PREVALENCE OF GASTROINTESTINAL PARASITES IN BLACKBUCK (*Antelope cervicapra* Linnaeus, 1758) OF BLACKBUCK CONSERVATION AREA, KHAIRAPUR, BARDIA, NEPAL

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Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

May 2023

DECLARATION

I hereby declare that the work presented in this thesis "**Prevalence of Gastrointestinal Parasites in Blackbuck (*Antelope cervicapra* Linnaeus, 1758) of Blackbuck Conservation Area, Khairapur, Bardia, Nepal**" 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 the reference to the author(s) or institution(s).

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RECOMMENDATION

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LETTER OF APPROVAL

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Muna Thapa entitled "**Prevalence of Gastrointestinal Parasites in Blackbuck (*Antelope cervicapra* Linnaeus, 1758) of Blackbuck Conservation Area, Khairapur, Bardia, Nepal**" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper parasitology.

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LIST OF ABBREVIATIONS

Abbreviated forms	Details of abbreviation
BCA	: Blackbuck Conservation Area
CI	: Confidence Interval
df	: degree of freedom
DNPWC	: Department of National Parks and Wildlife Conservation
GI	: Gastrointestinal
IUCN	: International Union for Conservation of Nature
i.e.	: That is
$K_2Cr_2O_7$	: Potassium dichromate
Km^2	: Square kilometer
NaCl	: Sodium Chloride
P	: Probability value
rpm	: Revolutions Per Minute
SNP	: Shuklaphanta National Park
sp.	: species
spp.	: several species
T. U	: Tribhuvan University
χ^2	: Chi square
µm	: micrometer

ABSTRACT

Blackbuck is a species of antelope native to the Indian subcontinent. This study aimed to investigate the prevalence, diversity, and concurrency of gastrointestinal (GI) parasites in the blackbuck population of the Blackbuck Conservation Area in Nepal. A total of 150 fecal samples of blackbuck were collected and examined using the iodine wet mount and concentration technique. The results revealed an overall prevalence of 96% for GI parasites, with females having a higher prevalence than males. Ten different genera of parasites belonging to protozoa, cestode, trematode, and nematode groups were identified with *Paramphistomum* sp. (55.33%) having the highest prevalence followed by *Strongyloides* sp. (52%), *Fasciola* sp. (36%), *Haemonchus* sp. (26%), *Moniezia* sp. (24%), *Trichostrongylu*s sp. (21.33%), *Eimeria* sp. (19.33%), *Entamoeba* sp. (15.33%), *Ascaris* sp. (8.67%) and *Trichuris* sp. (7.33%). The study also revealed mixed infection with one to six genera in each sample, with triple infections being the most prevalent. Most of the blackbucks exhibited light infection, while five specific parasite types showed heavy infection levels. These findings suggest a significant threat to the health and survival of the blackbuck population, highlighting the need for effective parasite control measures to prevent the spread of infection and improve overall health.

1. INTRODUCTION

1.1 Background

Parasites are ubiquitous in wildlife and an integral part of ecological communities. They are symbionts that coexist in harmony with their hosts (Botzler & Brown 2014; Rose et al. 2014). Intense parasitism can have significant effects on wildlife host populations, altering their reproductive success, fitness, and even their behavior (Aissa et al. 2021). Protozoa and helminths are the common gastrointestinal (GI) parasites in wild ruminants. Protozoa can be directly infectious when transmitted in the feces into the environment, whereas helminths require a maturation period in the soil to become infectious (Atanaskova et al. 2011). Intestinal parasites thrive in certain parts of the digestive tract, including the duodenum, ileum, cecum, and large intestine. Parasites must adapt to host feeding habits to live and multiply in the gastrointestinal tract (Arcari et al. 2000; Cuomo et al. 2009).

Threats to endangered species include habitat loss and change, hunting, and pollution, all of which are caused by humans, as well as the effects of introduced competitors and predators. Exposure to parasites, many of which are exotic and novel to endangered species, has emerged as a major threat to their survival in recent years (Hedrick et al. 2001). Nepal is an extremely diverse and unique nation with a vast array of landscapes, cultures, and wildlife (Tamang 2003). Blackbuck can be found in a diverse range of habitats, but it attains greatest densities in semi-arid grasslands (Bashistha et al. 2012). Blackbuck forages primarily during the day, but they occasionally do it at night. Their foraging activity is regulated by environmental conditions and seasonal changes (Choudhary & Chisty 2022). Nepal's National Red List (2011) listed blackbuck as critically endangered. Nepal's National Park and Wildlife Conservation Act, which came into effect in 1973 safeguards 27 types of mammals, including the blackbuck (Gyawali et al. 2020).

1.2 Species introduction

Blackbuck (*Antelope cervicapra*) is a graceful gazelle-like animal. It is regarded as the most attractive member belonging to the family Bovidae, which is classified in the order Artiodactyla and the class Mammalia. It is native to the subcontinent of South Asia and was once the most common wild animal in this region. Historically, blackbuck population were widespread over Bangladesh, Nepal, and Pakistan, but suffered a catastrophic population decline throughout the 20th century (Khanal & Chalise 2011; Meena et al. 2017). Blackbuck is the only existing member of the genus *Antelope*. Based on differences in coat color, horn length, and shape *Antelope cervicapra* are classified into four sub-species. *Antelope cervicapra cervicapra* is found in southern India, *Antelope cervicapra centralis* in central India, *Antelope cervicapra rupicapra* in northern India and Nepal, and *Antelope cervicapra rajputane* in northwestern India and Pakistan (Kumar & Zutshi 2013; Pant & Joshi 2019).

Species are diurnal ungulates with pronounced sexual dimorphism. Males have spiral antlers up to 79 cm which are absent in females. Males become progressively darker as they age, from tawny to strong brown to black. Females and young ones have yellow coloring on the front and back. Both sexes have white skin on their chins, lower legs, and chests. Eyes with a white ring around them. The body length of the species ranges from 100 - 150 cm, while the length of the tail ranges from 10 - 17 cm. The males weighed between 20- 57 kg, and the females weighed between 19- 33 kg (Roberts 1997; Sheikh & Molur 2004). There are two times of year when mating is at its peak: July to August and February to March. Females reach sexual maturity at around 15 months, and after a gestation period of around 6 months, they give birth to a single fawn. In the wild, they have a potential lifespan of 18 years (Long 2003).

There are an estimated 35,000 individuals living in the wild across India, while its populations in Nepal and Pakistan are regionally extinct and, on the IUCN, Red List, the species is designated as "Least Concern," and the Wildlife Protection Act (1972) places it in Schedule I (Pattnaik et al. 2021). Blackbuck number have experienced significant shift in Nepal due to predation and natural causes (Gyawali et al. 2020). Translocation was the first phase in the blackbuck recovery process in Nepal. In 2012, 28 blackbucks (22 from the Nepalgunj small zoo in two shifts and six from the central zoo in Lalitpur) were reintroduced to Hirapurphanta of Shuklaphanta National Park (SNP). The population in SNP has increased from 28 to 115, while the population in Khairapur, BCA, has increased from nine to 234 by 2020, suggesting that the translocation has been a great success (Pant & Joshi 2019; Bist et al. 2021).

1.3 Parasitic disease of Blackbuck

Disease can pose a significant threat to endangered species, sometimes causing sudden and unanticipated declines in local abundance (Cleaveland et al. 2002; Muoria et al. 2005).

Internal parasites such as *Haemonchus contortus*, *Trichostrongylus axei*, *Taenia hydatigena*, *Trichuris* sp., *Entamoeba* sp., *Eimeria* sp., *Paramphistomum* sp., *Fasciola* sp., *Moniezia* sp., *Ascaris* sp., *Strongyloides* sp., *Bunostomum* sp., and *Oxyuris* sp. have been identified in the blackbuck population. These parasites have the potential to infect various organs and systems of the blackbucks, including the gastrointestinal tract (Chaudhary & Maharjan 2017; Tahir et al. 2021).

Listeria monocytogenes, a food-borne pathogen, can cause listeriosis and mortality in blackbuck when it enters their bodies through ensilage herb that is stored in a slightly aerobic state. Another bacterial species, *Mycobacterium tuberculosis*, seriously affects them, but it has only been observed in captive blackbuck; wild blackbuck does not have this type of bacteria (Peters et al. 2020). Additionally, *Mycobacterium bovis*, which causes bovine tuberculosis, has been isolated from the lymph nodes of the thorax and abdomen of blackbucks and can lead to the death of these animals (Podhade et al. 2013; Akhtar et al. 2019). *Arcanobacterium pyogenes* has been linked to necrotizing pneumonia, mandibular osteomyelitis, peritonitis, and hepatic, pulmonary, renal, and subcutaneous abscessation in blackbuck (Portas & Bryant 2005). *Escherichia coli* is also found in the blackbuck members (Rathore et al. 2016).

Several parasites have been found in association with disease and mortality in *Antelope cervicapra*, including *Amphistoma* sp., *Neospora caninum*, *Camelostrongylus mentulatus*, *Strongyloides* sp., *Oesophagostomum* sp., *Strongyle* sp., *Trichostrongylus axei*, *T. probolurus*, *Toxoplasma gondii*, and *Trichuris* (Goossens et al. 2005; Fagiolini et al. 2010). *Balantidium coli*, *Nematodirus* spp., and *Wenyonella* spp. has been observed in a few species of blackbuck at Bikaner Zoo (Pilania et al. 2014). *Trypanosoma cruzi*-related ocular lesions and hemorrhagic parasitic conditions, specifically abomasitis and enteritis, are the result of infection by certain types of parasites such as *Haemonchus* spp., *Setaria* spp., and Trichostrongylids (Cruz-Hernandez et al. 2015).

1.4 Research questions

- What is the overall prevalence of gastrointestinal parasites in blackbuck within the conservation area?
- Is there a difference in the prevalence of gastrointestinal parasites between male and female blackbuck in the study area?

- Which species of gastrointestinal parasites are most found in blackbuck in the study area?

1.5 Objectives of the study

1.5.1 General objective

- To investigate the prevalence of gastrointestinal parasites in blackbuck of BCA, Khairapur, Bardia, Nepal.

1.5.2 Specific objectives

- To identify sex wise prevalence of gastrointestinal parasites.
- To determine the concurrency of parasitic infection of blackbuck.
- To assess the intensity of parasitic infection.

1.6 Rationale of the study

The primary reason for carrying this study is because blackbuck is an endangered as well as single population species in the wild in Nepal. The mission and goal of BCA is to protect the blackbuck population by solving resettlement problems and improving blackbuck conservation for the benefit of local and global communities. The importance of diseases as a potential threat to the conservation of endangered species is often overlooked or not given due consideration. The present study will aid in the formulation of appropriate strategies for sustaining the health and well-being of captive and wild blackbuck populations by providing baseline data on the parasitic burden in blackbuck of BCA.

2. LITERATURE REVIEW

Parasitic infections account for almost one third of total losses due to all animal diseases which are often overlooked because most infected animals exhibit few obvious clinical symptoms, and their effects are gradual and chronic (Gelot et al. 2016; Raza et al. 2007). In addition to poaching and habitat degradation, health issues have emerged as a significant threat to wildlife in recent years. Numerous infectious and non-infectious diseases, especially those of parasitic origin, affect wild animals (Akhter & Arshad 2006). Wildlife parasitic diseases are an essential area of study because they can have a significant impact on the health of wild animals and pose a public health risk (Liatis et al. 2017).

Wildlife protection has been implemented in many parts of the world through the establishment of parks and zoological gardens, where animals are under continuous stress and are susceptible to parasitic infection despite care and management. Studies have found that they are susceptible to a variety of coccidia, nematodes, and trematodes (Parsani et al. 2001). The existence of several parasitic infections of *Strongyloide*s spp., *Nematodirus* spp., *Balantidium coli*, *Eimeria* spp., *Trichuris* spp. and *Wenyonella* spp. was detected in blackbucks housed at the zoo in Bikaner, situated in Rajasthan, India (Pilania et al. 2014). An additional research study was undertaken to examine parasitic infections affecting wild herbivores in Chhatbir, Punjab. *Strongyle* was a highly prevalent parasite followed by *Trichuris* spp., *Eimeria* spp. and amphistomes. Most herbivores exhibited a mixed infection (Singh et al. 2006).

Mir et al. (2016) observed that 48% of the animals studied had mixed parasitic infections involving both helminths and protozoans. The study identified six distinct parasites, including s*trongyle*, *Strongyloides* spp., *Trichuris* spp., ascarid, coccidia and *Capillaria* spp. The effect of introducing wildlife species' diseases on local wildlife populations has become a world priority in recent years. This is very important when disease risks involve the livestock industries and human health (Krausman & Bleich 2013). *Haemonchus* spp., *Setaria* spp., and *Trichostrongylids* were linked to the fatalities in blackbuck in Mexico. Also, *Anaplasma marginale* was identified (Cruz-Hernandez et al. 2015).

Helminthiasis has a substantial economic impact and is found all over the world (Hossain et al. 2011; Lashari & Tasawar 2011). Thornton et al. (1973) identified a significant number of *Taenia hydatigena*, *Camelostrongylus mentulatus* and *Trichostrongylus probolurus*. Four trichostrongyles commonly found in cattle, sheep, and goats, *Haemonchus contortus*, *T. axei, T. colubriformis, and Nematodirus spathiger,* were detected in blackbuck. *Oesophagostomum* sp. and *Trichuris* sp. were additional gastrointestinal helminths. *Longistrongylus curvispiculum* was recovered from free ranging exotic antelope in Texas (Craig 1993). In Cholistan desert of Pakistan, 20% gastrointestinal infections by helminths were recorded where blackbuck shared *Haemonchus contortus* and *Trichostrongylus* spp. The most common parasites in the research region were helminths with direct life cycles (Farooq et al. 2012). In a survey conducted to ascertain the relationship between the incidence of helminthic infection and meteorological parameters, Fathima et al. (2017) found 29.26% of overall helminth infection and it was observed that combined infections of *Strongyles* and *Strongyloides* spp., and hookworms were present in the study population.

Khanal and Chalise (2011) found a high degree of similarity between herbivores sharing the same grazing fallow and parasitic incidence. Coccidia, *Paramphistomum*, *Ascari*s and *Strongyles* were the most prevalent types of parasites found in livestock and blackbucks that graze in the BCA. Furthermore, Pant and Joshi (2019) observed that parasite transmission between cattle and blackbuck was 67% prevalent in the Hirapurphanta. In cattle and blackbuck, the parasites *Eimeria* spp. and *Strongyle* spp. were very frequent.

Ten different forms of parasites were discovered in ruminants, according to Thapa and Maharjan (2015), along with gastrointestinal nematodes like *Strongyloides*, *Trichostrongylus*, *Trichuris*, *Ascaris*, *Haemonchus*, *Oxyuris*, larvae of bronchopulmonary nematode like *Dictyocaulus* sp. and *Muellerius* sp. were identified and coccidian parasites like *Eimeria* sp. and cestode parasites like *Moniezia* sp. were detected. Multiple parasite infections were more common. Pun (2018) discovered parasites from the protozoan and helminth groups in ruminant species housed in Nepal's central zoo. GI parasites were found in 59% of the animals, with blackbuck having the highest incidence of 19.85%. The sole protozoan found was *Eimeria* sp. and three nematode species were identified: *Trichostrongylus* sp., *Haemonchus* sp., and *Strongyloides* sp. Among the ruminant species tested, only single and double parasite infections were found. Statistical investigation revealed there was no significant variation in the co-occurrence of parasitic infections.

In comparable study, Chaudhary and Maharjan (2017) identified *Entamoeba* and *Eimeria* among protozoans, *Moniezia* among cestodes, *Paramphistomum* and *Fasciola* among trematodes and *Trichostrongylus, Ascaris*, *Strongyloides*, *Bunostomum*, *Haemonchus Trichuris* and *Oxyuris* among nematodes in the study conducted of blackbuck of BCA, Bardia and Shuklaphanta Wildlife Reserve, Kanchanpur. Multiple parasite infections were identified, and most blackbucks had light infections i.e., less than two ova or oocyst per field. Airee (2018) reported the highest occurrence of double infection (34.01%) followed by single, triple, and multiple infection and maximum number of spotted deer were infected with light infection. Similarly, Antelopes had considerably lower infection rates, according to Aissa et al. (2021), *Camelostrongylus mentulatus* and *Nematodirus spathiger* were detected alongside other *Trichostrongylus* spp. isolates. Ghimire and Bhattarai (2019) showed concurrent infection with more than two parasites, up to septuple infection among all the positive samples.

Ban (2012) reported coccidian, *Fasciola* sp., *Paramphistomum* sp., *Strongyle* sp., *Trichostrongylus* sp., *Strongyloides* sp., *Trichuris* sp., *Moniezia* sp., and *Schistosoma* sp. as the primary parasites affecting blackbuck. The infection rate in males and females were 65.21% and 29.34% respectively. Raza et al. (2014) observed higher prevalence of gastrointestinal helminth in male goats (81.1%) as compared to females (77%). On the contrary, opposite trends were observed in sheep. A related study demonstrated the prevalence of different gastrointestinal parasites did not vary significantly between male and female (Mpofu et al. 2020).

3. MATERIALS AND METHODS

3.1 Study area

BCA is located in western lowland terai within Gulariya municipality of Bardia district. Its geographical coordinates fall between 20^0 07' 54" to 28^0 17' 22" N latitude and 81^0 16' 48" to 81^0 22' 54" E longitude. In 2009, the Nepal government designated an area of 16.95 km^2 in Khairapur as BCA, which includes ward number 1, 2, 3 and 4 of Gulariya municipality. This was the first organized initiative by the government of Nepal to conserve the critically endangered blackbuck. It comprises a core area of 5.27 km^2 and a community development zone of 11.68 km^2. The Babai riverbed borders the area on three sides and most of the region is composed of marginal agricultural and grazing land.

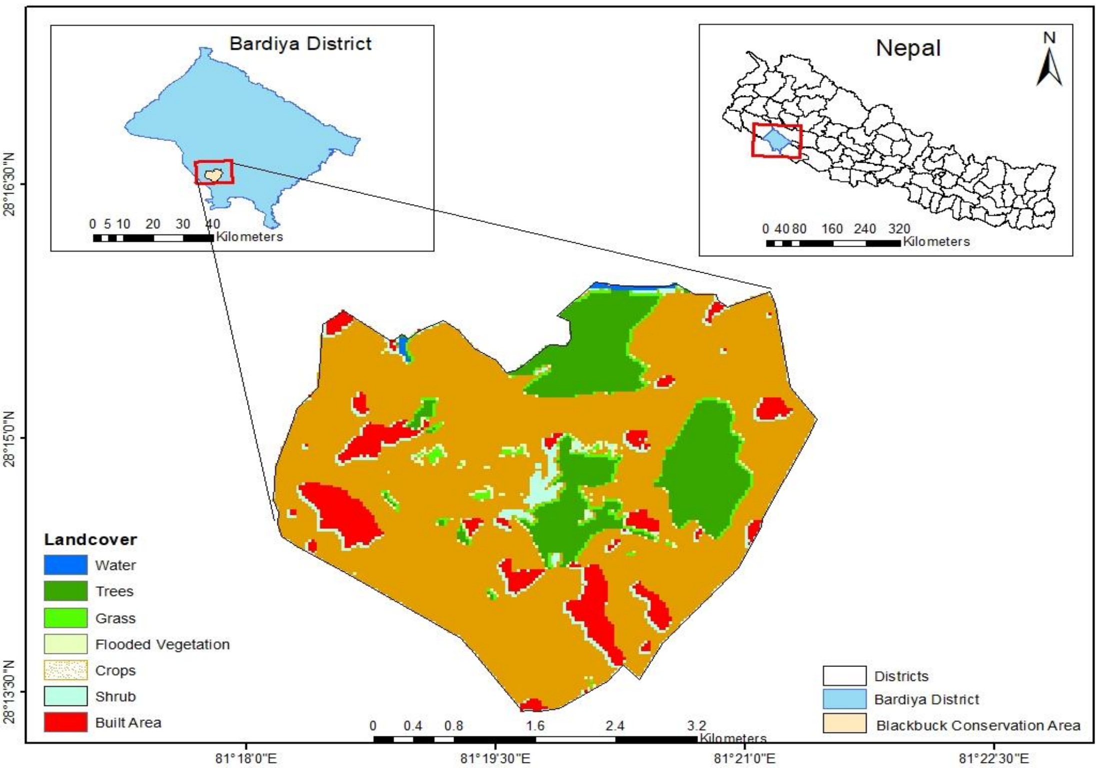

Figure 1: Map illustrating the geographic location of study area

3.2 Ethical considerations

To proceed ethically, approval was obtained from DNPWC and BCA ethics review committees.

3.3 Research design

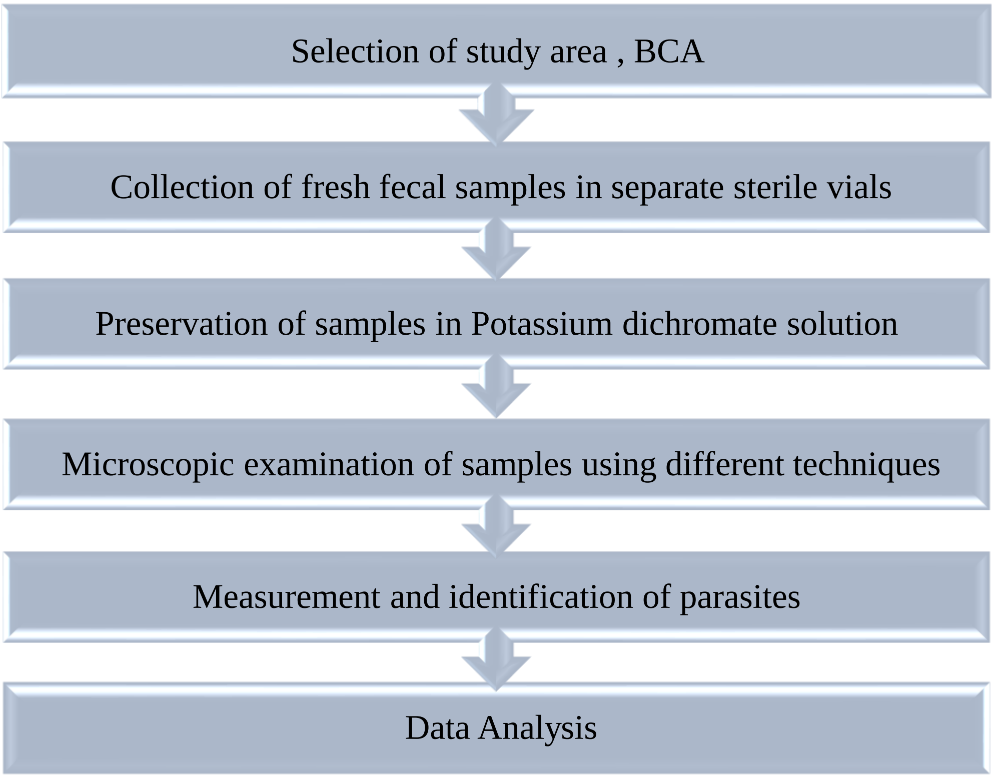

Figure 2: Flow chart showing entire study

3.3.1 Study period

The study was conducted from 19 August to 18 November of 2022.

3.3.2 Gender identification

To visually identify the sex of the blackbuck in a conservation area for fecal sample collection, certain physical characteristics and behavior were observed.

- **Horns:** Male blackbuck typically have long, spiral horns that are absent in females and young individuals.
- **Coloration:** Both male and female blackbucks have a distinctive color pattern. Males have rich dark brown to black upper body, while females have lighter brown coat.
- **Body size and shape:** Adult males are generally larger and more robust compared to females. They have a more muscular neck and shoulder region.
- **Genitalia:** In some cases, the presence of visible genitalia helped to identify the sex. Males have a prominent scrotum, while females have a urogenital opening.

- **Behavior:** Male blackbucks display territorial behavior and aggression. They establish and defend their territories, engaging in fights with other males to attract females. In contrast, female blackbucks are more social and tend to form herds consisting of females and young.

  When collecting fecal samples from the dominant male blackbuck, it was important to consider their defecation habits. The dominant male often selects specific locations within its territory for defecation. These locations were typically marked by scraping the ground or creating shallow depressions before defecating.

3.3.3 Sample collection

After determining the blackbuck's primary habitat, fresh fecal samples were collected in sterile vials immediately after the animal defecated in the early morning or at sunset. Samples were collected from each individual blackbuck separately, and they were mostly found in the vicinity of the conservation office and view towers of BCA. The fresh sample was moist, shiny, and dark in color, usually 1-2 cm in length. Each vial was labeled assigning a reference number.

3.3.4 Sample size

At the onset of the sample collection process, a population of blackbuck was 182. However, due to factors such as predation and challenges in accurately determining the sex of the fawns, a total of 150 fresh fecal samples were successfully collected from BCA.

3.3.5 Preservation of sample

Fecal samples were preserved in 2.5% potassium dichromate ($K_2Cr_2O_7$) solution after collection. It aids in preserving the morphology of protozoan parasites and inhibits the further development of certain helminth eggs and larvae.

3.3.6 Sample examination

All samples were tested in the Central Department of Zoology (CDZ) laboratory at T.U, Kirtipur. The eggs, cysts, oocysts, and larvae of various parasites were identified using morphology and quantitative estimation using Iodine wet mount technique and the concentration method (flotation and sedimentation).

3.3.6.1 Iodine wet mount technique

This approach is commonly used to detect protozoan eggs/cysts since Iodine makes them visible. A toothpick was used to stir the fecal sample. On a clean glass slide a solution of 1% Lugol's iodine and a sample of an emulsified toothpick head was placed. Then, a coverslip was delicately kept on top, and smear was thoroughly studied under a microscope (Swift, M4000-D) with 10X and 40X objective lenses (Zajac & Conboy 2012).

3.3.6.2 Flotation concentration method

Nematode and cestode eggs are lighter than trematode eggs, this technique is typically used to detect them. They float on a saturated solution of sodium chloride (NaCl). The mixture was prepared by combining about 3 g of fecal sample with 20 ml of distilled water. It was then filtered through a tea strainer after grinding in a mortar. The resulting filtrate solution was transferred into a centrifuge tube with a capacity of 15 ml and subjected to centrifugation for a duration of 5 minutes at 2000 rpm using a Remi R-303 centrifuge machine. Following this, the water was drained from the tube and saturated NaCl solution was added and centrifuged again. Then, a more saturated NaCl solution was added to generate a convex surface at the top of the tube. A cover slip was placed over the tube for a few minutes, after which it was mounted on a slide and examined under 10X and 40X magnification (Soulsby 2012).

3.3.6.3 Sedimentation concentration method

After examining the floated portion, the saturated salt solution was carefully removed from the test tube, and the sediment content was poured into a watch glass and delicately mixed. One drop was removed from the mixture to prepare a slide. The specimen was stained with a damp mounts solution containing iodine. Generally, this technique detects trematode eggs due to their heavy weight and large size (Soulsby 2012).

3.3.7 Measurement and Identification of eggs/cysts/oocysts

To estimate the precise size of parasite oocysts or eggs, the ocular micrometer's reference line (usually the 0 mark) was aligned with a suitable line on the stage micrometer. The perfectly overlapped lines were identified, and the number of divisions on the ocular micrometer occupied by the parasite oocysts or eggs was multiplied by the calibration factor (2.24µm). The micrometer used for measurement was the Erma Inc. ESM-11. The identification of eggs, oocyst and larvae was done by comparing their morphology, size, and color using references such as book (Zajac & Conboy 2012; Foreyt 2013), as well as other published and unpublished articles.

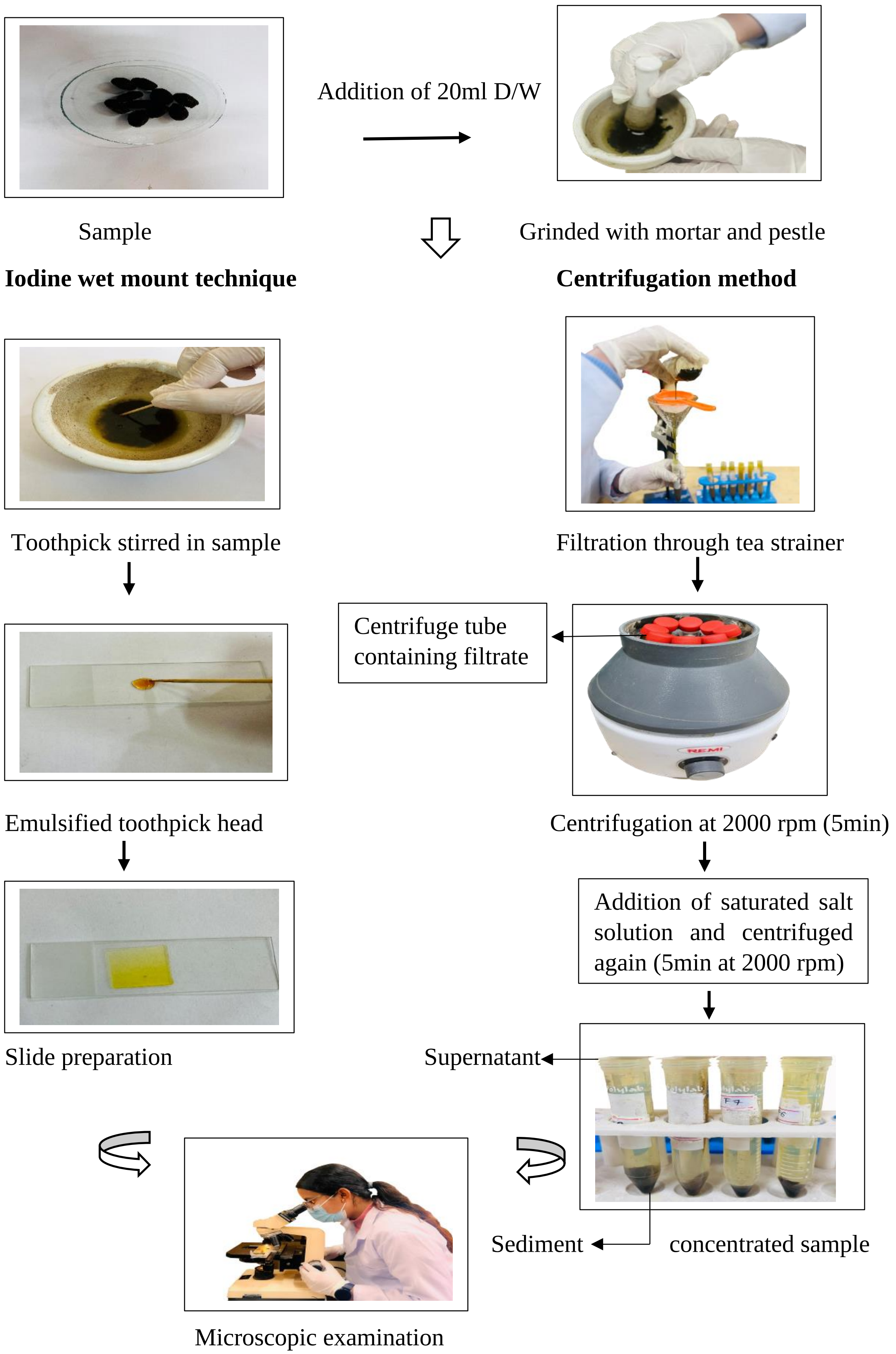

Figure 3: Flowchart illustrating laboratory analysis of sample

3.3.8 Intensity of parasites

The intensity of parasitic infection was evaluated by counting the number of eggs, oocysts, or larvae observed within each microscopic field. The infection was divided into four groups: light infection, mild infection, moderate infection, and heavy infection.

Light infection (+) = less than two egg/oocyst/larvae per field

Mild infection (++) = two or three egg/oocyst/larvae per field

Moderate infection (+++) = four or five egg/oocyst/larvae per field

Heavy infection (++++) = six or more egg/oocyst/larvae per field

3.3.9 Data analysis

IBM SPSS Statistics (version 28 © IBM Corporation) was used for the analysis after entering all the data into an Excel worksheet (version 2305). Chi-square (χ^2) test was used for statistical analysis of data. In each instance, a statistically significant difference was determined using a 95% confidence interval (CI) and threshold of p-value less than 0.05. The prevalence was computed using the formula:

Prevalence = $\frac{n}{N} \times 100$

where n = number of positive sample and

N = total number of fecal samples examined.

4. RESULTS

4.1 Overall prevalence of gastrointestinal parasites

Iodine smear and concentration technique was performed on a total of 150 samples. Among them, 144 tested positive for one or more gastro-intestinal parasites, indicating a prevalence of 96% in blackbuck of BCA.

Table 1: Overall prevalence of GI parasites in blackbuck

S.N.	Sex	Study sample	Infected	Total Prevalence
1.	Male	63	61	96%
2.	Female	87	83	
Total		N = 150	n = 144	

4.2 Sex wise prevalence of GI parasites

The prevalence of gastrointestinal parasites in females was higher i.e., 55.33% as compared to male which was 40.67%. There was no statistically significant variation in sex wise prevalence of GI parasites (χ^2 = 0.193, df=1, p = 0.661).

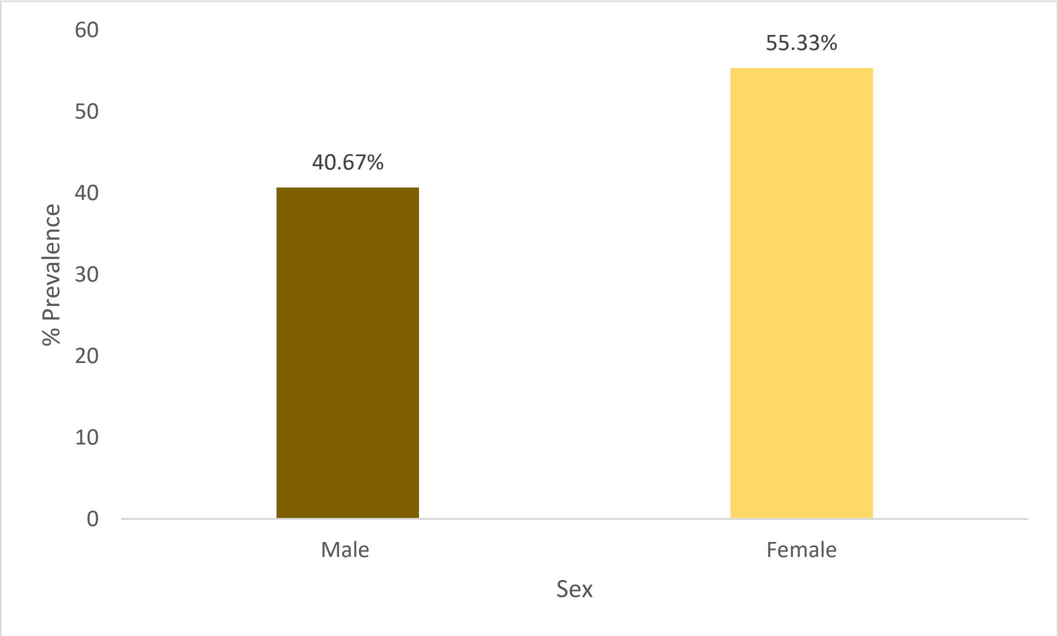

Figure 4: Sex wise prevalence of gastrointestinal parasites in blackbuck

4.3 Genera wise prevalence of GI parasites

Microscopic examination revealed 10 different genera of GI parasites with *Paramphistomum* sp. (55.33%) having highest prevalence and *Trichuris* sp. (7.33%) having the lowest. There was no statistically significant relationship between sex and prevalence by genus (χ^2 = 9.141, df= 9, p = 0.424).

Table 2: Genera wise prevalence of GI parasites in blackbuck

S.N.	Identified GI parasites	Number of infected samples		Total prevalence	χ^2 value	P value
		Male	Female			
1.	*Eimeria* sp.	11	18	19.33%	9.141	0.424
2.	*Entamoeba* sp.	9	14	15.33%		
3.	*Moniezia* sp.	15	21	24%		
4.	*Fasciola* sp.	23	31	36%		
5.	*Paramphistomum* sp.	36	47	55.33%		
6.	*Trichostrongylus* sp.	17	15	21.33%		
7.	*Ascaris* sp.	5	8	8.67%		
8.	*Haemonchus* sp.	23	16	26%		
9.	*Trichuris* sp.	4	7	7.33%		
10.	*Strongyloides* sp.	26	52	52%		

4.4 Class wise prevalence of GI parasites

In the present study, 144 samples were positive for the presence of gastrointestinal parasites belonging to ten different genera and four distinct groups, namely protozoa, cestode, trematode, and nematode.

4.4.1 Prevalence of protozoan parasites

Two different protozoan parasites identified, among them *Eimera* sp. showed higher prevalence than *Entamoeba* sp. There was no significant difference in prevalence of protozoan parasites (χ^2 = 0.692, df = 1, p = 0.405).

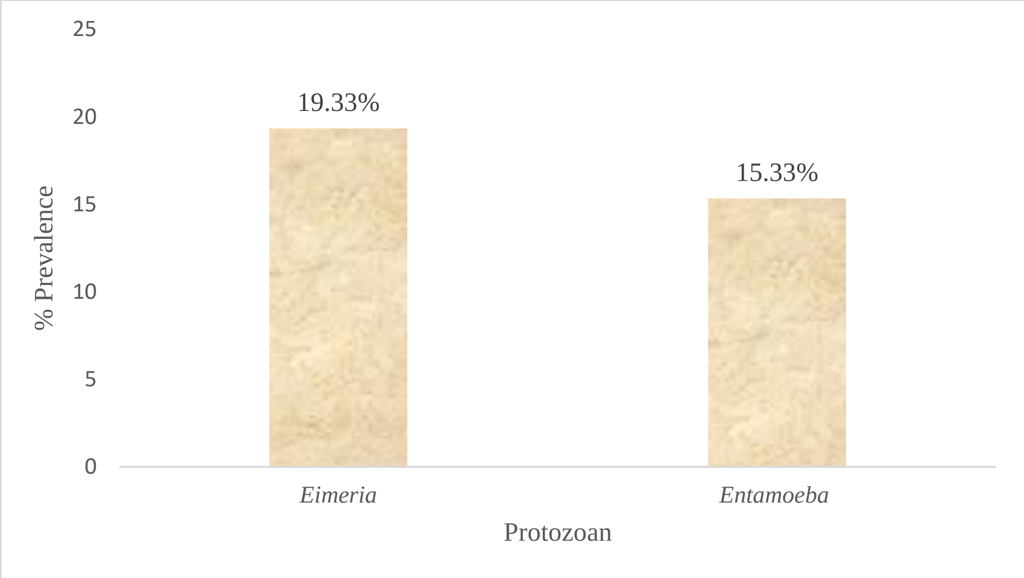

Figure 5: Prevalence of protozoan parasites in blackbuck

4.4.2 Prevalence of cestode parasites

Out of total samples examined, 36 (24%) samples were determined to be positive for cestode parasites. There was only one genus of cestode; the recovered cestode parasite was *Moniezia* sp.

4.4.3 Prevalence of trematode parasites

Among the analyzed samples, *Paramphistomum* sp. was more prevalent as compared to *Fasciola* sp. and statistically, there was significant variation in the occurrence of trematode parasites ($\chi^2 = 6.139$, $p = 0.013$).

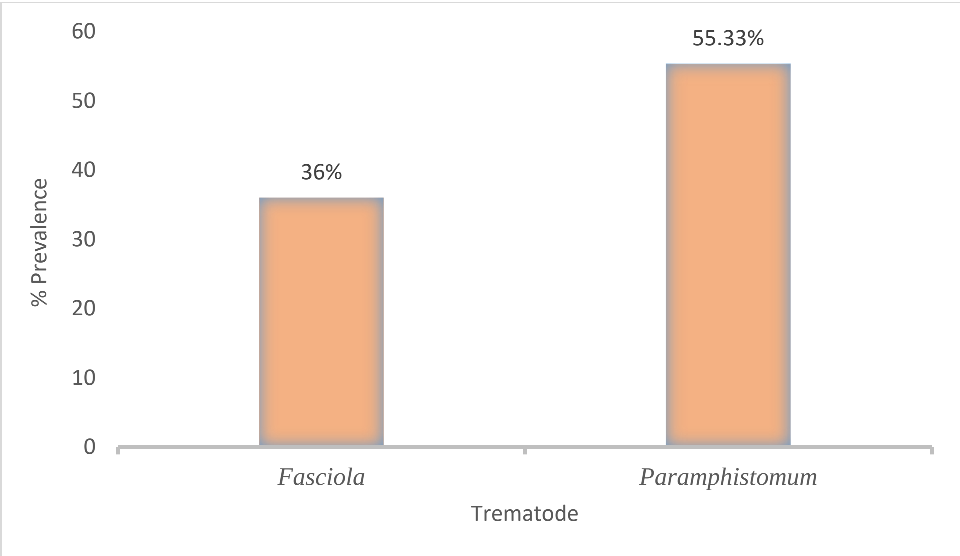

Figure 6: Prevalence of trematode parasites in blackbuck

4.4.4 Prevalence of nematode parasites

In 150 samples, five distinct nematode parasites were identified. *Strongyloides* sp. was found to have the maximum prevalence followed by *Haemonchus* sp., *Trichostrongylus* sp., *Ascaris* sp. and *Trichuris* sp. respectively. The statistical analysis revealed a significant difference in nematode parasite prevalence (χ^2 =84.775, df = 4, $p < 0.05$).

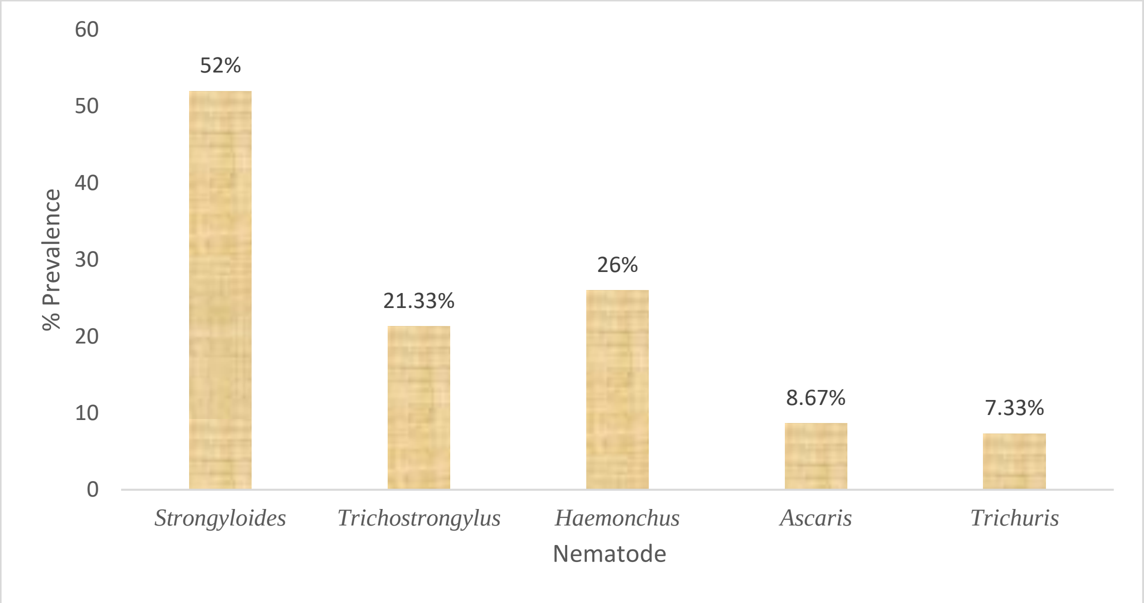

Figure 7: Prevalence of nematode parasites in blackbuck

4.5 Concurrency of parasitic infection

The positive samples revealed mixed infection with one to six genera in each sample. The mixed infection was categorized into four types: single, double, triple, and multiple. Out of all the samples, there were 21 samples with a single infection, 36 with two, 57 with three, and 30 with four or more. The highest occurrence was observed in triple infections, which had the most prevalent rate among them. The study showed there was statistically significant difference in the concurrency of parasitic infection (χ^2 = 19.5, df = 3, p = 0.0001).

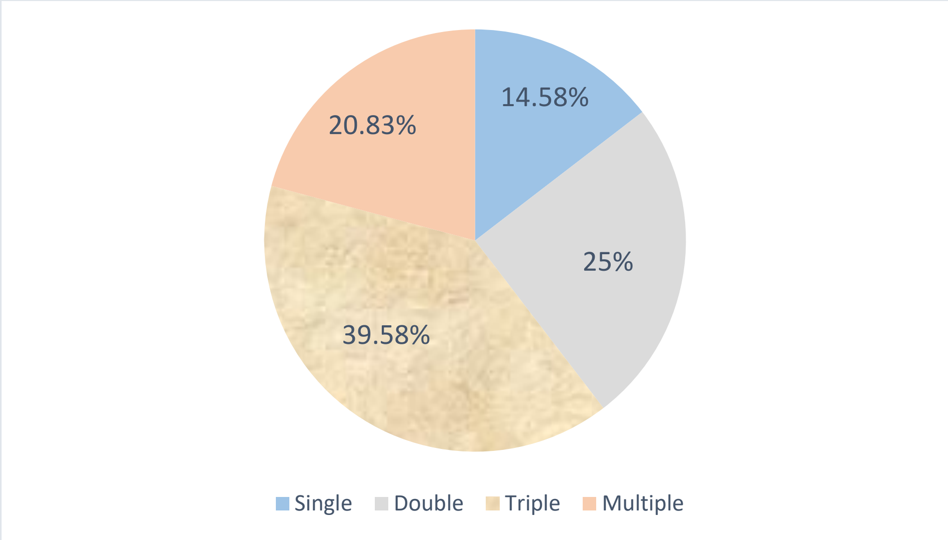

Figure 8: Concurrency of parasitic infection in blackbuck

4.6 Intensity of parasites

The severity of the parasite infection was evaluated by counting the number of eggs, or oocysts, and larvae that were discovered on each microscope slide. The greatest number of blackbucks were found to be affected with light infection. There were five distinct kinds of parasites that displayed heavy infection.

Table 3: Intensity of parasites in blackbuck

S.N.	Parasites	Light (+)	Mild (++)	Moderate (+++)	Heavy (++++)
1.	*Eimeria* sp.	-	-	7 (4.67%)	22 (14.67%)
2.	*Entamoeba* sp.	18 (12%)	5 (3.33%)	-	-
3.	*Moniezia* sp.	-	4 (2.67%)	13 (8.67%)	19 (12.67%)
4.	*Fasciola* sp.	17 (11.33%)	27 (18%)	10 (6.67%)	-
5.	*Paramphistomum* sp.	21 (14%)	30 (20%)	18 (12%)	14 (9.33%)
6.	*Trichostrongylus* sp.	18 (12%)	11 (7.33%)	3 (2%)	
7.	*Ascaris* sp.	9 (6%)	4 (2.67%)	-	-
8.	*Haemonchus* sp.	20 (13.33%)	12 (8%)	5 (3.33%)	2 (1.33%)
9.	*Trichuris* sp.	11 (7.33%)	-	-	-
10.	*Strongyloides* sp.	16 (10.67%)	29 (19.33%)	12 (8%)	21 (14%)

4.7 Identified gastrointestinal parasites in 40X magnification

Figure 9: *Eimeria* sp. (31× 18 µm)

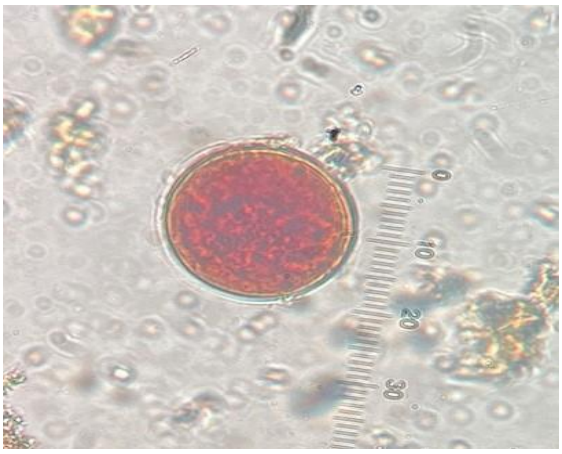

Figure 10: *Entamoeba* sp. (37 µm)

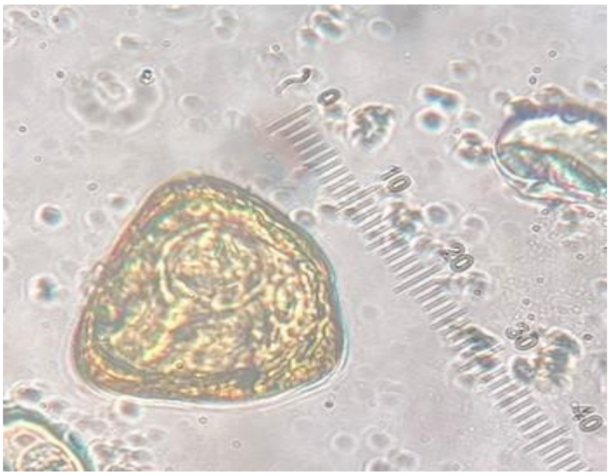

Figure 11: *Moniezia* sp.(63µm)

Figure 12: *Fasciola* sp. (132×78 µm)

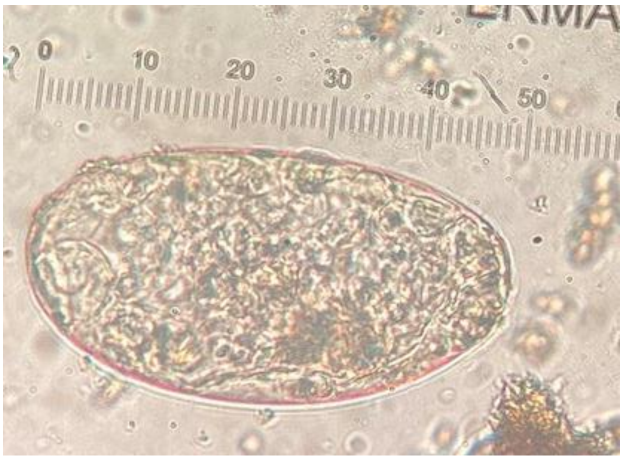

Figure 13: *Paramphistomum* sp. (137×81 µm)

Figure 14: *Trichostrongylus* sp. (92×39 µm)

Figure 15*: Haemonchus* sp. (77×46 µm)

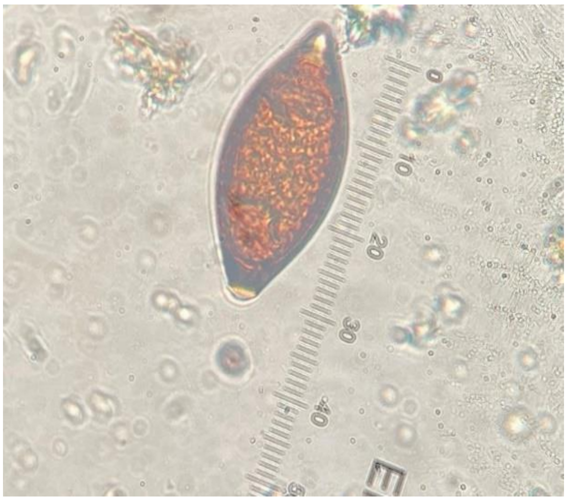

Figure 16: *Trichuris* sp. (72×29 µm)

Figure 17: *Strongyloides* sp. (87×56 µm)

Figure 18*: Strongyloides* larva

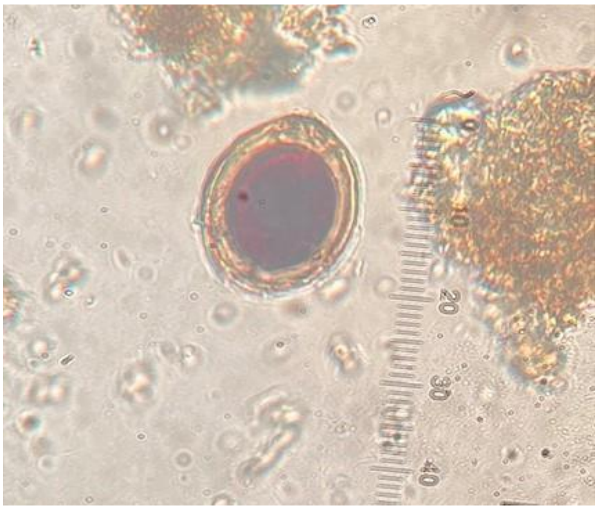

Figure 19: *Ascaris* sp. (48×31µm)

5.DISCUSSION

Blackbuck is a species of medium-sized ungulate that is native to the Indian subcontinent. Males of the species have spiraled horns and a glossy, dark coat, which gives them a distinctive appearance. Social and gregarious, blackbucks prefer open grasslands and scrub forests as their habitat. Despite their ecological and cultural importance, there is a limited understanding of the health and disease ecology of blackbuck (Milton et al. 2019; Prasad et al. 2020). The current study provides valuable insights into the prevalence and diversity of gastrointestinal parasites in blackbuck in a conservation area of Nepal.

The study indicated that parasite prevalence in blackbuck was 96%, which was higher than the findings of previous studies conducted by Pilania et al. (2014) and Chaudhary and Maharjan (2017) which reported prevalence rates of 81.81% and 90% respectively. Pellet samples from the blackbuck population revealed that 93.33% of the population and 100% of the livestock grazed were infected with at least one form of gastrointestinal parasite. The joint grazing ground of blackbuck and livestock increased the likelihood of parasite and disease transmission (Khanal & Chalise 2011). Whereas microscopic inspection of the samples in deer revealed overall parasitic incidence of 98% (Airee 2018). The absence of deworming practices in the blackbuck population of the conservation area can be a significant contributing factor to the higher prevalence of gastrointestinal parasites.

Host behavior is crucial in mediating parasite exposure (Ezenwa et al. 2016). In the current study, females had a greater prevalence of gastrointestinal parasites (55.33%) than males (40.67%). Abara et al. (2021) found that out of the total number of antelopes evaluated, 10 (38.5%) males and 16 (61.5%) females were infected. Physiological changes such as pregnancy and lactation may also weaken females' immune systems, making them more susceptible to parasitic infections. This finding was consistent with the results of another study conducted by Ban (2012), who found a higher prevalence of parasite infection in female animals compared to males. The foraging behavior of female blackbucks could be a possible explanation for the higher prevalence of parasite infection in this species. Females are frequently found in groups and have a propensity for prolonged grazing.

Based on finding blackbuck harbored several protozoan and helminth species with *Paramphistomum* sp. (55.33%) and *Strongyloides* sp. (52%) being the most identified parasites. The outcome of this research aligns with the results reported in prior studies carried out by Chaudhary and Maharjan (2017) and Khanal and Chalise (2011). In wildlife populations Singh et al. (2006) recorded that the most detected parasitic infection in herbivores was *Strongyle* spp. Fathima et al. (2017) also noticed *Strongyloides* sp. in the blackbuck population. Blackbucks are in constant contact with the soil, which is often contaminated with helminth eggs or larvae.

In addition, *Fasciola* sp. (36%) was also found to be prevalent in the blackbuck population in present day findings, which is in line with the findings of (Raza et al. 2007). *Fasciola hepatica* was found to be significantly most prevalent (Lashari & Tasawar 2011). 20.75% infection by *Fasciola* was detected. Several factors contribute to the high prevalence of this parasite, including the presence of suitable intermediate hosts and the ingestion of contaminated water and vegetation (Hossain et al. 2011).

Gastrointestinal nematodes such as *Trichostrongylus* and *Haemonchus* are a common cause of parasitic infections in both livestock and wildlife. Current study found a prevalence of 21.33% and 26% for *Trichostrongylus* and *Haemonchus* respectively. This figure was consistent with the earlier studies, (Cruz-Hernandez et al. 2015; Naz et al. 2021) found prevalence rate ranging from 23.81% to 30%. The prevalence may be attributed to species′ natural habitat, which is open grasslands or semi-arid regions. These environments are ideal for the survival and proliferation of these parasites, as they require a warm and humid climate to complete their life cycle.

According to the findings of the present study, *Moniezia* sp. was the only cestode in blackbuck accounting for 24% of overall prevalence which indicates that this tapeworm is relatively common in this host species. A study by Kar et al. (2007) reported a much lower prevalence of *Moniezia* spp. (3.1%) in goats which are related hosts. On the other hand, Farooq et al. (2012) and Airee (2018) reported higher prevalence. These findings suggest that the prevalence of *Moniezia* spp. in blackbuck may vary considerably depending on the geographic location and the presence of domestic animal reservoirs.

Among the other identified parasites, *Ascaris* sp. (8.67%) had the lowest prevalence rate followed by *Trichuris* sp. (7.33%). Parasitic infection in captive wild by Mir et al. (2016) showed *Trichuris* spp. (19%) and ascarid (10%). The lower prevalence rates in our study may be due to regional differences in parasite distribution and transmission, as well as differences in sampling method.

In the present-day study *Eimeria* sp. (19.33%) and *Entamoeba* sp. (15.33%) were also found. The identification of protozoan parasites, specifically *Eimeria* sp. and *Entamoeba* sp., in the blackbuck population in various studies conducted in different locations highlights their significance in the study area. Heuschele et al. (1986) found coccidia oocysts of the genus *Eimeria* in 2.7% of the specimens examined, while Pilania et al. (2014) reported a higher prevalence of 7.14% for *Eimeria* sp. in blackbucks at Bikaner zoo. Pun (2018) found *Eimeria* without micropyle in 26% and *Eimeria* with micropyle in 15% of the samples tested.

Additionally, *Eimeria* sp. was reported in fecal samples from blackbucks at Shuklaphanta National Park (Pant & Joshi 2019). Coprological analysis by Chouhan et al. (2021) revealed an overall prevalence of 22.78% for *Eimeria* sp. Furthermore, the study by Hassan et al. (2019) reported a prevalence of 26.22% for *Entamoeba* sp. The presence of *Entamoeba* sp. and *Eimeria* sp. in the blackbuck population suggests the potential for these parasites to cause disease and highlights the importance of regular monitoring and appropriate management practices to maintain the health of these animals. The variation in prevalence reported across studies may be attributed to differences in factors such as habitat, climate, and management practices.

The current study highlights that blackbuck frequently suffer from multiple parasitic infections, with a high proportion of mixed infections involving several parasite genera. The prevalence of single infections was found to be 14.58%, while double, triple, and multiple infections accounted for 25%, 39.58%, and 20.83% of cases, respectively. This result was consistent with the study by Airee (2018) who found mixed infections, 14.96% for single infections, 34.01% for double infections, 30.61% for triple infections, and 20.4%. Multiple infection might be due to proximity of blackbuck to domestic animals that may serve as a reservoir for a wide range of parasites.

Notably, Ghimire and Bhattarai (2019) found that samples examined were concurrently infected with more than two parasites, including up to seven different parasites, indicating a high parasite burden in the population and can have detrimental effects on host mortality. Dhakal et al. (2023) showed that multiple species of animals were infected with at least one type of GI parasites, with varying degrees of concurrent parasitic infections. Grazing in contaminated pastures and lack of effective management strategies could contribute to the acquisition of multiple parasites.

The current study conducted revealed that most of the blackbucks were affected with light parasitic infections, defined as less than two eggs or oocysts in each microscopic field. Thapa and Maharjan (2015) and Pangeni (2021) also found light infection in the examined sample. Achhami et al. (2016) detected low to moderate infestation in all identified parasites. It is significant to note that depending on the severity of the infestation, the effects of parasitic illnesses on blackbucks may change.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study revealed a high prevalence of gastrointestinal parasites in blackbuck, with 144 out of 150 samples being positive for parasites belonging to ten different genera and four distinct groups. Among the four groups, the nematode parasites showed the highest prevalence, with *Strongyloides* sp. being the most prevalent parasite, followed by *Haemonchus* sp., *Trichostrongylus* sp., *Ascaris* sp., and *Trichuris* sp., respectively. The protozoan parasites showed a moderate prevalence, with *Eimeria* sp. being more prevalent than *Entamoeba* sp. The prevalence of cestode parasites was relatively low, with *Moniezia* sp. being the only genus recovered, and the trematode parasites showed a significant variation in occurrence, with *Paramphistomum* sp. being more prevalent than *Fasciola* sp. The concurrent infection of multiple parasite genera in a single host further adds to the favorable environment for the coexistence of different genera. Interestingly, female blackbucks were found to have a higher frequency of gastrointestinal parasites than males. The intensity of infection varied greatly among different parasite genera, with some causing only light infections while others were associated with mild to heavy infections. This study emphasizes the need for effective parasite control measures to protect blackbuck populations from the detrimental effects of parasitic infections.

6.2 Recommendations

Based on the study's findings, the following recommendations are made to improve the management and health of the blackbuck population:

- It is crucial to regulate livestock grazing within the blackbuck conservation zone because both blackbuck and the livestock share a common risk of parasite transmission.
- Establishment of veterinary laboratories within conservation areas and wildlife reserves to enable regular diagnosis and treatment of parasitic diseases.

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ANNEX 1

Photo 1: Herd of blackbuck at BCA

Photo 2: Defecating position

Photo 3: Sample collection

Photo 4: Sample preservation

Photo 5: Male blackbuck

Photo 6: Female blackbucks

Photo 7: Male pellets

Photo 8: Female pellets

ANNEX 2

Preparation of 2.5% potassium dichromate solution (Arrington & Prophet 1992)

- The calculated amount of potassium dichromate was carefully weighed using a weighing balance with an appropriate precision. For example, to make 100 ml of the solution, 2.5 grams of potassium dichromate was needed (2.5% of 100 grams).
- The weighed potassium dichromate was added to a suitable container, such as a beaker, and a small volume of distilled water was added.
- The mixture was stirred using a glass rod until the potassium dichromate was completely dissolved.
- Once the potassium dichromate was fully dissolved, more distilled water was added to the container to achieve the desired final volume. For example, to prepare 100 ml of the solution, enough water was added to reach the 100 ml mark on a calibrated measuring cylinder.
- The solution was stirred gently to ensure complete homogeneity.

Preparation of saturated salt solution (Foreyt 2013)

- The volume of the solution needed was determined and the amount of NaCl required was calculated. For 1000 ml (1 liter) of water, 400 grams of NaCl was added to achieve saturation at room temperature. This corresponds to the maximum solubility of NaCl in water at that temperature.
- Using a weighing balance with an appropriate precision, carefully measured the calculated amount of NaCl and added it to a beaker.
- Distilled water was poured into a beaker containing NaCl and stirred the mixture using a glass rod. Continued stirring until the NaCl was completely dissolved. It was important to add enough water to dissolve all the NaCl without exceeding the container's capacity.

ANNEX 3

Population of Blackbuck in Conservation Area from Year 1975 to 2022

Years	Population	**Years**	Population	**Years**	Population
1975	9	**1991**	177	**2007**	131
1976	23	**1992**	150	**2008**	185
1977	38	**1993**	109	**2009**	191
1978	_	**1994**	111	**2010**	219
1979	_	**1995**	109	**2011**	225
1980	23	**1996**	257	**2012**	280
1981	38	**1997**	240	**2013**	287
1982	_	**1998**	101	**2014**	300
1983	66	**1999**	113	**2015**	274
1984	100	**2000**	113	**2016**	230
1985	130	**2001**	50	**2017**	230
1986	152	**2002**	53	**2018**	257
1987	164	**2003**	74	**2019**	201
1988	170	**2004**	92	**2020**	234
1989	177	**2005**	85	**2021**	173
1990	177	**2006**	107	**2022**	183

Source annual report: 2022/23, BCA