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SEASONAL VARIATION AND PREVALENCE OF GASTROINTESTINAL PARASITES IN CAPTIVE MAMMALS AT LAHORE ZOO

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ABSTRACT

This study was conducted at Lahore Zoo, Punjab, Pakistan from January to December 2023 to assess the prevalence of gastrointestinal parasites in 10 different captive wild mammalian species. The mammals including girrafe (*Giraffa*), vervet monkey (*Chlorocebus pygerythrus*), zebra (*Equus quagga*), lion (*Panthera leo*), leopard (*Panthera pardus*), Rhinoceros (*Rhinocerotidae*), wild boar (*Sus scrofa*), Chinkara deer (*Gazella bennettii*), urial (*Ovis vignei*) and nilgai (*Boselaphus tragocamelus*) were selected. A total of 100 fresh fecal samples were collected from the animal enclosures in polythene bags (6×4 inches) 2 times during study period. Coprological examination was done using qualitative analysis such as direct smear method, sedimentation method and flotation methods. The parasitic prevalence was as follows zebra 44.5%, followed by giraffes, rhinoceroses, and wild boars at 27.8%, nilgai, urial, lion and vervet monkey at 22.3%, chinkara 16.7% and leopard 11.2%. The prevalence of gastrointestinal parasites was as *Eimeria spp*. (3.9%), *Strongyloides spp*. (3.4%), *Trichuris spp*. (2.8%), *Giardia spp*., *Ascaris spp*., and *Strongyle spp*. (2.3%), *Entamoeba histolytica* (1.12%), *Trematode* eggs (1.12%), *Nematodirus* (1.12%), *Balantidium coli* (0.6%), *Haemonchus contortus* (0.6%), *Paramphistomum cervi* (0.6%), *Parascaris equorum* (1.12%) and *Cryptosporidium* (1.12%). The overall parasitic prevalence was 23.9%, with a noticeable seasonal variation, being higher in summer (31.2%) compared to winter (17.79%). These findings highlight the significant parasitic burden in captive wild mammals. It can be concluded that regular de-worming sessions, cleaning, disinfection of enclosures and proper disposal of fecal matter should be done to reduce the overall parasitic burden.

Keywords: Captivity, Zoo, Fecal examination, Parasites, Risk factor, zoonosis

INTRODUCTION

The wildlife is important for ecological balance, environmental control and have scientific values. The primary goal of zoological gardens are to increase the aesthetic, educational programs and wild animal conservation. In nature, there is a balance between the host and the parasite and animals have certain built-in defense against parasites. So, animals rarely experience dangerous conditions in case of parasitic infections (Niranjan et al. 2020). The issue of parasitic diseases can worsen and constitute a major threat to the animals when these wild species are housed in cramped quarters in wildlife parks (Hossain *et al.* 2021).

In their natural habitats, wild animals are typically exposed to a wide range of environmental conditions and interact with diverse ecosystems. This exposure helps them develop certain

adaptations and genetic resistance to the parasites present in their specific ecosystems. However, when these animals are confined to smaller, artificial environments such as zoos or wild parks, their exposure to parasites becomes more limited and potentially concentrated (Price & Stoinski 2007). Close proximity between individuals allows for easier transfer of parasites through direct contact or contaminated environments. Moreover, the stress of captivity can weaken animals' immune systems, making them more susceptible to infections. In zoo, high number of individuals in cage and stressful environment can lead to an increase in the prevalence and severity of parasitic infections. Occasionally, these infections can result in unexpected local fatalities, especially if the parasites are particularly virulent or if the animals' immune systems are compromised (Kleiman *et al.* 1996).

It's worth noting that modern zoos and wild parks strive to provide appropriate veterinary care and disease management protocols to mitigate these risks. The challenge of the interaction between wildlife and parasites remains a significant concern in captive settings. This is particularly concerning for endangered species that are already at risk (Wielebnowski 2003). Parasites can cause a range of problems for their hosts. They can directly impair the host's well-being and reproductive capacity through various pathological consequences. These consequences may include tissue destruction, loss of blood, congenital abnormalities, abortion, and in rare cases the death (Thawait *et al*. 2014).

Moreover, parasites can also indirectly affect the physical condition of the host by weakening their overall resistance (Niranjan et al. 2020). The major causes of gastrointestinal parasitism in captive animals is mostly due to contaminated food or water provided to them. It is important to note that different food sources can harbor various parasites or pathogens. Contaminated or improperly handled food can introduce these parasites into the animals' digestive systems, leading to the growth and establishment of parasites in their gastrointestinal tracts (Edwards, 2003). Parasites commonly found in food sources can include protozoans, nematodes (roundworms), cestodes (tapeworms), trematodes (flukes) and other microorganisms. These parasites can infect the gastrointestinal tract of animals and cause gastrointestinal parasitism (Barbosa *et al.* 2020; Conde *et al.* 2011;Escobar-Ibarra *et al.* 2020).

A recent research has shown that the gastrointestinal parasites in wild animals kept in zoos resemble those to people. It has also been stated that the prevalence parasite illnesses among wild species and confined wildlife is comparable to that of human. While there is a potential risk associated with zoonotic transmission of nematodes, cestodes, and trematodes (Adegbulu et al. 2015).

Deworming includes providing animals with different anthelmintic drugs like de-wormers to protect them from helminthes parasites. Different animals are given with different types and quantities of de-wormers after an interval of 3-6 months regularly (Akinboye *et al*. 2010). Wild mammals have a variety of de-wormers e.g. lion and leopard (de-worming powder or canivam, 5g/20 kg), deer family (albendazole or fenbendazole, 25ml/100 pounds), zebras (flubendazole or oxafex+flubendazole, 30-60 mg/kg of diet), giraffe (moxidectin or albendazole 1mg/kg), monkeys (ivermectin and albendazole combined, 7.5 mg/kg albendazole and 300 mg/kg ivermectin), rhinos (oxafex or albendazole 0.2 mg/kg) and wild boar (piperazine or fenbendazole, 4ml/5kg) (Opeyemi *et al.* 2022). Pariyar et al. (2021) reported that regular gastrointestinal parasite monitoring and control programs will help zoo animals stay healthy and grow while it will also be indirectly protecting the public's health. (Kolapo and Jegede 2017).

In order to improve the health of the animals, hygienic techniques should be used in zoological parks along with continuous periodic routine screening of fecal samples, regular deworming, reduction of intermediate host and quarantine for newly acquired animals. Additionally, the visitors should be prohibited from feeding animals to protect the zoo animals from parasites (Williams *et al.* 2021). The change in habitat from natural to captive place effects the animal's ecology. This change increases the susceptibility to diseases especially infections by parasites. Therefore, present study was planned to analyze the prevalence as well as the risk factors associated with gastrointestinal parasitism in selected

captive wild mammals. The change in habitat from natural to captive place effects the animal's ecology. This change increases the susceptibility to diseases especially infections by parasites. Therefore, present study was planned to analyze the prevalence as well as the risk factors associated with gastrointestinal parasitism in captive wild mammals

MATERIALS AND METHODS

Study area

The present one year study was conducted at Lahore Zoo, Punjab, Pakistan. Zoo started in 1860s as a menagerie but properly became a zoo in 1872. Presently, zoo houses around 1200 animals of 120 species including primates, big cats, deers, antelopes, reptiles and birds.

Samples collection

Fecal samples were collected in winter (February) and summer (June) before the de-worming session of animals. The deworming protocol includes the fecal screening and deworming of animals after almost every 3 months. Most commonly used dewormers include ivermectin, fenbendazole, albendazole, oxybendazole. The dose of dewormer varies for each animal. First sampling was done in February, 2023 while second sampling was done during June 2023. Overall, 10 mammals species were selected randomly to collect their fecal samples. The fecal samples of animals including Girrafe (*Giraffa*), vervet monkey (*Chlorocebus pygerythrus*), zebra (*Equus quagga*), lion (*Panthera leo*), leopard (*Panthera pardus*), Rhinoceros (*Rhinocerotidae*), wild boar (*Sus scrofa*), Chinkara deer (*Gazella bennettii*), urial (*Ovis vignei*) and nilgai (*Boselaphus tragocamelus*) were collected.

The fresh fecal samples were collected from the animal enclosures in which the animals were kept. A total of 10 fecal samples were collected in polythene bags (6"× 4") from the animals that are placed in individual (giraffe, rhino, and leopard) or in groups (lion, urial, zebra, chinkara, nilgai, boar & monkey). After proper labeling, samples were brought to the Postgraduate Lab, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Ravi Campus for further processing and analysis.

Coprological examination

Coprological examination includes qualitative analysis. Qualitative analysis includes direct smear method, sedimentation method and flotation method. 10 samples from each animal were examined through sedimentation method, flotation method and through direct smear method (Kolapo and Jegede 2017).

Qualitative examination

Direct smear method

With the use of a spatula, a small amount of feces was placed onto a slide. Then a few drops of distilled were added to create a transparent thin film. Then covered it with a coverslip to ensure that the smear remains uniform. The smear dyed using Lugol's iodine solution (10g potassium iodide and 5g iodine in 100 ml distilled water) and then examined under the microscope. At least 3 slides were examined for each sample (Niranjan et al. 2020).

Sedimentation method

To eliminate coarse fecal particles, a little amount of feces was placed in a mortar with some distilled water, correctly triturated with a pestle, and then filtered into a beaker using a tea strainer. The filtrate was poured into a centrifuge tube up to two-third full and centrifuged for 5 to 10 minutes at 2000 to 3000 rpm. After discarding the supernatant, distilled water was added to the tube once again, and it was centrifuged two to three times until the supernatant became clear. A drop from the sediment was then taken on a glass slide and viewed under a compound microscope. In this way 30 slides were prepared by winter samples and 30 from summer samples (Dibakou et al. 2021).

Flotation method

To eliminate coarse fecal particles, a little amount of feces was placed in a mortar with some distilled water, correctly triturated with a pestle, and then filtered into a beaker using a tea strainer. The filtrate was poured into a centrifuge tube up to two-third full and centrifuged for 5 to 10 minutes at 2000 to 3000 rpm. After discarding the supernatant, distilled water was added to the tube once again, and it was centrifuged two to three times until the supernatant became clear. Then the flotation and the sedimentation fluids were mixed and again centrifuged them at 1500 rpm for 1-2 minutes. Then tube was filled with flotation fluid up to the brim and was placed for 5-10 minutes after covering with glass slip. In the last, placed some sample on glass slide and observed under microscope. Another technique was applied for sedimentation which included the use of Eppendorf tubes. In this method, after triturating and filtering the sample, the liquid filtrate was added to Eppendorf tubes. Then cover slips were placed onto the mouth of the tubes for 15-20 minutes. Then these cover slips were placed onto slides and observed under microscopes (Ramdevi and Venu 2020).

Prevalence of parasites

The prevalence of parasites was calculated according to (Thrusfield and Metcalfe 2020);

$$\text{Prevalence P} = \frac{\text{Number of infected animals during the study period}}{\text{Total number of animals screened}} \times 100$$

Statistical analysis

The data were collected and structured for logical interpretation. Then data were compiled, tabulated and subjected to appropriate statistical analysis like frequency distribution and prevalence (Choi, 2017). For the association better qualitative variables chi square test was applied considering $p \leq 0.05$. All the statistical analysis was performed by using SAS software (version 9.1).

RESULTS AND DISCUSSION

Total 100 samples of 10 different captive wild mammalian species were collected in winter as well as in summer from Lahore zoo. Table 1 showing the details of parasites identified during study period. The seasonal variation was almost doubled in summer as compared to winter. Table 2 showing the seasonal prevalence of gastrointestinal parasites in captive mammals at Lahore Zoo during present study. The overall parasitic prevalence was 23.9%, with a noticeable seasonal variation, being higher in summer (31.2%) compared to winter (17.79%).

Table 1. Details of parasites identified during study period in captive mammals at Lahore zoo.

Parasite	Season	Method
Giardia	Winter + early summer	Direct smear method
Ascaris	Winter + summer	Sedimentation
Trichuris	Winter + summer	Sedimentation
Eimeria	Winter + summer	Flotation + direct smear
E. histolytica	Summer	Direct smear
Strongyloides	Winter + summer	Sedimentation + direct smear
Strongyle	Winter + summer	Direct smear + flotation
Trematode	Winter	Direct smear + flotation
Ostertagia	Summer dominant	Direct smear method
Balantidium coli	Summer	Flotation
Haemonchus spp.	Summer	Direct smear method
Paramphistomum cervi	Throughout year but cause infection in summer	Flotation
Nematodirus	Late spring and summer	Direct smear method
Parascaris equorum	Summer	Sedimentation
Cryptosporidium	Summer	Sedimentation

Table 2: Seasonal prevalence of gastrointestinal parasites in captive mammals at Lahore Zoo during present study.

		Winter								
Sr. No.	**Parasites**	**Nilgai**	**Lion**	**Leopard**	**Monkey**	**Rhino**	**Boar**	**Zebra**	**Urial**	**Giraffe**	**Chinkara**
	Giardia spp.	0	1	0	0	0	0	0	0	0	0
	Ascaris spp.	0	0	0	0	0	0	2	1	0	0
	Trichuris spp.	0	0	0	0	1	1	0	0	0	0
	Eimeria spp	1	0	0	0	0	0	0	0	2	0
	Entamoeba histolytica	0	0	0	1	0	0	0	0	0	0
	Strongyloides spp	1		0	1	1	0	0	0	0	0
	Strongyle type egg	0	0	0	0	0	0	1	0	0	0
	Trematode eggs	0	0	0	0	1	0	0	0	0	1
		Summer									
	Giardia spp.	2	1	0	0	0	0	0	0	0	0
	Ostertagia spp.	0	0	0	0	0	0	0	1	0	0
	Eimeria spp.	0	0	0	0	1	1	0	2	0	0
	Ascaris suum	0	0	0	0	0	0	0	0	1	0
	Trichuris spp.	0	0	1	0	0	0	0	0	2	0
	Balantidium coli	0	0	0	0	0	0	0	0	1	0
	Haemonchus spp.	0	0	0	0	1	0	0	0	0	0

	Strongyloides spp.	0	0	1	1	0	1	0	0	0	0
	Paramphistomum cervi	0	0	0	0	0	0	0	0	0	1
	Nematodirus spp.	0	0	0	0	0	0	0	0	0	2
	Strongylus spp	0	0	0	0	0	0	3	0	0	0
	Parascaris equorum	0	0	0	0	0	0	2	0	0	0
	Entamoeba histolytica	0	0	0	1	0	0	0	0	0	0
	Cryptosporidium spp.	1	1	0	0	0	0	0	0	0	0

Parasitic prevalence in animals were recorded as Lion (22.3%), Leopard (11.2%), Rhinoceros (27.8%), Vervet monkey (22.3%), wild boar (27.8%), Urial (22.3%), Chinkara (16.7%), Zebra (44.5%), Giraffe (27.8%) and Nilgai (22.3%) (Table 3 and Figure 1).

Tale 3: Parasitic prevalence (%) in selected mammals at Lahore zoo.

Sr. No.	Animals		No. of Parasites	Percentage Prevalence
	Common Name	Scientific Name		
1.	**Nilgai**	*B. tragocamelus*	4	22.3%
2.	**Lion**	*Panthera leo*	4	22.3%
3.	**Leopard**	*Panthera pardus*	2	11.2%
4.	**Vervet Monkey**	*C. pygerythrus*	4	22.3%
5.	**Rhinoceros**	*Rhinocerotidae*	5	27.8%
6.	**Wild Boar**	*Sus scrofa*	5	27.8%
7.	**Zebra**	*Equus quagga*	8	44.5%
8.	**Urial**	*Ovis vignei*	4	22.3%
9.	**Giraffe**	*Giraffa*	5	27.8%
10.	**Chinkara**	*Gazella benne*	3	16.7%

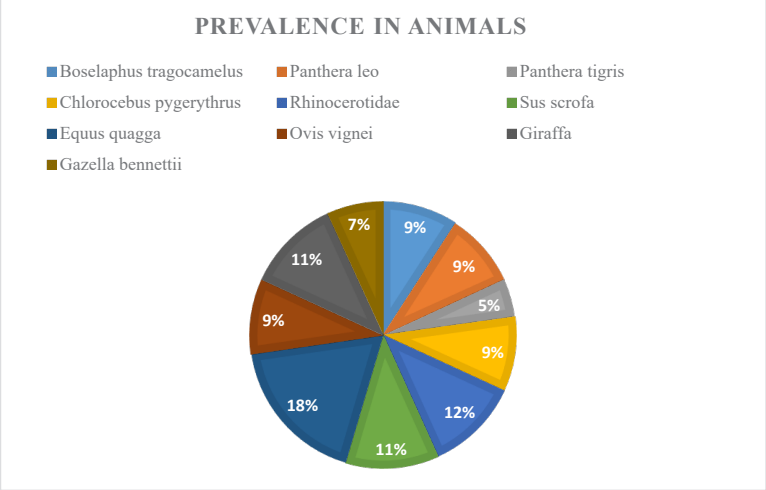

Figure 1: Parasitic prevalence in selected mammals at Lahore zoo.

Table 4 showing the parasitic prevalence at Lahore zoo during study period. Gastrointestinal parasites were observed along with their prevalences as *Giardia spp*. (2.3%), *Ascaris spp*. (2.3%), *Trichuris spp*. (2.8%), *Eimeria spp*. (3.9%), *Entamoeba histolytica* (1.12%), *Strongyloides spp*. (3.4%), *Strongyle spp*. (2.3%), *Trematode* eggs (1.12%), *Balantidium coli* (0.6%), *Haemonchus contortus* (0.6%), *Paramphistomum cervi* (0.6%), *Nematodirus* (1.12%), *Parascaris equorum* (1.12%) and *Cryptosporidium* (1.12%) (Figure 2 and 3). Results were statistically analyzed using chi square test. Table 5 representing the result of Chi Square test.

Table 4: Parasitic prevalence on the basis of parasites at Lahore zoo during study period.

Sr. No.	Parasite	No. of times identified	Prevalence (%)
1	*Giardia*	4	2.3%
2	*Ascaris*	4	2.3%
3	*Trichuris*	5	2.8%
4	*Eimeria*	7	3.9%
5	*E. hustolytica*	2	1.12%
6	*Strongyloides*	6	3.4%
7	*Strongyle*	4	2.3%
8	*Trematode*	2	1.12%
9	*Balantidium coli*	1	0.6%
10	*Haemonchus*	1	0.6%
11	*Paramphistomum*	1	0.6%
12	*Nematodirus*	2	1.12%
13	*Parascaris equorum*	2	1.12%
14	*Cryptosporidium*	2	1.12%

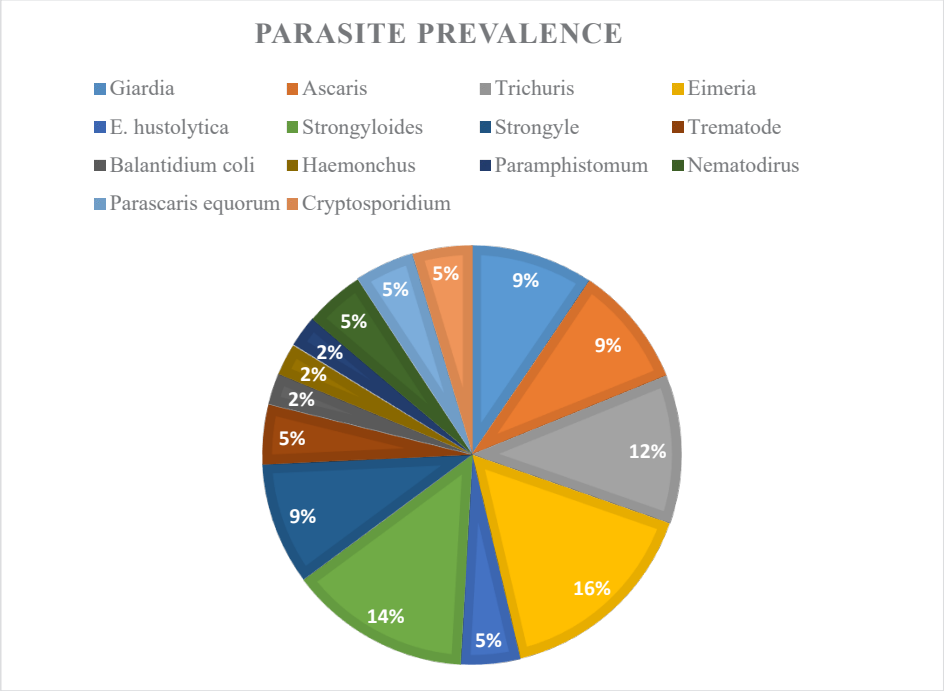

Figure 2: Parasite prevalence during present study at Lahore zoo

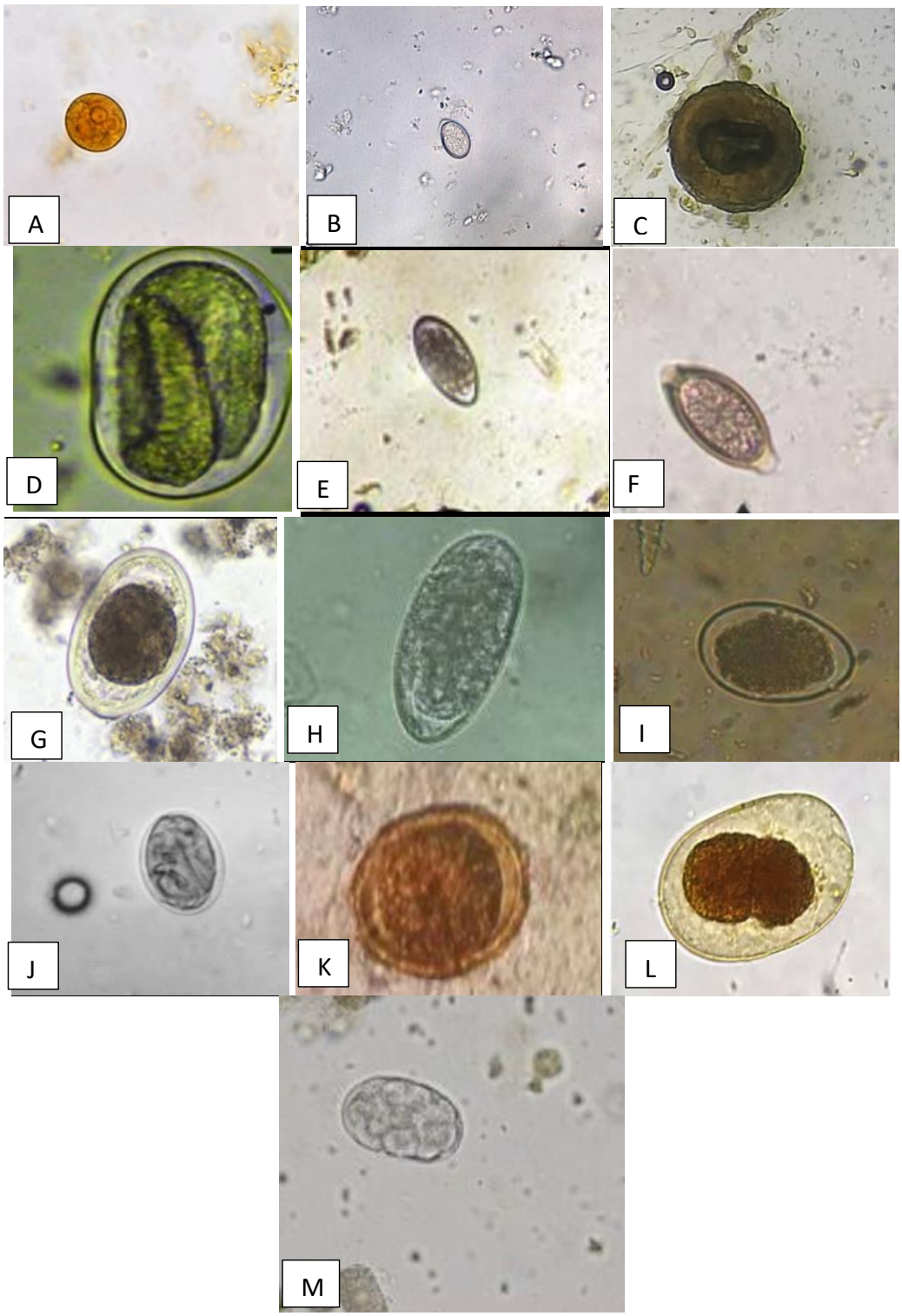

Figure 3: Gastrointestinal parasites identified during present study (A) *Entamoeba histolytica* (B) *Eimeria spp.* (C) *Cryptosporidium spp*. (D) *Nematode* (E) *Haemonchus contortus* (F) *Trichuris spp.* (G) *Ascaris spp*. (H) *Ostertagia spp*. (I) *Trematode eggs* (J) *Giardia spp*. (K) *Cryptosporidium* spp. (L) *Strongyloides spp.* (M) *Balantidium coli.*

Table 5: Chi-Square test.

Test	Value	df	Asymp. Sig. (20 sided)
Chi Square	210.386[a]	126	.000
a. 150 cells (100.0%) have expected count less than 5. The minimum expected count is .05.			

Zoos house wild animals primarily for entertainment and the protection of endangered species. When wild animals are taken into captivity (such as zoos and safari parks), their biology is affected by the abrupt exposure to an undesirable environment. This type of modification makes the body more susceptible to a variety of infectious diseases (such as viral, bacterial, fungal, and parasitic). The most prevalent of which are gastrointestinal (GI) parasite infections (helminthes and protozoa) in confined wild animals (Adeniyi *et al.,* 2015; Moudgil *et al.* 2015; Kolapo & Jegede, 2017; Carrera-Játiva *et al*., 2018). These infections pose serious impacts on the status, behavior, reproduction, and survival of wild animals (Thawait *et al*., 2014; Kvapil *et al*., 2017). The most important thing in protecting animals from worms and parasites includes deworming.

The findings of current revealed that parasites may have an impact on the health of captive animals. Few parasites such as *Trichuris spp., Ascaris* is also significant since in the absence of effective control methods they can harm the health of zoo staff members and guests. These species of gastrointestinal parasites were observed namely Giardia (2.3%), Ascaris (2.3%), Trichuris (2.8%), Eimeria (3.9%), *Entamoeba histolytica* (1.12%), Strongyloides (3.4%), Strongyle (2.3%), Trematode (1.12%), *Balantidium coli* (0.6%), Haemonchus sp. (0.6%), Paramphistomum (0.6%), Nematodirus (1.12%), *Parascaris equorum* (1.12%) and Cryptosporidium (1.12%) the results of current study are align with Niranjan *et al.* (2020).

A total of two species of gastrointestinal parasites in non-human primates (eggs/cysts/oocysts) were recovered namely Strongyloides sp. (13.33%) and Eimeria sp. (20%). Varadharajan and Kandasamy (2000) also observed the Strongyloides sp. in non-human primates at V.O.C. Park and Mini Zoo Coimbatore. Singh *et al*. (2009) also reported the Strongyloides sp. in langur at Mechendra Choudhury Zoological Park Chat Bir and Punjab. Similarly, Hossain *et al*. (2021) indicated in his investigations that helminthes and protozoan infections were 51.8% (29) and 35.7% (20) respectively with the overall rate of parasitic infection being 78.6% (44). *B. coli* protozoa made up 35.7% of the identified parasites, along with nematodes strongyles (26.8%), Ascaris species (3.6%), Dictyocaulus species (5.4%), Strongyloides species (7.1%), Trichuris species (3.6%), Capillaria species (5.4%), and trematode Fasciola species (5.4%). The results vary because they use both birds and animals instead of different species of mammals. 11 different types of gastrointestinal parasites were found, including one protozoan, Coccidia (54.76%), four cestodes, Hymenolepis spp. (55.36%), Spirometra spp. (24.40%), Diphyllobothrium spp. (32.14%), and Taenia spp. (12.5%), as well as six nematodes, Ascaris spp. (58.93%), Toxocara spp. into the findings of Liza *et al*. (2019). The difference in prevalence was very high because the major concern of author's study were carnivores only and there was change of temperature. In this investigation, the overall prevalence of intestinal parasitism was 63.9%, with helminth positivity at 27.8% and protozoa positivity at 36.1%. The most common species was Heterakis spp. (8.3%), followed by Ascaridia sp. (4.1%), and Capillaria spp. (5.6%). *Strongyloides avium* and *Strongyloides pavonis* larvae on the other hand were present in 2.8% and 4.1%, respectively of the samples under study (El-Shahawy and Abou 2015). The major difference among both studies was the investigation on birds only by other author and difference of temperature.

Conclusion and recommendations

The findings of present study highlight the significant parasitic burden in captive wild mammals. It can be concluded and recommended that regular de-worming sessions, cleaning, disinfection of enclosures and proper disposal of fecal matter should be done to reduce the overall parasitic burden.

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