Investigation on Prevalence of Parasitic Infection in Herbivores and Carnivores of Chattogram Zoo, Chattogram, Bangladesh

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Investigation on Prevalence of Parasitic Infection in Herbivores and Carnivores of Chattogram Zoo, Chattogram, Bangladesh

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List of abbreviations

Abbreviation	Elaboration
µm	Micrometer
40x	40 times magnification
GI	Gastrointestinal
Sp	Species

Abstract

Gastrointestinal parasites pose significant health risks to captive animals, whose confinement and stress increase susceptibility to infections. This study investigated the prevalence and causal agents of gastrointestinal parasitic infection in selected captive herbivores and carnivores of Chattogram Zoo, Chattogram. A total of 17 fecal samples from 11 species of captive herbivores and carnivores were screened using direct smear, sedimentation, and floatation techniques. The overall prevalence of gastrointestinal parasitic infection was 29.41%, with herbivores showing a higher prevalence (44.45%) compared to carnivores (12.5%). Six different types of parasites were identified in this study including *Monizia* sp., *Ostertagia* sp., *Trichuris* sp. *Chebartia* sp., *Trichostrongylus* sp., and *Oesophagostomum* sp. Mixed infection was found only in herbivores. Protozoa and trematodes were not detected in this study. The animals showed no obvious clinical symptoms, indicating a subclinical infection. However, among these parasites, *Ostertagia* sp. and *Oesophagostomum* sp. can cause severe clinical disease under immune-compromised or adverse environmental condition. This study suggests the need for further detailed epidemiological studies on species-specific parasite along with regular feces examination regardless of illness and an effective deworming schedule should be implemented to control parasitic infection in Chattogram Zoo.

Keywords: Chattogram Zoo, Gastrointestinal, Parasite, Prevalence

Chapter 1: Introduction

Over two-thirds of the worldwide wildlife population decreased as many wild species' natural habitats were changed, degraded, or occupied by the rising human population (Almond et al., 2020). That's why zoological gardens (zoo) and animal parks display wild animals in cages which serves as the conservation of endangered animals as well as increase awareness among people about wildlife and the environment (Reade and Waran, 1996). It also serves as a means of education, research, and recreation for the society and changes people's perspective regarding wild animal and their conservation (Carr and Cohen, 2011; Godinez and Fernandez, 2019). However, wild animals in captivity pose various challenges, requiring the control of infectious diseases and the maintenance of proper husbandry practices (Kirkwood, 1996). Parasites can be introduced into zoos in a variety of ways, including through contaminated or infected foods, intermediate or paratenic hosts, introducing parasitized animals, and by infected zoo staff and visitors (Panayotova-Pencheva, 2013). The incidence of parasitic infection in captive animals may vary depending on managemental strategies, parasitism, disease prevention and therapeutic interventions (Lim et al., 2008). Besides, animals maintained in small cages and constricted surroundings become stressed, which decreases their immune systems, leaving them more susceptible to parasitic diseases or even death due to high parasitic load which they might survived in nature (Van Wyk and Boomker, 2011). Regular deworming of captive animals normally reveals no notable signs or symptoms of parasitic illness (Parsani et al., 2001; Schieber and Štrkolcová, 2019). However, some endoparasites of wild animals might endanger the health of zoo staff and tourists. That's why knowledge of the illness state of the zoo animals and adequate screening are crucial for animal welfare and public health safety, as many parasites can infect several host species (Nath et al., 2021). Regular screening for gastrointestinal parasites and effective control measures, including timely diagnosis, prevention, and efficient intervention, can improve the health of zoo animals while preserving public health (Hallinger et al., 2018; Kolapo and Jegede, 2017). Lamentably, inadequate knowledge on diseases and parasites of captive animals is a significant drawback in

zoological facilities (Rahman et al., 2014). As a result, the purpose of this study is to identify the prevalence of gastrointestinal parasitic infection in captive wild animals, including selected herbivores and carnivores of Chattogram zoo in Bangladesh. The findings of this study have significance for developing a better knowledge of these animals' gastrointestinal parasite fauna and preventing the transmission of contagious parasitic illnesses among animals in the zoo.

Chapter 2: Materials and Methods

2.1 Study periods and sites

The study was conducted on wild mammals at Chattogram zoo, Chattogram, Bangladesh, between March and April, 2024. It is the only wildlife conservation, research, education, and recreation center in Chattogram district, located at 22°22′0″ N and 91°47′46″ E, with an area of 10.2 acres. There were about 620 animals belonging to 66 species which comprise herbivores, carnivores, birds, and reptiles. Herbivores were housed in open-top enclosures, while carnivores and birds were confined in close cages. Fecal examination was carried out only in suspecting disease condition by the caretakers. Enclosures were cleaned regularly, and the animals were dewormed twice a year using a broad spectrum anthelmintic.

2.2 Study population

Among the various animals, our study primarily focused on newly introduced and selected mammalian herbivores and carnivores. 11 species from both groups were included for the study with the number of animals and sample size listed in Table 1.

2.3 Sample collection and preservation

A total of 17 fecal samples were collected from 11 species of selected wild mammals. Among them, 9 samples from herbivores and 8 samples from carnivores were collected, respectively. Approximately 5-10 g of pooled fecal samples was obtained from each enclosure using sterile forceps before cleaning the cages in the morning with the

Table 1. List of animals and number of samples taken for the study

Species	No. of animals	No. of samples
Llama (*Lama glama*)	6	2
Hippopotamus (*Hippopotamus amphibious*)	2	2
Kangaroo (*Macropus* spp.)	2	2
Gayal (*Bos frontalis*)	3	1
Wildebeest (*Connochaetes taurinus*)	4	2
Tiger (*Panthera tigris)*	19	2
Lion (*Panthera leo*)	2	2
Asian palm cevet (*Paradoxurus hermaphrodites*)	2	1
Bengal fox (*Vulpes bengalensis*)	2	1
Fishing cat (*Felis chaus*)	4	1
Leopard cat (*Prionailurus bengalensis*)	4	1

assistance of the animal caretakers. During collection, particular care was taken to avoid any sort of contamination. Immediately after collection, the samples were placed in a dry, clean plastic container and labeled without any delay. The samples were preserved in 10% formalin and transported to the Parasitology laboratory of the Department of Pathology and Parasitology of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. They were stored at 4°C until laboratory examination.

2.4 Examination of fecal samples

The preserved samples were examined in the Parasitology laboratory of Department of Pathology and Parasitology at CVASU. The samples were homogenized, and filtration was done to remove the undigested, coarse fecal content. Qualitative tests including direct smears, sedimentation, and flotation techniques were conducted to detect parasitic infection.

Direct smear technique

A small amount of fecal suspension was placed on a clean glass slide and mixed with a few drops of water. Then, a cover slip was put over the smear which was observed under a microscope to detect the parasite egg or Oocyst (Soulsby, 1982).

Floatation technique

Approximately 3 g of feces was placed into a suitable container and thoroughly mixed with 50 ml of floatation fluid (saturated salt solution) using a stirring device for the test tube floatation technique. The homogenized fecal suspension was then poured through a tea strainer into another container in order to remove the larger fecal content. After that, the fecal suspension was poured into a test tube until a convex meniscus was formed. Finally, a glass cover slip was placed over the meniscus and allowed to stand still for 15 minutes. The cover slip was carefully lifted off and immediately placed on a clean glass slide for microscopic examination (Soulsby, 1982).

Sedimentation technique

Approximately 3 g of fecal material was transferred into a container and mixed with 50 ml of tap water by a stirring device for the simple sedimentation technique. Then the suspension was filtered to remove the coarse fecal material. After that, the filtrated suspension was transferrd into a test tube and allowed to settle down for five minutes. The supernatant was discarded carefully with a pipette. A small amount of the top layer of sediment was transferred to a microscope slide, and a cover slip was placed and examined under a microscope (Soulsby, 1982).

Chapter 3: Results

3.1 Overall prevalence of parasitic infection in animals

A total of 17 fecal samples were examined, and 5 samples were found to be infected with different types of parasites. At least one intestinal parasite was identified in each positive fecal sample of the animals. The overall prevalence of parasitic infection was 29.41%. The prevalence of parasitic infection was higher in herbivores (44.45%) compared to carnivores (12.5%) (Table 2.). Helminths infection was the most prevalent 100% type of parasitic infection. Among the helminths, nematodes were predominant (83.3%), followed by cestodes (16.7%) (Figure 1.). Carnivores were infected only with nematodes (100%), whereas herbivores were infected by both nematodes (83.3%) and cestodes (16.7%) (Figure 2.). No trematodes and protozoa were identified during the study. These findings suggest that helminth infections are more prevalent among the studied animals, with nematodes being the dominant parasitic group.

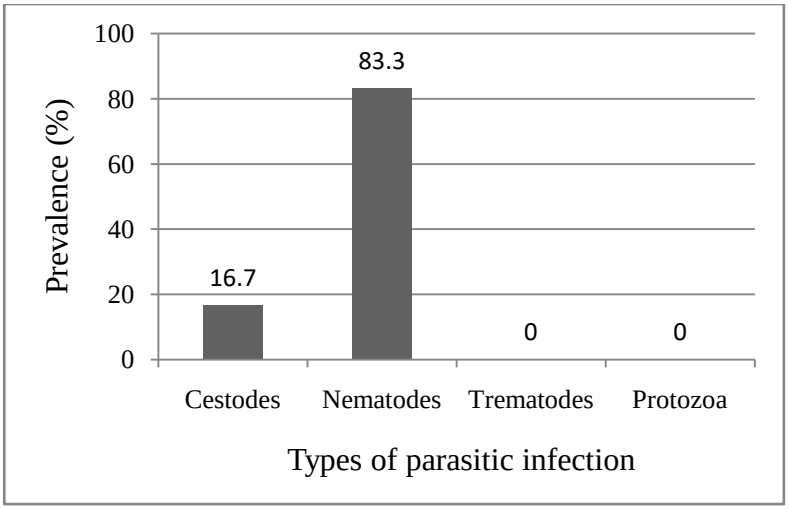

Figure 1. Overall prevalence of parasitic infection in animals of Chattogram zoo

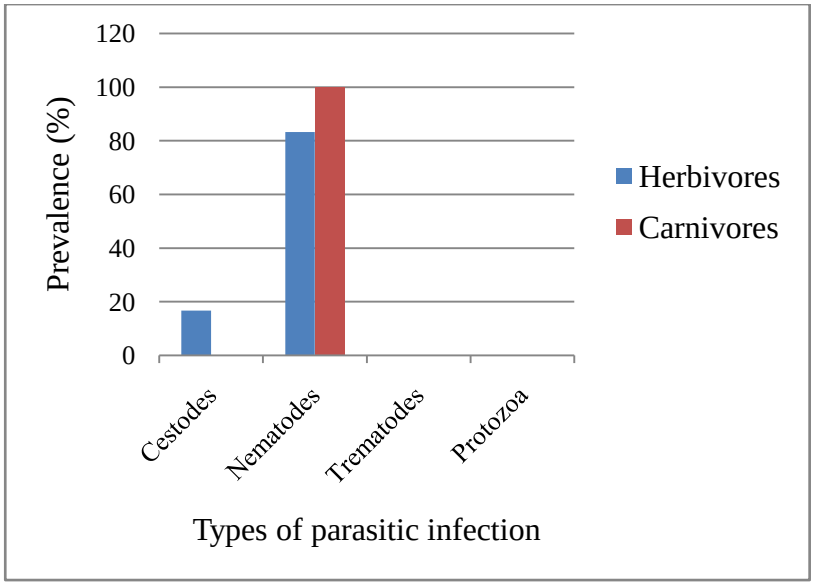

Figure 2. Prevalence of parasitic infection in herbivores and carnivores at Chattogram zoo

Table 2. Overall prevalence of parasitic infection in herbivores and carnivores at Chattogram zoo

Species	Total no. of sample	Total no. of positive case	Prevalence (%)
Herbivores	9	4	44.45%
Carnivores	8	1	12.5%
Total	17	5	29.41%

3.2 Prevalence of different gastrointestinal parasite in herbivores and carnivores at Chattogram zoo

Among the selected herbivores, the prevalence of gastrointestinal parasites was 100% in both Llama and Wildebeest, while in carnivores only the Bengal fox (100%) was shown to be infected (Table 3.). The gastrointestinal parasites of Llama include *Monizia* sp. (50%), *Ostertagia* sp. (100%), *Trichuris* sp. (50%), and *Chebartia* sp. (100%). We identified *Trichostrongylus* sp. (100%) and *Oesophagostomum* sp. (100%) in Wildebeest. Only *Trichuris* sp. (100%) was found in Bengal fox (Table 4.). Mixed

infections were found only in Llama and Wildebeest (Table 5.). Prevalence of the mixed infection was *Monizia* sp. and *Trichuris* sp. (50%), *Chebartia* sp. and *Ostertagia* sp. (100%) and *Trichostrongylus* sp. and *Oesophagostomum* sp. (100%).

Table 3. Overall prevalence of parasitic infection in captive animals at Chattogram Zoo

Species	No. of sample	No. of positive sample	Prevalence (%)
Llama	2	2	100
Hippopotamus	2	0	0
Kangaroo	2	0	0
Gayal	1	0	0
Wildebeest	2	2	100
Tiger	2	0	0
Lion	2	0	0
Asian palm cevet	1	0	0
Bengal fox	1	1	100
Fishing cat	1	0	0
Leopard cat	1	0	0

Table 4. Prevalence of different gastrointestinal parasites in captive animals

Name of the animal	Name of parasite	No. of positive case (No. of sample)	Prevalence (%)
Llama	*Monizia* sp.	1(2)	50
	Ostertagia sp.	2(2)	100
	Trichuris sp.	1(2)	50
	Chebartia sp.	2(2)	100
Wildebeest	*Trichostrongylus* sp.	2(2)	100
	Oesophagostomum sp.	2(2)	100
Bengal fox	*Trichuris* sp.	1(1)	100

Table 5. Prevalence of mix infection

Name of the animal	Parasites	No. of case	Prevalence (%)
Llama	*Monizia* sp + *Trichuris* sp.	1(2)	50
	Chebartia sp. + *Ostertagia* sp.	2(2)	100
Wildebeest	*Trichostrongylus* sp. + *Oesophagostomum* sp.	2(2)	100

3.3 Diameter of eggs of different gastrointestinal parasites in different zoo animal

The published literature was used to identify the morphological characteristics of helminth eggs (Ruiz Hurtado, 2016; Traversa et al., 2011; Van Wyk and Boomker, 2011). The diameters (length by width) in µm of egg of different gastrointestinal parasites were measured in the present study. The diameter of egg of *Monizia* sp. (68 x 61) µm, *Ostertagia* sp. (79 x 54) µm, *Trichuris* sp. (72 x 34) µm, *Chebartia* sp. (87 x 45) µm in Llama, *Trichostrongylus* sp. (72 x 49) µm and *Oesophagostomum* sp. (68 x 51) µm in Wildebeest and *Trichuris* sp. (87 x 40) µm in Bengal fox were measured.

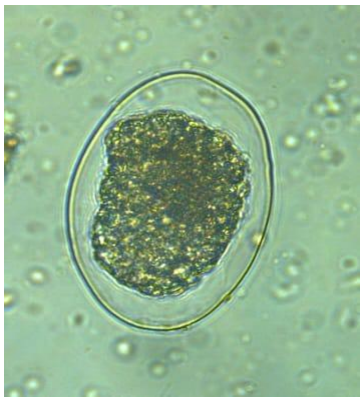

Figure 3. Egg of *Ostertagia* sp. of Llama (40x)

Figure 4. Egg of *Monizia* sp. of Llama (40x)

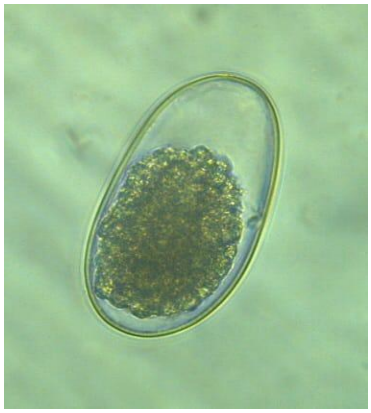

Figure 5. Egg of *Chebertia* sp. of Llama (40x)

Figure 6. Egg of *Trichuris* sp. of Llama (40x)

Figure 7. Egg of *Trichostrongylus* sp. of Wildebeest (40x)

Figure 8. Egg of *Oesophagostomum* sp. of Wildebeest (20x)

Figure 9. Egg of *Trichuris* sp. of Bengal fox (40x)

Chapter 4: Discussion

Gastrointestinal (GI) parasite infections harm wild animals' life and wellbeing. Monitoring GI parasitism (helminths and protozoa) is crucial for improving animal management and health in captivity. Zoo animals often become infected by various endoparasites (Adetunji, 2014; Lim et al., 2008; Mir et al., 2016; S. M. Rahman et al., 2014; Sprenger et al., 2018), and in this study, several parasite species were identified in selected herbivores and carnivores of Chattogram zoo. The overall prevalence of GI parasitic infection was 29.41% which was close to previous findings (28%) made by Atanaskova et al. (2011). Our findings conflict with other studies where lower prevalence of endoparasitic infection in Macedonia (21%) and Servia (17%) in wild animals at zoos were reported (Atanaskova et al., 2011; Lalošević et al., 2007), as well as higher prevalence of GI infection were also recorded in India (46.2%), Spain (72%), Nigeria (76%), and Bangladesh (76% and 78%) , respectively (Cordón et al., 2008; Opara et al., 2010; Rahman et al., 2014; Raja et al., 2014; Thawait et al., 2014). This difference may be due to geographic conditions, climates, captivity conditions, husbandry practices, and feeds and feeding practices, which might influence the prevalence of parasitic infection.

In this study, the prevalence of helminths infection (100%) was more common than protozoan infections, which is consistent with a previous study where 100% helminths infection was recorded (Rahman et al., 2023). Some similar studies with higher helminths infection than protozoal infection were also reported in an Italian and Malaysian zoo (Fagiolini et al., 2010; Lim et al., 2008). However, it contradicts the findings of Levecke et al. (2007) and Cordón et al. (2008), who found that protozoal infection was higher than helminthic infection. Among the helminths, nematode infections were more common than other helminth infections, which was in agreement with prior studies (Rahman et al., 2014). It is because of their direct life cycle, which does not require any intermediate hosts for completion of their life cycle, and their ability to transmit through feed and water contamination (Mir et al., 2016). In contrary, trematodes and some cestodes need at least

one intermediate host for life cycle completion. This might be the reason for the lower occurrence of cestode and absence of trematode infection in the zoo (Atanaskova et al., 2011; Mir et al., 2016). The GI parasitic infection in herbivores was recorded 44.45% in this study, which is similar to previous studies (Lim et al., 2008; Wahed, 2004), where they found 45.7% and 44.5% infection rate in herbivores, respectively. Higher prevalence (54.3%) in herbivores were found in Romania (Dărăbuş et al., 2014), while lower prevalence (30.8%) was also recorded in Rangpur zoo (Khatun et al., 2014). In carnivores, the prevalence was 12.8%. Higher (72.7%, 97.3%) and lower (7%) infection rate in carnivores were recorded by other researchers (Khatun et al., 2014; Lim et al., 2008; Kvapil et al., 2017). Geographical and environmental factors are the reasons for the variation in the findings. Among herbivores Llama and wildebeest were infected with GI parasites. Llama was affected with *Monizia* sp., *Ostertagia* sp., *Trichuris* sp., and *Chebartia* sp. which was previously reported in other studies (Cafrune et al., 1999; Navone and Merino, 1989; Ruiz Hurtado, 2016). Cestode infections might result from untreated fodder contaminated by intermediate hosts, such as snails. *Trichostrongylus* sp. in wildebeest is not very common but it can be found in captive herbivores (Horak et al., 2021). The difference in findings might be due to enclosure structure and feed habit. The *Trichuris* sp. was commonly encountered in fox which is supported by Magi et al. (2016).

In zoos, controlling these parasites should largely focus on reducing infection pressures through the use of anthelmintics. Although the animals were treated with antiparasitic medications on periodic schedules, the infection rate seen in this study suggests that existing preventative methods are insufficient or that a large percentage of reinfections occur. Lack of quarantine for appropriate time, insufficient fecal examination before introduction as well as free-ranging rodents and birds, might also be sources of parasite infection. Zoo workers might also lead to transmission by acting as a vector and disseminating parasites through footwear, clothing, and contaminated water or food (Adetunji, 2014; Otegbade and Morenikeji, 2014). Furthermore, the regular use of broad-spectrum anthelmintics combined with inadequate doses and administration techniques may contribute to the development of resistance to anthelmintics (Shalaby, 2013). In a

study done by Young et al. (2000), it was found out that several gastrointestinal roundworms in captive wild ruminants shown increased resistance to widely used anthelmintics such as levamisole and ivermectin. To assure anthelmintic efficacy, parasite control methods must be revised, with a higher emphasis on surveillance through periodic parasitological assessments (Nath et al., 2021). In conclusion, the supplied information highlight the significance of periodically conducting copro-parasitological examinations in zoos, in order to find effective measures by the zoo technician to enhance the health and well-being of animals (da Silva et al., 2022).

Limitations

This study has a few drawbacks. One notable disadvantage of this study is the small sample size. Due to logistical restrictions, the sample size may not accurately represent the Chattogram zoo's overall captive wild animal population. This study did not investigate species-specific differences, which might be critical for understanding the dynamics of parasite infections in confined wild animals. The study mostly used regular parasitological approaches (fecal examinations), to identify parasites. The use of molecular methods for species-level parasite identification might offer more specific information about the parasites. A more extensive study incorporating a larger number of species would offer a more detailed picture of gastrointestinal parasitic prevalence in the zoo.

Conclusion

In this study, a considerable prevalence of endoparasites (29.41%) was observed in captive wildlife at Chattogram Zoo. As there was no recorded mortality and clinical evidence, and the animals were apparently healthy during the period of investigation, the incidence even in high standards at Chattogram zoo indicates subclinical infection, which indicates a potential risk of pathogenic outbreaks under stressful or immune-compromised conditions. Zoo animals act as both hosts and reservoirs for several zoonotic parasites. More detailed and year-long study on parasitic infections is required to better understand the epidemiology of parasitism and to enhance parasitic infection prevention. Our finding provides critical insights into the parasitic species affecting zoo animals, which could aid in improving animal health and mitigating parasitic risks in similar captive environments.

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