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Research Article

Parasitic infection in blackbuck (*Antilope cervicapra* Linnaeus, 1758) of Blackbuck Conservation Area, Bardiya and Shuklaphanta Wildlife Reserve, Kanchanpur, Western Nepal

Ram Bahadur Chaudhary and Mahendra Maharjan*

Central Department of Zoology, Tribhuvan university, Kathmandu, Nepal

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 parasitic infection in blackbuck of BCA and SWR. A total of 150 and 70 fresh faecal samples of blackbuck were collected from BCA and SWR respectively and examined by floatation, sedimentation and Stoll's counting technique. The overall prevalence of GI parasites was found to be 90.00%. The protozoan and helminthic parasitic prevalence revealed 55% and 89% respectively in both the study area. Blackbuck of BCA were found to be infected with 12 different species of parasites which includes *Entamoeba* sp. (20%) and *Eimeria* sp. (45.33%) among protozoa; *Paramphistomum* sp. (25.33%) and *Fasciola* sp. (17.33%) among trematodes; *Moniezia* sp. (14%) among cestode; *Trichostrongylus* sp. (75.33%), *Ascaris* sp. (57.33%), *Haemonchus* sp. (18%), *Strongyloides* sp. (16%), *Bunostomum* sp. (12.67%), *Trichuris* sp. (6%) and *Oxyuris* sp. (4.67%) among nematodes; while blackbuck of SWR were found to be infected with 10 different parasitic genera, i.e. *Entamoeba* sp. (8.57%) and *Eimeria* sp. (51.43%) among protozoa; *Paramphistomum* sp. (38.57%), *Fasciola* sp. (21.43%) and *Schistosoma* sp. (7.14%) among trematodes; *Trichostrongylus* sp. (55.71%), *Ascaris* sp. (38.57%), *Haemonchus* sp. (14.28%), *Strongyloides* sp. (12.86%) and *Bunostomum* sp. (2.86%) among nematodes. The present findings provide some baseline information on the parasitic burden in Blackbuck and help to formulate appropriate strategies to mitigate the endo-parasitic problem of blackbuck in SWR and BCA.

Key words: Blackbuck, GI parasites, wildlife, Blackbuck Conservation Area, Shuklaphanta Wildlife Reserve

Introduction

Blackbuck (*Antilope cervicapra*), locally known as Krishnasaar, is an elegant, gazelle-like animal regarded as the most handsome member of the Bovidae family and order 'Artiodactyla'. According to the coat, color, length and the shape of the horn, there are four sub-species of *Antilope cervicapra,* i.e. *Antilope cervicapra cervicapra*, *A. cervicapra centralis*, *A. cervicapra rupicapra* and *A. cervicapra rajputane* (Corbett & Hill, 1977). The blackbuck is Near Threatened which belongs to IUCN Red List of Threatened Species (IUCN, 2008).

The blackbuck is typically distributed in India, Pakistan and Nepal (Lydekker, 1924). Ranjitsinh (1989) 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 & Burton, 1987). About 43,600 Blackbucks were introduced to Argentina and the USA (Mallon & Kingswood, 2001). In Nepal, before the malaria eradication programme, blackbuck was commonly found in Eastern and Western Tarai; 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, Nepalgunj mini zoo and semi-captive enclosures at Mrigasthali, Kathmandu (Khanal, 2006). Now, blackbucks are also restricted to Hirapur phanta of SWR, Kanchanpur (DNPWC, 2012). Twenty eight blackbucks were re-introduced to Hirapur phanta of SWR, Kanchanpur (DNPWC, 2012). In 2012, the population of blackbuck was estimated to be 287 in blackbuck Conservation Area, Khairapur (Shah, 2012).

The animals suffer from a variety of infectious and non-infectious diseases, particularly that of parasitic origin (Akhter & Arshad, 2006; Lama et al., 2015). The major concern with wild ruminants

*Corresponding author, email address: mmaharjan@cdztu.edu.np

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 parasitic infections like coccidiosis, paramphistomiasis, fascioliasis, schistosomiasis, taeniasis nematodiasis (Thornton et al., 1973). Mortality and health problem diagnosed mainly in young adult male and pregnant blackbucks include food shortage and malnutrition, overcrowding and parasitism in USA (Thornton et al., 1973). 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 endo-parasites. The major gastro-intestinal parasites reported in blackbuck includes Coccidia, *Taenia hydatigena, Camelostrongylus* sp., *Haemonchus* sp., *Trichostrongylus* sp., *Nematodirus* sp., *Oesophagostomum* sp., *Trichuris* sp., *Paramphistomum* sp., *Schistosoma* sp., *Moniezia* sp. (Ban, 2012; Frooq et al., 2012; Khanal, 2006). Out of these reported parasites, *Camelostrongylus mentulatus* is most dreadful nematode to blackbuck and also associated with chronic emaciation and death of dominant adult males (Flach & Swell, 1987).

Materials and Methods

Study area

The study was conducted in BCA of Khairapur, Bardiya district and SWR of Kanchanpur district Nepal. The blackbuck Conservation Area is the only one conservation area of Tarai region, which is located at Khairapur, Bardiya district in Western Nepal. It covers an area of 16.95 km^2 which includes the core habitat of 5.27 km^2 with circumference of 10.25 km and peripheral area of villages and settlements spreading over 11.68 km^2. The SWR is a protected area, located in the Far western low-land Tarai towards the southwest edge of Nepal. It covers 305 km^2 of open grassland, forests, riverbed, and tropical wetlands at an altitude of 174 to 1,386 meters. The blackbuck habitat is situated in Hirapur Phanta of SWR (Fig. 1).

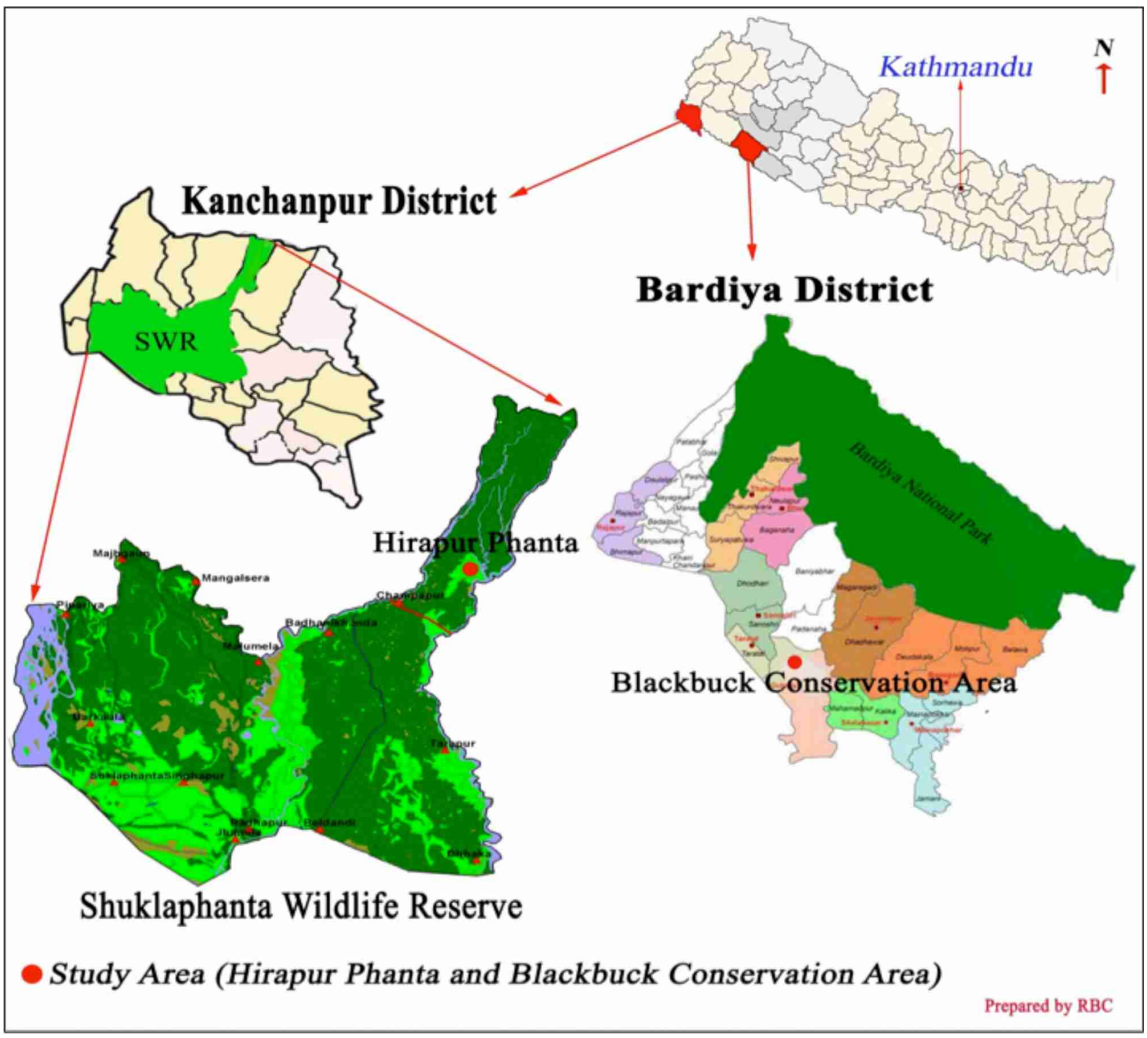

Figure 1 Location of blackbuck conservation area (Map not to scale)

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 district from June to August 2013.

Sample collection method
At first, the major habitat of blackbuck was determined and observed the position of defecation and urination according to sex, in order to collect the fresh faecal samples with help of blackbuck's caretaker. In the next day early morning or sunset time, proper care was taken when an animal defecated and sex was identified, then the fresh sample was collected in sterile vial from field immediately to prevent contamination (Plate 1, Photo 1-6).

Preservation of samples
Collected faecal samples of blackbuck were preserved in 2.5% potassium dichromate that helps in maintaining the morphology of protozoan parasites and preventing further development of some helminthes eggs and larvae.

Microscopic examination
All samples were examined at the laboratory of Central Department of Zoology, Tribhuvan University, Kirtipur. 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 ova dilution technique to determine eggs per gram (EPG), cyst per gram (CPG), oocyst per gram (OPG) of faeces (Soulsby, 1982).

Data analysis
The association of parasitic infection between BCA and SWR as well as among the endo-parasites calculating the chi-square values and probability (P- value) at 95% confidence level at various degree of freedom (d.f) using the software SPSS version 19.

Results and Discussion

Blackbucks of both the study area (BCA and SWR) were found to be infected with protozoan as well as helminthes parasites. The overall prevalence of parasites was found to be 90.0% in BCA and SWR. The highest prevalence may be due to favorable climatic conditions, constant exposure of infestation and availability of infective stage larvae on the grazing ground by animals (Lama et al., 2015; Opara et al., 2010). Furthermore, samples were taken during rainy season, i.e. from June to August. This might be the reason for the highest prevalence of parasites. Sufficient rainfall and moisture during wet season favor the survival of infective larvae in pasture and higher probability of uptake of the infective larvae leading to higher prevalence rate (Kuchai et al., 2010). The characteristics of cyst, oocysts and eggs of the identified parasites are shown in Table 1.

Protozoan parasitic infection in blackbucks
Protozoan parasitic infection in blackbucks was found to be 55.33% and 55.71% in BCA and SWR respectively. In both of the study area, fawns were infected more (60%) compared to both the sexes of blackbucks. Even though, the difference was statistically significant in BCA (P= 0.001), it was insignificant in SWR (P= 0.667). The blackbucks of both the study area were highly infected with *Eimeria* sp. parasites than *Entamoeba* sp. (Fig. 2). The result indicated the maximum fawns were infected by *Eimeria* sp. with and without micropyle group. The distribution of each species of protozoan parasites are not statistically significantly different in between BCA and SWR (P=0.958). Due to coccidiosis, the infected hosts generally exhibit loss of appetite, weakness, diarrhoea, particularly in fawns than the adults, resulting in the compromised immune system (Ghimire et al., 2008) indicating its high impact on the survival of Bblackbuck.

Trematodes
Prevalence of trematode parasitic infection observed in blackbuck of BCA and SWR were 34.67% and 50% respectively. These prevalence rates are almost comparable with the results presented earlier (Ban 2012; Khanal, 2006). Blackbucks of both study area were found highly infected with intestinal fluke, *Paramphistomum* sp. in compared to liver-fluke, *Fasciola* sp. However, infection of blood-fluke (*Schistosoma* sp.) was found only among the blackbucks of SWR (Fig. 3). It indicates that appropriate snail intermediate hosts exist in both study area. *Paramphistomum* sp. and *Fasciola* sp. have been reported earlier from Blackbucks (Ban, 2012; Rao & Acharjyo, 1972; Rimal, 2011). The result revealed high prevalence of trematode parasitic infection in adult blackbucks of both sexes than the fawns.

Cestodes
The cestode parasites identified in the faecal examination of wild ruminants of various part of the world are *Taenia hydatigena, Moniezia expansa and M. benedeni* (Bogale et al., 2014; Frooq et al., 2012; Kuzmina et al., 2010; Mohammad et al., 2012) and in Nepal (Ban, 2012; Thapa, 2013). However, the present study hasn't found *Taenia* sp. in any samples, but genus *Moniezia* has been isolated in some samples. The prevalence rate of *Moniezia* sp. was observed to be 14% in blackbuck of BCA, which is higher than the earlier reports (Ban, 2012).

Nematodes
Blackbucks were found highly infected by nematode parasites than others. Out of the total samples examined, 89.33% samples of BCA and 81.43% samples of SWR were positive for gastrointestinal nematode parasites. Among them, *Trichostrongylus sp.* (75.55%) was the highly prevalent in Blackbuck of BCA followed by *Ascaris* (57.33%), *Haemonchus* sp. (18%), *Strongyloides* sp. (16%), *Bunostomum* sp. (12.67%), *Trichuris* sp. (6%) and *Oxyuris* sp. (4.67%) (Fig. 4). The nematode *Haemonchus contortus* (wire

Table 1 Characteristics of cyst, oocyst and eggs of blackbuck parasities

Class	Genera	Size (μm)	Characteristics	Plate No.	Photo No.
Sporozoa	*Eimeria* sp.	34.5 + 19.5 (without micropile)	small with morula located centrally or sub-centrally or completely filled up, Micopile onone side.	2	1 & 2
Sarcodina	*Entamoeba* sp.	30 + 6 (diameter)	small, rounded or spherical having four nucleus	2	3
Trematoda	*Fasciola* sp.	135 + 15 x 79.5 + 4.5	Oval, unsegmented thin shell containing an ovum and cluster of yolk cells, morula sub-central, operculum usually indistinct	2	4
	Paramphistomum sp.	165 + 5 x 82.5 + 7.5	operculum in one pole, containing five blastomeres surrounded by about 50 yolk cells, morula centrally or somewhat sub centrally	2	5
	Schistosoma sp.	147 + 3 x 75	spindle shaped, flattened at one side, greatly elongated slender posterior spine at another end	2	6
Cestoda	*Moniezia* sp.	70.5 + 4.5	triangular or; somewhat irregular having a circular or pear shaped (pyriform) apparatus at one end	2	7
Nematoda	*Trichostrongylus* sp.	81 + 6 x 48 + 12	oval or bean shaped, central mass usually in 8-12 cell stages,	3	1
	Ascaris sp.	31.5 + 7.5	spherical, granular contents and unsegmented, thick aleveolated albuminous shell	3	2
	Stongyloides sp.	88.5 + 7.5 x 52.5 + 7.5	Small, oval with round edges or ellipsoidal, thin shelled containing fully developed larvae	3	3 & 4
	Trichuris sp.	75 x 36	unsegmented embryo, barrel shaped with a transparent plug at either pole	3	5
	Haemonchus sp.	80 + 5 x 48 + 12	oval, thin shelled, hyaline, elongated, large and round, segmented (embryonated) when deposited	3	6
	Bunostomum sp.	95 + 5 x 46.5 + 1.5	4-8 blastomeres, thick and rectangular walls.	3	7
	Oxyuris sp.	112.5 + 7.5 x 55.5 + 19.5	bean shaped, embryonic mass surrounded by sticky fluid	3	8

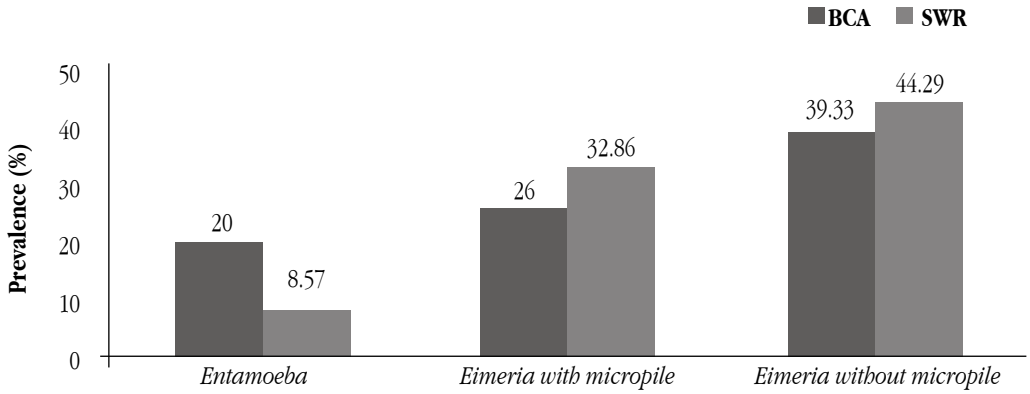

Figure 2 Protozoan parasitic infections in blackbucks of BCA and SWR

worm) is one of the most important pathogens for blackbuck (Thorton et al., 1973), producing a disease known as haemonchiasis which can cause anemia, growth loss, edema, emaciation, and even death (Roberts & Janovy, 2005). Three species of *Trichostrongylus* encountered in blackbuck include *Trichostrongylus axei, T. colubriformis* and *T. probolurus* (Thornton et al., 1973) and *T. retortaeformis, T. vitrinus* and *T. capricola* in other wild ruminants (Kuzmina et al., 2010; Mayo et

al., 2013; Mohammd et al., 2012). Three species of *Trichuris* sp. that have been described in previous reports include *T. globulosa, T. cervicaprae* and *T. ovis* (Goossens et al., 2005; Thornton et al., 1973,). In the present study, *Trichuris* sp. (6%) was isolated from blackbuck of BCA only. This shows that blackbuck of BCA are more infected with *Trichuris* sp. as compared to 4.19% and 3.26% recorded by Bogale et al. (2014) and Ban (2012), respectively.

This result shows that the blackbuck Conservation Areas are highly infected with *Ascaris* sp. eggs. A characteristic *Ascaris* sp. egg was also found to be distributed in many faecal samples of the present study. Likewise, the prevalence of *Bunostomum* sp. in the present study was found to be 12.67% and 2.86% in blackbuck of BCA and SWR respectively. The prevalence (4.67%) of *Oxyuris* sp. was observed in faecal samples of blackbuck of BCA only. This is much lower than result of Thapa (2013) who revealed 88.24% prevalence in Himalayan Thar and 70.59% prevalence in barking deer.

Mixed infection

Maximum blackbucks of the BCA were found to be infected with multiple parasitic infections in which combination of species like *Paramphiostomum* sp., *Ascaris* sp. and *Trichostrongylus* sp. were common (Fig. 5). While in SWR, double parasitic infection was most common as compared to triple and multiple infections. Among them, combination of coccidian parasites and nematode parasite are the most common. 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. The present result was similar to the findings of Thapa (2013) who described the mixed infection as the most common in Himalayan Thar and barking deer.

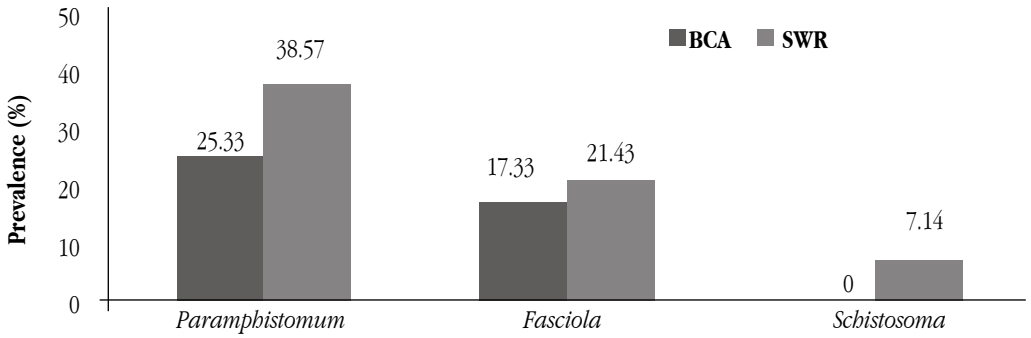

Figure 3 Trematode parasitic infection in blackbucks of BCA and SWR

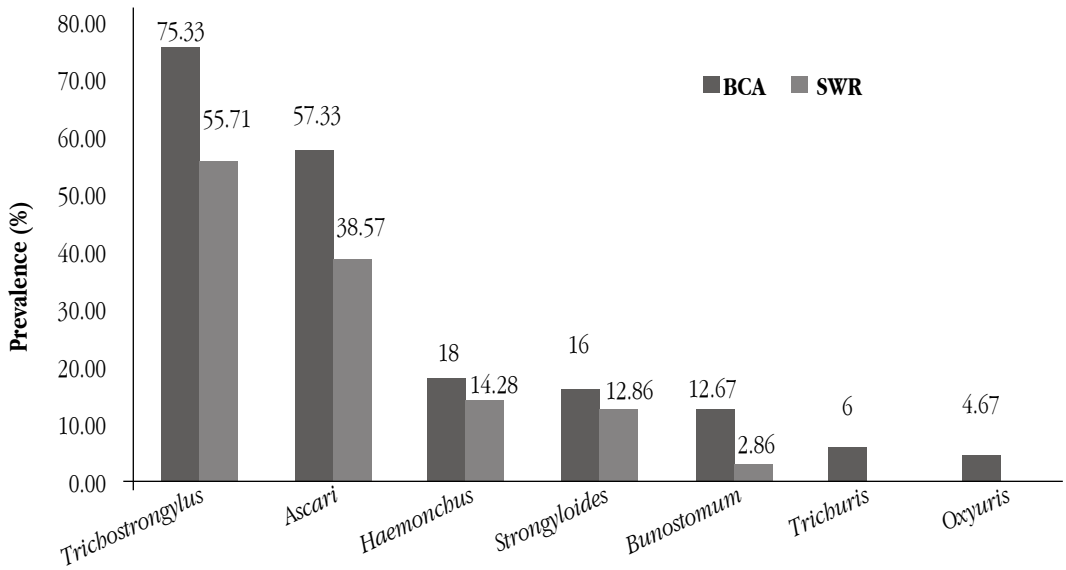

Figure 4 Nematode parasitic infections in blackbucks of BCA and SWR

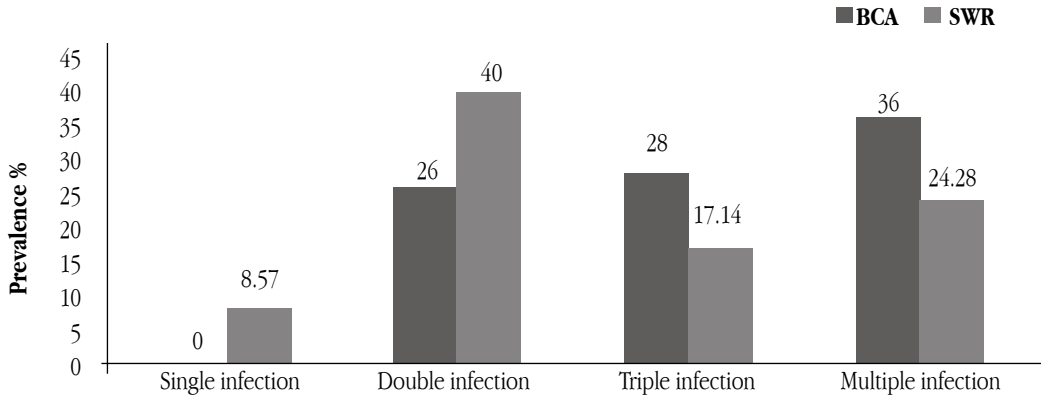

Figure 5 Mixed parasitic infection in BCA and SWR

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 (Table 2). 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* sp. was 700, followed by *Ascaris* sp (400), *Strongyloides* sp, *Haemonchus* sp, *Bunostomum* sp, *Trichuris* sp and *Oxyuris* sp.

Table 2 Parasitic load in blackbuck of BCA and SWR

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

Conclusion

Blackbucks of both BCA and SWR were found to be highly infected with protozoan, trematode, cestode and nematode parasites. Parasitic genera identified infecting blackbucks includes *Eimeria* sp. and *Entamoeba* sp. among protozoans, while *Paramphistomum* sp., *Fasciola* sp. and *Schistosoma* sp. among trematodes; *Trichostrongylus* sp. *Ascaris* sp., *Haemonchus* sp., *Strongyloides* sp., *Trichuris* sp., *Oxyuris* sp. and *Bunostomum* sp. among nematodes and *Moniezia* sp. among cestode parasites. Mixed parasitic infection was found common in blackbucks of both the study area due to high contamination of pasture lands. The highest parasitic load was found to be 700 revealed by *Trichostrongylus* sp. among all GI parasites. The result indicated that blackbucks are highly susceptible to endo-parasites. Therefore, proper pasture management programme and control strategy should be conducted in BCA and SWR in order to upgrade the health status and conservation of blackbuck in Nepal.

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Plate 1: Photoes 1. Facal pellet of male, 2. Faecal pellet of female, 3. Faecal pellet of fawn,
4. defecating position of female, 5. Defecating position of male, 6. Defecating position of fawn

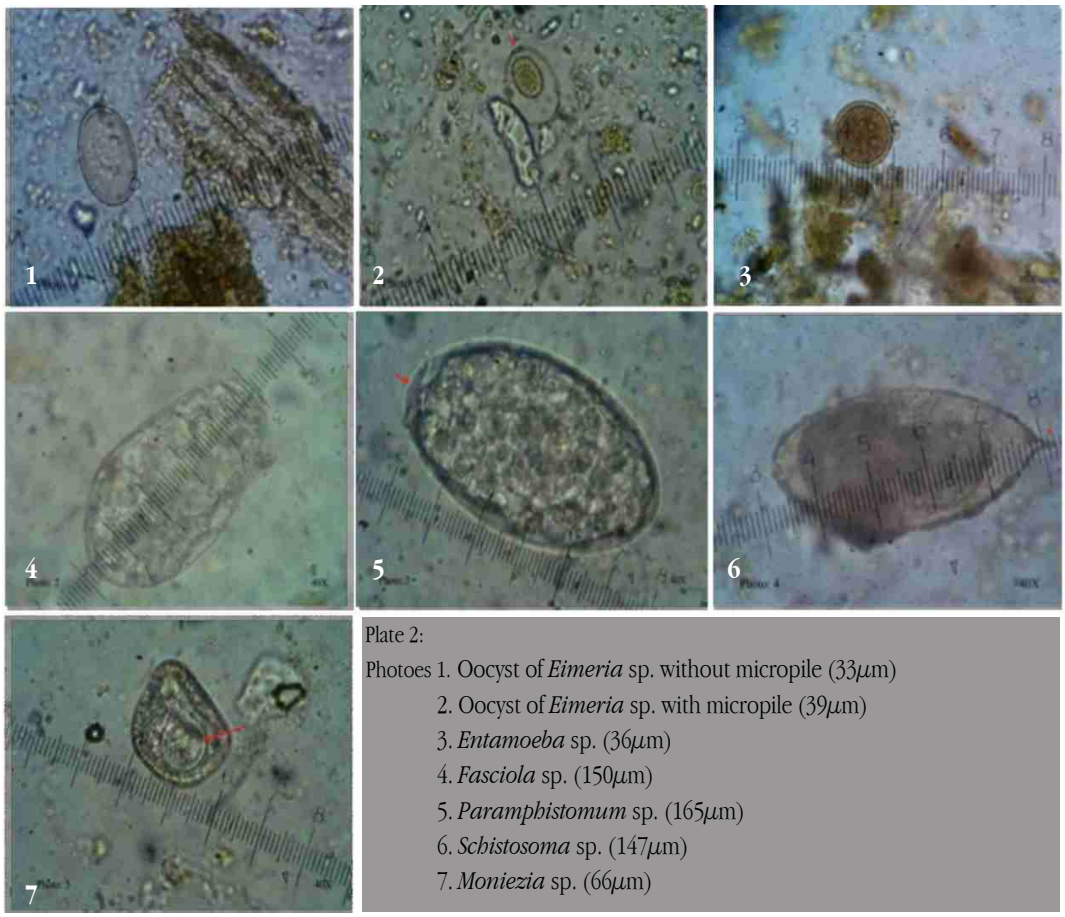

Plate 2:

Photoes 1. Oocyst of *Eimeria* sp. without micropile (33μm)
2. Oocyst of *Eimeria* sp. with micropile (39μm)
3. *Entamoeba* sp. (36μm)
4. *Fasciola* sp. (150μm)
5. *Paramphistomum* sp. (165μm)
6. *Schistosoma* sp. (147μm)
7. *Moniezia* sp. (66μm)

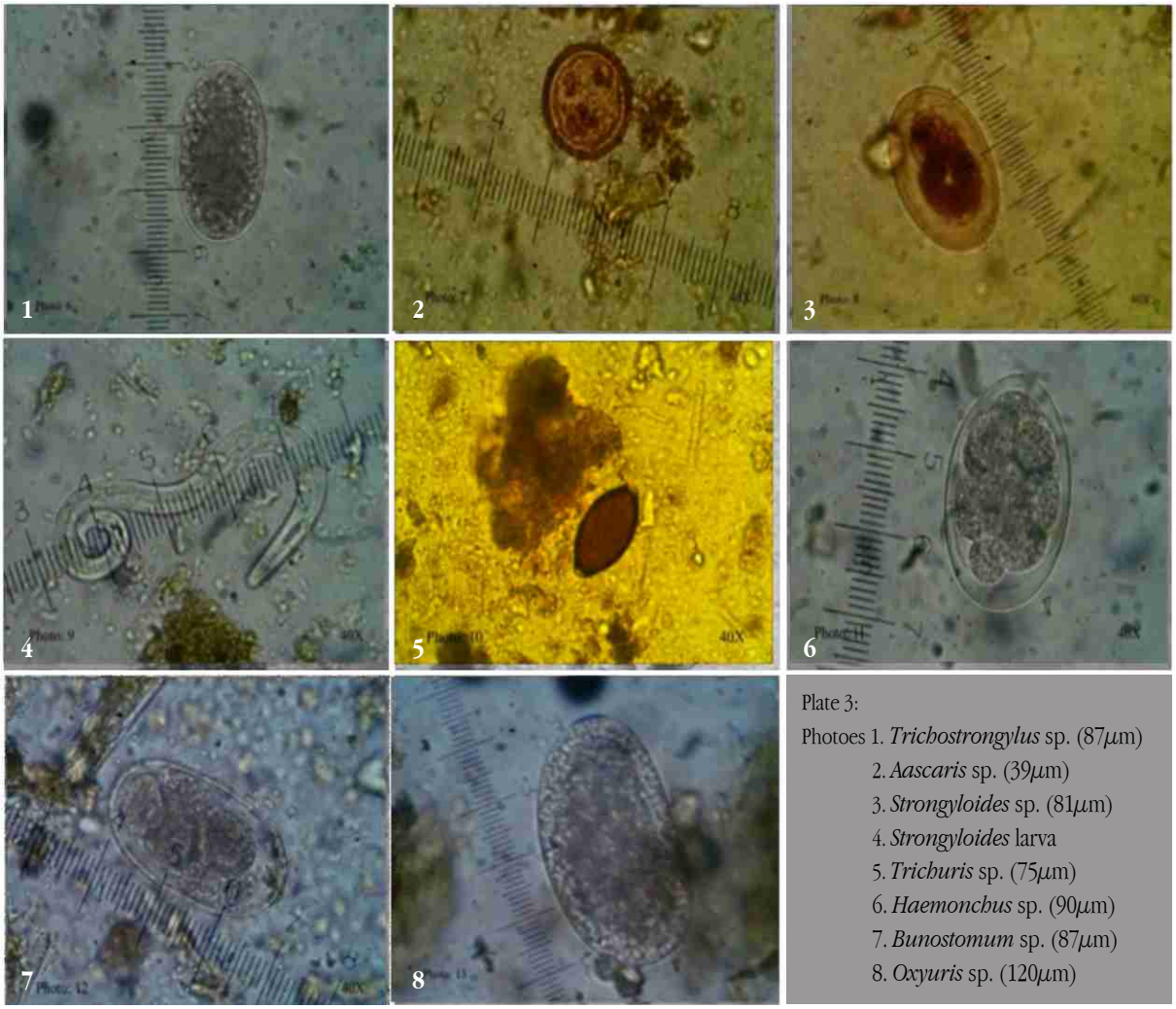

Plate 3:

Photoes 1. *Trichostrongylus* sp. (87μm)
2. *Aascaris* sp. (39μm)
3. *Strongyloides* sp. (81μm)
4. *Strongyloides* larva
5. *Trichuris* sp. (75μm)
6. *Haemonchus* sp. (90μm)
7. *Bunostomum* sp. (87μm)
8. *Oxyuris* sp. (120μm)