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Parasitic infection in captive wild animals of Nainital zoological park, India

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Abstract

The research work was conducted from September, 2016 to April, 2017. A total of ninety-nine (n=99) faecal samples were randomly collected from the captive animals. The overall prevalence of intestinal parasitic infection in the present study was observed 28.28%, with 20.20% samples positive for helminths, 7.07% samples positive for protozoans and 1.01% samples positive for mixed infection. In case of Sambar Deer *Strongyle* sp. (40%) was more prevalent than *Trichuris* sp. (20%). *Eimeria* sp. (50%) was most prevalent than *Muellerius* sp. and *Capillaria* sp. (25%) in Gorals. Prevalence of *Capillaria* sp. and *Eimeria* sp. observed in Markhor and Spotted Deer was 100% and 50%, respectively. In present study, the prevalence of *Toxocara cati* and *Toxocara canis* was also observed 33.33% and 50% in Leopard and Himalayan black bear, respectively. In case of Tibetan wolf, *Isospora* sp. was observed and recorded 50% prevalence. In total of fifty-five Common Peafowl (birds) samples, ten samples (18.18%) were found positive for *Ascaridia galli*. Screening of captive wild animals at regular intervals is needed to assess the gastrointestinal parasites to alert the zoo authorities to take up proper preventive measures.

Keywords: Captive wild animals, gastro-intestinal parasites, Nainital zoological park

1. Introduction

India has a big variety of wildlife as well as a long history and custom of conservation. The wildlife has play important role in ecological balance and cleaning of the environment. A zoo is an ex-situ form of conservation, where wild animals are placed in enclosures for the exhibition. The main principle of Zoological Park is as creative, informative and protection of the wild animals (Varadharajan and Pythal, 1999) [28]. In wild situation, animals have a little bit natural immunity against the intestinal parasites and other infectious agents.

But when these animals are kept in captivity these parasites causes many serious problems for wild captive animals due to stressed conditions under the enclosure and their immunity reduced. Due to these reasons day by day unexpected fall in a number of wild animals is going on (Muoria *et al.*, 2005) [15]. Parasites cause direct and indirect effects on these captive animals. Parasites can directly affect the host existence and reproduction via pathological effects such as damage of tissue, loss of blood, hereditary deformities, abortion, rarely death and indirectly affecting the physical condition by declining the host's resistance.

Due to some these serious effects of intestinal parasites on captive animals, so it is important to control these parasites. Control and prevention programmes for wildlife mainly depends on economic resources and public health structures, reduction of parasitic load, control of animal reservoirs and vectors, improved diagnostic methods, environmental and ecological changes, human behaviours and education of the people that are involved in the wildlife and domestic animals chain (Chomel, 2008) [2]. However, planned prevention and control programme for captive animals can be done by regular screening of faecal samples, periodic regular deworming of the animals, decreasing the intermediate hosts, quarantine period for newly acquired animals and improved hygiene practices should be followed in the Zoological Parks for better health of the animals. There should also be compulsory policy that visitors should not be allowed to feed these captive animals, thus improving the health of the captive animals from parasitic diseases (Adegbulu *et al.*, 2015) [1]. Parasitic diseases can also be checked by preventing the contact between wild animals and domestic animals because wild animals acts as a reservoir host for most of the parasites (Gupta *et al.*, 2011) [7]. Keeping in view the above facts, the present study was undertaken to identify the prevalence and intensity of gastro-intestinal parasites in captive animals of Nainital Zoological Park.

2. Materials and Methods

2.1 Study duration and area

The present study was conducted from September, 2016 to April, 2017. Experiment was carried out at Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Pantnagar and Nainital Zoological Park. The Zoological Park is located about 2 kilometres uphill from Tallital bus station in Nainital on the Sher Ka Danda hill at a height of 2,100-2,150 meter (6,890-7050ft) above sea-level between Shivalik and middle Himalayas mountain range (coordinates: 29.381°N 79.469°E).

2.2 Selection of animals

The study covered all age groups and both sexes of captive animals found in Zoological Park, Nainital. Herbivores, carnivores, non-human primates and different types of wild birds were selected for the study.

2.3 Collection of samples

A total of ninety-nine (n=99) faecal samples were collected from Nainital Zoological Park. Samples were collected randomly from the heap of faecal mass preferably freshly voided by the animals with the help of spatula into clean sterile collection vials. The samples were collected by the caretaker of the Zoo animals, which were marked with the time, date of collection, species of animal, sex and animals cage number. The labelled samples were transported to laboratory in thermacol box containing ice packs to avoid the hatching of the eggs of parasites then the samples were stored at refrigerator (6 °C) temperature till further use.

2.4 Coprological examination

Faecal samples were examined in the laboratory of the Department of Veterinary Parasitology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar as well as in the diagnostic laboratory of Zoo and processed for qualitative and quantitative examination. The qualitative examination was done by the direct smear examination method and concentration methods (Zajac and Conboy, 2012)^[29] and quantitative examination was done by using Modified McMaster egg counting technique to find out egg per gram (EPG)/cyst per gram (CPG)/oocyst per gram (OPG) of faeces as described (Soulsby, 1982)^[25]. Sporulation of coccidian species was done in a 2.5% potassium dichromate solution.

2.4.1 Qualitative examination

2.4.1.1 Direct smear examination method

A small quantity of faeces was taken on a clean slide. Then few drops of distilled water were added and spread in a small area to make a thin film. Then a cover slip was placed over it for uniformity of the smear. For carnivores and non-human primates, a wet smear was stained with Lugol's iodine solution then examined under compound microscope. At least three slides from different parts of the faecal samples were examined before conclusion.

2.4.1.2 Sedimentation method

A small quantity of faeces was taken in a mortar then distilled water was added and triturated properly with the help of a pestle then strained with a tea strainer into a beaker to remove coarse faecal material. The filtrate was filled into a centrifuge tube up to two-third of the tube and centrifuged at 2000-3000 rpm for 5-10 minutes. Then a drop of the sediment was taken on a clean slide and examined under a compound microscope

(Zajac and Conboy, 2012)^[29]. This method was mostly useful for the examination of eggs of trematodes.

2.4.1.3 Floatation method

A small quantity of faeces was taken into a mortar then distilled water was added and triturated properly with the help of pestle, was strained with a tea strainer into a beaker to remove coarse faecal material. The filtrate was poured into a centrifuge tube upto two-third of the tube and centrifuged at 2000-3000 rpm for 5-10 minutes. Then sediment was mixed with floatation fluid in a centrifuge tube and again centrifuged at 1500 rpm for 1-2 minutes, then the tube was kept in erect stand and floatation fluid was added with dropper up to the brim of the tube and a cover slip was placed over it so that it touches on its surface with the fluid and was allowed to stand for 15 minutes. Then cover slip was gently lifted and placed on a slide and was examined under compound microscope (Zajac and Conboy, 2012)^[29]. This method is mostly useful for the examination of eggs of nematodes and cestodes.

2.4.2 Quantitative examination

2.4.2.1 Modified Mc Master method

It is used for counting the number of eggs/cysts/oocysts per gram of faeces. For this study, one gram of faecal sample was weighed and triturated in a mortar with the help of pestle after adding 14 ml of floatation fluid and was sieved through a tea strainer and transferred into a test tube of 20 or 30 ml capacity and faecal suspension was uniformly mixed with the help of dropper. Then McMaster egg counting chamber of volume 0.3 ml was charged with prepared faecal suspension and allowed to settle. Eggs of gastro-intestinal nematodes were counted under compound microscope (MAFF)^[13].

2.4.2.2 Identification of coccidian oocysts

Coccidian oocysts when passed in faeces were unsporulated and were not differentiated. Therefore, the culture of faecal sample for sporulation of coccidian oocysts is very much important for diagnosis as well as for epidemiological study. From the collected faecal sample, a small quantity of faeces were taken in a petridish and 2.5% potassium dichromate solution was added in it and was incubated at 27 °C temperature for a day to a week to allow the development of sporocysts and sporozoites. Aeration with the help of a pasture pipette was done regularly to supply oxygen to the oocysts. A drop of the suspension was examined microscopically to check complete sporulation.

3. Results and Discussion

3.1 Overall prevalence of gastro-intestinal parasites in Zoo animals

A total of ninety nine (n=99) faecal samples were collected from Zoo animals (eighteen herbivores, twenty four carnivores, two non-human primates and fifty five different types of wild birds). The overall prevalence of intestinal parasitic infection in the present study was observed 28.28% with 20.20% samples positive for helminths, 7.07% samples positive for protozoans and 1.01% samples positive for mixed infection (Table 1). These findings are similar with the earlier reports of Engh *et al.* (2003)^[5] who reported 28.57% prevalence at Masai Mara National Reserve, Kenya. This finding is higher than the reports of Shibashi *et al.* (2003), Singh *et al.* (2006), Khan *et al.* (2014) and Li *et al.* (2015)^[22, 23, 9, 11] who observed the prevalence 25%, 25.70%, 15.84% and 26.51% respectively. In contrast, this finding is lower

than the reports of Cordon *et al.* (2008), Lim *et al.* (2008), Opara *et al.* (2010) and Gurler *et al.* (2010) [4, 12, 16, 8] who observed that the prevalence was 88.7%, 36.4%, 56.3% and 42.4% respectively. The overall prevalence in the Zoological Park, Nainital is low due to lesser number of animals, lesser enclosed captive area, less stress on animals, situation of the zoo at high altitude, lesser contamination with different stages of the parasites and good hygiene practices. Attendants cleaning the cages and enclosures of these wild animals could act as vehicle for cross transmission of parasites among the animals and keepers. Thus, hygiene of attendants and cages may also be responsible for differences in results. Results of this study indicated that helminth infections were more as compared to protozoans and mixed infection in captive animals. This could be due to more favourable condition of Nainital region for the development of different stages of helminth parasites.

3.2 Prevalence of gastro-intestinal parasites in herbivores

In present study, the prevalence of helminths (27.77%) was higher than protozoa (16.66%) and 5.55% with mixed infection (Table 1). Pilarczyk *et al.* (2005) [20] and Lim *et al.* (2008) [12] also observed similar trend but different prevalence as 52% and 27.5%, 67% and 35%, positive with helminths and protozoans respectively.

3.3 Prevalence of gastro-intestinal parasites in carnivores

The prevalence of helminths infection was more than protozoan infection in carnivores i.e. 20.83% and 4.16% respectively (Table 1). Varadharajan and Kandasamy (2000); Parasani *et al.* (2001) and Lim *et al.* (2008) [27, 18, 12] observed prevalence of helminths and protozoans as 58% and 6%, 50% and 18.8% and 34.5% and 21.8%, respectively. However, it is contrary to the studies of Gomez *et al.* (2000); Levecke *et al.* (2007) and Cordon *et al.* (2008) [6, 10, 4] who observed higher protozoan infection compared to helminthic infection as 54% and 25%, 65% and 30%, 43% and 28% respectively. The differences in prevalence of parasites may be due to geographic conditions, husbandry practices and source of feeds, method of sample collection and the use of anthelmintic in the particular Zoo animals.

3.4 Prevalence of gastro-intestinal parasites in non-human primates

In present study, non-human primates were infected with protozoans (50%) only (Table 1). There was no infestation with helminths. Similarly Radhy *et al.* (2012) [21] observed 86% prevalence of protozoans in non-human primates at Al-Zawra Zoological Park, Baghdad.

3.5 Prevalence of gastro-intestinal parasites in wild birds

Out of 55 samples collected from Pheasants, 18.18% samples were positive with helminth infection and 3.63% positive for protozoan infection (Table 1). Patel *et al.* (2000) [19] observed 20.75% samples were positive with helminths and 17.92% with protozoan infection in some wild birds at Kamla Nehru Zoo, Ahmedabad and Sayyajibaug Zoo, Vadodara. Otegbade and Morenikeji (2014) [17] observed overall prevalence of 21.9% in birds at Zoological Park, South-West Nigeria.

The association between host (carnivore and herbivore animals) and parasites (helminth and protozoa) is considered to be statistically non-significant ($p > 0.05$).

Table 1: The overall prevalence of intestinal parasitic infections among various animals

Animals	Sample size	Helminth Positive (%)	Protozoa Positive (%)	Mixed Infection (%)	Total Positive (%)
Carnivores	24	05 (20.83)	01 (4.16)	00	06 (25.00)
Herbivores	18	05 (27.77)	03 (16.66)	01 (5.55)	09 (50.00)
Non-human primates	02	00	01 (50.00)	00	01 (50.00)
Pheasant	55	10 (18.18)	02 (3.63)	00	12 (21.81)
Total	99	20	07	01	28
Overall prevalence (%)	-	20.20	7.07	1.01	28.28

3.6 Prevalence and intensity of different gastro-intestinal parasites observed in herbivore animals

In present study, different gastro-intestinal parasites were recovered in herbivore animal's sample. In case of Sambar Deer *Strongyle* sp. (40%) (Fig. 1) was more prevalent than *Trichuris* sp. (20%) (Fig. 2). *Eimeria* sp. (50%) (Fig. 3) was most prevalent than *Muellerius* sp. and *Capillaria* sp. (25%) (Fig. 6) in Gorals. Prevalence of *Capillaria* sp. and *Eimeria* sp. observed in Markhor and Spotted Deer was 100% and 50%, respectively (Table 2). Cook *et al.* (1979) and Mir *et al.* (2016) [3, 14] recorded prevalence of *Strongyle*, *Capillaria* sp. and *Trichuris* sp. as 22.7%, 5%, 4.5% and 67%, 10%, 19% respectively. The highest EPG/CPG/OPG was counted in case of *Eimeria* sp. (500) followed by *Strongyle* (300), *Capillaria* sp. (100) and *Trichuris* sp. (100) (Table 2). The intensity of different gastrointestinal parasites in herbivore animals were calculated and found lower intensity than the findings of Singh *et al.* (2009) [24], who observed the intensity of different parasites in range from 100-7500. It may be due to regular screening of faecal samples and periodic deworming of the animals with suitable anthelmintics in the Zoological Park, Nainital.

3.7 Prevalence and intensity of different gastro-intestinal parasites observed in carnivores

In present study, the prevalence of *Toxocara cati* (Fig. 4) and *Toxocara canis* (Fig. 5) was observed 33.33% and 50% in Leopard and Himalayan black bear, respectively. In case of Tibetan wolf, *Isospora* sp. (Fig. 8) was observed and recorded 50% prevalence (Table 2) *Toxocara cati* (10%) and *Toxocara canis* (5.50%) were reported in Leopard and Bear respectively (Thawait *et al.*, 2014) [26]. *Toxocara cati* (6.70%) were reported in Royal Bengal Tiger from Rajkot, Zoological Park (Parasani *et al.*, 2001) [18] and M.C. Zoological Park, Chhatbir, Punjab (Singh *et al.*, 2006) [23]. EPG/CPG/OPG were also calculated and ranged from 150-450. The highest EPG/CPG/OPG was counted in case of *Toxocara cati* (450) followed by *Toxocara canis* (300) and *Isospora* sp. (150) (Table 2). The intensity of different parasites was found lower than the study of Singh *et al.* (2009) [24], who observed intensity in range of 100-7500. It may be due to hygiene measures adapted at Zoo, regular examination of faecal samples and periodic deworming of the animals.

3.8 Prevalence and intensity of different gastro-intestinal parasites identified in non-human primates

Out of two samples, one sample was found positive with *Eimeria* sp. (50%) with CPG 350 (Table 2). Singh *et al.* (2009) [24] also observed protozoans in non-human primates.

3.9 Prevalence and intensity of different gastro-intestinal parasites observed in wild birds

In total of 55 Common Peafowl samples, ten samples (18.18%) (Table 2) were found positive with *Ascaridia galli* (Fig. 7) and two samples were found positive for *Eimeria* sp. (3.63%). Patel *et al.* (2000) [19] observed *Ascaridia galli*

(20.75%) in some wild birds at Kamla Nehru Zoo, Ahmedabad and Sayyajibaug Zoo, Vadodara. *Ascaridia galli* (12.50%) in Common Peafowl were also reported in the study of Otegbade and Morenikeji (2014) [17]. In the present study, the EPG for *Ascaridia galli* was recorded 250 and for *Eimeria* sp. was 500 (Table 2).

Table 2: Prevalence and intensity of different gastro-intestinal parasites in captive animals

Name of animals	Name of Parasites	Number of positive case (Number of samples)	Prevalence (%)	EPG/OPG/CPG
Sambar deer (Herbivore)	<i>Strongyle</i> sp.	02 (05)	40	300
	<i>Trichuris</i> sp.	01 (05)	20	100
Goral (Herbivore)	<i>Muellerius</i> sp.	01 (04)	25	300
	<i>Capillaria</i> sp.	01 (04)	25	100
	<i>Eimeria</i> sp.	02 (04)	50	500
Spotted deer (Herbivore)	<i>Eimeria</i> sp.	01 (02)	50	500
Markhor (Herbivore)	<i>Capillaria</i> sp.	01 (01)	100	100
Leopard (Carnivore)	<i>Toxocara cati</i>	03 (09)	33.33	450
Himalayan black bear (Carnivore)	<i>Toxocara canis</i>	02 (04)	50	300
Tibetan wolf (Carnivore)	<i>Isospora</i> sp.	01 (02)	50	150
Non-human primates	<i>Eimeria</i> sp.	01 (02)	50	350
Common Peafowl (Wild Birds)	<i>Ascaridia galli</i>	10 (55)	18.18	250
	<i>Eimeria</i> sp.	02 (55)	3.63	500



Fig 1: Strongyle type egg



Fig 2: *Trichuris* sp. egg



Fig 3: *Eimeria* sp. oocyst (Sporulated coccidia oocyst)

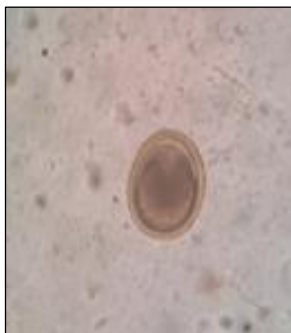


Fig 4: *Toxocara cati* egg



Fig 5: *Toxocara canis* egg

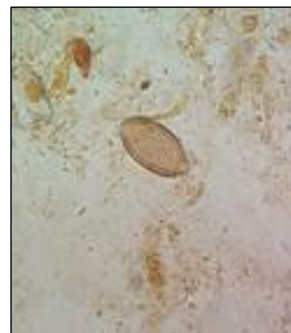


Fig 6: *Capillaria* sp. egg



Fig 7: *Ascaridia galli* egg

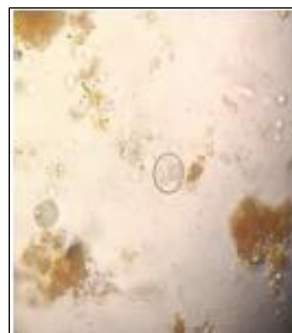


Fig 8: *Isospora* sp. oocyst



Fig 9: Unsporulated oocyst of coccidia

4. Conclusion

From the results of present study, it can be concluded that gastrointestinal helminth parasites are more prevalent than protozoa in captive animals of Nainital, Zoological Park. The result of present study suggests that regular screening of faecal samples of captive animals is required. In this way proper diagnosis of parasitic infestation will help in saving the harmful effects of these parasites in captive animals. Proper management, routine monitoring of parasitic infestations, treatment of the affected animals and the use of specific anthelmintics can significantly help for the control of gastrointestinal parasites in Zoological Parks. It is further suggested that a long-term epidemiological study of parasitic infection is needed so as to understand the parasitism and prevent possible recurrence of existing infection in captive animals. There is also need to examine the prevalence of vectors and intermediate hosts. Such studies will provide a clear concept of parasitic infection in captive animals there by help in proper prevention and treatment of parasitic infections. Therefore, a detailed study related to parasites of captive animals should be carried out to get a clear picture of parasitism in India. There is need for identification of parasites and diagnosis of parasitic diseases using molecular techniques and pathophysiology of different helminth species.

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