First record of Cyclospora and Cryptosporidium species in the Himalayan Goral {Naemorhedus goral (Hardwicke, 1825)} from Nepal

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Research note

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Abstract

Objectives: This study was carried out to detect the various gastrointestinal parasites in the fecal samples of the Himalayan goral Naemorhedus goral (Hardwicke, 1825) from a forest patch of Rumsi area, the Seti River basin, Tanahun district, Nepal.

Results: A total of 17 fecal samples (89.47%) were positive for different parasites in which the prevalence of protozoa was 52.63%, and that of helminths was 73.68%. The positive rates of different parasites showed the following orders as Entamoeba spp. (52.63%), Spirocerca spp. (52.63%), Angiostrongylus (36.84%), Cryptosporidium (26.31%), Cyclospora (26.31%), Strongyle (26.31%), Eimeria (10.52%), Trichostrongylus (10.52%), Muellerius capillaris (10.52%), and Blastocystis (5.26%). Although all of the above parasites are firstly reported from the fecal samples of goral in Nepal, the presence of Cyclospora and Cryptosporidium species suggests that these coccidia may directly affect the survival of the Himalayan goral. Further molecular evidences of causal association with Cyclosporiasis and Cryptosporidiosis should be established in these animals.

Introduction

Cryptosporidium and *Cyclospora* are obligate intracellular apicomplexan parasites that inhabit in the epithelium of the intestine or bile duct of various vertebrates and invertebrates. Both parasites cause coccidiosis and can be asymptomatic, followed by acute diarrhea or symptomatic being experienced by prolonged diarrhea. Both are water-, soil-, food-, and fecal-borne in nature and can be detected by applying acid-fast and hot-safranin staining techniques (1, 2). Although *Cryptosporidium* can be zoonotic (3), there are no reports that illustrate the zoonotic nature of *Cyclospora* spp. Up to date, nineteen species of *Cyclospora* have been described in reptiles, insectivores, snakes, rodents, primates (4), and recently in dairy cattle (5, 6), monkeys (7), red pandas (8), and male goats (9). There are 26 species of *Cryptosporidium* spp. recognized as valid, although several genotypes or other species have been reported to infect domesticated and wild animals around the world (10).

The Himalayan goral *Naemorhedus goral (*Hardwicke, 1825) (Order: Artiodactyla; Family: Bovidae; Subfamily: Caprinae) is listed as Near Threatened on the IUCN Red List globally and nationally (11–13) and Appendix I in CITES category (https://www.cites.org/eng/app/appendices.php, accessed on: August 13, 2019). This goral is a medium-sized mammal endemic to the mountains of central East Asia including Nepal, Bhutan, China, India, and Pakistan with a normal distribution range from 500 to 3500masl (12). Although gorals are the most important prey species of the leopard (14), it has been usually hunted by humans because it is regarded as one of the most delicious meat. Thus, in addition to illegal hunting, the habitat fragmentation, disturbance, and intensive livestock grazing are critical threats to Himalayan gorals (12, 13, 15). However, diseases caused by parasites are one of the significant threats to Himalayan goral around the world. For example, deaths due to tapeworm infestation, pneumonia, gastroenteritis, and hepatitis have been reported in captive populations (16). In Pakistan, overhunting, natural disasters, predators, parasites, and diseases have been indicated to be the declining factors of

their populations (17). However, in Nepal, this type of study in gorals has been lacking. Therefore, this study has been conducted to list these pathogens in the Himalayan goral, and principally, during the research, we have first recorded the presence of oocysts of *Cyclospora* and *Cryptosporidium* in the stool of this mammal from a forest patch of Rumsi area, the Seti River basin, Tanahun district, Nepal.

Methodology

Study area

The study was conducted in the Rumsi area (500–1000m asl) of Seti River basin of Tanahun district in western Nepal which has a steep and gentle slope and a dense mixed forest with grasses *(Additional file 1).* Most people depend on the agriculture and animal husbandry accompanied by the rearing of goats, sheep, cattle, and buffaloes. This livestock are either feral or carried to the forest for grazing, and notably, the local people collect firewood, fodders, and thatch grasses.

Study design, sample collection, and sample examination

The study was cross-sectional descriptive type. A total of 19 fresh samples of Himalayan goral as soon as defecation (from 6 AM to 11 AM) were purposively collected from the Rumsi forest area of Tanahun from April 2019 to May 2019. The sample was put in sterile vials containing 2.5% potassium dichromate solution and was transported to the laboratory (18). Previous laboratory techniques of processing and examination of parasites (9, 19–21) were applied. The fecal filtrate was directly examined at 2.5% potassium dichromate, 0.9% saline solution, and Lugol's Iodine. For observing helminth eggs and coccidian oocysts (e.g., *Eimeria),,* floatation of fecal sample at concentrated NaCl (45%w/v) at1200 revolution per minute, rpm x5 minutes was carried out (18).

For sedimentation technique, one ml of filtrate and l3ml of 0.9% NaCl were mixed in a 15ml centrifuge tube the mixture was centrifuged (1200 rpm x5 minutes). Two drops of the settled solution were kept on a glass slide containing Lugol's iodine, and parasitic stages were examined on the microscope. For acid-fast staining, a portion of sediment obtained after centrifugation (1200rpm x5 minutes) of 2–5gm sample at formal ether solution was used to prepare in the slide and allowed to dry at room temperature. Then, it was fixed with absolute methanol (2 minutes), and stained with Carbol Fuchsin (10 minutes), washed with distilled water, and distained by acid alcohol for 2 minutes. It was restained with Malachite Green (3 minutes), rinsed with distilled water, and was allowed to dry (18). The slide was observed at x1000 magnification using immersion oil.

All the sample were observed under light microscope (Optika Microscopes Italy, B–383PLi) at a total magnification of x40, x100, x400, and x1000 and images (1280 pixels x 720 pixels) were taken using SXView 2.2.0.172 Beta (Nov 6, 2014) Copyright (C) 2013–2014 and sizes were measured using lmageJ 1.51k (National Institute of Health, USA). Based on few works of literatures

(https://parasitology.cvm.ncsu.edu/m_keys/ruminant/parasite/strongyle.html, accessed on August 15, 2019) (9, 18, 19, 22–24), parasites were analyzed and identified.

Data were analyzed using Prism 5 for Windows, Version 5.00, March 7, 2007. The size of the parasites was expressed in the range, mean±standard error (SE), and median, coefficient of variation and at 10% and 90% percentiles, otherwise stated.

Results

Firstly, a total of 34 *N. goral* were observed based on the direct observation in the field, and it was validated by the knowledge of the local people *(Additional file 1).* A total of 19 pellet samples were collected from the steep landscape (range: 525–682m asl) containing mixed forest with trees like *Shorea robusta, Adina cordifolia, Terminalia alata, Schima wallichii, Castanopsis indica, Quercus* sp., *Michelia excelsa* and herbs like *Phoenix humilis, Eulaliopsis binate.,* it was observed that a total of 183±7.25 (range: 134–230) numbers of solid pellets (shape ranging from rolling to large) in a group (area: 20×20 centimeter squares) were found to be defecated by each *N. goral (Additional file 1).* Microscopic studies showed that a total of 17 fecal samples (89.47%) were positive for different parasites in which the prevalence of protozoa was 52.63% and that of helminths was 73.68%. The positive rates of different parasites showed the following orders as *Entamoeba* spp. (52.63%), *Spirocerca* spp. (52.63%), *Angiostrongylus* (36.84%), *Cryptosporidium* (26.31%), *Cyclospora* (26.31%), Strongyle (26.31%), *Eimeria* (10.52%), *Trichostrongylus* (10.52%), *Muellerius capillaris* (10.52%), and *Blastocystis* (5.26%) *(Table 1) (Fig.1, 2, Additional file 1).* The percentage of mixed infection patterns for doubled, tripled, quadrupled, and quintupled infections was 36.84%, 26.31%, 21.05%, and 5.26% respectively. Among five positive fecal samples for *Cyclospora,* it occurred as quadruple mixed infections in four samples and quintupled parasites in one sample. In contrast, *Cryptosporidium* showed triple infections in one, quadruple infections in three, and quintupled infections in one sample.

Following acid-fast staining, the photographs of the *Cyclospora* oocysts (range: 7–11μ, median: 8μ, mean±SE: 8.5±0.1μ, Coefficient of variation: 13.0%, 10% percentile: 7μ, 90% percentile: 10 μ, n = 80) and the oocysts of *Cryptosporidium oocysts (*range: 3–7μ, median: 4μ, mean±SE: 4.5±0.08μ, Coefficient of variation: 25%, 10% percentile: 3μ, 90% percentile: 6μ, n = 180) were analyzed *(Additional file 2).*

Discussion

This study firstly showed the presence of *Cyclospora* and *Cryptosporidium* species in the stool sample of the Himalayan goral in Nepal. In this country, in addition to public health, *Cyclospora* spp. have been reported to be critical for veterinary health because it has been isolated from both domestic and street dogs, chicken in ecozonal region near forest areas, and rhesus monkeys *(Macaca mulatta)* wandering via the forest region (25), red panda in community forest (8) and goats brought for meat purposes from different areas (9), one or more of hosts like mice, rats, dogs, and chicken (2, 26, 27) although *Cyclospora* in domestic animals and birds were not detected previously (2, 28). In addition to this Himalayan country,

this coccidian has been reported in cattle from China (5), calf from Japan (29), monkeys from China (7, 30), Ethiopia (31), and captive primates from Europe (32) although no reports on the stool of various cattle, birds, and wild animals from Haiti (33). Thus, though risks of this coccidian have been implicated in food-borne, soil-borne, water-borne, and fecal-borne transmissions (34–36), in the absence of detailed epidemiologic and molecular evidence, it is not easy to link this coccidian with zoonosis in goral. It has been shown that potential competition between goral and other ungulates like domestic cattle, Northern red muntjac (*Muntiacus vaginalis),*, and Himalayan serow (*Capricornis thar)* for living space, escape cover, water sources, salt licks, and forage species (37). Importantly, cross-transmission of *Cyclospora* via contact with fecally-contaminated feces of domestic animals brought for grazing or interacting livestock or nearby human may occur in the place, and likelihood of goral to act as a natural or paratenic host may exist in this area.

The role of *Cryptosporidium* spp. in food-borne, water-borne, travel-related, and community outbreaks globally has been reviewed (38) with a clear-cut seasonal outbreak in the people of Nepal (1). This coccidian has been shown to be zoonotically associated in both immunocompetent and immunocompromised patients both in the developing and developed world (3). In this context, several hosts including felines, canines, and bovids may play in the transmission of the parasites. The current study area is prone to the cross-transmission of these coccidia because of the usual visits by the local goats, sheep, cattle, and buffaloes, however, gorals to act as natural hosts for *Cryptosporidium* spp. cannot be ruled out. Further molecular pieces of evidence might explain our answers.

In the current study, the rate of GI observation was 89.47% which was higher than that reported from Pakistan (75%) (39). In Pakistan, various parasites like *Fasciola hepatica, Thysaniezia* spp*., Moniezia expansa, Nematodirus spathiger, Gaigeria pachyscelis, Trichostrongylus* spp*., Haemonchus contortus, Bunostomum trigonocephalum, Cotylophoron cotylophorum, Fascioloides magna,* and *Ostertagia circumcincta* have been reported to occur in goral (39). Our reports of various lists of other protozoa like *Entamoeba* spp., *Blastocystis,* and *Eimeria* and nematodes like *Spirocerca, Angiostrongylus,* Strongyle*, Trichostrongylus,* and *Muellerius* suggest that goral in the study area are severely affected. As gorals are both browsers and grazers (37), the transmission of these pathogens is probable via ingestion of larva or cyst or oocysts stages remained in grasses. In these contexts, feral goats, dogs, and domestic animals might play critical roles. Although it is not known whether the currently reported parasites are acquired via domestic animals or are natural, some of them are highly pathogenic. Postmortem examinations of reports of the deaths of 17 gorals between 1976 and 1999 listed that they were died by infectious, digestive, and respiratory diseases and one newborn goral's death was linked to coccidiosis during this period (40). The presence of the protozoan and helminth parasites might be a critical issue in the current studied populations and an integrated study involving the samples of human, wildlife, and domestic livestock can provide a better link of parasitism.

Conclusion

The current study enlightens the possible spread of these parasitic species among feral domestic animals, wildlife, and the goral and may partly contribute to the decision-making process and policy for management of goral and consequently to the veterinary and public health.

Limitations

This study has addressed the GI parasitism of only 19 fecal samples of about 35 gorals. We have confirmed *Cyclospora* and *Cryptosporidium* based on direct wet mount and acid-fast staining techniques. However, compared to molecular techniques, our methodologies are less standard.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information files (additional file).

Competing interests

The authors declare that they have no conflict of interest. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Author’s Contributions

BB and TBT supervised JNA who performed field survey and collected the samples. RBA and TRG performed laboratory assays. JNA and TRG prepared the manuscript and all authors read and approved it.

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Tables

Table 1 Gastrointestinal parasites in the Himalayn goral, *Naemorhedus goral* (Hardwicke, 1825) in Tanahun, Nepal.

Parasites	Positive Numbers	% positive (N=19)
Entamoeba spp.	10	52.63.%
Spirocerca spp.	10	52.63%
Angiostrongylus spp.	7	36.84%
Cryptosporidium spp.	5	26.31%
Cyclospora spp.	5	26.31%
Strongyle	5	26.31%
Eimeria spp.	2	10.52%
Trichostrongylus spp.	2	10.52%
Muellerius capillaris	2	10.52%
Blastocystis spp.	1	5.26%

Figures

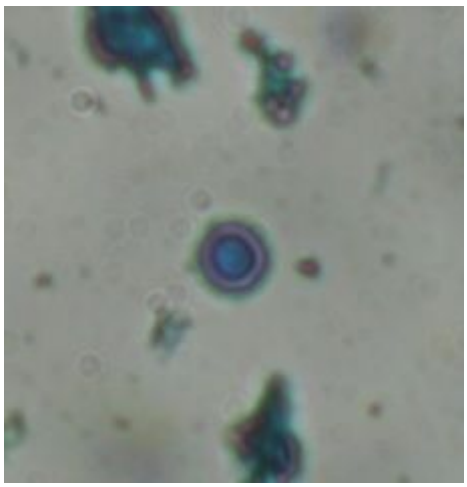

Figure 1

Oocyst of Cryptosporidium sp. after acid-fast staining (4X4µm, at 1000x).

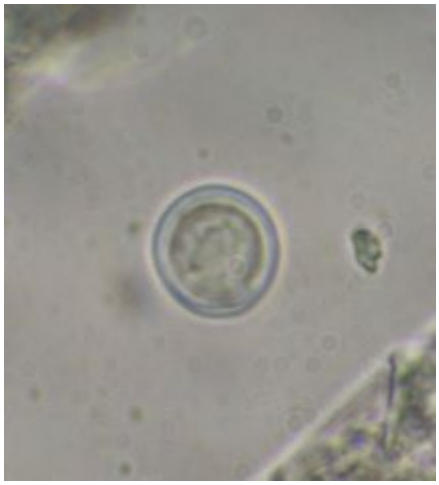

Figure 2

Oocyst of Cyclospora sp. after acid-fast staining (9X8µm, at 1000x).

Supplementary Files

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