RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 5: 587-593 (May 2022)

Prevalence of Gastrointestinal Helminth Parasites of Himalayan goral (*Naemorhedus goral*) in Kazinag National Park, Jammu and Kashmir, India

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ABSTRACT

Background: Natural populations of ungulates are threatened by pathogens and parasites with gastrointestinal parasites being the most widespread. Parasitic infestation is one of the major problems in the management and a growing concern for the conservation of threatened wild animal species. The current study was undertaken to evaluate prevalence of gastrointestinal helminth parasites in Himalayan goral (*Naemorhedus goral*) in Kazinag National Park (KNP) of Kashmir.

Methods: From May 2018 to June 2020, a total of 463 fresh faecal samples of Himalayan goral were collected from Kazinag National Park. The samples were processed by the standard sedimentation and floatation techniques.

Result: Out of 463 faecal samples examined, 236 were found infected with GIT helminthes. In the present study five helminth species were found including, four nematodes [*Haemonchus contortus* (35.70%), *Trichuris ovis* (23.40%), *Strongyloides* spp. (20.80%) and *Trichostrongylus* spp. (12.30%)] and one cestode [*Moneizia expansa* (8.00%)]. Based on the severity of infection, 80.6% of goral positive samples were severely infected (epg>1,500), 9.4% heavily infected (epg = 1,100-1,500), 3.3% moderately infected (epg=800-1,000) and 6.8% mildly infected (epg=500). Epidemiological prevalence of GIT helminthes in goral was profoundly influenced by different seasons. The maximum helminth infection was observed in summer season and minimum in winter (p=0.03). Mixed infection was observed in 206 fecal samples with a prevalence of 47.24%.

Key words: GIT helminthes, Himalayan goral, Kazinag national park, Prevalence, Season.

INTRODUCTION

The Himalayan goral (*Naemorhedus goral*: family Bovidae, order Artiodactyla, class Mammalia), a cliff-dwelling ungulate with goat-like appearance in subfamily Caprinae (Abbas *et al.,* 2011) is an oriental species, endemic to the Himalayan mountains with a distribution range extending in Nepal, Bhutan, Pakistan and north India (Prater, 1980; Mirza,1998; Valdez, 2011). The species is basically a grazer and prefers feeding on grassy ridges and precipitous rocky slopes, but seeks protection under rock overhangs and dense forests (Hayssen and Van Tienhoven, 1993). The species is listed as Near-threatened (IUCN, 2020) and placed in Appendix I of CITES due to its population decline which has been accredited to various anthropogenic pressures (Duckworth and MacKinnon, 2008).

In present scenario, wildlife in any habitat is reeling under great pressure due to numerous factors like stress, climatic variations and anthropogenic activities such as habitat loss, habitat deterioration and poaching. However, diseases especially parasitism of wild ungulates, act as a possible detrimental factor for population decline, pose significant threat to their populations and may be a potent agent of extinction (Woodroffe, 1999; Daszak *et al.,* 2000). Different genera of parasites that inhabit gastrointestinal tract of ungulates cause various diseases (Cordero and Rojas, 1999) resulting in huge economic deficits through reduced food consumption leading to diminutive growth and mortality (Waller, 2006). Gastrointestinal helminth parasites infecting ungulates have profound impacts on their productivity. Parasites infecting such ungulates include coccidian parasites, nematodes, cestodes and trematodes (Onaga *et al.,* 2009; Komaronny, 2010). The wild animals serve as a reservoir of various intestinal parasites including nematodes and trematodes (Richards *et al.*1995; Karbowiak *et al.*,1999) and interactions of these wild animals in different ecosystems execute considerable role in hauling of parasites among different species (Bengis *et al.,* 2002; Smith *et al.,* 2009). Additionally, people living in rural communities are at greater risk of becoming infected due to their close contact with freely roaming wild ungulates around the villages. Gastrointestinal parasitic infestations may induce population

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How to cite this article: Dar, J.A., Fazili, M.F., Bhat. B.A., Ahmad, R. and Wani, I.N. (2022). Prevalence of Gastrointestinal Helminth Parasites of Himalayan goral (*Naemorhedus goral*) in Kazinag National Park, Jammu and Kashmir, India. Indian Journal of Animal Research. 56(5): 587-593. DOI: 10.18805/IJAR.B-4342.

Submitted: 24-10-2020 **Accepted:** 05-02-2021 **Online:** 30-03-2021

decline (Pedersen *et al.,* 2007), however, the decline of population of wildlife due to parasitism is not well documented.

Parasitic diseases are very common in wildlife although low intensity infestations are generally asymptomatic (Anderson and May, 1979). However, heavy infections infect multiple organs and organ systems of the host body particularly intestines (Loukopoulos *et al.,* 2007) which leads to intestinal ulceration and loss of blood and nutrient uptake (Prestwood and Kellogg, 1971), therefore making the animal weak and susceptible to other diseases and death in severe cases (Maublanc *et al.,* 2009). Bovid wildlife is often observed to be susceptible to gastrointestinal infections of domestic livestock with higher rate of susceptibility in limited habitat ranges (Halvorsen, 1986). Migratory herders, locally known as Bakkarwals, migrate seasonally from subtropic plains of Jammu to subalpine and alpine pastures of Kashmir valley. They arrive in the park around late spring with about 15,000 sheep and goats to utilize the seasonal green and nutritious flush in the area during summer and early autumn (Ahmad *et al.,* 2017). Although a number of studies have documented the gastrointestinal parasites of ruminants of Kashmir (Dhar *et al.*, 1982; Lone *et al.*, 2012, 2016; Tariq *et al.*, 2008), there is so far no scientific report on the parasitic infestations of ruminants of Kazinag National Park especially Himalayan goral. Considering the gaps in the literature on Himalayan goral and its gastrointestinal parasites in the region, the present study was carried out to identify the possible GIT helminthes of this threatened goat in Kazinag National Park, Kashmir, India. The findings would be useful for planning conservation measures for protection of this wild ungulate.

MATERIALS AND METHODS

Study area

This study was conducted in the Kazinag mountain range (34°10′0″N and 74°2′0″E) situated in northwestern Himalaya, Jammu and Kashmir, India (Fig 1). It is located in the North-West Himalayan Biogeographic Zone (2A) (Rodgers and Panwar, 1988). Encompassing an area of 90.88 km^2, the park was carved out from three protected areas *viz.* Lacchipora Wildlife Sanctuary, Limber Wildlife Sanctuary and Naganari Conservation Reserve (Fig 1). The elevational range of our study site was between ca. 1800 and 4700 m asl. The region is characterized by severe to moderately cold temperatures during winters and moderate temperatures in summer. The maximum temperature in the region goes up to 30°C during summers and the minimum temperature goes down to -5°C. At higher elevations, the minimum temperatures can go down to -15°C. The vegetation in general is temperate coniferous, sub-alpine and alpine type (Champion and Seth, 1968) dominated by Pine (*Pinus wallichiana*), Deodar (*Cedrus deodara*) and Fir (*Abies pindrow*) in the lower and middle altitudes. At higher altitudes, the subalpine forest is dominated by Birch (*Betula utilis*) and mixed forests where as the alpine vegetation is dominated by Juniper (*Juniperus squamata*) and alpine meadows. The lower areas of riverine forests are overshadowed by Horse Chestnut (*Aesculus indica*) and cranberry bush (*Viburnum grandiflorum*). There are temperate grasslands with rolling terrain at lower elevations. Kazinag harbors other large mammals such as musk deer (*Moschus cupreus*), markhor (*Capra falconeri*), Himalayan brown bear (*Ursus arctos*), Himalayan black bear (*Ursus thibetanus*), Himalayan langur (*Semnopithecus ajax*), common leopard (*Panthera pardus*), yellow-throated martin (*Martes flavigula*) and pheasants such as the western tragopan (*Tragopan melanocephalus*) and cheer pheasant (*Catreus wallichii*).

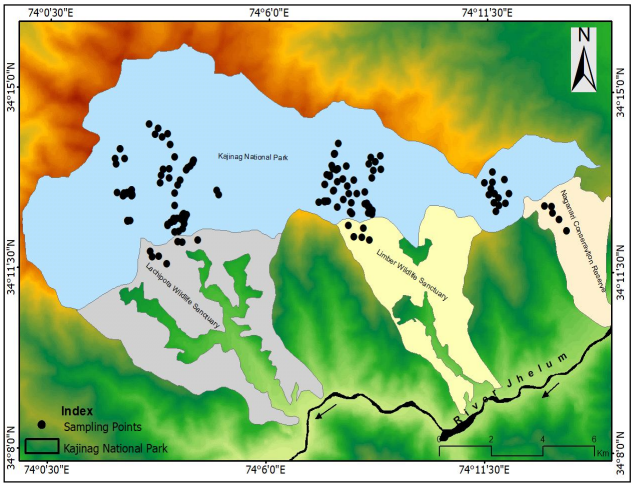


Fig 1: Locations of sampling sites in Kazinag National Park, Jammu and Kashmir.

Faecal examination

A total of 463 faecal samples from May 2018 to June 2020 were collected (Fig 1) from Kazinag National Park between altitudinal range of 1900 m asl and 3600 m asl. Approximately 20 gm of fresh fecal samples, coated with moist mucous, were collected with gloved hands to prevent contamination in every sampling. The pellets of goral were differentiated from markhor, musk deer, sheep and goat on the essence of morphological characters *viz.* dimension, shape and size (Ashraf *et al.,* 2014). A least possible distance of 300-400 m was kept in consecutive sampling to avoid repetition of sampling *i.e.* samples from same individual. Samples were kept separately in 5.0 ml sterile vials in 10% neutral formalin solution. Preserved samples were carried to the Biodiversity Conservation Research Laboratory, Department of Zoology, University of Kashmir. Fecal samples were examined in the first instance by naked eyes for adult parasites and tapeworm segments. Sample of 10-20 g was placed in a petri-dish and diluted with water, ten times of its volume. Thorough mixing was done to make a suspension of required consistency and observation for the presence of any parasite was done (Soulsby, 1982). For observation and diagnosis of gastro-intestinal tract (GIT) helminth eggs, larvae and protozoan cysts, fecal samples were subjected to floatation, sedimentation and Mc Master Test (Sloss *et al.*, 1994; Urquhart *et al.*,1996; Bogale *et al.,* 2014).

Eggs were identified on morphological appearance and size with the help of keys developed by Soulsby (1982) and Zajac and Conboy (2012). The prevalence of each species of parasite in a group of hosts was determined by the number of infected hosts divided by the total number of hosts in the group and expressed as percentage. Based on the severity index defined by Soulsby (1982), parasitic infestations in goral were categorized as mild, moderate, heavy and severe if the fecal egg counts were less than 500, 800-1000, 1100-1500 and more than 1500 respectively and were used to extrapolate the intensity of worm infection. Mean abundances of each of the species was detected.

Data analysis

Chi square test was employed to measure the impact of different seasons on parasitism of goral. Eggs were calculated as number of eggs per gram of feces with their respective means. The data was analyzed using Statistical packages MS-Excel 2019 and MINITAB software version 13.2 with confidence level held at 95% and $P<0.05$ for significance.

RESULTS AND DISCUSSION

An epidemiological investigation on GIT helminthes was conducted among naturally infected goral from the Kazinag National Park in Kashmir Valley. Of the 463 analyzed samples, 236 were found positive for gastrointestinal parasites with an inclusive prevalence of 50.97%. Five genera, comprising of four nematodes and one cestode were identified with prevalence of 92.2% and 7.8% respectively. The parasites in decreasing order of prevalence (%) were *Haemonchus contortus* (35.7%), *Trichuris ovis* (23.4%), *Strongyloides* spp. (20.8%), *Trichostrongylus* spp. (12.3%) and *Moniezia expansa* (8.0%) and their eggs per gram (epg) are shown in Fig 2. Parasitic prevalence differed significantly (χ^2=16.6, df=3, p=0.014) with highest prevalence of 70.86% during summer and lowest (36.79%) in winter. Infection was moderate in spring (40.35%) and autumn (52.58%) (Table 1). Mixed infections were revealed in 49.24% of fecal samples of goral. Based on the severity index defined by Soulsby (1982), 80.6% of goral samples were severely infected, 9.4% heavily infected, 3.3% moderately infected and 6.8% mildly infected. The maintenance of elevated infection rate of helminth parasites in the park might be associated with lack of any parasitic helminthes intervention program and the managing system in the national park where many small ruminants were permitted to graze together on small plots of land throughout the year which facilitates contamination of grazing grounds.

The overall intensity of infection was very low as was apparent from the low/moderate faecal egg counts in different seasons (Table 1). A great deviation in temperature occurs and a grazing interruption in winter disrupts the continuous cycle of infection between host and pasture, so that the transmission of parasites does not occur throughout the year. The grazing grounds of Kazinag National Park are, therefore, contaminated during the summer seasons only by grazing of other ruminants and thus results in higher prevalence of infection during these months.

Prevalence of helminth parasites reported in our study was lower than that reported earlier by different workers *viz.* 81.17% by Pandit *et al.* (2003), 61.64% by Tariq *et al.* (2008a) and 53.59% by Lone *et al.* (2016) in ruminants of Kashmir. Nematodes have been found extremely prevalent in wild ungulates (Rana *et al.,* 2015) and most common GIT helminthes in ruminants of Kashmir (Lone *et al.,* 2012). During present study the overall parasitic prevalence was 50.97% and nematodes were by far the most dominant helminth group (92.2%) infecting goral, followed by cestodes (8.0%), while trematodes were not detected. The absence

Table 1: Influence of seasons on the prevalence of gastrointestinal helminth infection in goral in KNP, Kashmir.

Season	Examined	Infected (%)	Mean EPG
Spring	114	46 (40.35)	1263.3±191.24
Summe	127	90 (70.86)	1828.3±276.15
Autumn	116	61 (52.58)	1643.2±152.67
Winte	106	39 (36.79)	182.0±38.89
Total	463	236 (50.97)	P=0.03

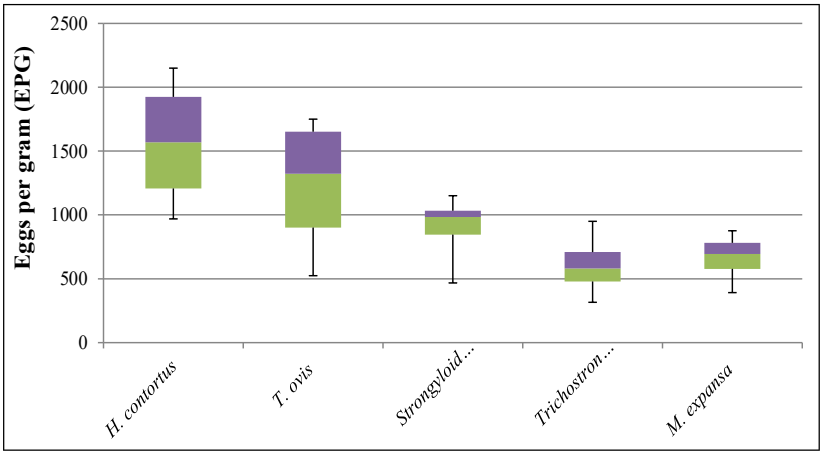


Fig 2: Box plot model for eggs per gram of faeces of GIT parasites of Himalayan goral.

of trematodes may be due the fact that these parasites involve an intermediate host for their transmission (Atanaskova, 2011). This is a high altitude zone and snow covered during winter months with an average winter temperature of -15°C to 5°C and summer temperature not above 30°C. For the establishment of trematodes to a given environment, availability of suitable snail habitat and favorable climatic conditions are needed (Urquhart *et al.,* 2003). Infestation with trematodes has also been seen rarely in wild ungulates (Boomker, 1990; Van Wyk and Boomer, 2011; Karmaliyev, 2019). The trematodes are restricted to only warm and moist regions, where mollusk intermediate hosts occur (Horak, 1981).

The highest prevalence of nematode infection in goral can be explained by the fact that all the nematodes infecting goral have simple lifecycle. Most nematode species do not require any intermediate host for the completion of their life cycle and are transmitted by feco-oral route through contaminated food, water and soil (Gulland and Fox, 1992; Thawait *et al.*, 2014). Such a higher prevalence of nematodes than trematodes and cestodes found in our study is in agreement with the findings from all over the world (Vlasoff, 2001; Fikru *et al.,* 2006; Sissay *et al.,* 2007). *H. contortus*, being a tropical and subtropical parasite, was found to be exceedingly prevalent during present study although in temperate climatic zone of Indian subcontinent. The enhancement in temperature due to trending global climatic variations may be one of the probable factors behind their higher occurrence in this temperate climatic zone. The higher prevalence of *H. contortus* could be correlated to its comparatively concise generation interval and a huge number of eggs (10,000) laid per day for several months (Raza *et al.,* 2014).This nematode has also been found highly prevalent in developed countries of Europe with a prevalence rate of 77% in Switzerland and Italy (Rinaldi *et al.*, 2015). It has also been reported from India (Katoch, 1999) and Pakistan (Raza *et al.,* 2012). Animals with chronic infections, with this helminth, may suffer from anemia and weight loss (Islam *et al.,* 2008) and severe infestations can cause death of the young ones and adult animals within short time (Jamal *et al.,* 2016). Prevalence of *Moniezia expansa* was found moderate. The occurrence of *Moniezia* in ruminants may be attributed to consumption of oribatid mite (with mature cysticercoids in it) while grazing (Slinitson, 1931). The *Moniezia* infections are generally not detrimental and asymptomatic even when the tapeworms are present in huge numbers (Elliott, 1986). However, intestinal obstruction, diarrhea and weight loss may be caused by heavy infection.

The data put together for seasonal estimation of GIT infection unveiled definite seasonal patterns of infection in goral for a period of one year with highest infection during summer and lowest in winter (Fig 3). This is in accordance with the findings of Lone *et al.* (2016) who reported that there were definite seasonal patterns of infection in Hangul with highest infection rate in summer and lowest in winter. A considerable seasonal variation was recorded in fecal egg counts which was influenced by months of the year with significant differences (P=0.03). The highest prevalence (70.86%) and high fecal egg counts (1828.3±276.1) were observed in summer season (Table 1). Presence of sufficient humidity in summer season favors the survival of infective larvae in pastures and enhances their probability of uptake by the ungulate grazers, thus leading to a higher prevalence rate (Sissay *et al.,* 2007). During summer important feeding grounds of goral in Kazinag National Park are occupied by the domestic livestock of nomads (Bakkarwals) and locals which are important source of helminth infection and may lead to transmission of diseases and helminth infestations to goral (Ahmad *et al.*, 2016). The alpine and subalpine grazing grounds of this protected area are, therefore, contaminated by eggs and larvae of helminth species, which pass out along with the fecal pellets of domestic livestock, during summer season, resulting in higher prevalence of infection. Borkovcova *et al.* (2013) also reported that wild ungulates are exposed to a number of infections particularly when they share grazing grounds with domestic livestock. There was a considerable reduction in fecal egg counts from pre-winter (December) to mid-winter (January) period. The low infection reported in this period may be due to the unavailability of intermediate hosts and freezing temperature which results in hypobiosis of nematodes in the host (Ogunsuri and Eysker,1979; Gibbs, 1986). The observations are in agreement with the findings of previous workers (Vlasoff *et al.,* 2001; Shahadat *et al.,* 2003; Tariq *et al.*, 2008; Lone *et al.*, 2012) who reported low winter prevalence of GIT helminthes in other ruminants of Kashmir. The trend of seasonal variations of GIT infestations reported in the present study is in accordance with the findings of various authors (Vlasoff *et al.,* 2001; Khajuria and Kapoor, 2003; Lateef *et al.,* 2005; Tariq *et al.,* 2008a). Mixed infection with multiple helminthes was prevalent in 47.08% fecal samples. Mixed infections cause more drastic diseases than infection with only one species (Terefe *et al.,* 2004; Talpar *et al.,* 2009) because of the impairment of the host's immune system (Wang *et al.,* 2006).

The present findings reveal that grazing wild ungulates are susceptible to receive a high level of GIT infection during the moist months of the year. Such infections become more detrimental for the health of the hosts in harsh winter season due to inadequate nutritional status. Thus, climatic conditions have pronounced impact in the commencement of helminthic infections in Himalayan goral. It is, therefore, necessary to work more on this important issue.

It was noticeable that all helminth parasites encountered during this survey were also recorded in other ruminants despite the habitat diversity, extensive range and grazing area. However, all being ungulates are sharing the same grazing and watering sites, particularly in the summer season; this might predispose wildlife to infestation with all these parasites and explain their high susceptibility to such parasites. However, experimental studies with the goral cannot be simulated owing to its threatened and scheduled status.

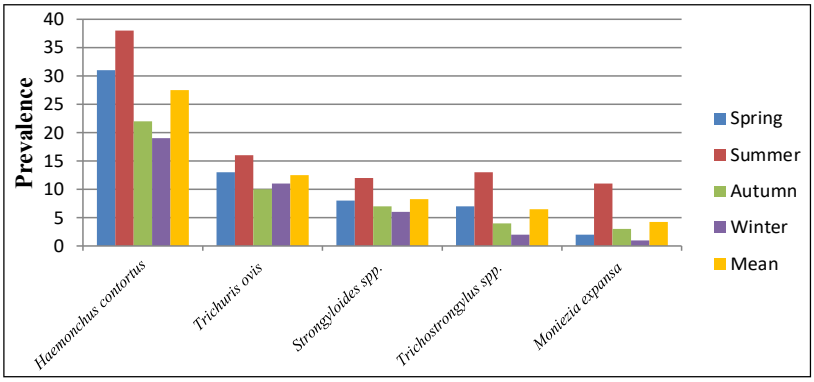
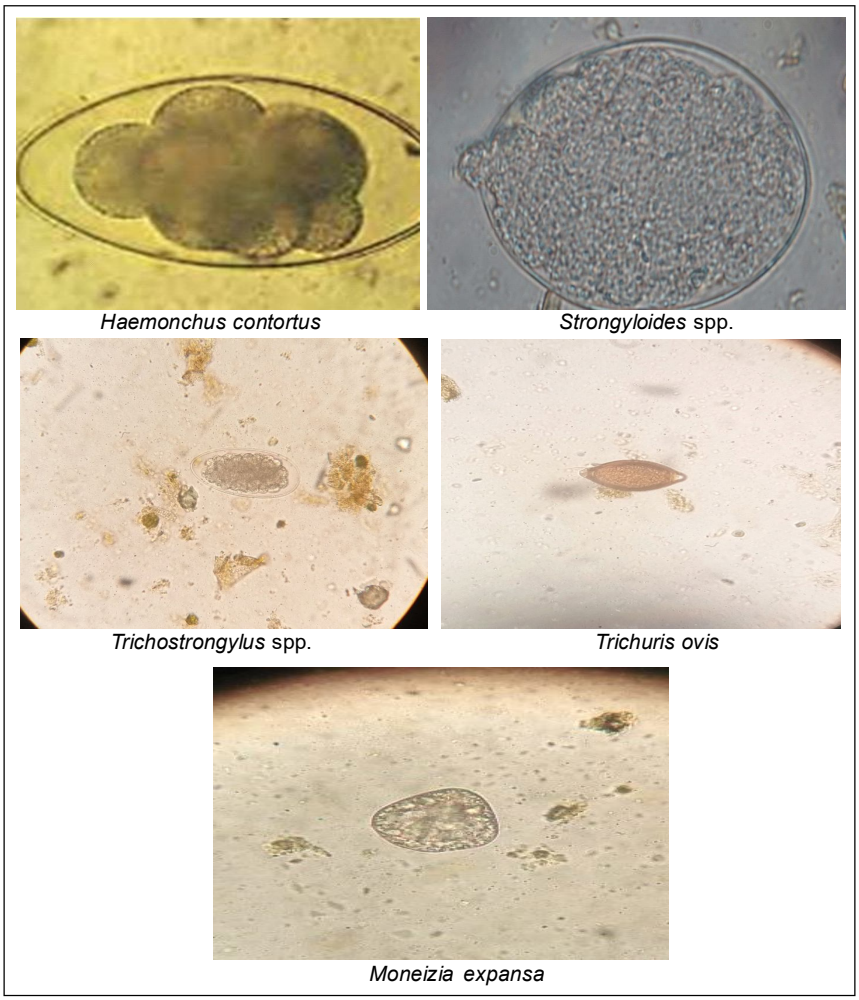


Fig 3: Seasonal and mean prevalence of various GIT parasites of Himalayan goral as assessed by fecal examination.



Photographs of identified parasites

CONCLUSION

The present study indicated that quantitative fecal egg counts are imperative for monitoring degree of parasitic infestations and recognizing problems. The nematode species recorded in this study are known to be of pathogenic nature. Our study contributes baseline data on the paradigm of parasitic infection in Himalayan goral, providing a first step towards population health and disease risk estimation for conservation and management plans. The helminth fauna of other ruminants particularly domestic sheep and goats (Pandith *et al.,* 2003 and Tariq *et al.,* 2008; Lone *et al.,* 2012) are similar to goral and their access to national park should be checked properly to prevent pasture contamination. Gastrointestinal parasite classification by fecal analysis is flaccid by its very nature. However, it is the only censurable method to approach threatened species. Future studies

using molecular analysis and opportunistic necropsies are desired to augment our classification of the gastrointestinal parasites of goral. The possibility of protozoan diseases and bacterial infection in goral and other wild ungulates in the park is of utmost importance and needs to be investigated.

ACKNOWLEDGEMENT

Thanks are due to SERB-DST for providing financial assistance under the project number CRG/2019/002369. The authors acknowledge the Department of Zoology, University of Kashmir for providing laboratory and other facilities. The authors would like to thank the Department of Wildlife Protection, Jammu and Kashmir for providing permission for the smooth conduction of work.

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