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) at 1200 revolution per minute, rpm x 5 minutes was carried out (18).

For sedimentation technique, one ml of filtrate and 13ml of 0.9% NaCl were mixed in a 15ml centrifuge tube the mixture was centrifuged (1200 rpm x 5 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 x 5 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 ImageJ 1.51k (National Institute of Health, USA). Based on few works of literatures
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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References

1. Ghimire TR, Mishra PN, Sherchan JB. The seasonal outbreaks of Cyclospora and Cryptosporidium in Kathmandu, Nepal. Journal of Nepal Health Research Council. 2005;3(1):39–47.

2. Ghimire TR, Mishra PN, Sherchan JB. Epidemiology of Cyclospora cayetanensis in HIV/AIDS patients in Kathmandu, Nepal. Journal of Nepal Health Research Council. 2008;6(12):28–37.

3. Xiao L, Feng Y. Zoonotic cryptosporidiosis. Pathogens and Disease. 2008;52(3):309–23.

4. Lainson R. The genus Cyclospora (Apicomplexa: Eimeriidae), with a description of Cyclospora schneideri n.sp. in the snake Anilius scytale scytale (Aniliidae) from Amazonian Brazil—a review. Memorias do Instituto Oswaldo Cruz. 2005 Apr;100(2):103–10. PubMed PMID: 16021295. Epub 2005/07/16. eng.

5. Li G, Xiao S, Zhou R, Li W, Wadeh H. Molecular characterization of Cyclospora-like organism from dairy cattle. Parasitology research. 2007 Apr;100(5):955–61. PubMed PMID: 17206510. Epub 2007/01/09. eng.

6. Xiao SM, Li GQ, Zhou RQ, Li WH, Yang JW. Combined PCR-oligonucleotide ligation assay for detection of dairy cattle-derived Cyclospora sp. Veterinary parasitology. 2007 Nov 10;149(3–4):185–90. PubMed PMID: 17850971. Epub 2007/09/14. eng.

7. Li N, Ye J, Arrowood MJ, Ma J, Wang L, Xu H, et al. Identification and morphologic and molecular characterization of Cyclospora macacae n. sp. from rhesus monkeys in China. Parasitology research. 2015 May;114(5):1811–6. PubMed PMID: 25673080. Pubmed Central PMCID: PMC5784403. Epub 2015/02/13. eng.

8. Lama ST, Lama RP, Regmi GR, Ghimire TR. Prevalence of intestinal parasitic infections in free-ranging Red Panda Ailurus fulgens Cuvier, 1825 (Mammalia: Carnivora: Ailuridae) in Nepal. Journal of Threatened Taxa. 2015;7(8):7460–4.

9. Romero-Castañón S, Ferguson BG, Güiris D, González D, López S, Paredes A, et al. Comparative parasitology of wild and domestic ungulates in the Selva Lacandona, Chiapas, Mexico. Comparative Parasitology. 2008;75(1):115–27.
10. Ryan U, Fayer R, Xiao L. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology. 2014 Nov;141(13):1667–85. PubMed PMID: 25111501. Epub 2014/08/12.

11. Amin R, Baral HS, Lamichhane BR, Poudyal LP, Lee S, Jnawali SR, et al. The status of Nepal’s mammals. Journal of Threatened Taxa. 2018;10(3):11361–78.

12. IUCN. The IUCN Red List of Threatened Species: International Union for Conservation of Nature and Natural Resources (IUCN); 2019. Available from: https://www.iucnredlist.org/species/14296/4430073.

13. Jnawali SR, Baral, H. S., Lee, S., Acharya, K. P., Upadhyay, G. P., Pandey, M., Shrestha, R., Joshi, D., Laminchhane, B. R., Griffiths, J., Khatiwada, A. P., Subedi, N., Amin, R. (compilers) The Status of Nepal Mammals: The National Red List Series. Department of National Parks and Wildlife Conservation, Kathmandu, Nepal 2011. Available from: http://awsassets.panda.org/downloads/nepal_redlist_low_09_06_2012_1.pdf.

14. Lovari S, Ventimiglia M, Minder I. Food habits of two leopard species, competition, climate change and upper treeline: a way to the decrease of an endangered species? Ethology Ecology & Evolution. 2013;25(4):305–18.

15. Shakeel U, Minhas RA, Awan MS, Bibi SS, Iftikhar N. Conservation Status of Himalayan Grey Goral (*Naemorhedus goral bedfordi*) Hardwicke, 1825 in Machiara National Park, Azad Jammu and Kashmir, Pakistan. Pakistan Journal of Zoology. 2015;47(5).

16. Mead Jl. *Nemorhaedus goral*. Mammalian Species. 1989 (335):1–5.

17. Perveen F, Khan A, Shah AH. Hunting and Trapping Pressures on the Himalayan Goral, *Naemorhedus goral* (Hardwicke) (Artiodactyla: Bovidae) in Kohistan, Pakistan. American Journal of Zoological Research. 2013;1(1):5–11. PubMed PMID: doi:10.12691/ajzr–1–1–2.

18. Ghimire TR, Bhattarai N. A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal. Journal of Parasitic Diseases. 2019:1–10.

19. Zajac AM, Conboy GA, Greiner EC, Smith SA, Snowden KF. Fecal Examination for the Diagnosis of Parasitism. In: Zajac AM, Conboy GA, editors. Veterinary Clinical Parasitology. 8th ed. UK: John Wiley & Sons; 2012. p. 3–169.

20. Dryden MW, Payne PA, Ridley R, Smith V. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet Ther. 2005;6(1):15–28.

21. Basnett K, Nagarajan K, Soundararajan C, Vairamuthu S, Rao GVS. Morphological and molecular identification of *Cyclospora* species in sheep and goat at Tamil Nadu, India. Journal of parasitic diseases. 2018;42(4):604–7.
22. Taylor MA, Coop RL, Wall RL. Parasites of sheep and goats. In: Taylor MA, Coop RL, Wall RL, editors. Veterinary Parasitology. 4th ed: John Wiley & Sons, Ltd; 2016. p. 436–523.

23. Koudela B, Boková A. Coccidiosis in goats in the Czech Republic. Veterinary Parasitology. 1998;76(4):261–7.

24. Chartier C, Paraud C. Coccidiosis due to *Eimeria* in sheep and goats, a review. Small Ruminant Research. 2012 2012/03/01/;103(1):84–92.

25. Chu DM, Sherchand JB, Cross JH, Orlandi PA. Detection of *Cyclospora cayetanensis* in animal fecal isolates from Nepal using an FTA filter-base polymerase chain reaction method. The American journal of tropical medicine and hygiene. 2004 Oct;71(4):373–9. PubMed PMID: 15516629. Epub 2004/11/02. eng.

26. Sherchand JB, Cross JH. Emerging pathogen *Cyclospora cayetanensis* infection in Nepal. The Southeast Asian journal of tropical medicine and public health. 2001;32 Suppl 2:143–50. PubMed PMID: 12041579. Epub 2002/06/04. eng.

27. Sherchand JB, Cross JH, Jimba M, Sherchand S, Shrestha MP. Study of *Cyclospora cayetanensis* in health care facilities, sewage water and green leafy vegetables in Nepal. The Southeast Asian journal of tropical medicine and public health. 1999 Mar;30(1):58–63. PubMed PMID: 10695790. Epub 2000/03/01. eng.

28. Ghimire TR, Ghimire LV, Shahu RK, Mishra PN. *Cryptosporidium* and *Cyclospora* infection transmission by swimming. Journal of Institute of Medicine. 2010;32(1):43–5.

29. Yamada M, Hatama S, Ishikawa Y, Kadota K. Intranuclear coccidiosis caused by *Cyclospora* spp. in calves. Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2014 Sep;26(5):678–82. PubMed PMID: 25012083. Epub 2014/07/12. eng.

30. Zhao G-H, Cong M-M, Bian Q-Q, Cheng W-Y, Wang R-J, Qi M, et al. Molecular characterization of *Cyclospora*-like organisms from golden snub-nosed monkeys in Qinling Mountain in Shaanxi province, northwestern China. PloS one. 2013;8(2):e58216-e. PubMed PMID: 23469155. Epub 02/28. eng.

31. Eberhard ML, da Silva AJ, Lilley BG, Pieniazek NJ. Morphologic and molecular characterization of new *Cyclospora* species from Ethiopian monkeys: *C. cercopithecus* sp.n., *C. colobi* sp.n., and *C. papionis* sp.n. Emerging infectious diseases. 1999 Sep-Oct;5(5):651–8. PubMed PMID: 10511521. Pubmed Central PMCID: PMC2627716. Epub 1999/10/08. eng.

32. Marangi M, Koehler AV, Zanzani SA, Manfredi MT, Brianti E, Giangaspero A, et al. Detection of *Cyclospora* in captive chimpanzees and macaques by a quantitative PCR-based mutation scanning approach. Parasites & vectors. 2015 May 15;8:274. PubMed PMID: 25972100. Pubmed Central PMCID: PMC4456053. Epub 2015/05/15. eng.
33. Eberhard ML, Nace EK, Freeman AR. Survey for *Cyclospora cayetanensis* in domestic animals in an endemic area in Haiti. J Parasitol. 1999 Jun;85(3):562–3. PubMed PMID: 10386455. Epub 1999/07/01. eng.

34. Koumans EH, Katz DJ, Malecki JM, Kumar S, Wahlquist SP, Arrowood MJ, et al. An outbreak of cyclosporiasis in Florida in 1995: a harbinger of multistate outbreaks in 1996 and 1997. The American journal of tropical medicine and hygiene. 1998 Aug;59(2):235–42. PubMed PMID: 9715939. Epub 1998/08/26. eng.

35. Madico G, McDonald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 1997 May;24(5):977–81. PubMed PMID: 9142805. Epub 1997/05/01. eng.

36. Bern C, Hernandez B, Lopez MB, Arrowood MJ, de Mejia MA, de Merida AM, et al. Epidemiologic studies of *Cyclospora cayetanensis* in Guatemala. Emerging infectious diseases. 1999 Nov-Dec;5(6):766–74. PubMed PMID: 10603209. eng.

37. Chaiyarat R, Laohajinda W, Kutintara U, Nabhitabhata J. Ecology of the goral (*Naemorhedus goral*) in Omkoi Wildlife Sanctuary Thailand. Nat Hist Bull Siam Soc. 1999;47:191–205.

38. Putignani L, Menichella D. Global distribution, public health and clinical impact of the protozoan pathogen *Cryptosporidium*. Interdisciplinary perspectives on infectious diseases. 2010;2010. PubMed PMID: 20706669. Pubmed Central PMCID: PMC2913630. Epub 2010/08/14. eng.

39. Rana MA, Ahmad I, Jabeen F, Naureen A, Munaza S. Comparative study of endo-parasites from fecal samples of sambar (*Rusa unicolor*) and goral (*Naemorhedus goral*) in captivity. Journal of Biodiversity and Environmental Sciences (JBES). 2015;6(5):399–408.

40. Shin N, Kwon S, Lee G, Kim Y, Kweon O, Kim D. Retrospective survey on the mortality of gorals at Everland Zoological Gardens (1976–1999). Korean Journal of Veterinary Clinical Medicine. 2000;17(2):515–8.

**Tables**

**Table 1** Gastrointestinal parasites in the Himalayan 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).
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