Assessment of gastrointestinal parasitic infections and anthelmintic efficacy in captive animals: A Study at Chattogram zoo, Bangladesh

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Assessment of gastrointestinal parasitic infections and anthelmintic efficacy in captive animals: A Study at Chattogram zoo, Bangladesh

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ABBREVIATIONS

Abbreviations	Elaborations
%	percentage
Ctg	Chattoram
Etc.	Et cetera
GI	Gastrointestinal

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Abstract

A coprological study was undertaken to assess prevalence of gastrointestinal (GI) parasite infection and efficacy of anthelmintic in herbivore, primates and birds at Chattogram zoo, Bangladesh. Total 40 samples examined where the highest prevalence (100%) was found in birds (Emu and Turkey) prior to anthelmintic treatment. Nematodes (27.5%) were the most prevalent parasite with high infection in birds (100%) and carnivore (85.71%) where trematode (12.5%) were common in herbivore while cestode were completely absent in the studied animals. Anthelmintic treatment was done with triclabendazole (25%), levamisole (37.5%) and fenbendazole (37.5%) twice yearly. The prevalence was significantly decreased in those animals except wild beast (*Connochaetes taurinus*), samber deer (*Rusa unicolor*), and barking deer (*Muntiacusmuntjak*) after the treatment. Triclabendazole was administered in herbivore where llama (*Lama glama)* showed significant change than other animals. In carnivore, levamisole dramatically reduced GI parasite load while other animals were treated with fenbendazole which significantly reduced the GI parasite infection. Development of an appropriate and effective anthelmintic program for captive-wild animals to identify the optimal time of the year and frequency of anthelmintic other epizootiological parameters.

Keywords: Anthelmintic efficacy, Chattogram zoo, gastro-intestinal parasite, prevalence.

Chapter 1
Introduction

Zoological garden (Zoo) is an ex-situ facility where animals are displayed in cages or enclosures for education, research, conservation, rescue, rehabilitation and recreation purposes Thawait et al., (2014). Zoo populations are unique and important sources for studying wildlife and their habitats preserving endangered species through captive breeding and reintroduction programs Schulte Hostedde and Mastromonaco (2015). Furthermore, maintaining animals in zoos can benefit scientific investigations, environmental education, and learning the biological behavior of species (Morezzi et al, 2021). In Bangladesh, a few zoological gardens, safari parks, and environmental parks have been built serving as a valuable source of entertainment for people of all ages and conversations of endangered species (Khatun et al., 2014). The study was conducted at the Chattogram Zoo, a small zoo with 68 animal species established in 1988 and has a total area of 10.2 acres. There are several zoos in Bangladesh, whose salient purpose, is to conserve wildlife, especially endangered species native to Bangladesh and this end, the Chattogram Zoo's management has successfully bred tigers and snakes in captivity. In Chattogram Zoo, the first white tiger born in Bangladesh in 2018. It is the sole source of entertainment for the people of Chattogram; the second largest city of Bangladesh. Furthermore, student of kindergarten as well as school have the opportunity to truly experience anything of their bookish knowledge. Moreover, zoos are also crucial for university students and wildlife researchers by providing a controlled environment for studying animals, supporting conservation efforts, and advancing research on animal health and behavior.

However, zoo animals can be suffered from different pathogenic diseases by pathogens (Bacteria, Virus and Parasite) when they are kept in captivity with inadequate or unhealthy management and environment, among those pathogens especially parasites, that can cause injury and even death of animals de Melo et al, (2022). Parasitic infections are any illness or condition caused by living and reproduction of parasites in the host body. There are three chain links such as the interrelationship among the parasites, the hosts and the outer surroundings that account for the development and spreading of parasitic diseases.

No animal is immune to parasites, although low-grade infections frequently cause resistance. According to husbandry practices, illness prevention strategies, parasite-host interactions, and therapy administered, the prevalence of parasites in captive animals may vary (Hasan et al., 2021). In captive conditions, parasitic diseases constitute a major problem for animals and create severe illness and vulnerability to GI parasites (Hossain et al. 2021).

However, it has been shown in zoos globally that, a common prevalence of gastrointestinal parasites in various animal species. For instance, studies conducted in zoos in India, Africa, and Europe have indicated that captive animals are frequently infected with nematodes, cestodes, trematodes, and protozoans. A study by Thawait et al. (2014) in Nandan Van Zoo, India, found a high prevalence of GI parasites in captive wild animals, where nematodes were the most common parasites. In Egypt, a study by Khalafalla et al. (2011) showed that captive carnivores and herbivores harbored various parasitic infections, especially nematodes and protozoans. Similarly, in the Dhaka Zoo, Bangladesh, Barmon et al. (2011) reported the presence of GI parasites in most zoo animals examined, with *Strongyles spp.* and *Ascarids* sp*p.* being the most commonly found parasites. These studies highlight the universal nature of parasitic infections in zoo settings, with significant overlap in the types of parasites across different regions. A study by Uddin et al. (2017) at the Dhaka National Zoo showed that most animals had infections caused by nematodes, cestodes, and protozoa. Another study by Islam et al. (2018) found similar results in Rangpur Zoo, where various GI parasites were detected in carnivores, herbivores, and omnivores. The climate of Chattogram, which is humid and tropical, creates an ideal environment for parasites to thrive, increasing the risk of infection in zoo animals. This underscores the need for ongoing research and monitoring to assess the parasitic burden on the zoo's animal population.

Anthelmintics (anti-parasitic drugs) are one of the most common methods for controlling gastrointestinal parasites in zoo animals. However, over time, the misuse or overuse of these drugs can lead to anthelmintic resistance, reducing their effectiveness. Studies have reported instances of drug resistance, especially in the case of nematodes, when the same class of drugs is repeatedly used. A study by Walker et al. (2014) highlighted the global challenge of anthelmintic resistance in both livestock and zoo animals, emphasizing the need for rotating drug classes and integrating alternative treatments, such as herbal anthelmintics, where possible. global Localized studies on the efficacy of anthelmintic drugs in zoos are limited in Bangladesh, but similar trends in drug resistance are likely to be present in zoos like Chattogram. So, our study aims-

1.To identify and classify the gastrointestinal parasites present in different species of animals at Chattogram Zoo through fecal examination.

2. To determine the prevalence of specific gastrointestinal parasites within the zoo in the carnivores, herbivores, primates and aves.

3. To evaluate the efficacy of anthelmintic treatments currently used in the zoo for controlling parasitic infections.

Chapter 2

Materials and Methods

Study Area, period and animal selection

The study was conducted at Chattogram Zoo. Various animals were randomly selected for the study including wild herbivores, carnivores, birds and primates from March to September, 2024.

Target population

Chattogram zoo houses a total number of over 320 animals including mammals, reptiles, and
birds. The study included herbivores, carnivore, primates and birds housed at the zoo (Table 1). A total of 40 samples were collected in pre and post anthelmintic treatment in six-month intervals.

Table 1: Categorization of animals under study

Name of species	No. of examined animals	Percentage (%)
Herbivores	25	62.5
Carnivores	7	17.5
Primates	4	10
Birds	4	10

Collection and preservation of samples

Freshly voided fecal samples were collected from the animals with the assistance of the zoo caretakers. Immediately after collection, the samples were transferred to a collection vial containing 10% formalin. Then, samples were brought to the Parasitology lab of Department of Pathology and Parasitology and were stored at 4 degrees Celsius till coproscopy.

Examination of fecal sample

The sample was examined in the parasitology laboratory of Department of Pathology and Parasitology, Chattogram Veterinary and Animal Sciences University. According to morphologic characteristics eggs of different parasite were identified through performing different qualitative parasitological examinations such as direct smear, sedimentation and floatation technique.

Direct Smear Technique

A qualitative method of examining feces that allows for the identification of helminth eggs and larvae is the direct smear. To create a thin smear, a tiny quantity of excrement and a few droplets of water were placed on a sterile slide. Then, carefully place a cover slip over the smear and examine the parasites' distinctive morphology under a microscope at varying resolutions (10x at first, and then 40x) (Soulsby, 1982).

Flotation technique

Flotation technique is used for the detection of Nematode and Cestode eggs, ova, cyst and oocyst of protozoa having low specific gravity. It can be performed by using flotation fluids having a specific gravity range of 1.18 to 1.364. In this case, test tube flotation was performed by using a salt sugar solution (SG 1.28). Approximately, 5gm of feces in 50 ml of flotation fluid and mix thoroughly by a stirring device. Then the resulting suspension was poured into another with a tea strainer. The suspension was poured into a test tube until a meniscus is formed and a cover slip was placed over the meniscus. It is allowed to stand for 15-30 minutes. The cover slip was lifted off carefully and placed on microscope carefully, then observed at 40x and 100x magnifications (Soulsby,1982).

Sedimentation Technique

Sedimentation is the technique for detecting eggs of trematode as they are large and heavy. It can be performed simple and centrifugal methods. Approximately 5gm of feces were taken into a container. Added 40-50 ml of tap water into the container and mix thoroughly with a stirring device. Then, filtered the suspension through a tea strainer and poured filtered material into test tube allowing to sediment 15-30 minutes or centrifuge at 1500 rpm for 5 minutes. After that, discard the superannuate very carefully. Then re-suspend the sediment in 5 ml water and allow to settle for 5 minutes. The supernatant was removed carefully. A small amount of top layer sediment was placed on a microscope slide by the use of pipette. A cover slip was applied on microscope slide and observed under a microscope at 40x magnification (Soulsby,1982).

Data Analysis

The obtained data were stored in a Microsoft Excel 2016 spreadsheet. Then, the data were sorted for errors and inconsistencies, coded, and checked for integrity in Microsoft Excel 2016. Afterward, the data were exported to STATA-IC-1 to examine the prevalence of GI parasite and anthelmintic efficacy in the studied animals. The p-value of 0.05 was considered a significant cut-off value.

Figure 1: Conducting coproscopy

Chapter 3

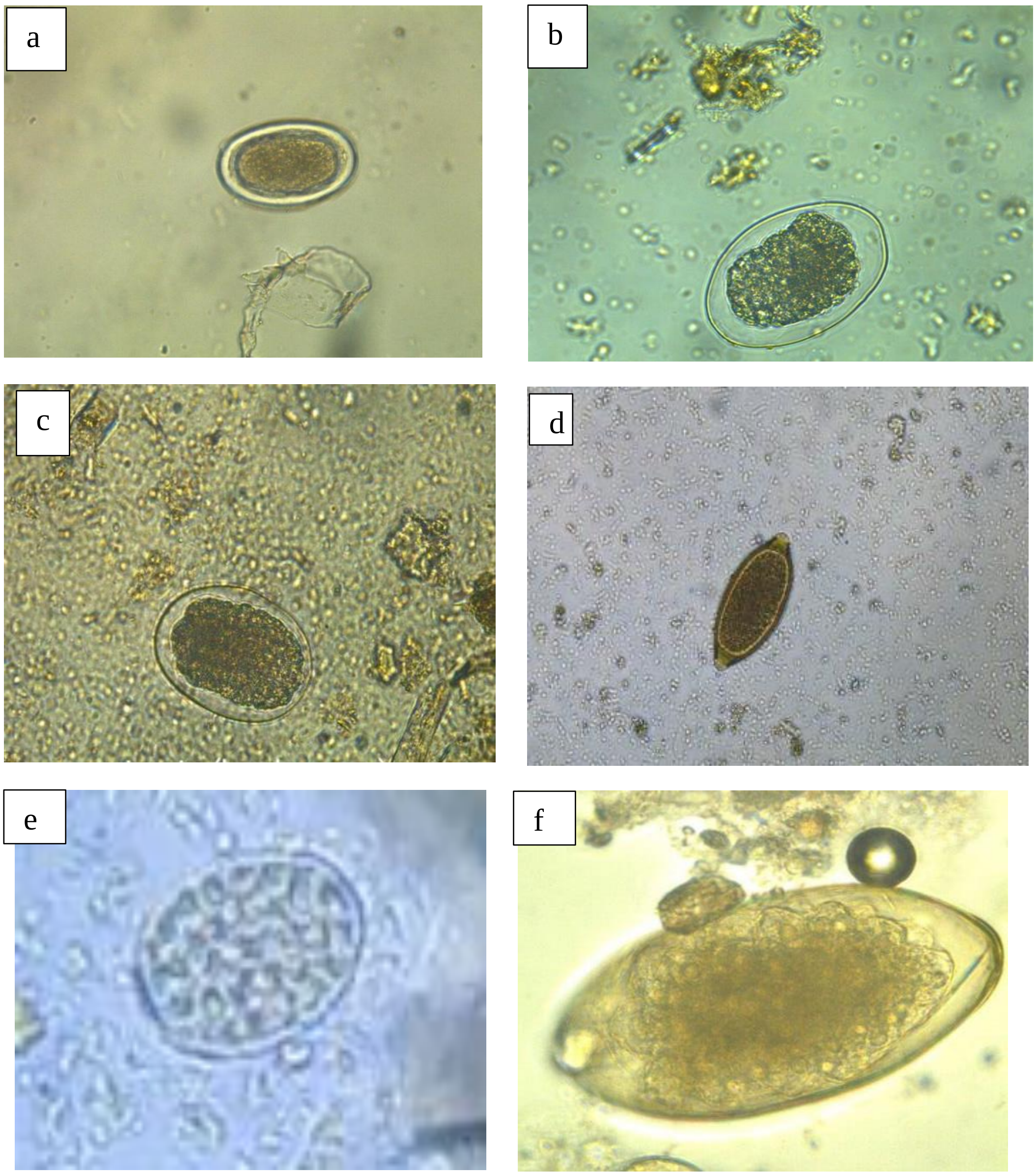
Results

Among studied animals, birds have the greatest GI parasite infection rate of any species (100%), followed by carnivores (85.71%) and herbivores had the lowest infection rate (68%) before anthelmintic use (Table 2).

Table 2: GI parasitic infection status in different species prior to anthelmintic use

Categories	No. of examined animals	No. of infected animals	Percentage (%)
Herbivores	25	17	68
Carnivores	7	6	85.71
Primates	4	3	75
Birds	4	4	100

Figure 2 exhibited the morphological characteristics of eggs under of different paraistes found by coproscopy. The egg of *Ascaridia spp.* was thick-shelled, oval, and smooth-surfaced, containing a single-cell zygote. *Ostertagia spp.* eggs were thin-shelled, oval, and contain a morula with multiple blastomeres. The eggs of *Uncinaria spp.* were thin-shelled, oval, and have a clear space surrounding the morula. *Trichuris spp.* eggs were distinctively lemon-shaped, thick-shelled, and possess polar plugs at both ends. *Oesophagostomum spp.* eggs were thin-shelled, oval, and contain a segmented morula. The eggs of fasciola were large, oval, operculated, and have a golden-brown shell.The eggs of *Capillaria spp*. was barrel-shaped, thick-shelled, and has bipolar plugs. *Paramphistomum spp.* eggs were large, oval, operculated, and pale grayish in color. *Strongylus spp.* eggs were thin-shelled, oval, and contain a segmented morula. *Toxocara spp*. eggs are spherical, thick-shelled, and have a finely pitted surface.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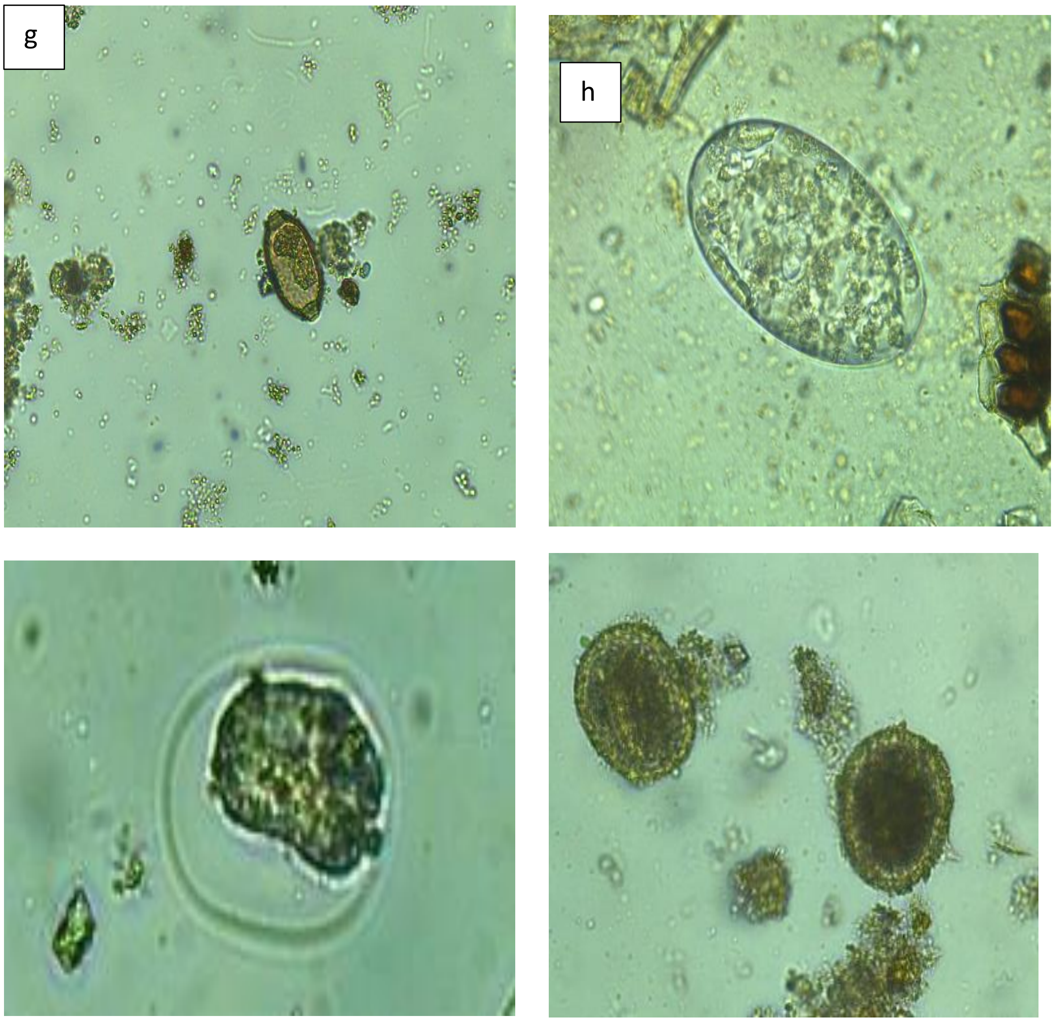

Figure 2: Morphology of eggs of different parasites

a)Ascaridia spp., b) Ostertagia spp., c) Uncinaria spp., d) Trichuris spp. e) Oesophagostomum spp., f) *Fasciola spp g) Capillaria spp., h) Paramphistomum spp., i) Strongylus spp., j) Toxocara spp.*

Nematodes were the most common GI parasite found during post-anthelmintic administration. Moreover, barking deer were found to have a mixed trematode and nematode infestation. No animals were exhibiting cestode infection (Table 3).

Table 3: Pre-treatment distribution of gastrointestinal parasites

Species	No. of examined sample	GI parasite		
		Cestode	Trematode	Nematode
Llama	4	-	-	1
Wild beast	4	-	1	-
Gayal	2	-	1	-
Horse	2	-	-	1
Zebra	2	-	-	1
Sambar deer	4	-	1	-
Spotted deer	4	-	1	-
Barking deer	4	-	1	1
Lion	2	-	-	1
Tiger	2	-	-	1
Fox	1	-	-	1
Wild Cat	2	-	-	1
Monkey	4	-		1
Turkey	2	-	-	1
Emu	2	-	-	1

Paramphistomum spp. (Figure 2) was fully prevalent in Gayal, while in other herbivores the infection rate ranging from 50% to 75%. Besides, horses and zebras were found completely susceptible to Ostertegiasis; while *Toxocara spp*. infections were found in all carnivores. Monkeys were found to be susceptible to oesophagostomiasis (75%). Moreover, ascaridiasis and capillariasis is fully (100%) prevalent among the birds under the study (Table 4). Moreover, table 4 reveals that mixed infection was also found in wild beasts, barking deer, tiger, fox and turkey.

Table 4: GI parasite observed prior to anthelmintic treatment

Species	GI Parasite	positive case	percentage (%)
Llama	*Trichuris spp.*	(2)4	50
Wild beast	*Paramphistomum spp.*	(3)4	75
	Fasciola spp.	(2)4	50
Gayal	*Paramphistomum spp.*	(2)2	100
Horse	*Ostertagia spp.*	(2)2	100
	Strongylus spp.	(2)1	50
Zebra	*Ostertagia spp.*	(2)2	100
Samber deer	*Paramphistomum spp.*	(2)4	50
Spotted deer	*Paramphistomum spp.*	(2)4	50
Barking deer	*Paramphistomum spp.*	(2)3	66.66
	Ostertagia spp.	(1)3	33.33
Lion	*Toxocara spp.*	(2)2	100
Tiger	*Toxocara spp.*	(2)2	100
	Uncinaria spp.	(1)2	50
Fox	*Toxocara spp.*	(1)1	100
	Trichuris spp.	(1)1	100
Wild cat	*Toxocara spp.*	(1)2	50
Monkey	*Osepgaostomum spp.*	(3)4	75
Turkey	*Capillaria spp.*	(2)2	100
	Ascaridia spp.	(2)2	100
Emu	*Capillaria spp.*	(2)2	100

Following anthelmintic treatment, there was a considerable reduction in GI parasites load in the study population. Whereas nematodes were detected only in barking deer, trematodes were found in wild beasts and sambar deer (Table 5).

Table 5: Post-treatment distribution of gastrointestinal parasites

Species	No. of examined samples	Presence of parasite		
		Cestode	Trematode	Nematode
Lama	4	-	-	-
Wild beast	4	-	1	-
Gayal	2	-	-	-
Horse	2	-	-	-
Zebra	2	-	-	-
Sambar deer	4	-	1	-
Spotted deer	4	-	-	-
Barking deer	4	-	-	1
Lion	2	-	-	-
Tiger	2	-	-	-
Fox	1	-	-	-
Wild Cat	2	-	-	-
Monkey	4	-	-	-
Turkey	2	-	-	-
Emu	2	-	-	-

Following anthelmintic treatment, most animals showed no evidence of gastrointestinal parasite infections. However, a few exceptions were observed: wild beasts had a prevalence rate of 50%, sambar deer 25%, and barking deer 33.33% (table 6), but infection rates were notably lower compared to those observed prior to anthelmintic treatment showed in table 4.

Table 6: GI parasite observed post-anthelmintic treatment

Species	GI parasite	positive case	Prevalence (%)
Lama	-	0	0
Wild beast	*Paramphistomum spp.*	(2)4	50
Gayal	-	0	0
Horse	-	0	0
Zebra	-	0	0
Samber deer	*Paramphistomum spp.*	(1)4	25
Spotted deer	-	0	0
Barking deer	*Ostertagia spp.*	(1)3	33.33
Lion	-	0	0
Tiger	-	0	0
Fox	-	0	0
Wild cat	-	0	0
Monkey	-	0	0
Turkey	-	0	0
Emu	-	0	0

Table 7 showed that anthelmintic treatment reduced the GI parasite load in the captive animals under study. Significant reduction of GI parasite observed in wild lama (p= 0.014). However, in the other animals the infection reduced non-significantly.

Table 7: Comparative analysis of gastrointestinal parasite prevalence pre and post-anthelmintic treatment

Species	Pre-anthelmintic treatment (positive case)	Post-anthelmintic treatment (positive case)	p-value
Llama	2	0	**0.014**
Wild beast	3	2	0.829
Gayal	2	0	0.24
Horse	2	0	0.24
Zebra	2	0	0.24
Sambar deer	3	1	0.363
Spotted deer	2	0	0.244
Barking deer	3	1	0.805
Lion	2	0	0.244
Tiger	2	0	0.244
Fox	1	0	0.5
Wild cat	1	0	1
Monkey	3	0	0.241
Turkey	2	0	0.244
Emu	2	0	0.244

At Chattogram Zoo, various categories of anthelmintics were administered to different animal species. Fenbendazole, levamisole, and triclabendazole were employed to treat gastrointestinal parasites, with levamisole and fenbendazole being the most commonly used (Table 8).

Table 8: Usage Patterns of anthelmintic in Chattogram zoo

Anthelmintic	**No. of species**	Percentage (%)
Triclabendazole	10	25
Levamisole	15	37.5
Fenbendazole	15	37.5

Chapter 4

Discussion

The current study conducted at Chattogram Zoo evaluated the prevalence of gastrointestinal (GI) parasitic infections across a range of zoo animals, as well as the effectiveness of anthelmintic treatments in reducing these infections. The results showed in table 2 underscore presence of GI parasites in captive animals, with birds showing the highest prevalence (100%) of parasitic infections, followed by carnivores (85.71%), primates (75%), and herbivores (68%). These findings were consistent with similar studies conducted in other zoos globally (Amouei et al. 2018; Barmon et al. 2014) which reported high rates of parasitic infections in zoo animals due to factors like close confinement, shared enclosures, and environmental conditions that facilitate parasite transmission. In this study, the climate of Chattogram, characterized by high humidity and a tropical environment, likely played a role in sustaining and propagating parasitic infections, particularly in birds and herbivores.

In figure 2, the observed morphology of the eggs found in the captive animals was consistent with the characteristics described in Soulsby (1982).

The study also revealed a predominance of nematodes as the most common GI parasite before anthelmintic treatment, affecting both carnivores and birds, with a particular prevalence in *Toxocara spp.* in carnivores and *Capillaria spp*. in birds. Trematodes, notably *Paramphistomum spp.*, were commonly observed in herbivores like wild beasts, sambar deer, and barking deer, which are known to be more susceptible to these parasites (table 4). The complete absence of cestode infections across all species was an interesting finding, suggesting either a lack of exposure or environmental factors that prevent the establishment of these parasites in the zoo population. Mixed parasitic infections were found in several species, notably in wild beasts, barking deer, and some carnivores, underscoring the complexity of parasitic dynamics in zoo animals. These findings were in line with Hossain et al. (2022).

Llamas (*Lama glama*) exhibited the most significant reduction in GI parasite load, with a p-value of 0.014, indicating a statistically significant reduction in parasitic prevalence after the administration of triclabendazole (table 7). This aligns with findings from similar studies on camelids, where triclabendazole is known for its effectiveness in treating trematode infections, particularly *Paramphistomum spp.* The remarkable reduction in parasite load in llamas supports the targeted use of this anthelmintic for camelid species (Hossain et al. 2022; Kouadio et al. 2021). However, for other species such as wild beasts, sambar deer, and barking deer, although a reduction in parasite load was observed, the infections were not eliminated. Table 6 showed that wild beasts had a 50% prevalence, sambar deer showed 25%, and barking deer had 33.33% prevalence post-treatment, suggesting possible resistance, reinfection, or suboptimal dosing. This highlights the need for tailored treatment plans based on specific species' susceptibilities and potential variations in drug efficacy (Hasan et al., 2021).

The primary aim of the study was to assess the efficacy of anthelmintic treatments, which included triclabendazole, levamisole, and fenbendazole. These drugs were chosen based on their effectiveness against the common GI parasites found in the zoo animals. The most commonly used anthelmintics were levamisole and fenbendazole, both administered to 37.5% of the species and triclabendazole, which was used for 25% of species, particularly herbivores (table 8). The treatment frequency was biannual, with notable improvements observed in most of the treated animals. Ganager et al. (2019) also observed such pattern of anthelmintic usage. The use of levamisole for carnivores proved effective in reducing nematode infections, with *Toxocara spp.* being the predominant parasite in this group. Fenbendazole, meanwhile, was successful in reducing infections in herbivores and carnivores, particularly against nodular worms like *Strongylus spp.* and *Ostertagia spp.* (Ganager et al. 2019*;* Panayotova et al.2023*;* Yuskiv et al. 2024). Fenbendazole has a broad spectrum of activity against GI helminths, making it suitable for diverse species, but the persistence of infections in certain species, as seen with the barking deer and wild beasts, suggests potential drug resistance or the need for more frequent dosing in these animals.

The efficacy of anthelmintics observed in this study is consistent with other studies globally, which report levamisole and fenbendazole as effective treatments against common GI parasites in zoo animals. However, some studies have noted the emergence of anthelmintic resistance (Von-Samson et al.2021) particularly in nematodes, when drugs are overused or misused in captive populations. This highlights the need for rotational use of anthelmintic drugs and regular monitoring to avoid the development of resistance, which can reduce the effectiveness of treatments over time. Additionally, environmental factors, such as the zoo's sanitation practices, the level of overcrowding, and the feeding habits of the animals, play a critical role in parasite transmission (Morgan et al., 2007). Further studies on these factors could help refine the zoo's parasitic control programs.

The study's findings also highlight the importance of species-specific anthelmintic protocols, which account for the unique parasitic profiles and treatment responses of different animal groups (Hansen et al., 2016). While anthelmintic treatments reduced parasite loads in most species, it is clear that certain animals may require more tailored management, including different dosages or drug combinations, and periodic reevaluation to ensure sustained efficacy. Additionally, mixed infections and the persistence of parasites in certain species underscore the complexity of parasite management in a zoo setting and the need for continued research and adaptive management strategies.

Conclusion

This study reinforces the importance of regular and targeted anthelmintic treatment in reducing GI parasitic infections in captive zoo animals. While the treatments were largely effective, certain species, such as wild beasts, barking deer and sambar deer, demonstrated persistent infections post-treatment, highlighting the need for more specialized approaches. Triclabendazole proved particularly effective in llamas, while levamisole and fenbendazole showed significant promise in carnivores and herbivores. Future studies should explore rotational drug protocols**,** the impact of environmental management, and potential drug resistance to optimize parasitic control strategies in zoos.

Limitations

This study focuses on coproscopy to identify specific parasitic eggs without integrating molecular or genetic analysis. The sample size was small it was quite difficult to interpret the exact parameters related to drug efficacy. Due to time limitations, all diagnostic tools could not be used and could not count parasitic eggs per gram. For better evaluation, the quantitative test is recommended.

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Biography

I am, Ummey Sahibunnesa, daughter of Md Abu Talab and Taskin Ara Begum. I have passed my Secondary School Certificate (SSC) examination in 2016 followed by Higher Secondary Certificate (HSC) examination in 2018 under the Chattogram education board. Now, I am an intern student of Chattogram Veterinary and Animal Sciences University (CVASU) under the faculty of Veterinary Medicine (FVM). I have major interest on animal medicine, zoonotic diseases, anti-microbial resistant, public health significance, vaccine and its potentiality. After completing my study, I would like to work in the development of livestock sectors, zoonotic diseases and antimicrobial resistance. Moreover, I want to play a role in the conservation of livestock resources, public health promotion and advancement of medical knowledge in keeping with the principles of veterinary medical ethics.