digestive were identified by Darabus et al. (2014) in Romania.

2.2 In National Context

2.2.1 Endoparasites of wild ruminants

It includes both protozoan and helminthes parasites are as follows:

a. Protozoan parasites

Protozoan parasites like *Coccidia, Eimeria, Cryptosporidium, Isospora,* etc. Khanal (2006) and Ban (2012) have been reported coccidian parasites in blackbuck from Khairapur, Bardiya. Thapa 2013 has been reported two types of *Eimeria* in the faecal samples of Himalyan Thar and Barking deer from Rara National Park such as *Eimeria*

with micropile and *Eimeria* without micropile that seems to be non-pathogenic but during large numbers, it causes watery diarrhoea, weight loss, weakness, etc.

b. Helminths

It contains trematode, cestode and nematode parasites which are found in the liver, bile duct as well as digestive system of both wild and domestic ruminant's animals. They are:

i. Trematodes

These are leaf like parasites including *Paramphistomum, Fasciola, Schistosoma, Dicrocoelium,* etc. *Paramphistomum* sp. is the most important pathogen that has been reported in blackbuck (Khanal 2006 and Ban 2012), wild elephant (Karki and Manandhar 2008), captive elephant (Shrestha et al. 2011) and deer (Rimal 2011). *Fasciola* sp. in captive elephant (Shrestha et al. 2011) and blackbuck (Ban 2012) have been documented from different parts of Nepal. Karki and Manandhar (2008) was identified *Fasciola jacksoni* in wild elephant. *Schistosoma* sp. has been observed in faeces of various ruminants like wild elephant (Karki and Manandhar 2008) and blackbuck (Ban 2012). Likewise *Dicrocoelium* sp. has also been recovered in wild elephant (Karki and Manandhar 2008).

ii. Cestodes

It is a ribbon like tapeworms such as *Moniezia, Taenia,* etc. that are found in the gut of ruminants and sometimes in human being. Karki and Manandhar (2008), Ban (2012) and Thapa (2013) have been encountered *Moniezia* sp. in wild elephant, blackbuck, Himalayan Thar and Barking deer respectively.

iii. Nematodes

The *Haemonchus* sp. (wire worm) is the blood feeding parasites that causing anaemia, edema (bottle jaw), weakness, etc. in bovids. It has been identified by Rimal (2011) in deer, Thapa (2013) in Himalayan Thar and Barking deer. Similarly, *Trichostrongylus* sp. has been described in deer (Rimal 2011), blackbuck (Ban 2012) and Himalyan Thar (Thapa 2013). Ova of *Ascaris* were found in blackbuck (Khanal 2006), captive elephant (Shrestha et al. 2011), Himalayan Thar and Barking deer (Thapa 2013). Eggs of *Strongyloides* sp. were recorded in blackbuck (Ban 2012) and Himalayan Thar (Thapa 2013). *Strongyle* type is strongylid nematode which is observed in the faecal matter of

blackbuck (Khanal 2006 and Ban 2012) from Khairapur, Bardiya. Eggs of *Chabertia* sp. were identified from wild elephant (Karki and Manandhar 2008). *Trichuris* sp. has also been identified in different ruminants including deer (Rimal 2011), blackbuck (Ban 2012), Himalayan Thar and Barking deer (Thapa 2013). Ova of *Oesophagostomum* sp. were revealed from deer by Rimal (2011). *Oxyuris* sp. was reported from Himalayan Thar and Barking deer (Thapa 2013). *Dictyocaulus* sp. and *Muellerius* sp., filarial nematodes are parasites of respiratory system recovered in the faecal samples of Himalayan Thar and Barking deer (Thapa 2013).

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in two areas; BCA of Khairapur, Bardiya and SWR of Kanchanpur.

3.1.1 Blackbuck Conservation Area (BCA)

The Blackbuck Conservation Area is the only one conservation area of Terai region, which is located at Khairapur in Bardiya district of western Nepal. The BCA is located at a distance of 30 km east of Bardiya National Park. It was established in 6th March 2009 (23th Falgun 2065 B.S.) and was declared in 3th Chaitra 2065 by Government of Nepal to conserve the blackbuck. It covers an area of 16.95sq.km which includes the core habitat of 5.27 sq. km with circumference of 10.25 km and peripheral area of villages and settlements spreading over 11.68 sq. km.

Blackbuck habitat is situated in Pataha phanta. The old babai riverbed, locally known as "Sarju River", runs along north, west and south boundary of the study area. The area is mostly marginal agricultural land and grazing land bordered on the three sides by old riverbed and on the other side by scrub jungle (Lehmkuhl 1979). Topography of the study area is more or less flat land, sloping towards the South with an elevation of average 152 m above mean sea level.

3.1.2 Suklaphanta Wildlife Reserve (SWR)

The SWR is a protected area, located in the far-western lowland Terai on the southwest edge of Nepal. It covers 305 sq. km (118 sq. mi) of open grassland, forests, riverbed and tropical wetlands an altitude of 174 to 1,386 meters (571 to 4,547 ft). It is situated in Kanchanpur district which lies between 80^{o}25'0" South and 28^{o}35'0" North. It extends north of the east west highway to create a corridor for seasonal migration of wildlife into the sivalik hills. The Syali River forms the eastern boundary, southward to the international border with India, which demarcates the reserve's southern and western boundary. The blackbuck habitat is situated in Hirapur phanta of SWR. This area has most suitable habitat with well managed short grass for blackbuck, bordered on the three sides by forest and other side by Syali River. The Hirapur phanta is located at a distance of 4 km north of Mahendra highway or Arjuni phanta (Sub-headquarter). Now there are

two predator-proof fenced areas of 7.5 and 4.5 hectares in Hirapur phanta to enhance their survival before adapting to the wild habitat.

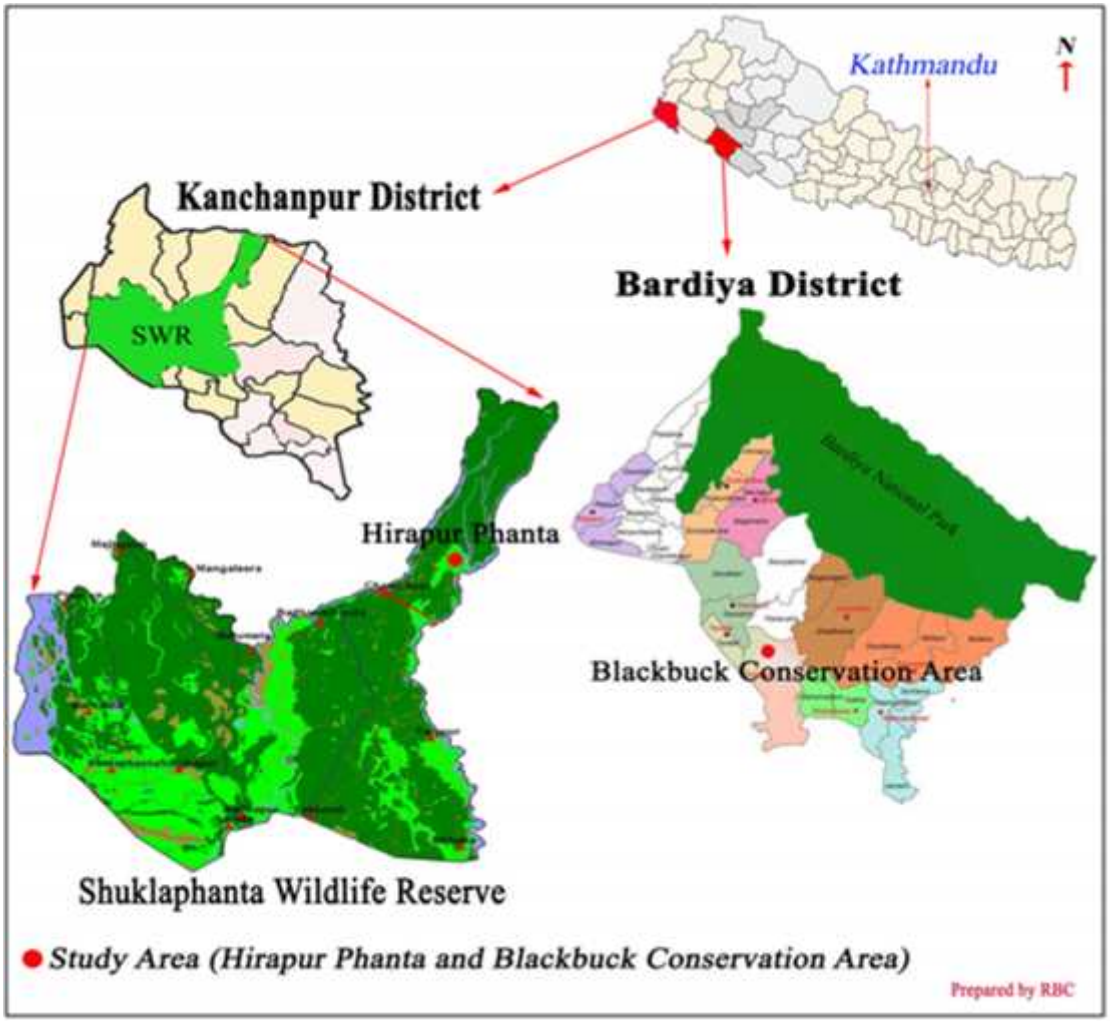


Figure: 1 **Map shows location of study area**

3.2 Materials

The materials used during research work have been listed below:

3.2.1 Materials for Laboratory

I. Beakers

II. Motor/Pistle

III. Glass rods

IV. Slides

V. Cover slips

VI. Volumetric flask

VII. Tea strainer

VIII. Measuring cylinder

IX. Droppers

X. Toothpicks

XI. Cello tape

XIII. Centrifuge tubes

XIV. Incubator

XV. Electric balance

XVI. Refrigerator

XVII. Electric microscope

XVIII. Cottons

XIX. Gloves and Mask

XX. Stage micrometer

XXI. Oculomicrometer

3.2.2 Materials for field

I. Sterile vials

II. Binocular

III.Camera

3.3 Chemicals

I. Potassium dichromate ($K_2Cr_2O_7$)

II. Distilled water (D/W)

III. Normal saline

IV. Saturated NaCl solution

V. Saturated ZnCl solution

VI. Methylene blue

VII. Lugol's Iodine solution.

3.4 Study Design

The cross-sectional study was designed to access the parasitic infection among blackbuck of BCA and SWR. The study includes a) Selection of animal's habitat. b) Collection of fresh pellet samples in sterile vials. c) Preservation of faecal samples in 2.5% Potassium dichromate solution. d) Examination of faecal samples by using floatation, sedimentation and Stoll' counting technique. e) Identification and measurement of eggs/cysts of different parasites.

3.4.1 Sample size

Out of total 220 fresh faecal samples of blackbuck, 70 samples were collected from Hirapur phanta of SWR, Kanchanpur and 150 samples from BCA, Khairapur, Bardiya from June to August 2013.

3.4.2 Sample collection method

At first, the pellets of blackbuck as well as their position of defecation and urination according to sex to collect the fresh faecal samples with help of blackbuck's caretaker (Plate: 4-11). In the next day early morning or sunset time, proper care was taken when an animal defecated and sex was identified, then the fresh pellet sample was collected in sterile vial from field immediately to prevent contamination (Plate: 12 & 13). After collection of faecal sample, the samples containing vial were marked.

3.4.3 Preservation of samples

Collected faecal samples of blackbuck were preserved in 2.5% Potassium dichromate that help in maintaining the morphology of protozoan parasites and preventing further development of some helminthic eggs and larvae (Plate: 14).

3.5 Microscopic Examination

All samples were examined at the laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur (Plate: 15). The samples were processed for microscopic examination. The ova/cysts/oocysts and larvae of different parasites were identified according to the morphology and quantitative estimation by using saline and iodine wet mount and concentration methods (floatation and sedimentation) and Stoll's counting technique to determine EPG/CPG/OPG of faeces (Soulsby 1982).

3.5.1 Saline wet mount

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

3.5.2 Iodine wet mount

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

3.5.3 Concentration method

Eggs/cysts are often low number in faeces that they are difficult to be detected in direct smears or mounts. Therefore, this procedures were performed which incudes floatation and sedimentation techniques (Soulsby 1982).

Plate: 1 Male pellets

Plate: 2 Fawn pellets

Plate: 3 Female pellets

Plate: 3 Female pellets

Plate: 4 Urinating position of Male

Plate: 5 Defecating positon of Male

Plate: 6 Urinating position of female

Plate: 7 Defecating position of female

Plate: 8 Urinating position of male fawn

Plate: 9 Defecating position of male or urinating position of female fawn

Plate: 10 Defecating position of female fawn

Plate: 11 Help in detection of blackbuck's pellet with Mr. Hari Ram Yadav

Plate: 12 Collection of female pellets

Plate: 13 Collection of male pellets

Plate: 14 Sample preservation

Plate: 15 Sample examination

Plate: 16 Fighting between adult males

Plate: 17 Jumping of blackbucks in SWR

a. Floatation technique

Floatation involves suspending the specimen in a medium of high density than that of the helminthes eggs and protozoans cysts. The eggs and cysts float to the top and were collected by taking a cover slip on the surface of the meniscus at the top of tube. Then it was transferred into the slide and examined under light microscope at 10X and 40X. Following floatation techniques were applied i.e. saturated salt and Zinc sulphate floatation techniques (Soulsby 1982).

b. Sedimentation technique

Concentration of intestinal parasites by this technique, using either gravity or centrifugation leads to a good recovery of protozoan cysts and helminthes eggs. Both eggs and cysts of parasites were settled and concentrated at the bottom because they have high density than the suspending medium. The residues were tested using saline wet mount and iodine wet mount (Soulsby 1982).

3.5.4 Modified Stoll's counting technique

The fecal sample was first well mixed and then 3 grams of feces were weighed with the help of a weighing balance and put in 100 ml graduated beaker. The beaker was then filled with water up to 45 ml mark. The feces were thoroughly mixed with water by a stirrer. The mixture was strained with a tea strainer. The strained mixture was again shaken and 0.15ml of suspension was taken onto a slide and covered with a cover slip. Then the slide was placed under a microscope and intestinal parasitic eggs were identified and counted. Total number of eggs of parasites found in the slide was multiplied by 100 to get the eggs per grams of faeces (Soulsby 1982).

3.6 Measurement of Diameter of Eggs/Ocysts/Cysts

The eggs/ocysts/cysts were identified in faecal samples of blackbuck, on the basis of their shape and size. The length and width of eggs/ocysts/cysts were measured by using a micrometer (Cable 1965).

3.7 Data Analysis

Since the study was mainly focused on identification of different gastrointestinal parasites, the data were analyzed by using MS-Excel 2007 and SPSS 19.

4. RESULTS

4.1 Gastrointestinal Parasites of Blackbuck in BCA

During the study period, a total of 150 faecal samples of blackbuck (76 males, 51 females and 23 fawns) were taken from Blackbuck Conservation Area, Khairapur, Bardiya and examined by using floatation, sedimentation and Stoll's counting techniques. Gastrointestinal parasites were found to be 90%.

4.1.1 Protozoan parasites

Out of 150 samples, 55.33% samples were found to be positive for protozoan parasites. In this study, 82.61% fawns, 62.75% females and 42.11% males were found to be infected with protozoan parasites. Among the intestinal protozoan parasites identified, *Eimeria* without micropile (39.33%) showed the highest prevalence followed by *Eimeria* sp. with micropile (26%) and *Entamoeba* (20%). According to sex, the present study highlights that these parasites were most prevalent in fawns than females and males (Table: 1). It shows that the existence of these parasites among fawn blackbuck. Protozoan parasites are statistically significant between sexes ($P= 0.001$, $\chi 2= 13.437$ at d.f. = 2).

Table:1 Protozoan parasites

S.N.	Class	Parasites	Male (%) n=76	Female (%) n=51	Fawn (%) n=23	Total (%) n=150
1.	Sarcodina	*Entamoeba*	8 (10.53%)	15 (29.41%)	7 (30.43%)	30 (20.00%)
2.	Sporozoa	*Eimeria* with micropile	17 (22.37%)	14 (27.45%)	8 (34.78%)	39 (26.00%)
		Eimeria without micropile	29 (38.16%)	19 (37.25%)	11 (47.83%)	59 (39.33%)

4.1.2 Trematodes

Prevalence of trematodes observed in blackbuck of BCA was 34.67% during the study. There were two different trematode parasites identified with high prevalence of *Paramphistomum* (25.33%) compared to *Fasciola* (17.33%). Sexwise parasitic infections revealed that 38.16% males, 37.25% females and 17.39% fawns were found to be

infected**.** It was found that there was no significant difference between sexes with regard to the prevalence of trematodes in blackbuck of BCA (P= 0.166, $\chi 2$= 3.590 at d.f. = 2). *Paramphistomum* (27.63%) was observed in male while *Fasciola* (21.56%) in female followed by fawns as shown in Table: 2.

Table: 2 Trematodes

S.N.	Parasites	Male (%) n=76	Female (%) n=51	Fawn (%) n=23	Total (%) n=150
1.	*Paramphistomum*	21 (27.63%)	14 (27.45%)	3 (13.04%)	38 (25.33%)
2.	*Fasciola*	14 (18.42%)	11 (21.56%)	1 (4.35%)	26 (17.33%)

4.1.3 Cestodes

Out of 150 samples examined, only one species of cestode (*Moniezia*) was recovered in 14% samples of blackbuck of BCA. This study showed that females (17.65%) and fawns (17.39%) were highly infected by *Moniezia* followed by males (10.53%). Statistically, there was no significant difference between sexes (P= 0.462, $\chi 2$= 1.545 at d.f. = 2).

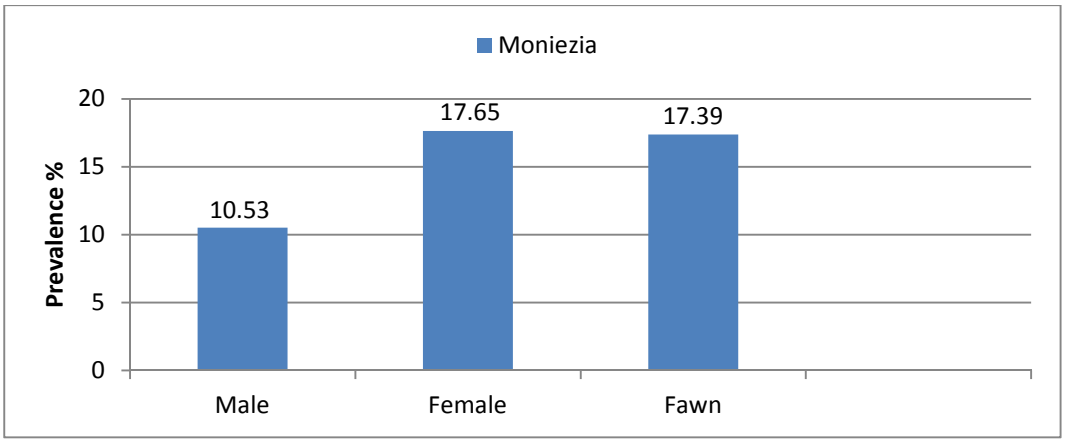


Figure: 2 Cestode (*Moniezia)* in blackbuck of BCA

4.1.4 Nematodes

In 150 samples, 89.33% samples were found to be positive for seven different nematode parasites. Among them, *Trichostrongylus* (75.55%) was the highly prevalent in blackbuck of BCA followed by *Ascaris* (57.33%), *Haemonchus* (18%), *Strongyloides* (16%), *Bunostomum* (12.67%), *Trichuris* (6%) and *Oxyuris* (4.67%). According to sexes, 90.79%

males, 90.20% females and 82.61% fawns were found to be infected with these nematodes which were not statistically significant (P= 0.522, $\chi 2$=1.300 at d.f. = 2). *Trichostrongylus*, *Haemonchus* and *Bunostomum* were most prevalent in males, *Ascaris* in females and *Strongyloides, Trichuris* and *Oxyuris* in fawns as shown in Table: 3.

Table: 3 Nematodes

S.N.	Parasites	Male (%) n=76	Female (%) n=51	Fawn (%) n=23	Total (%) n=150
1.	*Trichostrongylus*	64 (84.21%)	37 (72.55%)	12 (52.17%)	113 (75.33%)
2.	*Ascaris*	36 (47.37%)	37 (72.55%)	13 (56.52%)	86 (57.33%)
3.	*Haemonchus*	17 (22.37%)	9 (17.65%)	1 (4.35%)	27 (18.00%)
4.	*Strongyloides*	12 (15.79%)	8 (15.69%)	4 (17.39%)	24 (16.00%)
5.	*Bunostomum*	18 (23.68%)	0.000	1 (4.35%)	19 (12.67%)
6.	*Trichuris*	3 (3.95%)	4 (7.84%)	2 (8.69%)	9 (6.00%)
7.	*Oxyuris*	1 (1.32%)	4 (7.84%)	2 (8.69%	7 (4.67%)

4.1.5 Mixed infection

Blackbucks of BCA (90%) were found to be infected with different GI parasites. Single infection was not found in any samples. Multiple infections showed the highest rate (36%) among three infections. Double infection was most prevalent in males, triple in fawns and multiple in females observed in this study. Double, triple and multiple infections were statistically significant in between sexes (P= 0.000, $\chi 2$= 2.226 at d.f. = 6 for double, P= 0.000, $\chi 2$= 2.209 at d.f. = 6 for triple and P= 0.000, $\chi 2$= 2.258 at d.f. = 6 for multiple).

Table: 4 Types of infection

S.N.	Type of infection	Male (%)	Female (%)	Fawn (%)	Total (%)
1.	Single	-	-	-	-
2.	Double	23 (30.26%)	10 (19.61%)	6 (26.09%)	39 (26.00%)
3.	Triple	20 (26.32%)	14 (27.45%)	8 (34.78%)	42 (28.00%)
4.	Multiple	26 (34.21%)	23 (45.10%)	5 (21.47%)	54 (36.00%)

4.1.6 Intensity of parasitic infection

In BCA, heavy parasitic infection was considered in those samples which has 6 or more ova or oocyst observed per field. Among the total positive samples, *Eimeria* (0.67%) belongs to sporozoa; *Paramphistomum* (0.67%) belongs to trematoda and *Trichostrongylus* (2%) and *Ascaris* (1.33%) belongs to nematoda revealed heavy infection. While maximum numbers of blackbuck were infected with light infection which was considered < 2 ova or oocyst observed per field. Similarly, slightly less but not minimum numbers of blackbuck were found to be infected with mild i.e. 2-4 ova/oocyst per field than light and minimum numbers of blackbuck to be infected with moderate infection i. e. 4-6 ova/oocyst per field than mild.

Table: 5 Intensity of parasitic infection

Class	Parasites	Light (+)	Mild (++)	Moderate (+++)	Heavy (++++)
Sarcodina	*Entamoeba*	18 (12%)	12 (8.00%)	-	-
Sporozoa	*Eimeria*	40 (26.67%)	23(15.33%)	4 (2.67%)	1 (0.67%)
Trematoda	*Paramphistomum*	16 (10.67%)	17(11.33%)	4 (2.67%)	1 (0.67%)
	Fasciola	14 (9.33%)	9 (6.00%)	3 (2.00%)	-
Cestoda	*Moniezia*	13 (8.67%)	6 (4.00%)	2 (1.33%)	-
Nematoda	*Trichostrongylus*	70 (46.67%)	32(21.33%)	8 (5.33%)	3 (2.00%)
	Ascaris	47 (31.33%)	30 (20%)	7 (4.67%)	2 (1.33%)
	Haemonchus	17 (11.33%)	8 (5.33%)	2 (1.33%)	-
	Stongyloides	20 (11.33%)	3 (2.00%)	1 (0.67%)	-
	Bunostomum	17 (11.33%)	2 (1.33%)	-	-
	Trichuris	8 (5.33%)	1 (0.67%)	-	-
	Oxyuris	6 (4.00%)	1 (0.67%)	-	-

4.2 Gastrointestinal Parasites of Blackbuck in SWR

During this study, a total of 70 faecal samples of blackbuck were collected from Hirapur phanta of Shuklaphanta Wildlife Reserve, Kanchanpur and examined by using previously menframed techniques. Gastrointestinal parasites were found to be 90%.

4.2.1 Protozoan parasites

Out of 70 samples examined, protozoan parasites were found to be 55.71%. Among them 57.14% males, 50% females and 64.29% fawns were found to be positive for one or other types of protozoan parasites. Although protozoan infection were not statistically significant in between sexes (P= 0.667, $\chi 2$= 0.811 at d.f. = 2). Among them *Eimeria* sp. without micropile (44.29%) was most prevalent protozoan in blackbuck followed by *Eimeria* sp. with micropile (32.86%) and *Entamoeba* (8.57%). *Entamoeba* seriously infects the females than the males and fawns while *Eimeria* infect the fawns than males and females (Table: 6).

Table: 6 Protozoan parasites

S.N.	Class	Parasites	Male (%) n=28	Female (%) n=28	Fawn (%) n=14	Total (%) n=70
1.	Sarcodina	*Entamoeba*	2 (7.14%)	3 (10.71%)	1 (7.14%)	6 (8.57%)
2.	Sporozoa	*Eimeria* with micropile	7 (25.00%)	9 (32.14%)	7 (50.00%)	23 (32.86%)
		Eimeria without micropile	13 (46.43%)	11 (39.29%)	7 (50.00%)	31 (44.29%)

4.2.2 Trematodes

Three different trematodes were identified in 50% positive samples. *Paramphistomum* (38.57%) showed most prevalent among these trematode parasites. Among them 64.29% females were found to be more infected than males (50%) and fawns (42.86%) which is statistically significant (P= 0.000, $\chi 2$= 1.647 at d.f. = 6). Males were mostly infected by *Fasciola* while females by *Paramphistomum* followed by fawns but infection rate of *Schistosoma* were similar in all sexes (Table: 7).

Table: 7 Trematodes

S.N.	Parasites	Male (%) n=28	Female (%) n=28	Fawn (%) n=14	Total (%) n=70
1.	*Paramphistomum*	11 (39.29%)	14 (50.00%)	2 (14.29%)	27 (38.57%)
2.	*Fasciola*	8 (28.57%)	5 (17.86%)	2 (14.29%)	15 (21.43%)
3.	*Schistosoma*	2 (7.14%)	2 (7.14%)	1 (7.14%)	5 (7.14%)

4.2.3 Nematodes

A total of five different nematode parasites were identified in 81.43% positive samples. Among them *Trichostongylus* (55.71%) was the most prevalent namatodes in blackbuck followed by *Ascaris* (38.57%), *Haemonchus* (14.28%), *Strongyloides* (12.86%) and *Bunostomum* (2.86%). According to sexes 78.57% males, 82.14% females and 85.71% fawns were found to be positive for nematode parasites. Sexwise distribution of the nematode parasites showed statistically significant difference (P= 0.000, $\chi 2$= 1.507 at d.f. =6). *Trichostrongylus* and *Strongyloides* were most prevalent in females, *Ascaris* in males while *Haemonchus* in fawns but *Bunostomum* shows similar prevalence in both males and females.

Table: 8 Nematodes

S.N.	Parasites	Male (%) n=28	Female (%) n=28	Fawn (%) n=14	Total (%) n=70
1.	*Trichostrongylus*	14 (50.00%)	17 (60.71%)	8 (54.14%)	39 (55.71%)
2.	*Ascaris*	13 (46.43%)	8 (28.57%)	6 (42.86%)	27 (38.57%)
3.	*Haemonchus*	4 (14.29%)	3 (10.71%)	3 (21.43%)	10 (14.28%)
4.	*Strongyloides*	2 (7.14%)	5 (17.86%)	2 (14.29%)	9 (12.86%)
5.	*Bunostomum*	1(3.57%)	1 (3.57%)	0.000	2 (2.86%)

4.1.4 Mixed infection

Out of 90% positive samples, the different types of infections were encountered in blackbuck of SWR. Table No. 9 showed that the rate of double infections was found to be

40% followed by single (8.57%), triple (17.14%) and multiple (24.28%). Double infection was most prevalent in fawns than females and males while triple in males than females and fawns but single and multiple were same in both males and females than fawns. All types of infections showed significant difference among the sexes (P= 0.000, $\chi 2$= 2.252 at d.f. = 6 for single, P= 0.000, $\chi 2$= 2.244 at d.f. = 6 for double, P= 0.000, $\chi 2$= 2.219 at d.f. = 6 for triple, P= 0.000, $\chi 2$= 2.202 at d.f. = 6 for multiple).

Table: 9 Types of infection

S.N.	Type of infection	Male (%)	Female (%)	Fawn (%)	Total (%)
1.	Single	3 (10.71%)	3 (10.71%)	0.000	6 (8.57%)
2.	Double	9 (32.14%)	12 (42.86%)	7 (50.00%)	28 (40.00%)
3.	Triple	6 (21.43%)	4 (14.29%)	2 (14.29%)	12 (17.14%)
4.	Multiple	7 (25.00%)	7 (25.00%)	3 (21.43%)	17 (24.28%)

4.2.5 Intensity of parasitic infection

In SWR, heavy parasitic infection was considered in those samples which has 6 or more ova or oocyst observed per field. Among the total positive samples heavy infection was not found in any samples but *Eimeria* (5.71%) belongs to sporozoa; *Paramphistomum* (4.29%) and *Fasciola* (2.86%) belongs to trematoda and *Trichostrongylus* (7.14%) and *Ascaris* (2.86%) belongs to nematode revealed moderate infection which was considered 4-6 ova/oocyst observed per field. While maximum numbers of blackbuck were infected with light infection i.e. < 2 ova or oocyst observed per field and slightly less but not minimum numbers of blackbuck were found to be infected with mild infection i.e. 2-4 ova/oocyst per field.

Table 10: Intensity of parasitic infection

Class	Parasites	Light (+)	Mild (++)	Moderate (+++)	Heavy (++++)
Sarcodina	*Entamoeba*	2 (4.86%)	4 (5.71%)	-	-
Sporozoa	*Eimeria*	20 (28.57%)	12 (17.14%)	4 (5.71%)	-
Trematoda	*Paramphistomum*	14 (20.00%)	10 (14.29%)	3 (4.29%)	-
	Fasciola	8 (11.43%)	5 (7.14%)	2 (2.86%)	-
	Schistosoma	4 (5.71%)	1 (1.43%)	-	-
Nematoda	*Trichostrongylus*	22 (31.43%)	12 (17.14%)	5 (7.14%)	-
	Ascaris	15 (21.43%)	10 (14.29%)	2 (2.86%)	-
	Haemonchus	8 (11.43%)	2 (2.86%)	-	-
	Stongyloides	7 (10.00%)	2 (2.86%)	-	-
	Bunostomum	2 (2.86%)	-	-	-

4.3 Comparative Analysis of GI Parasites of Blackbuck in Between BCA and SWR

4.3.1 Protozoan parasites

The protozoan parasites in blackbuck of BCA and SWR were compared. Among two protozoan parasites encountered, *Entamoeba* was found to be most prevalent in blackbuck of BCA compared to SWR. While with regard to *Eimeria* sp., both with micropile and without micropile *Eimeria* were comparatively higher in SWR than BCA. Statistically there were not significant difference in protozoan infection between BCA and SWR i.e. ($P= 0.958$, $\chi2= 0.003$ at d.f. = 1).

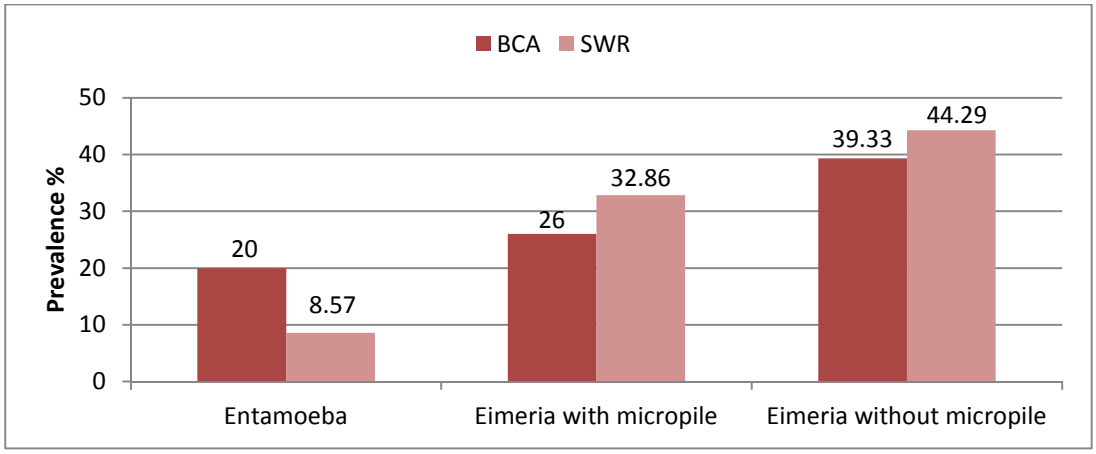


Figure: 3 Comparative studies of protozoan parasites in between BCA & SWR

4.3.2 Trematodes

Among three different trematodes identified i.e. *Paramphistomum*, *Fasciola* and *Schistosoma*, all of these parasites were found to be more prevalent in blackbuck of SWR than blackbuck of BCA. While *Schistosoma* sp. was only reported from blackbuck of SWR. Trematode parasitic infection was statistically significant in between BCA and SWR (P= 0.030, $\chi 2$=4.694 at d.f. = 2).

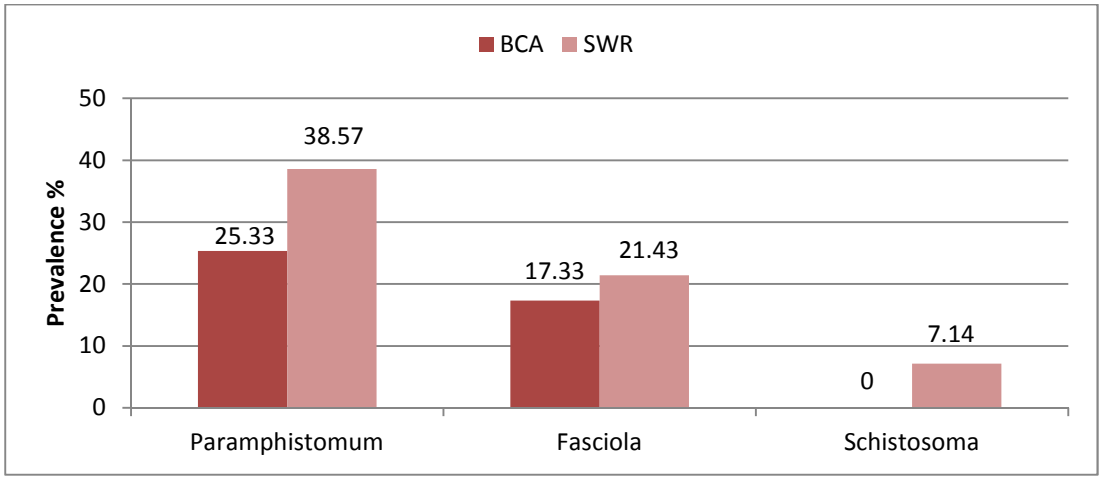


Figure: 4 Comparative studies of trematodes in between BCA and SWR

4.3.3 Nematodes

Seven different nematodes were observed during the study. All of these nematodes showed highest prevalence in blackbuck of BCA than blackbuck of SWR. While two nematodes, *Trichuris* and *Oxyuris* were only reported in blackbuck of BCA. All of these

nematodes were statistically significant in between BCA and SWR (P= 0.000, χ2= 4.331 at d.f. = 4).

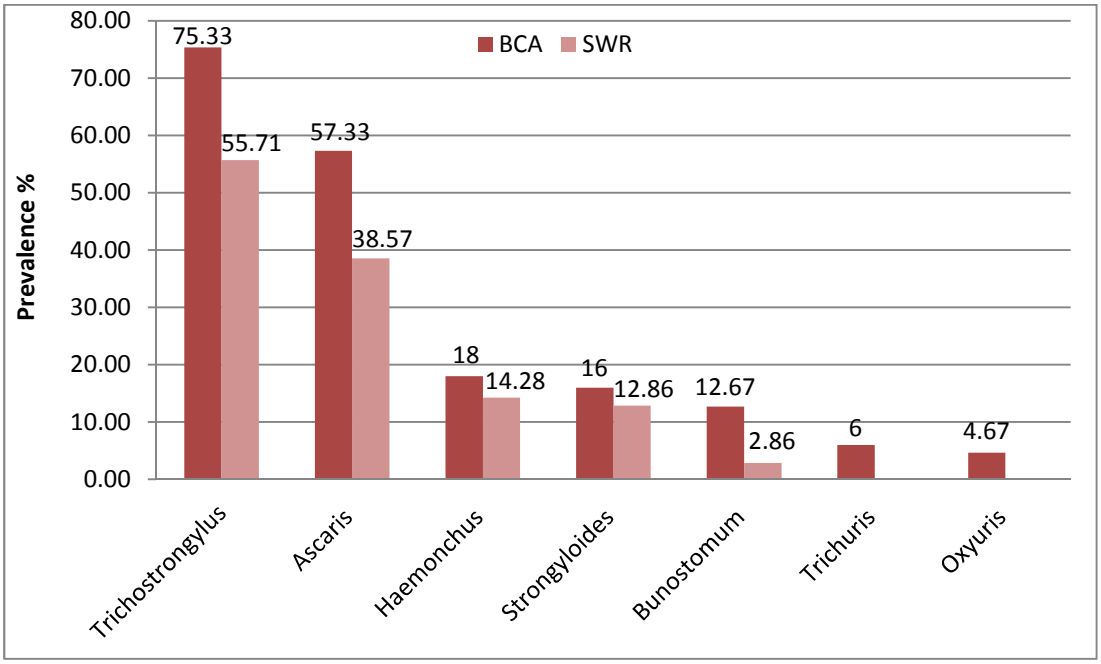


Figure: 5 Comparative studies of nematodes in between BCA and SWR

4.3.4 Mixed infection

Double infection was most prevalent in blackbuck of SWR (40%) than BCA (26%) while multiple (36%) in BCA as compared to the SWR. Single and double infections were significant (P= 0.000, χ2= 13.218 at d.f. = 1 for single and P= 0.036, χ2= 4.417 at d.f. = 1 for double) but triple and multiple infections were not significant (P= 0.081, χ2= 3.038 at d.f. = 1 for triple and P= 0.083, χ2= 2.996 at d.f. =1 for multiple) in between BCA and SWR.

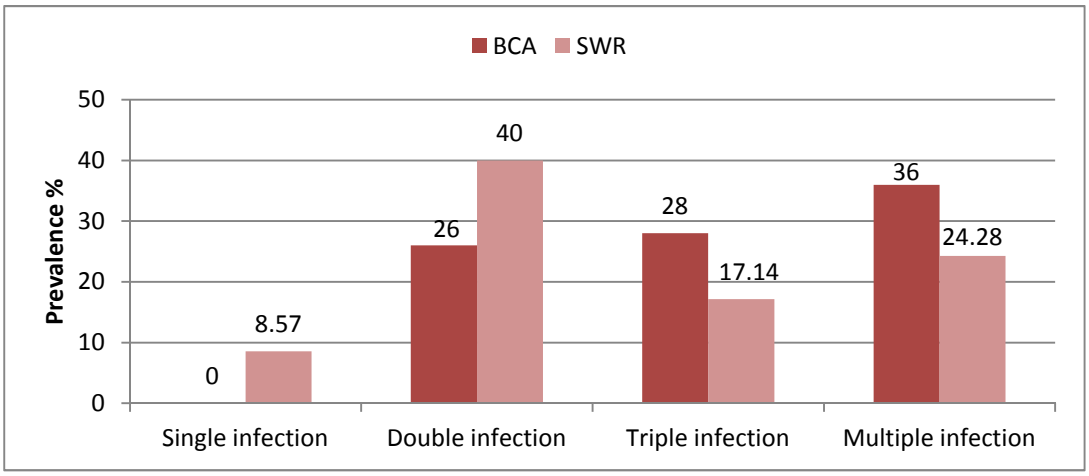


Figure: 6 Comparative studies of mixed infection in between BCA and SWR

4.4 Parasitic Load in Blackbuck of BCA and SWR

The parasitic load in blackbuck was assessed based on the number of eggs/oocysts. The mean of EPG/OPG/CPG was calculated in all of the positive samples and the ranges are shown in Table: 11. Among protozoans, the highest CPG (300) was found in blackbuck due to *Eimeria* sp. than *Entamoeba* sp. Among trematodes, the highest parasitic load of *Paramphistomum* sp. was found to be 450 followed by *Fasciola* sp. and *Schistosoma* sp. while among nematodes, the highest EPG of *Trichostrongylus* was 700 which is followed by *Ascaris* (400), *Strongyloides*, *Haemonchus*, *Bunostomum*, *Trichuris* and *Oxyuris*.

Table 11: Parasitic load in blackbuck of BCA and SWR

S.N.	Class	Name of genera	Parasitic load (EPG/OPG/CPG)	
			Range	Average
1.	Sarcodina	*Entamoeba*	200-300	250
2.	Sporozoa	*Eimeria*	200-400	300
3.	Trematoda	*Paramphistomum*	200-700	450
		Fasciola	100-600	350
		Schistosoma	100-300	200
4.	Cestoda	*Moniezia*	100-600	350
5.	Nematoda	*Trichostrongylus*	100-1300	700
		Ascaris	300-500	400
		Haemonchus	100-400	250
		Stongyloides	200-400	300
		Bunostomum	100-200	150
		Trichuris	100	100
		Oxyuris	100-300	200

4.5 Diameter of Eggs/Cysts of Different GI Parasites of Blackbuck

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

- ***Eimeria* sp.**

Diameter of oocyst of *Eimeria* sp. was 34.5 ± 19.5 µm according to without micropile (Plate 18) while 27 ± 12 µm with micropile (Plate 19). Eggs are small in size, pink in colour and contain morula which is located centrally or sub-centrally or completely filled up. Micopile occur in one side.

- ***Entamoeba* sp.**

Eggs are small, rounded or spherical in shape having four nucleus and measure 30 ± 6 µm in diameter (Plate 20; Photo: 1).

- ***Fasciola* sp.**

Eggs are 135 ± 15 × 79.5 ± 4.5 µm in size, large, oval in shape, yellowish brown in colour, thin shelled, unsegmented containing an ovum and cluster of yolk cells, morula located sub-centrally, operculum usually indistinct (Plate 20; Photo: 2).

- ***Paramphistomum* sp.**

The eggs of it is 165 ± 5 × 82.5 ± 7.5 µm in size, operculum in one pole, pale grey or greenish in color, contains five blastomeres surrounded by about 50 yolk cells, morula located centrally or somewhat subcentrally (Plate 20; Photo: 3).

- ***Schistosoma* sp.**

Eggs are 147 ± 3 × 75 µm in size, spindle shaped, flattened at one side, greatly elongated with straight slender posteror spine at another end (Plate 20; Photo: 4).

- ***Moniezia* sp.**

Eggs are triangular or quadriangular in shape; somewhat irregular having a circular or pear shaped (pyriform) apparatus at one end and measure 70.5 ± 4.5 µm in size (Plate 20; Photo: 5).

- ***Trichostrongylus* sp.**

An egg are 81 ± 6 × 48 ± 12 µm in size, oval or kidney bean shaped with thin and transparent outer shell and wrinkled inner membrane, bilaterally symmetrical, colourless, central mass usually in 8-12 cell stages or multisegmented and varies from 16-32 in number. The space between the egg shell and embryonic mass is relatively conspicuous. One side is more rounded than the other or somewhat both sides rounded (Plate 21; Photo: 6).

- ***Ascaris* sp.**

Eggs are 31.5 ± 7.5 µm in size, nearly spherical, yellowish brown, granular contents and unsegmented, thick aleveolated albuminous shell (Plate 21; Photo: 7).

- ***Strongyloides* sp.**

Eggs are small, measure 88.5 ± 7.5 × 52.5 ± 7.5 µm in size, oval with round edges or elipsoidal, thin shelled and contain fully developed larvae that can be seen under low power (Plate 21; Photo: 8).

- ***Trichuris* sp.**

An eggs are in 75 × 36 µm size, contains unsegmented embryo, brown in colour, barrel shaped with a transparent plug at either pole (Plate: 21; Photo: 10).

- ***Haemonchus* sp.**

Eggs are oval, thin shelled, hyaline, elongated, larger and rounder than *Ostertagia* and measure 80 ± 5 × 48 ± 12 µm in size. They are already segmented (embryonated) when deposited and resemble with those of *Trichostrongylus* (Plate 22; Photo: 11).

- ***Bunostomum* sp.**

Eggs are medium to large containing 4-8 blastomeres, sometimes the walls becomes thick and rectangular and measure 95 ± 5 × 46.5 ± 1.5 µm in size (Plate 22; Photo: 12).

- ***Oxyuris* sp.**

Eggs are bean and oval shaped having segmented embryonic mass surrounded by stiky fluid and measure 112.5 ± 7.5 × 55.5 ± 19.5 µm in size (Plate 22; Photo: 13).

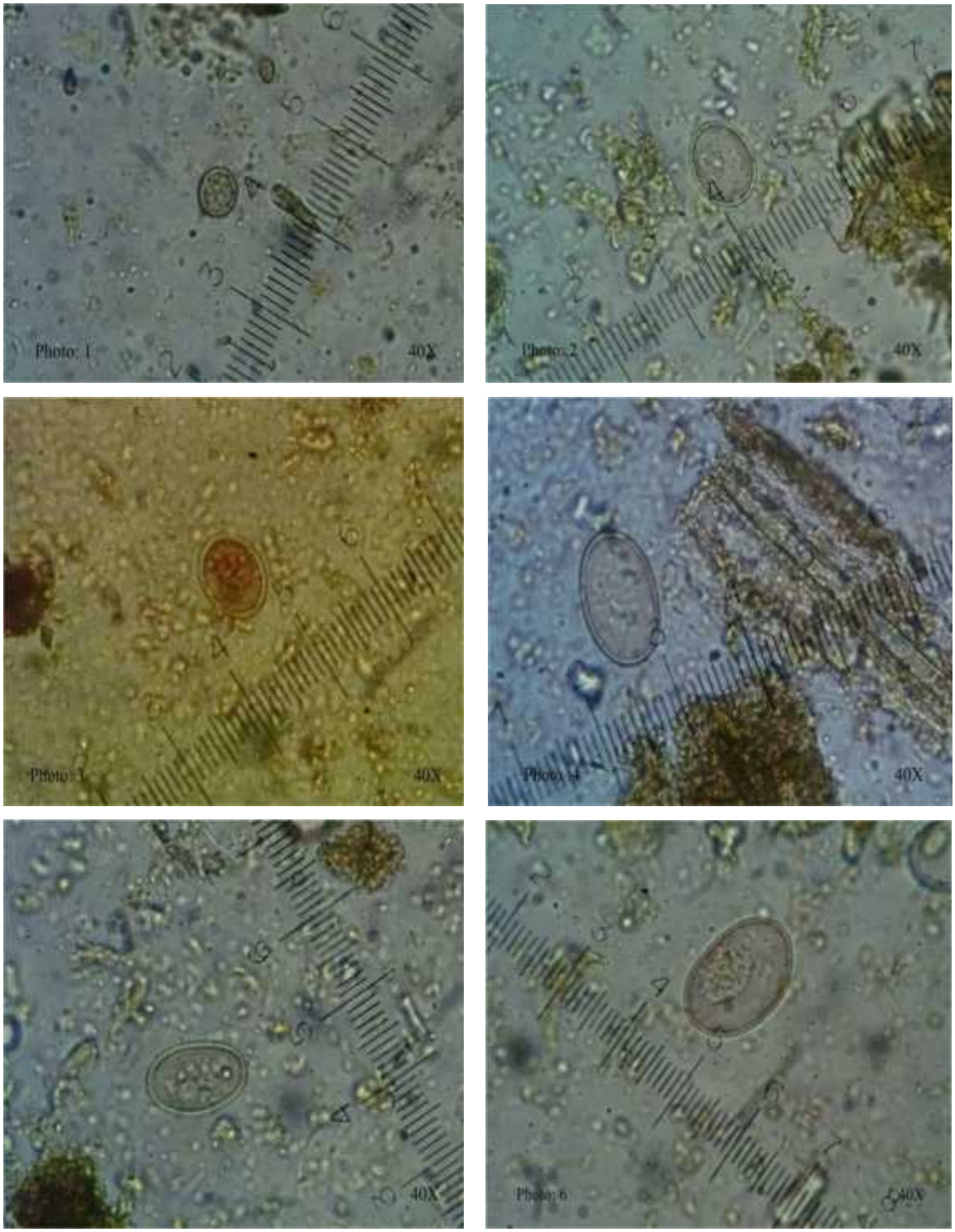


Plate 18: Oocysts of *Eimeria* sp. without micropile; Photo: 1 (15µm), Photo: 2 (33µm), Photo: 3 (30µm), Photo: 4 (45µm), Photo: 5 (36µm) and Photo: 6 (54µm).

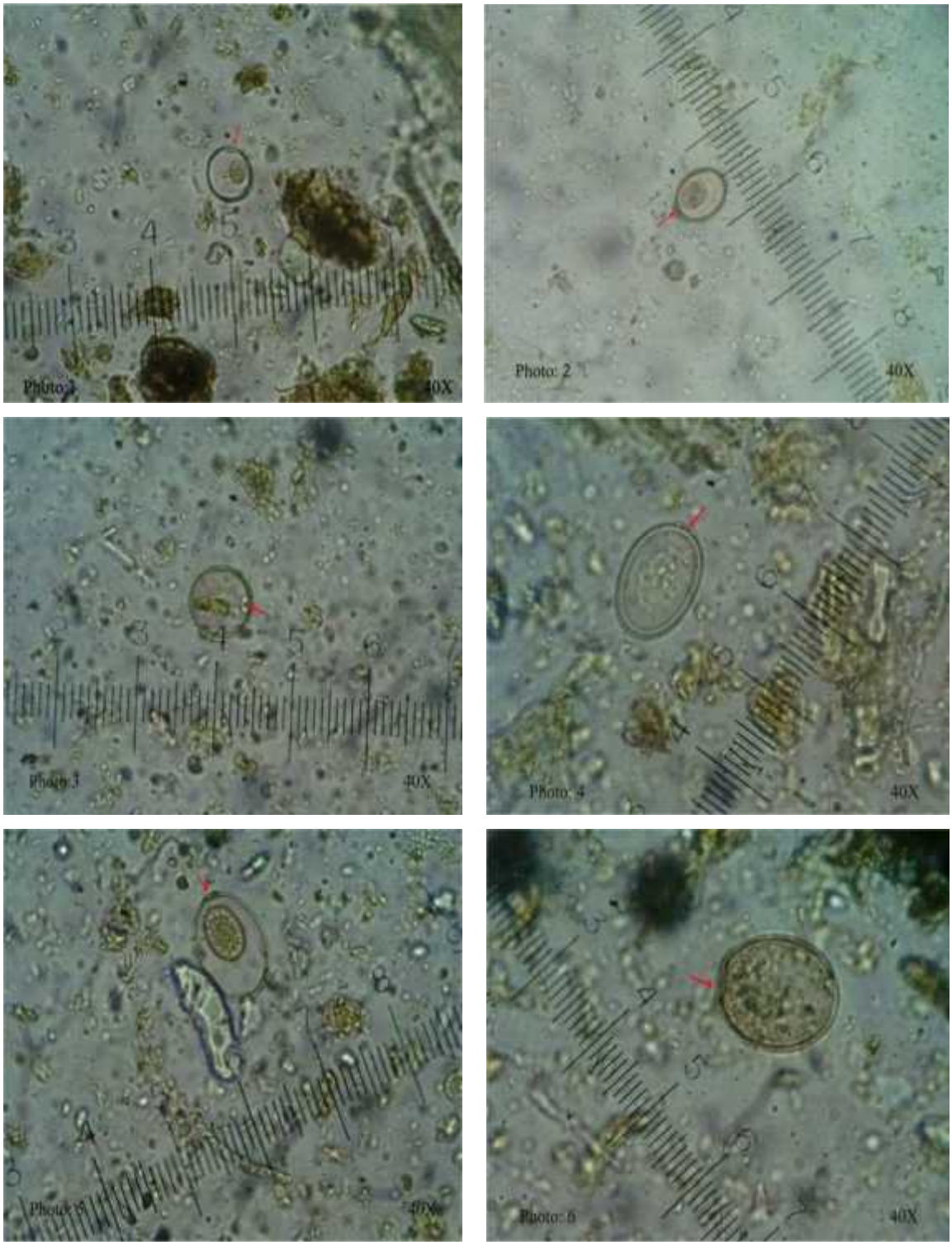


Plate 19: Oocysts of *Eimeria* sp. with micropile; Photo: 1 (15µm), Photo: 2 (18µm), Photo: 3 (21µm), Photo: 4 (39µm), Photo: 5 (30µm) and Photo: 6 (33µm)

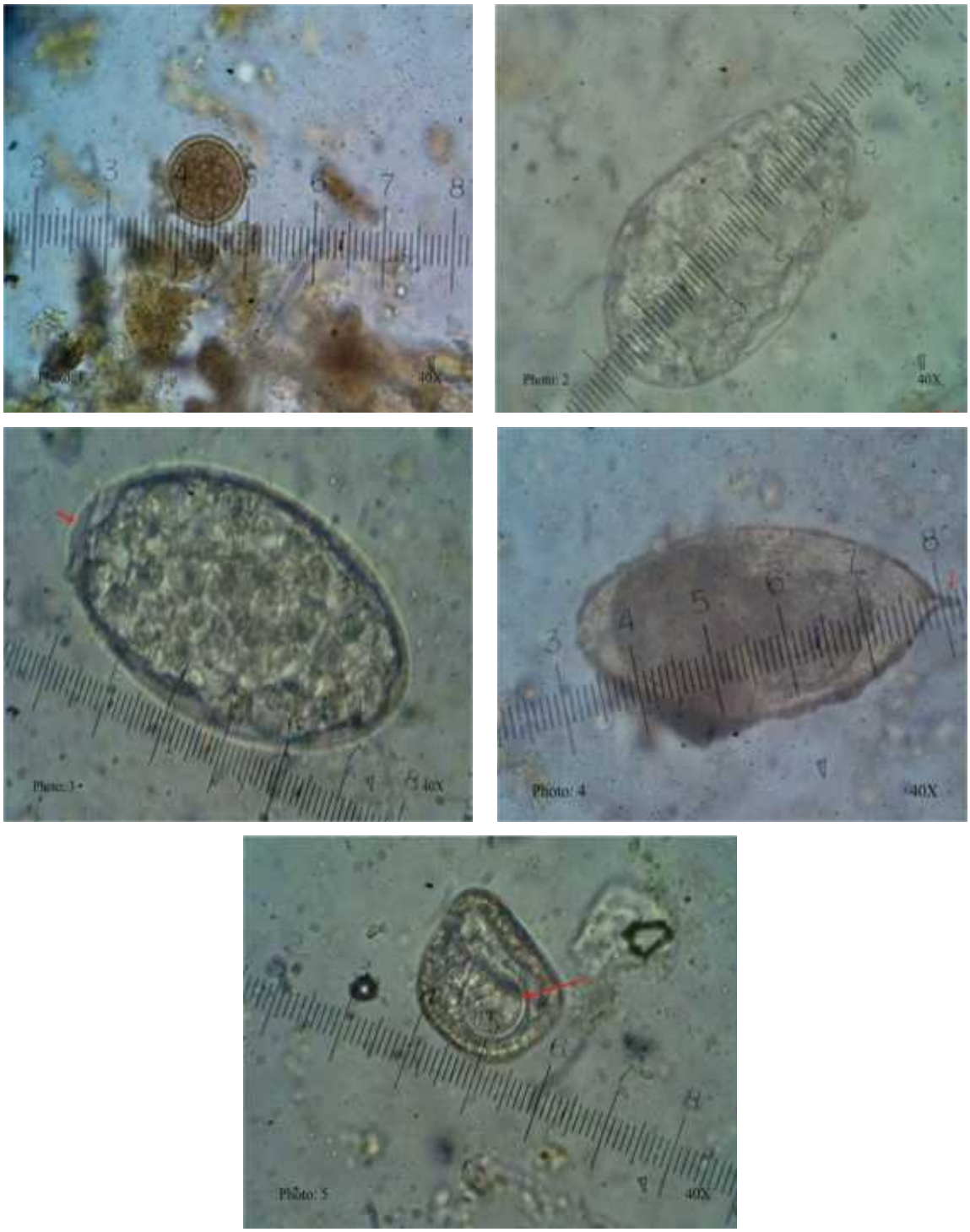


Plate 20: **Cysts/Eggs of different parasites** Photo:1 *Entamoeba* sp. (36µm), Photo: 2 *Fasciola* sp. (150µm), Photo: 3 *Paramphistomum* sp. (165µm), Photo:4 *Schistosoma* sp. (147µm), Photo: 5 *Moniezia* sp. (66µm).

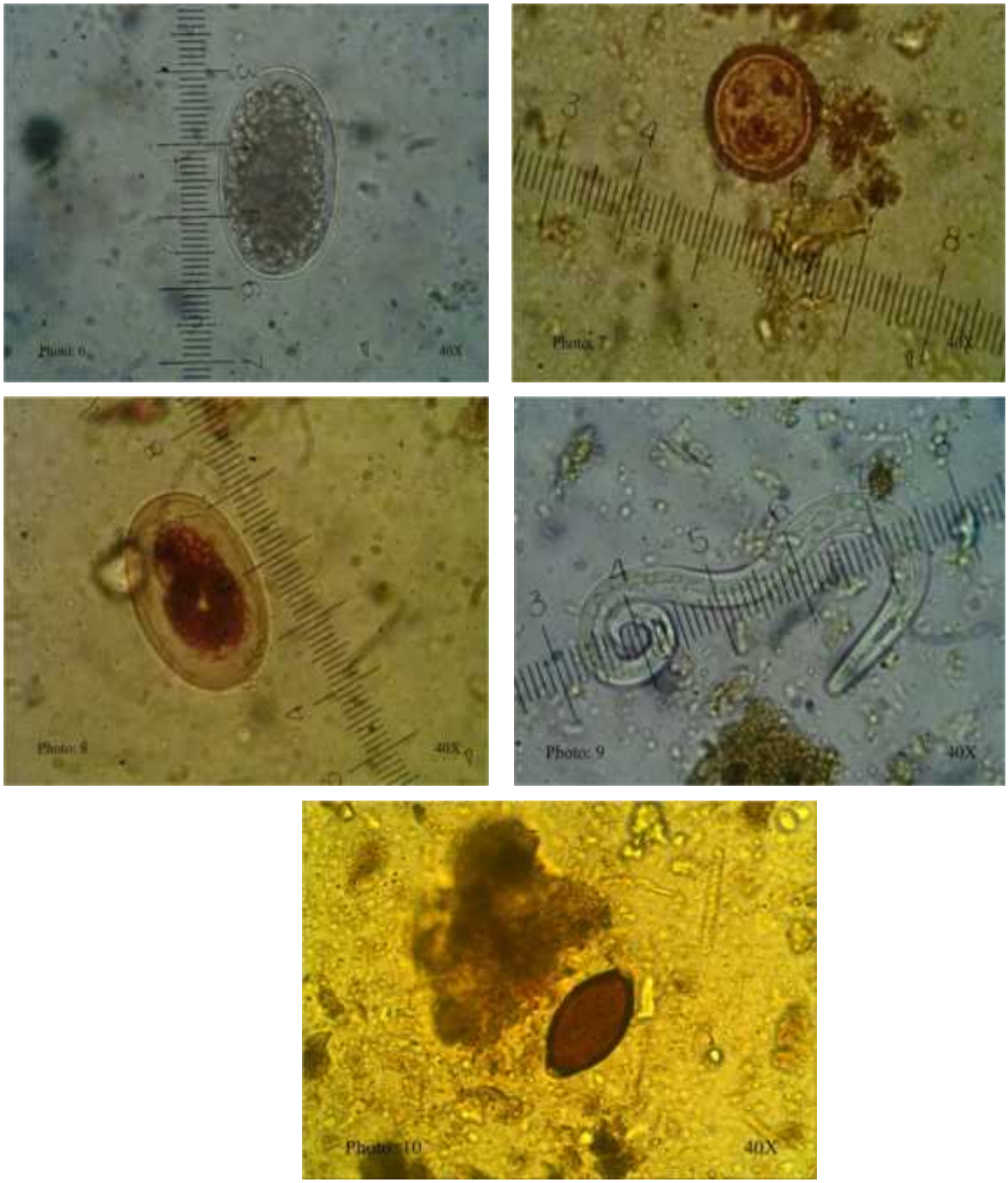
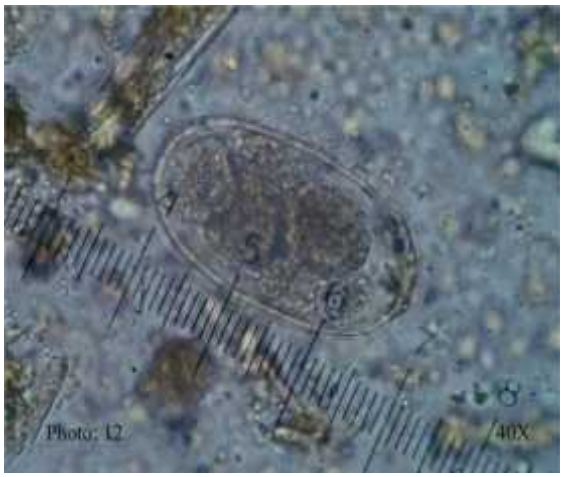


Plate 21: **Eggs of different nematodes;** Photo: 6 *Trichostrongylus* sp. (87µm), Photo: 7 *Aascaris* sp. (39µm), Photo: 8 *Strongyloides* sp. (81µm), Photo: 9 *Strongyloides* larva and Photo: 10 *Trichuris* sp. (75µm).



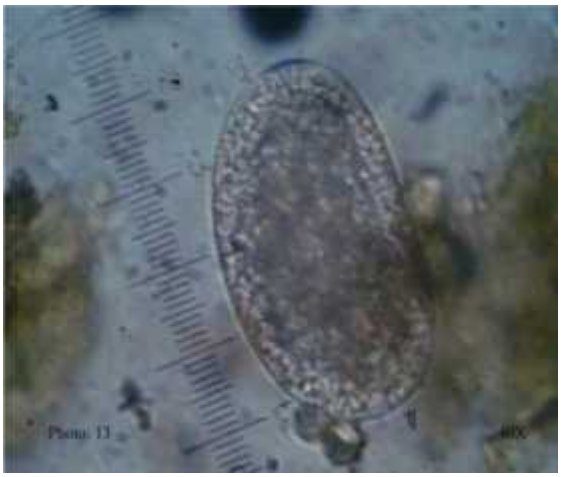


Plate 22: Eggs of different nematodes; Photo:11 *Haemonchus* sp. (90µm), Photo: 12 *Bunostomum* sp. (87µm) and Photo: 13 *Oxyuris* sp. (120µm).

5. DISCUSSION

Blackbuck (*Antilope cervicapra*) is near threatened elegant gazelle like animal (IUCN 2008) regarded as the most handsome member of the "Bovidae" family. The blackbuck (*Antilope cervicapra*) is locally known as Krinashar. It is endemic to the South Asian continent where it is the sole reprehensive of the genus "*Antilope*". It was once the most ubiquitous of the larger wild animals of this region. It is hoofed mammals or ungulates having two or four weight bearing toes on each foot. Hence, the name even toed ungulates. Blackbuck is one of the protected species under National Park and Wildlife Conservation Act 1973.

In many parts of the world, the protection of wild life has been undertaken through establishment of protected or conservation areas including parks and wildlife or nature reserves. Protected or conservation areas are locations which receive protection because of their recognized natural, ecological or cultural values. It is essential for biodiversity conservation, often providing habitats and protection for hunting for threatened and endangered species. According to IUCN (2008), protected area is a geographical space, recognized, dedicated and managed through legal or other effective means, to achieve the long term conservation of nature with associated ecosystem services and cultural values. There are over 161,000 protected or conservation areas in the world while just over 1842 national parks in the world (Protected Planet 2010, www.en.m.wikipedia.org).

In Nepal, 10 national parks, 3 wildlife reserves, six conservation areas and one hunting reserve have been established for the protection of wildlife (DNPWC 2012). A conservation area represents human's sublime idea of giving space and resource for the other species to live in an un-interfered manner (DNPWC 2011). A total of 181 mammal species have been recorded to occur in Nepal (DNPWC 2012).

In Nepal, Blackbuck Conservation Area (BCA), one of the six conservation areas is located at Khairapur of Bardiya district covering 16.95 sq. km. This is the first organized effort to conserve the blackbuck (*Antilope cervicapra*). But now they are also conserved in two predator-proof fenced areas of 7.5 and 4.5 hectares in Hirapur phanta of Shukalaphanta Wildlife Reserve (SWR) to ensure their survival before adapting to the wild habitat. Blackbucks were found in India, Pakistan and Nepal (Lydekker 1924). About 43,600 blackbucks were introduced to Argentina and the USA (Mallon and

Kingswood 2001).and about 45,000 was reported in India (Ranjitsinh 1989). Twenty eight blackbucks were reintroduced to Hirapur phanta of SWR, Kanchanpur (DNPWC 2012). In 2012, the 278 blackbucks were estimated in Blackbuck Conservation Area, Khairapur, Bardiya (Shah 2012). At the time of study period, 300 number of blackbucks were found in BCA, Khairapur, Bardiya whereas 38 blackbucks in SWR, Kanchnapur.

In the present study, the gastrointestinal parasites were found to be 90% in blackbuck of BCA and SWR. This prevalence rate was low as compared to 97.06% and 100% GI parasites (Thapa 2013). But it was higher than 85%, 76.92, 76.6%, 73.02% and 72.5% observed by Seong-Jun et al. (2011), Rahman and Mustafizur (2012), Opara et al. (2010), Ban (2012) and Corden et al. (2008) respectively. The high prevalence rates may be due to the fact that they took faecal samples from different host. In the present study, 55.33% samples were found to be positive for protozoan parasites in blackbuck of BCA while 55.71% in blackbuck of SWR which showed comparatively similar to 55.77% protozoans reported by Rahaman and Mustafizur (2012). The present prevalence of protozoans were found to be less than 63.9%, 80.8%, 73.53% and 82.24% protozoans reported by Mohammad et al. (2012), Tovassoli et al. (2010) and Thapa (2013) while higher than the reports of Maesano et al. (2014), Vazquez et al. (2009) and Fagiolini et al. (2010) who revealed 50%, 35% and 25% respectively. With regrd to helminthes, 90% blackbuck of BCA and 88.57% blackbuck of SWR were found to be infected in this study which showed lowest prevalence rate as compared to 92.4% and 100% helminths reported by Kuzmina et al. (2010) and Aukstikalniene et al. (2013) while higher than 82.2%, 85.63%, and 87.5% recorded by Opara et al. (2010), Bogale et al. (2014) and Mayo et al. (2013) respectively.

In this study, highest prevalence of parasitic infection was found to be 92.16% female followed by male (90.79%) and fawn (82.61%) in blackbuck of BCA while 92.86% female followed by male (89.29%) and fawn (85.71%) in blackbuck of SWR. The present study is comparatively similar to the result of Khanal (2006) who reported that maximum numbers of female were infected with GI parasites than male and fawn but different to the report of Ban (2012) who revealed that male were highly infected followed by female and fawn. Besides these, Farooq (2009) and Farooq et al. (2012) reported as the female were highly infected than male which also close match to the result of present study.

From the economic and sanitary points of view, coccidian parasites are the most prevalent among protozoa. *Eimeria* oocysts have been reported in wild ruminants (Singh et al. 2009, Arslon and Sari 2010, Fagiolini et al. 2010, Seong-Jun et al. 2011, Saud et al. 2012, Maesano et al. 2014, Darabus et al. 2014). Diez et al. (2010) was reported seven *Eimeria* species from Galicia, Mohammad et al. 2012 reported *Eimeria saudiensis* from Suadi Arabia and Tovassoli et al. 2011 reported one or more *Eimeria* species from NW Spain. According to the previous reports, *Eimeria* can't be differentiating into species level in present study because it needs culture to identify the species of this parasite. But this parasite can be differentiating into two types of *Eimeria* (i.e. with micropile and without micropile) on the basis of morphological structure. In the present study, *Eimeria* was found to be 45.33% in blackbuck of BCA and 51.43% in blackbuck of SWR. Only, the rates of *Eimeria* in blackbuck of SWR was similar to the report of Maesano et al. (2014) who revealed as 50% which is higher than the prevalence of *Eimeria* of blackbuck of BCA. The prevalence of *Eimeria* of blackbuck of BCA and SWR were lowered as compared to 80.48%, 63.9% observed by Tovassoli et al. (2010) and Mohammad et al. 2012 but higher than 17.3% obtained by Corden et al. (2008). Due to coccidiosis, the infected ruminants generally exhibit loss of appetite, weakness, diarrhoea, etc. The diameter of oocyst of *Eimeria* sp was 34.5 $\pm$ 19.5µm according to micropile while 27 $\pm$ 12µm without micropile observed in many faecal samples of blackbuck of BCA and SWR.

At least 13 species of *Cryptosporidium* have been reported infecting mammals (Taylor et al. 2007, Radostits et al. 2008, Skerrett and Holland 2001, Corden et al. 2008, Lim et al. 2008, Fagiolini et al. 2010, Abu Samra et al. 2011, Castro-Hermida et al. 2011, Mohammad et al. 2012 and Radhy et al. 2013). It has been reported from Ireland, Spain, Malaysia, Italy, South Africa, Galicia, Saudi Arabia and Iraq. Blackbucks rose at BCA, Khairapur, Bardiya and SWR, Kanchanpur were negative for this parasites.

Some reports have indicated the presence of *Giardia, Isospora, Cyclospora* and *Blastocystis* in wild ruminants from Spain (Corden et al. 2008), Southeast Nigeria (Opara et al. 2010), Galicia (Castro-Hermida et al. 2011), and Iraq (Radhy et al. 2013). It is a potential pathogen in livestock causing diarrhoea, weight loss, poor condition and lethargy (Hunter and Thompson 2005). Olson et al. (2004) reported a high level of

Giardia infection in young livestock, especially calves. Blackbuck of BCA and SWR in Nepal were not infected with *Giardia, Isospora, Cycolospora and Blastcystis* sp.

Entamoeba sp has been recorded in captive mammals from Southeast Neigeria (Opara et al. 2010). In the present study, this parasite is also reported in blackbuck of BCA and SWR with prevalence rate 20% and 8.57% respectively. The size of *Entamoeba* (24-36µm) obtained in the present study corresponds to the size of *E. coli* (10-33µm), *E, histolytica* (10-15µm) and *E. muris* (9-20µm). Further analysis and comparisions with *E. coli*, *E. histolytica* and *E. muris* are required before a specific identification can be proposed. *Blantidium coli* are a ciliate and a normal inhabitant of intestine of wild ruminants, probably capable of becoming somewhat pathogenic under favorable conditions. It has been identified by Varadharajan and Kandasamy (2000) from India, Hossain (2012), Roki B Ur Raja (2012), Rahman and Mustafizur (2012) from Bangladesh. But the prevalence of *Blantidium coli* was not recorded in the present study. It means that Blackbuck Conservation Areas cannot be contaminated with this parasite.

The trematode species described from wild ruminants are *Paramphistomum cervi, Fasciola hepatica, Dicrocoelium dendriticum, D. lanceolatum* and *Schistosoma* sp. in the world. The prevalence of trematodes was observed in BCA (34.67%) as compared to SWR (50%) during study. These present prevalence rates were found to be more than 38.46% and 23.81% trematodes reported by Khanal (2006) and Ban (2012) but much lower than 90% tremaodes reported by Karki and Manandhar (2008). *Fasciola* sp. has been identified in India (Mandal et al. 2000, Singh et al. 2009), Southeast Nigeria (Opara et al. 2010), Pakistan (Farooq et al. 2012), Bangladesh (Rahman and Mustafizur 2012, Roki B Ur Raja 2012, Hossain 2012) and Nepal (Shrestha et al. 2011 and Ban 2012). Wahed et al. (2004) and Pakistan by Farooq (2009) were recovered *Fasciola hepatica* from Egypt, Karki and Manandhar (2008) reported *F. jacksoni* from Nepal. The prevalence rate 30% observed by Karki and Manandhar (2008) was higher than the present study (17.33% in blackbuck of BCA and 21.43% in blackbuck of SWR with average size $135 \pm 15 \times 79.5 \pm 4.5$µm). This prevalence rate was comparatively similar to19.23% obtained by Rahman and Mustafizur (2012) and Roki B Ur Raja (2012) but higher than 13.5% and 3.6% revealed by Mandal et al. (2000) and Ban (2012).

Paramphistomum sp. was reported from India (Mandal et al. 2000, Singh et al. 2009 and Borghare et al. 2009), Latvia (Ruta et al. 2009), Bangladesh (Kanungo et al. 2010,

Rahman and Mustafizur 2012, Roki B Ur Raja 2012), Italy (Fagiolini et al. 2010) and Nepal (Khanal 2006, Karki and Manandhar 2008, Rimal 2011, Shrestha et al. 2011 and Ban 2012). *Paramphistomum cervi* was reported by Kuzmina et al. (2010) in Ukraine, Aukstikalniene et al. (2013) in Lithuania. The *Paramphistomum* was 25.33% in blackbuck of BCA and 38.57% in blackbuck of SWR. The 26.09% of *Paramphistomum* obtained by Ban (2012) was comparatively similar to present prevalence rate (25.33%) in blackbuck of BCA duo to similar host and study area but less than 38.57% in blackbuck of SWR. The present rate of *Paramphistomum* was comparatively similar to 36.54% and 38.46% recorded by Rahman and Mustafizur (2012) and Khanal (2006). The present study was high as compared to previous rates 15.6%, 10.9% and 10% encountered by Mandal et al. (2000), Kuzmina et al. (2010), Aukstikalniene et al. (2013), Karki and Manandhar (2008). The size of this egg was found to be 165 $\pm$ 5 × 82.5 $\pm$ 7.5µm.

Schistosoma sp. have been encountered in Pakistan (Farooq et al. 2012), Southeast Nigeria (Opara et al. 2010). In this study, this parasite was found to be 7.14% in blackbuck of SWR only. This prevalence rate was much lower than 90% reported by Karki and Manandhar (2008) because they took their samples from different hosts while higher than 3.26% by Ban (2012) due to different study area. The size of this parasitic egg was also measured (147 $\pm$ 3 × 75µm). Likewise, *Dicrocoelium lanceolatum* was recorded in Egypt (Wahed et al. 2004), Romania (Darabus et al. 2014) and *Dicrocoelium dendriticum* identified by Aukstikalniene et al. (2013) in Lithuania whereas in Nepal, *Dicrocoelium* sp. reported by Karki and Manandhar (2008). This parasite can't be isolated in the present study.

The cestode species identified in the faecal examination of wild ruminants are *Taenia hydatigena, Moniezia expansa and M. benedeni* from US, Spian, Ukraine, Saudi Arabia, Egypt, Pakistan, Ethiopia (Thornton et al. 1973, Vazquez et al. 2009, Kuzmina et al. 2010, Mohammad et al. 2012, Wahed et al. 2004, Farooq 2009, Farooq et al. 2012 and Bogale et al. 2014) whereas Nepal (Karki and Manandhar 2008, Ban 2012 and Thapa 2013). However, in the present study *Taenia* sp. hasn't observed in any samples but genus *Moniezia* has been isolated in some samples. *Moniezia* sp. with average size (70.5 $\pm$ 4.5µm) was observed as 14% in blackbuck of BCA only which is higher than the earlier reports of Ban (2012), Bogale et al. (2014), Karki and Manandhar (2008) who revealed as 11.96%, 7.19% and 5% respectively. The present study was much lower than 70.59% and 47.06% *Moniezia* recorded by Thapa (2013). Because they were collected faecal samples

from various wild and captive ruminants and examined. It may conclude that these ruminants are harboring less number of infected mites (intermediate host) in the field.

All the species of nematodes were isolated from the faeces of wild and captive ruminants. The nematodes were found be 89.33% in BCA and 81.43% in SWR which were lower than 90.3%, 85.03% and 100% nematodes reported by Valcarcel et al. (2002), Thapa (2013), Bogale et al. (2014) while lower than 20%, 44.4%, 56.92% and 76.47% nematodes recorded by Farooq (2009), Wahed et al. (2004), Khanal (2006) and Thapa (2013). *Haemonchus contortus* (wire worm) is one of the most important pathogens for blackbuck (Thornton et al. 1973), producing a disease known as haemonchiasis which can cause anaemia, growth loss, edema, emaciation, etc and even death (Roberts and Janovy Jr. 2005). It was recovered in Poland (Cicek et al. 2003), India (Meshram et al. 2007, Borghare et al. 2009), Pakistan (Farooq 2009, Farooq et al. 2012), Ukraine (Kuzmina et al. 2010), Bangladesh (Kanungo et al. 2010), Iraq (Saud et al. 2012) and Spain (Mayo et al. 2013) while from Nepal (Rimal 2011 and Thapa 2013). In the present study, 18% and 14.28% of *Haemonchus* with average size (80 $\pm$ 5 × 48 $\pm$ 12µm) in blackbuck of BCA and SWR respectively. The rate (13.80%) of *Haemonchus* recorded by Meshram et al. 2007 was comparatively similar to 14.28% *Haemonchus* in blackbuck of SWR but less than 18% in blackbuck of BCA of present study. The rates (57.6% and 25%) as revealed by Kuzmina et al. (2010) and Borghare et al. (2009) were higher than present study. Thapa (2013) reported 5.88% and 2.94% in Himalayan Thar and Barking deer have been lowered as compared to the present rates.

Three species of *Trichostrongylus* have been encountered in blackbuck such as *Trichostrongylus axei, T. colubriformis, T. probolurus* (Thornton et al. 1973) and *T. retortaeformis, T. vitrinus* and *T. capricola* in other wild ruminants (Cicek et al. 2003, Kuzmina et al. 2010, Mohammad et al. 2012, Goossens et al. 2005, Mayo et al. 2013). *Trichostrongylus* sp. has also been reported in Central Spain (Garcia- Romero et al. 2000), Latvia (Ruta et al. 2009), Iraq (Saud et al. 2012), Pakistan (Farooq 2009 and Farooq et al. 2012) and Nepal (Rimal 2011). The larva of *T. colubriformis* was also recorded in Egypt by Wahed et al. (2004). *Trichostrongylus* eggs (81 $\pm$ 6 × 48 $\pm$ 12µm) were isolated with prevalence rate 75.33% in blackbuck of BCA and 55.71% in blackbuck of SWR in the present study. The prevalence rate (65%) obtained by Vazquez et al. (2009) was lower than 75.33% in blackbuck of BCA but higher than 55.71% in blackbuck of SWR in this study. On the other hand, the much lower prevalence rates i.e.

26.09%, 19.64%, 11.76%, recorded by Ban (2012), Hossain (2012), Thapa (2013) and Meshram et al. (2007) respectively than present study.

Although the present finding has been apparently in healthy blackbuck, some nematodes are known to be pathogenic. The most important nematode species are *Camelostrongylus* and *Nematodirus*. These nematodes have been isolated after postmortem from abdomen and small intestine of blackbuck (Thornton et al. 1973, Flach 1986, Flach and Sewell 1987) and from faecal matter of wild ruminants (Goossens et al. 2005, Kuzmina et al. 2010, Farooq et al. 2012, Mayo et al. 2013) but no specific diagnosis was given and none of these nematodes were encountered in the present study. Similarly, three species of *Oesophagostomum* such as *O. radiatum, O. dentatum, O. venulosum* were identified in the faecal samples of different wild as well as captive herbivores (Thornton et al. 1973, Garcia-Romero et al. 2000, Valcarcel et al. 2002, Cicek et al. 2003, Wahed et al. 2004, Goossens et al. 2005, Borghare et al. 2009, Tovassoli et al. 2010, Kuzmina et al. 2010, Rimal 2011, Farooq et al. 2012). This parasitic egg hasn't observed in this study. This study showed that blackbuck raising area in Nepal does not contaminated with these three parasites.

Three species of *Trichuris* have been described in previous reports including *T. globulosa, T. cervicaprae* and *T. ovis* (Thornton et al. 1973, Varadharajan and Kandasamy 2000, Goossens et al. 2005, etc). In the present study, *Trichuris* (6%) with average size (75×39µm) was isolated from blackbuck of BCA only. The prevalence of *Trichuris* in the present study was comparatively similar to 5.4%, 5.1% and 5% as obtained by Lim et al. (2008), Corden et al. (2008), Vazquez et al. (2009) and Aukstikalniene et al. (2013). Likewise, 41.5%, 25%, 13.9%, 11.76%, 8.82% and 8.5% of *Trichuris* reported by Tovassoli et al. (2010), Maesano et al. (2014), Mohammad et al. (2012), Thapa (2013) and Meshram et al. (2007) higher than present study. This study showed that blackbuck of BCA were more infected with *Trichuris* as compared to 4.19%, 3.26% and 1.8% recorded by Bogale et al. (2014), Ban (2012) and Singh et al. (2009).

Ascaris sp. has been reported from wild herbivores in India (Mandal et al. 2000), Southeast Nigeria (Opara et al. 2010), Bangladesh (Rahman and Mustafizur 2012), Ethiopia (Bogale et al. 2014) and Nepal (Khanal 2006 and Thapa 2013) with prevalence rates i.e. 5.3%, 3.57%, 16.92%, 17.65% and 52.94% of *Ascaris* respectively. The present rate of *Ascaris* was higher (57.33% in BCA and 38.57% in SWR) as compared to all

previous finding rates except 52.94% which is less than 57.33% and higher than 38.57%. This result showed that the Blackbuck Conservation Areas were highly infected with *Ascaris* eggs. *Ascaris* egg with size (31.5 ± 7.5µm) was also found to be distributed in many faecal samples of the present study. Likewise, *Bunostomum* in the present study was found to be 12.67% and 2.86% with average size (95 ± 5 × 46.5 ± 1.5µm) in blackbuck of BCA and SWR respectively. The present rates were much lower than 34.5% reported by Lim et al. (2008). The rates (10.9%, 10% and 4%) of *Bunostomum* sp. were lowered as compared to 12.67% in BCA cases but higher than 2.86% in SWR cases. In this study, *Oxyuris* with average size (112.5 ± 7.5 × 55.5 ± 19.5µm) was observed (4.67%) in faecal samples of blackbuck of BCA only. This is much lowered than result of Thapa (2013) who revealed as 88.24% in Himalayan Thar and 70.59% in Barking deer.

Strongyloides sp belong to the family strongyloididae was identified in the faecal samples of different wild ruminanats (Varadharajan and Kandasamy 2000, Ruta et al. 2009, Seong-Jun et al. 2011, Farooq et al. 2012, Saud et al. 2012, Hossain 2012). Besides Mandal et al. (2000), Meshram et al. (2007), Singh et al. (2009) and Bogale et al. (2014) reported the prevalence of *Strongyloides* are 11.5%, 7.1%, 5.39% and 4.5% respectively. These rates were comparatively lowered than the present study. The present prevalence of *Strongyloides* was 16% in blackbuck BCA and 12.86% in blackbuck of SWR that are less than the earlier reports of Meshram et al. (2007), Aukstikalniene et al. (2013) and Thapa (2013) who recorded as 31.5%, 25% and 64.71%. This parasitic eggs size (88.5 ± 7.5 × 52.5 ± 7.5µm) was also reported in the present study.

Several nematodes like *Capillaria, Cooperia, Chabertia, Ostertagia, Spiculopteragia, Dictyocaulus, Muellerius, Protostrongylus* and *Strongyles* have been reported in various wild ruminants by different researchers in global and national context. In the present study, none of these nematodes are isolated in the faecal matter of blackbuck of BCA and SWR.

In the present study, the mixed parasitic infections were found to be more common in blackbuck due to high contamination of pasture by grazing of domestic animals and human. Single infection was observed 8.57%, double (40.00%), triple (17.14%) and multiple (24.28%) in blackbuck of SWR while double (26.00%), triple (28.00%) and multiple (36.00%) but not found single infection in blackbuck of BCA. The present result was similar to the finding of Kunungo et al. (2010), who documented that infection are

majority in deer and Thapa (2013) who described the mixed infection was most common in Himalyan Thar and Barking deer. Mixed infection rate was observed as 15% single, 20% double, 15% triple, 30% quadruple and 5% quinruple (Seong-Jun et al., 2011) which are higher than single infection, lower than double infection, higher than triple infection of present study. But quadruple and quinruple infection were not observed in present study.

The intensity of different parasites in blackbuck of BCA and SWR was observed in this study. According to result, maximum numbers of blackbuck were found to be infected with light infection which is asymptomatic condition and can not cause the diseases in animals while less numbers of blackbuck were infected with heavy infection revealed by *Trichostrongylus, Ascaris, Paramphistomum* and *Eimeria in* BCA only. The heavy infection is symptomatic condition and cause serious diseases in animals. The result of present study was similar to the report of Thapa (2013) due to same measurement techniques.

The diameter of eggs/cysts/ocysts of different gastrointestinal parasites found in the present study is similar with the finding of Soulsby (1982). The diameter of eggs of *Trchostrongylus* was 81 $\pm$ 6 × 48 $\pm$ 12µm that are similar with the finding (72.5 × 43.5µm) of Roki B Ur Raja (2012) and Soulsby (1982). But the diameter of eggs of *Fasciola* sp. (135 $\pm$ 15 × 79.5 $\pm$ 4.5µm) is comparatively larger than the record of Roki (2012) who measured as 87 × 43.5µm but similar to the record of Soulsby (1982) who measured as 130-150 × 63-90µm. Likewise, the diameter of eggs of *Paramphistomum* sp. (165 $\pm$ 5 × 82.5 $\pm$ 7.5µm) is comparatively similar to the record of Roki (2012) and Soulsby (1982) who measured as 165 × 95µm. The diameter of eggs of *Strongyloides* sp. (88.5 $\pm$ 7.5 × 52.5 $\pm$ 7.5µm) is comparatively larger than the record of Roki (2012) who measured as 58 × 29 µm but *Ascaris* sp. (31.5 $\pm$ 7.5µm) is lower than 70µm recorded by Roki (2012). On the other hand, interestingly the result of present study revealed that the diameter of ocyst of *Eimeria* was similar to the report of Thapa (2013) but others are completely different. This variation in size of different parasitic eggs with the previous finding might be due to the method of measurement, strains of the parasite, species of the host and climatic factors.

In the present study, on the basis of comparative analysis, the blackbuck of SWR was highly infected with *Eimeria* with or without micropile among protozoan parasites than

that of blackbuck of BCA because the pasture was highly contaminated with animal's faecal matter containing oocyst of *Eimeria* which is the most common parasite of both wild and domestic animals. They are directly or indirectly contact with different types of wild and domestic animals like cattle, goats, buffaloes, dogs, deer, etc that defecate in the grazing field also.

Likewise, the blackbuck of SWR was found to be mostly infected with *Paramphistomum* among trematodes than blackbuck of BCA. This difference might be due to location of animal's area, availability of intermediate host near the areas and the source of feeds. The probable cause of this trematode infection was strongly connected with mud snails that live in the water resources; river, pond, lake, etc. that located in the conservation area and wildlife reserve as well as to eaten short green grass which may be contaminated with metacercaria. The chance of contamination is also higher as the areas located in the marshy land.

The figure: 5 showed that prevalence of all nematodes were high in BCA as compare to SWR due to highly contamination of Blackbuck Conservation Area with different species of nematodes. Nematodes are free living parasites that survive in free environments. Grazing on contaminated a pasture that drastically increases the chance of infections with infective stage of nematodes to the ruminants. Ruminants get infection through ingestion of contaminated grass with L3 larvae (Hagberg 2008).

The figure: 6 showed that the double infection was found to be highest (40%) in blackbuck of SWR than BCA (26%) while multiple (36%) infections in BCA as compared to SWR. This difference might be due to high conflict between blackbucks, different animals and human.

In the present study, the parasitic load of different parasites of blackbuck was calculated.The mean of EPG/OPG was measured and the range of lowest and highest parasitic load were recorded i.e. 100 due to *Trichuris* and 700 due to *Trichostrongylus*. The parasitic load of different parasites was similar with the finding of Singh et al. (2009) and Roki B Ur Raja (2012). As a whole, all gastrointestinal parasites were most prevalent to the blackbuck in Nepal.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the present study, it is clear that gastrointestinal parasites are highly prevalent (90.00%) in blackbuck of BCA and SWR, Nepal. Finding of this study showed that gastrointestinal protozoan parasites were more prevalent in both SWR and BCA while 90% blackbucks of BCA were found to be highly infected with helminths than SWR. So that helminths infections were comparatively more common than the protozoan infection in blackbuck of BCA and SWR. Among helminths, nematodes were more prevalent followed by trematodes and cestodes in blackbuck of both BCA and SWR. This prevalence rate showed that blackbucks are highly susceptible to endoparasites. The GI parasites were found to be more prevalent in female as compared to male and fawn. Double infection was more prevalent in SWR (40%) than BCA while multiple in blackbuck of BCA (36%) as compared to SWR. Twelve different parasitic genera were identified in blackbuck of BCA such as *Entamoeba* and *Eimeria* among protozoa; *Paramphistomum* and *Fasciola* among trematodes; *Moniezia* among cestode; *Trichostrongylus*, *Ascaris*, *Haemonchus*, *Strongyloides*, *Bunostomum*, *Trichuris* and *Oxyuris* among nematodes. Besides these parasites, *Schistosoma* among trematodes was reported but *Moniezia* among cestode; *Trichuris* and *Oxyuris* among nematodes were not observed in SWR. Out of all these identified GI parasites, *Trichostrongylus* showed the highest prevalence in BCA and SWR. *Eimeria* (0.67%), *Paramphistomum* (0.67%) and *Trichostrongylus* (2%) and *Ascaris* (1.33%) revealed heavy infection in BCA only. Among protozoans identified, *Entamoeba* was found to be most prevalent in BCA compared to SWR while *Eimeria* was comparatively higher in SWR than BCA. Three trematodes i.e. *Paramphistomum, Fasciola* and *Schistosoma* were found to be more prevalent in SWR than BCA while *Schistosoma* was only reported from SWR. *Moniezia* was only observed in blackbuck of BCA. All of seven different nematodes were comparatively higher in BCA than SWR. The highest parasitic load was found to be 700 revealed by *Trichostrongylus* among all GI parasites.

6.2 Recommendations

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

- The veterinary laboratory should be established in the conservation areas and wildlife reserves for the regular diagnosis of parasitic diseases and treatment.
- Proper pasture management programme should be conducted on blackbuck conservation areas for the parasites control.
- Conservation areas and wildlife reserves should be strictly prohibited for domestic animals since they are the major source of infection for blackbuck.
- In order to control the transmission of gastrointestinal parasitic disease, strengthening of the National Animal Health and Development Law is necessary.

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APPENDICES

A. Before Study Period, Population of Blackbuck (*Antilope cervicapra*)

i. In Blackbuck Conservation Area, Khairapur, Bardiya

Years	No. of blackbuck	Years	No. of blackbuck	Years	No. of blackbuck
1975	9	1988	170	2001	56
1976	23	1989	177	2002	74
1977	38	1990	177	2003	92
1978	-	1991	177	2004	85
1979	-	1992	150	2005	107
1980	23	1993	109	2006	131
1981	38	1994	111	2007	185
1982	-	1995	109	2008	191
1983	66	1996	101	2009	219
1984	100	1997	113	2010	212
1985	130	1998	113	2011	287
1986	152	1999	50	2012	278
1987	164	2000	53	2013	300 (Study period)

(Source: Annual Report 2011/12, BCA)

ii. In SWR, Kanchanpur at the time of reintroduction from different zoo

S.N.	From	No. of Male	No. of Female	No. of Fawn	Total
1.	Nepalgaunj Mini Zoo	6	16	-	22
2.	Central Zoo	3	2	1	6

(Source: DNPWC 2012)

B. During Study Period 2013, Population of Blackbuck

S.N.	Study Area	No. of Male	No. of Female	No. of Fawn	Total
1.	BCA	117	154	29	300
2.	SWR	13	17	13	38