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RESEARCH ARTICLE

Coprological survey of gastrointestinal parasites of mammals in Dehiwala National Zoological Gardens, Sri Lanka

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Abstract: A cross sectional, coprological survey on gastrointestinal (GI) parasites of captive mammals in the Dehiwala National Zoological Gardens was carried out in 2014. Fresh faecal samples from all the mammal species belonging to nine orders: Primates, Carnivora, Perissodactyla, Artiodactyla, Proboscidea, Erinaceomorpha, Lagomorpha, Rodentia and Diprotodontia were analyzed. Intensity of parasite infections was determined using the McMaster technique. Of the 70 samples, 44 (62.9%) were positive for one or more GI parasites. A total of 13 types of GI parasite eggs, cysts and/or oocysts of *Trichuris* sp., *Strongyloides* sp., *Toxocara* sp., *Spirometra* sp., *Moniezia* sp., *Nematodirus* sp., *Giardia* sp., *Blastocystis* sp., *Balantidium* sp., *Entamoeba* spp., strongyle type eggs, hookworm, and coccidian oocyts were observed. The most common stage was strongyle type egg (17.1%) followed by cysts of *Entamoeba* spp. (14.3%). Of the infected individuals, 25% had mixed infections. A higher prevalence of helminths (81.8%) compared to protozoans (47.7%) was observed but this difference was not statistically significant (Chi square test; $p>0.05$). There was no significant difference in the prevalence of infection among the captive bred, imported or wild caught individuals (Chi square test; $p>0.05$). Mammals of seven orders were infected with GI parasites but lagomorphs and diprotodonts did not have any parasites. Among the herbivores, strongyle type, *Moneizia, Entamoeba* and coccidian infections were common while *Nematodirus* sp. in a porcupine and *Spirometra* sp. in a flying squirrel were rare. Common parasites of carnivores were, *Toxocara* and *Entamoeba* but *Blastocystis* sp. in coati was a rare infection. *Trichuris* and *Giardia* infections were common in Primates. High worm burden was evidenced in silver leaf monkey, Hamadryas baboon, African lion, black rhino, pony, porcupine and flying squirrel. Although regular deworming is carried out, results of this survey highlight the importance of faecal analysis before administering deworming and applying a more targeted approach to manage the pathogenic species. This study provides baseline data on the GI parasites of all the mammal orders at Dehiwala Zoological Gardens.

Keywords: Captive mammals, GI parasites, Helminths, Protozoans, Sri Lanka, Zoological Gardens.

INTRODUCTION

Zoological gardens play an important role in the promotion of animal biodiversity by protecting endangered species (Kelly and English, 1997). Since animals are kept in confined areas, parasitic diseases constitute one of the major problems in zoological gardens around the world due to high environmental contamination (Rao and Acharjyo, 1984). Unlike in the wild, stress conditions caused by captivity can diminish the resistance to parasite diseases (Geraghty *et al*., 1982; Gracenea *et al*., 2002; Cordon *et al*., 2008). Occurrence of parasites in captive animals in zoological gardens might vary according to husbandry practices, disease prophylactic measures, parasite-host interactions and treatment administrated (Lim *et al*., 2008). Captive animals do not show alarming signs of parasitism, if regular de-worming practices are carried out in zoological gardens (Parsani *et al.*, 2001). However, Parsani *et al.*, 2001 further argue that some captive animals do show clinical signs due to parasites even if they are regularly dewormed and some will have no clinical signs even if they are never dewormed and this depends more on the parasite host interactions than deworming practices. Parasites can be brought into a zoological garden by many ways:

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through animal food, (contaminated fruits and vegetables, infected meat or fish, etc), intermediate and paratenic hosts (snails, ants, cockroaches and other insects, rodents, etc.), newly acquired parasitized animals and through infected zoo staff and visitors (Pencheva, 2013).

Many species of helminths and protozoans are known to infect mammals. Helminths such as *Strongyloides*, strongyles, *Trichuris*, *Nematodirus*, *Toxocara*, *Moniezia* and protozoan parasites such as *Giardia*, *Balantidium*, *Entamoeba* and coccidians are GI parasites commonly found in captive mammals worldwide. The presence of these parasites in the host may induce morbidity and even mortality (Nath *et al*., 2012). They can also act as the reservoir of parasites for the domestic mammals and some of these infections can be zoonoses which can spread to the humans (Bogale *et al*., 2014).

The Dehiwala National Zoological Gardens (referred to as Dehiwala Zoo here onwards) was established during the early years of the 20th century and is one of the oldest zoos in Asia. It is a pioneer institute that possesses, manages and conserves wild animals and displays the animal collections to the public. The Zoo is located in the heart of Colombo city, the largest city and the commercial capital of Sri Lanka with a population of 4.6 million in the metropolitan area. Though the apparent objective of setting up this Zoo was to exhibit animals, it is now treated as an animal welfare facility, involved in educating the community and an *ex situ* conservation center for endangered species (Dehiwala National Zoological Garden website). Because of the space limitation, many captive animals are caged in close proximity to one another and therefore they may succumb to parasitic infections. In a previous study, faecal samples of 13 species of captive primates at Dehiwala Zoo had been examined and reported many GI parasites including many protozoans: *Cryptosporidum* sp., *Balantidium* sp., *Blastocyst* sp., *Entamoeba* sp., *Giardia* sp., and coccidian and nematodes: the larvae of hook worm and the eggs of *Ascaris*, strongyle and *Trichuris* (Gunasekara *et al*., 2012). A few years earlier in 2009, Fernando and Udagama-Randeniya studied the GI parasites and ectoparasites of captive reptiles at Dehiwala zoo. The present coprological survey was carried out to determine the GI parasites of all the mammal orders in Dehiwala Zoo.

MATERIALS AND METHODS

Study site and study animals

At the time of survey, Dehiwala National Zoological Gardens comprised 70 species of mammals belonging to nine orders: 18 primates, 19 carnivores, six perissodactyles, 18 artiodactyles, two proboscideans, one lagamorph and one diprodont, one erinaceomorph and four rodent species. These mammals were distributed throughout the zoo enclosure providing maximum possible space per individual. Climatically, Dehiwala comes under the wet zone of Sri Lanka (6°51'-24°5″N and 79°52'-22°4″E) with a mean annual temperature of 27°C.

De-worming is carried out once in every three months and the food given to each animal is subjected to regular inspections by veterinary surgeons (personal communication with the Chief Veterinarian at Dehiwala Zoo). Background information about each mammal was collected using a questionnaire, which gathered information on the age, sex, physical location of the animal in the Zoo, details on de-worming (last date of deworming and type of drugs given), and a brief history of the animals' origin (whether the animal was brought from another zoo/country or born at the Zoo). The physical condition (fur coat, lethargy, appetite of the animals) at the time of sampling was also noted. Samples of the mammals living in groups (eg. monkeys) were taken randomly without considering a particular individual, assuming that if there is a single infected individual in that group the others were infected as well.

Collection of samples

Fresh faecal samples from all the captive mammal species at the Dehiwala National Zoological Gardens were collected from March to October 2014. Approximately 10 g of faeces was collected in the morning about 8.00 am before the cages were cleaned by the keepers. Each animal was sampled once during the study and if they lived in groups (eg. monkeys, deer) one sample from the group was taken. For herbivores and other less aggressive animals, the faecal sample was collected directly from the rectum. Samples from carnivores and other

aggressive mammals were directly picked up off the ground with the help of the caretaker. The samples were collected into small plastic seal bags. During a visit about 20 samples were collected at random whenever there was a fresh sample of a particular mammal was available and the sampling was done in every month until a final target of all the mammal species in Dehiwala Zoo was reached. Samples were brought to the laboratory in a cooler and were stored at 4°C until analysis. Processing of the samples was completed within a week in the parasitology laboratory, in the Department of Veterinary Pathobiology at the Faculty of Veterinary Medicine at University of Peradeniya using a modified salt floatation method, Sheather's sucrose floatation method, direct saline and iodine mounts. Nematode cultures were set up for some species to obtain DNA for molecular analysis to identify up to species level.

Modified salt floatation method

Three grams of faeces was measured and was taken into a 50 ml capped centrifuge tube. Then the volume was made up to 50 ml by adding 47 ml of distilled water, and mixed thoroughly using a wooden applicator. The suspension was centrifuged at 3000 g for 20 min and the supernatant was discarded. The pellet was washed twice by re-suspending in distilled water, followed by two centrifugations at 3000 g for 20 min until a clear supernatant was obtained. The pellet was emulsified with saturated salt, mixed thoroughly and was centrifuged again for another 20 min at 3000 g at room temperature. Approximately 5 ml of the top meniscus was aspirated and added to 15 ml centrifuge tube. The total volume was made up to 15 ml by adding distilled water and centrifuging for 10 min at 1370 g at room temperature. This was repeated and finally 1 ml of the suspension with the pellet at the bottom of the tube was mixed with distilled water and transferred to a 1.5 ml Eppendorf® tube using a Pasteur pipette. Distilled water was added to make it up to 1.5 ml and the tubes were centrifuged for 10 min at 1150 g in the microcentrifuge. The supernatant was discarded and the pellet was thoroughly mixed with 0.5 ml of distilled water. Using about 0.1 ml of the suspension, each microscopic slide was prepared and covered with a cover slip without staining. Five smears were observed from each sample under the light microscope (Olympus CH 31, Phillipines). Eggs of different parasite species were identified and number of eggs in 0.5 ml was estimated and the number of eggs per gram of faeces (EPG) was calculated assuming the method had concentrated all the eggs in the 3 g of faeces into 0.5 ml.

Sheather's sucrose floatation method

Protozoan cyst and oocysts isolation was done by Sheather's sucrose floatation method. Saturated sucrose was prepared and same steps of the modified salt floatation method mentioned above were followed replacing the floatation fluid by saturated Sheather's sucrose solution. Cysts and oocysts of particular protozoan species in each sample were calculated as cysts per gram (CPG) or oocysts per gram (OPG) of faeces.

Quantitative analysis

Initially, relative estimation EPG, CPG and OPG of faeces was carried out using iodine smears and observing under the light microscope. Later, more accurate counts were taken using the McMaster technique (Wood, 1995) and results were compared with that of the modified salt floatation and Sheather's sucrose floatation methods.

Nematode cultures

Freshly harvested eggs of single infections of *Toxocara* sp. and *Trichuris* obtained from the faecal samples of the African lion (*Panthera leo*) and Hamadryas baboon (*Papio hamadryas*), respectively were cultures according to Rajapakse *et al*., (1992) to identify the species using DNA analysis. Eggs were stored in petri dishes in 0.1 N sulphuric acid at a depth of 0.5 cm in room temperature (25°C) to incubate. In the course of this incubation, the culture was rocked gently once a day to ensure aeration. Embryonated eggs containing infective larvae were washed three times in distilled water by centrifugation at 1150 g for 10 min to remove sulphuric and the organic matter. One ml of the suspension was transferred to five 50 ml plastic centrifuge tubes. To each tube 10 ml of saturated calcium hypochlorite solution was added at room temperature. Every five minutes, one tube with suspension was diluted to 50 ml with distilled water, in order to prevent any further de-coating action in the egg shell by the calcium hypochlorite solution. Eggs at the butt of each tube were observed under microscope to select the tube with eggs at the suitable de-coating

stage with larvae coming out of eggs. Then the selected tubes were centrifuged at 1150 g for 10 min. After removal of the supernatant, suspensions were washed (1150 g for 10 min) and the hatched larvae at the butt of the tube were collected.

Molecular Identification by DNA extraction and PCR amplification

Identification of *Toxocara* and *Trichuris* was confirmed by extracting DNA from cultures or eggs in single infections. Genomic DNA was extracted using DSBIO DNA extraction kit and PROMEGA protocol following the manufacturer's instructions. The DNA obtained from eggs or larvae was re-suspended in 13 µl of distilled water. The ribosomal second internal transcribed spacer (ITS2) and the mitochondrial cytochrome oxidase subunit 1 (CO1) regions were amplified using polymerase chain reaction (PCR) for helminth parasites. Amplification reactions were performed in a final volume of 25.0 µl containing primers, deoxynucleoside triphosphates (dNTPs, 0.2 mM), *Taq* polymerase and aliquot of DNA template. The nematode genus specific primers used were ITS2 3S-FW:5'-CGG TGG ATC ACT CGG CTC GT-3' and CO1 FH5-FW:5'-TTT TTT GGG CAT CCT GAG GTT TAT-3'were used which amplify ribosomal DNA and mitochondrial DNA. Conditions in the PCR (Gene Amp PCR system 9700, Singapore) were as follows: initial de-naturation (94°C for 3 min), followed by 35 cycles. Each cycle included a denaturation (94 °C for 1 min) annealing (50°C for 1 .5 min) and an extension (72°C for 1 min. The annealing programme was completed with a final extension (72°C for 5 min).

Amplified PCR products were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide. Then, the PCR positive samples were subjected to sequencing to identify the species of the two parasites.

Data Analysis

Prevalence of infections was calculated for each mammal species as a percentage. The differences in the prevalence of GI infections between helminths and protozoans and the prevalence of infection among the captive bred, imported or wild caught individuals were analysed using a Chi square test in Minitab software (version 15).

RESULTS

Prevalence of GI parasites

Faecal samples of 70 mammal species belonging to nine orders were analysed, of which 44 (62.9%) were infected with one or more GI parasites (Table 1). Eleven individuals had mixed infection (25.1%; Tables 1 and 2). Individuals of seven orders were infected with GI parasites while the other two orders: lagomorphs and diprotodonts did not harbor any GI parasites (Table 2). Among the infected orders, all the perisodactyles, probodcideans and erinaceomorphans sampled were infected (100%), while artiodactyles had the lowest prevalence (44.4%). Overall, helminth infections were more common (81.8%) compared to the protozoan infections (47.7%; Table 2) but the difference in the infection was not statistically significant (Chi square test χ^2= 0.078; p>0.05). Moreover, there was no significant difference in the prevalence of infection among the captive bred, imported or wild caught individuals (Chi square test χ^2= 0.022; p>0.05).

Eggs, cysts and oocysts estimates from iodine smears, salt and sucrose floatation were comparable to those of the McMaster counts and therefore the EPG, CPG and OPG counts given in Table 1 were from the McMaster technique. Hatched out larvae of *Toxocara* and *Trichuris* from faecal cultures confirmed the eggs identified from the faecal samples of the African lion (*Pantheraleo*) and Hamadryas baboon (*Papiohamadryas*), respectively. Although data from PCR protocols confirmed the two nematode genera identified through light microscopy, the sequencing was not successful due to insufficient band size in the gel and therefore identifying the two nematodes to species level was not possible.

Types of parasites and their intensities

A total of 13 different types of species/faecal stages of parasites were identified in mammals at the Dehiwala Zoo (Figure 1). In addition, two unidentified larval stages were recorded from black rhino and flying squirrel and these could be the hatched out larvae of the nematodes infections found in these two hosts and therefore they were not considered for calculations.

Table 1: Background information, type of gastrointestinal parasites and the intensity of infection in the mammal species at Dehiwala Zoo

Mammal species & Order	History*	Caged as Group or Single	Sex ratio (M:F)	Age	Type of Infection	Intensity (EPG/CPG/OPG)§
ORDER PRIMATES						
Patas monkey (*Erythrocebus patas*)	Imported	Group	1:1	Adult	*Trichuris* sp.	200
Chimpanzee (*Pan* sp.)	Captive born	Group	2:4	All**	*Trichuris* sp.	600
Orangutan (*Pongo* sp.)	Captive born	Group	2:1	Juvenile, adult	-	-
Gibbbon (*Hylobates* sp.)	Captive born	Group	1:2	Adult	-	-
Japanese monkey (*Macaca fuscata*)	Imported	Group	2:4	Adult	*Trichuris* sp.	500
Silver leaf monkey (*Trachypithecus cristatus*)* **	Captive born	Group	4:8	All**	*Trichuris* sp.	600
					Giardia sp.	5,800
White handed gibbon (*Hylobates lar*)	Imported	Group	1:1	Adult	-	-
Torque monkey (*Macaca sinica*)	Captive born	Group	2:6	Juvenile, adult	-	-
Mangabey monkey (*Cercocebus* sp.)* **	Imported	Group	1:3	Adult	*Giardia* sp.	500
					Entamoeba sp.	1100
Purple faced leaf monkey (*Trachypithecus vetulus*)* **	Wild captured	Group	4:10	All**	*Trichuris* sp.	700
					Giardia sp.	600
					Entamoeba sp.	1500
Capuchin monkey (*Cebus* sp.)	Imported	Group	2:4	Adult	-	-
Spider monkey (*Ateles* sp.)	Imported	Group	1:2	Adult	*Giardia* sp.	700
Grey langur (*Semnopithecus* sp.)	Wild captured	Group	2:0	Adult	-	-
Ring tailed lemur (*Lemur catta*)	Imported	Single	1:0	Juvenile	Strongyle type eggs	600
Hamadryas baboon (*Papio hamadryas*)	Captive born	Group	3:5	Juvenile, adult	*Trichuris* sp.	5100
White nosed monkey (*Cercopithecus* sp.)	Imported	Single	1:0	Adult	*Trichuris* sp.	500
Chinese monkey (*Macaca* sp.)	Imported	Group	1:2	Adult	-	-
Brown lemur (*Eulemur*)	Imported	Group	1:3	Adult	Strongyle type eggs	400
ORDER CARNIVORA						
African lion (*Panthera leo*)	Imported	Group	4:2	Adult	*Toxocara* sp.	6300
Jungle cat (*Felis chaus*)	Captive born	Group	3:3	Adult	*Strongyloides* sp.	600
Fishing cat (*Prionailurus viverrinus*)	Wild captured	Group	2:3	Adult	*Toxocara* sp.	1000
Otter (*Lutra* sp.)	Wild captured	Single	1:1	Adult	*Entamoeba* sp.	1200
Coati (*Nasua* sp.)* **	Imported	Single	1:0	Adult	*Strongyloides* sp.	700
					Blastocystis sp.	500
Skunk (*Conepatus* sp.)	Imported	Single	1:0	Adult	-	
Brown bear (*Ursus arctos*)	Imported	Group	1:1	Adult	*Entamoeba* sp.	600
Sloth bear (*Ursus ursinus*)* **	Wild captured	Group	3:3	Adult	Hookworm	300

					Entamoeba sp.	800
Jackal (*Canis aureus*)	Captive born	Group	4:10	Adult	-	
Bengal tiger (*Panthera tigris*)	Imported	Group	2:2	Adult	*Toxocara* sp.	500
White tiger (*Panthera tigris*)	Imported	Group	1:1	Adult	*Toxocara*sp.	400
Meerkat (*Suricata suricatta*)	Imported	Group	1:2	Adult	Hookworm	200
Rusty spotted cat (*Prionailurus rubiginosus*)	Captive born	Group	2:3	Adult	-	
Sri Lankan palm civet (*Paradoxurus* sp.)	Wild captured	Single	1:0	Adult	*Entamoeba* sp.	600
Sea lion (*Zalophus* sp.)	Imported	Group	1:2	Adult	*Entamoeba* sp.	600
Leopard (*Panthera pardus*)	Wild captured	Group	2:4	Adult	*Toxocara* sp.	800
Ocelot (*Leopardus* sp.)	Captive born	Single	1:0	Adult	-	
Ring tailed civet (*Bassariscus* sp.)	Wild captured	Single	1:0	Adult	-	
Golden palm civet (*Paradoxurus zeylonensis*)	Wild captured	Group	1:1	Adult	-	
ORDER PERISODACTYLA						
Wild horse (*Equus przewalskii*)	Imported	Group	2:1	Adult	Strongyle type eggs	300
Black rhino (*Diceros bicornis*)***	Imported	Group	1:2	Juvenile, adult	Strongyle type eggs	2300
					Balantidium sp.	900
Donkey (*Equus africanus*)	Captive born	Group	1:2	Adult	*Moniezia* sp.	200
Pony (*Equus caballus*)	Captive born	Group	1:1	Adult	Coccidia oocysts	3100
Zebra (*Equus* sp.)	Captive born	Group	2:3	Juvenile, adult	Strongyle type eggs	500
Mule (*Equus* sp.)	Captive born	Single	1:0	Adult	Strongyle type eggs	600
ORDER ARTIODACTYLA						
Dual humped camel (*Camelus* sp.)	Imported	Single	1:0	Adult	*Trichuris* sp.	700
Nilagai (*Boselaphus* sp.)	Imported	Group	2:0	Adult	-	
Arabian oryx (*Oryx leucoryx*)	Captive born	Group	3:4	Juvenile, adult	-	
Spotted deer (*Axis axis*)***	Captive born	Group	15:40	All**	Strongyle type eggs	300
					Entamoeba sp.	500
Lechwe (*Kobus leche*)	Captive born	Group	12:20	All**	-	
Mouse deer (*Moschiola* sp.)	Captive born	Group	4:10	All**	Strongyle type eggs	200
Wild boar (*Sus scrofa*)	Wild captured	Group	1:1	Adult	-	
Buffalo (*Syncerus* sp.)	Captive born	Group	3:7	Juvenile, adult	Coccidia oocysts	200
Pygmy hippopotamus (*Cheropsis* sp.)	Imported	Group	6:4	Juvenile, adult	-	
Nile hippopotamus (*Hippopotamus amphibius*)	Imported	Group	2:1	Adult	-	
Barking deer (*Muntiacini* sp.)	Wild captured	Group	1:2	Adult	*Moniezia* sp.	400

Scimitar oryx (*Oryx dammah*)	Imported	Group	3:4	Juvenile, adult	*Strongyloides* sp.	100
Sambar (*Cervus unicolor*)	Captive born	Group	5:12	All**	*Strongyloides* sp.	400
Guanaco (*Lama guanicoe*)	Captive born	Group	4:8	All**	-	
Greater kudu (*Tragelaphus* sp.)	Captive born	Group	3:10	Juvenile, adult	-	
Giraffe (*Giraffa* sp.)	Captive born	Group	0:4	Adult	-	
Sable antelope (*Hippotragus* sp.)	Imported	Group	1:0	Adult	*Moniezia* sp.	200
Japanese deer (*Cervus nippon*)	Captive born	Group	4:8	Adult	-	
ORDER PROBOSCIDEA						
African elephant (*Loxodonta africana*)* **	Imported	Single	1:0	Adult	Strongyle type eggs	500
					Entamoeba sp.	700
Asian elephant (*Elephas maximus*)	Captive born	Group	1:6	Juvenile, adult	Unidentified protozoan cysts	1000
ORDER ERINACEOMORPHA						
Hedgehog (*Erinaceus* sp.)	Imported	Single	0:1	Adult	*Toxocara* sp.	2800
ORDER RODENTIA						
Porcupine (*Hystrix* sp.)* **	Wild captured	Group	1:3	Adult	Strongyle type eggs	900
					Strongyloides sp.	800
					Nematodirus sp.	3300
Maara (Dolichotis sp.)	Imported	Group	1:2	Adult	-	
Guinea pig (*Cavia* sp.)* **	Captive born	Group	12:20	All**	Strongyle type eggs	600
					Entamoeba sp.	900
Flying squirrel (*Pteromyini* sp.)* **	Captive born	Single	1:0	Adult	Strongyle type eggs	500
					Spirometra sp.	2100
ORDER LAGOMORPHA						
Rabbit (*Lepus* sp.)	Captive born	Group	4:6	All**	-	-
ORDER DIPROTODONTIA						
Wallaby (*Dorcopsis* sp.)	Imported	Group	2:3	Juvenile, adult	-	-

*whether captive born, imported or captured from the wild; ** babies, juveniles and adults; *** mixed infections, § EPG= eggs per gram; CPG= cysts per gram; OPG= oocysts per gram

Table 2: Prevalence of different types of gastrointestinal parasites in seven mammalian orders in the Dehiwala Zoo

	Parasite species	Percentage prevalence of infections (n)							Overall (70)*
		Primates (18)	Carrnivora (19)	Perisodactyla (6)	Artiodactyla (18)	Proboscidea (2)	Erinaceomorpha (1)	Rodentia (4)	
Protozoa	*Giardia* sp.	22.2 (4)	-	-	-	-	-	-	5.7 (4)
	Blastocystis sp.	-	5.3 (1)	-	-	-	-	-	1.4 (1)
	Entamoeba sp.	11.1 (2)	26.3 (5)	-	5.6 (1)	50.0 (1)	-	25.0 (1)	14.3 (10)
	Balantidium sp.	-	-	16.7 (1)	-	-	-	-	1.4 (1)
	Unidentified cysts	-	-	-	-	50.0 (1)	-	-	1.4 (1)
	Coccidia oocysts	-	-	16.7 (1)	5.6 (1)	-	-	-	2.9 (2)
Protozoan infections		**22.2 (4)**	**31.6 (6)**	**33.3 (2)**	**11.1 (2)**	**100 (2)**	**-**	**25.0 (1)**	**47.7 (21)**
Helminth	*Trichuris* sp.	38.9 (7)	-	-	5.6 (1)	-	-	-	11.4 (8)
	Strongyle type eggs	11.1 (2)	-	66.7 (4)	11.1 (2)	50.0 (1)	-	75.0 (3)	17.1 (12)
	Strongyloides sp.	-	10.5 (2)	-	11.1 (2)	-	-	25.0 (1)	7.1 (5)
	Toxocara sp.	-	26.3 (5)	-	-	-	100.0 (1)	-	8.6 (6)
	Hookworm	-	10.5 (2)	-	-	-	-	-	2.9 (2)
	Spirometra sp.	-	-	-	-	-	-	25.0 (1)	1.4 (1)
	Moniezia sp.	-	-	16.7 (1)	5.6 (2)	-	-	-	4.3 (3)
	Nematodirus sp.	-	-	-	-	-	-	25.01(1)	1.4 (1)
Helminth infections		**50.0 (9)**	**47.4 (9)**	**83.3 (5)**	**33.3 (6)**	**50.0 (1)**	**100 (1)**	**75.0 (3)**	**81.8 (36)**
Mixed infections		**16.7 (3)**	**10.5 (2)**	**16.7 (1)**	**5.6 (1)**	**50.0 (1)**	**-**	**75.0 (3)**	**25.0 (11)**
Total		**61.1 (11)**	**68.4 (13)**	**100 (6)**	**44.4 (8)**	**100 (2)**	**100 (1)**	**75.0 (3)**	**62.9 (44)**

n= number of hosts infected; * including the individuals from the two orders that did not have any infections

Note: Individuals of two orders: Lagomorpha and Diprotodonta did not have any parasites

The most common type of infection was strongyle type eggs (17.1%), followed by *Entamoeba* sp. (14.3%) and *Trichuris* sp. (11.4%) and the least common infections were the *Spirometra* sp. in flying squirrel, *Nematodirus* sp. in porcupine, *Blastocystis* sp. in coati and unidentified protozoan cysts in the Asian elephant. All of these infections were recorded only in one host species (prevalence 1.4%). Among different orders, carnivores were mostly infected with *Toxocara* sp. and *Entamoeba* spp. with a prevalence of 26.3% in both cases, while in artiodactyles, rodents, perissodactyles and proboscedeans the most commonly encountered stage was strongyle type eggs.

Seven species of Primates including Patas monkey, chimpanzee, Japanese monkey, silver leaf monkey, purple faced monkey, Hamadryas baboon and white nosed monkey were infected with *Trichuris* sp. and recorded low EPG of less than 1000 except Hamadryas baboon which had a high count of 5100 EPG (Table 1). According to the established infection intensity categories of World Health Organization (WHO, 1987) for soil transmitted nematodes (STNs) including *Trichuris trichiura* infection was defined as light (1–999 EPG) moderate (1,000–9,999) or heavy (10,000 EPG). Four species of primates were infected with *Giardia*: silver leaf monkey, mangabey monkey, purple faced leaf monkey and spider monkey. Of these, silver leaf monkey had a mixed infection of both *Trichuris* and *Giardia w*ith a CPG of 5800. Orangutan, gibbon, white handed gibbons, toque monkey, grey langur and Chinese monkey did not have any infections. The two lemur species: ringed tail and brown lemur had only strongyle type eggs at low intensities 600 or less EPG. Out of primates, only purple faced monkey and Mangabey monkey had *Entamoeba* infections where both had mixed infections with *Giardia* sp. and *Trichuris* sp. (Table 1).

In the carnivores, the most prevalent parasites were *Toxocara* and *Entamoeba* where five mammal species were infected with these two parasites. African lion, fishing cat, Bengal tiger, white tiger and leopard were infected with *Toxocara* and otter, brown bear, sloth bear, Sri Lankan palm civet and sea lion were infected with *Entamoeba*. Hedgehog (Order Erinaceomorpha) was also infected with *Toxocara.* Except in white tiger (400 EPG) all others had high intensity of *Toxocara* infections (>500 EPG) and African lion and fishing cat had EPG counts of 6300 and 1000, respectively. *Blastocystis* infection was recorded only in meerkat. Some carnivores: skunk, jackal, rusty spotted cat, Ocelot, ring tailed civet and golden palm civet did not harbor any GI parasites.

Among the herbivores, donkey, sable antelope and barking deer were infected with the tapeworm *Moneizia* while pony and buffalo had coccidian infections. *Nematodirus* sp. in porcupine (EPG= 3,300) and *Spirometra* sp. in flying squirrel (EPG=2,100) can be considered rare infections. Black rhino reported high intensity of strogyle type with 2300 EPG.

DISCUSSION

Results of this coprological survey show that more than half of the mammals (62.9%) at the Zoo were infected with one or more GI parasites. Similar prevalence levels of GI parasites have been reported from captive mammals in other zoos in Rangpur Recreational Garden in Bangladesh (Khatun *et al*., 2014) and Zoo Safari of Fasano in Italy (Fagiolini *et al*., 2010). A higher percentage of mammals at the Dehiwala Zoo were infected with helminths (81.8%) than protozoans (47.7%) irrespective of administration of regular deworming. The common practice at the Zoo is that all the mammals are treated with anthelmintics every three months irrespective of the parasite burden or the type of infection. Treatment should be given only to animals suffering from heavy parasitism of pathogenic species to avoid the development of drug resistance. Most animals that can tolerate existing incidental infections should be left untreated. A coprological analysis should be carried out to determine the types of parasites and the worm burden before administering the anthelmintics. Testing for infection and only treating when infection reaches a threshold would likely to reduce development of antiparasite resistance. Treatment might be on a herd basis when it comes to herbivores that occur in large herds or an individual basis especially the rare mammals. For rare species, since the number of individuals in the zoo is limited and valuable, testing and treatment on individual basis is essential. Higher helminth infections have also been reported in other zoo animals by many authors: Rangpur Recreational Garden in Bangladesh (Khatun *et*

al., 2014) and Zoo Safari of Fasano in Italy (Fagiolini *et al*., 2010). Opara *et al*., (2010) in Nekede Owerri zoological garden in Southeast Nigeria while Varadharajan and Kandasamy (2000) in V.O.C. Park and Mini Zoo, Coimbatore.

Present study reported six nematodes: *Trichuris* sp., *Strongyloides* sp., *Toxocara* sp., *Nematodirus* sp., hookworm (Superfamily Ancylostomatidea) and strongyle type eggs (Superfamily Strongyloidea), five protozoans: *Giardia* sp., *Blastocystis* sp., *Entamoeba* spp., *Balantidium* sp. and coccidians, two cestodes: *Moniezia* sp. and *Spirometra* sp. while there were no trematode infections. A sedimentation technique has to be applied to detect the eggs of trematodes as these eggs are heavier. Trematodes require one or more intermediate host(s) for their transmission and therefore are less likely to accumulate in a captive environment (Tandon *et al*., 2005; Atanaskova *et al*., 2011). Since most of common GI protozoans and nematodes spread by the faecal-oral route, infections are spread in areas with inadequate sanitation and poor hygiene. Only *Monezia* infection involves an intermediate host, a mite which occurs commonly in pastures where herbivores graze.

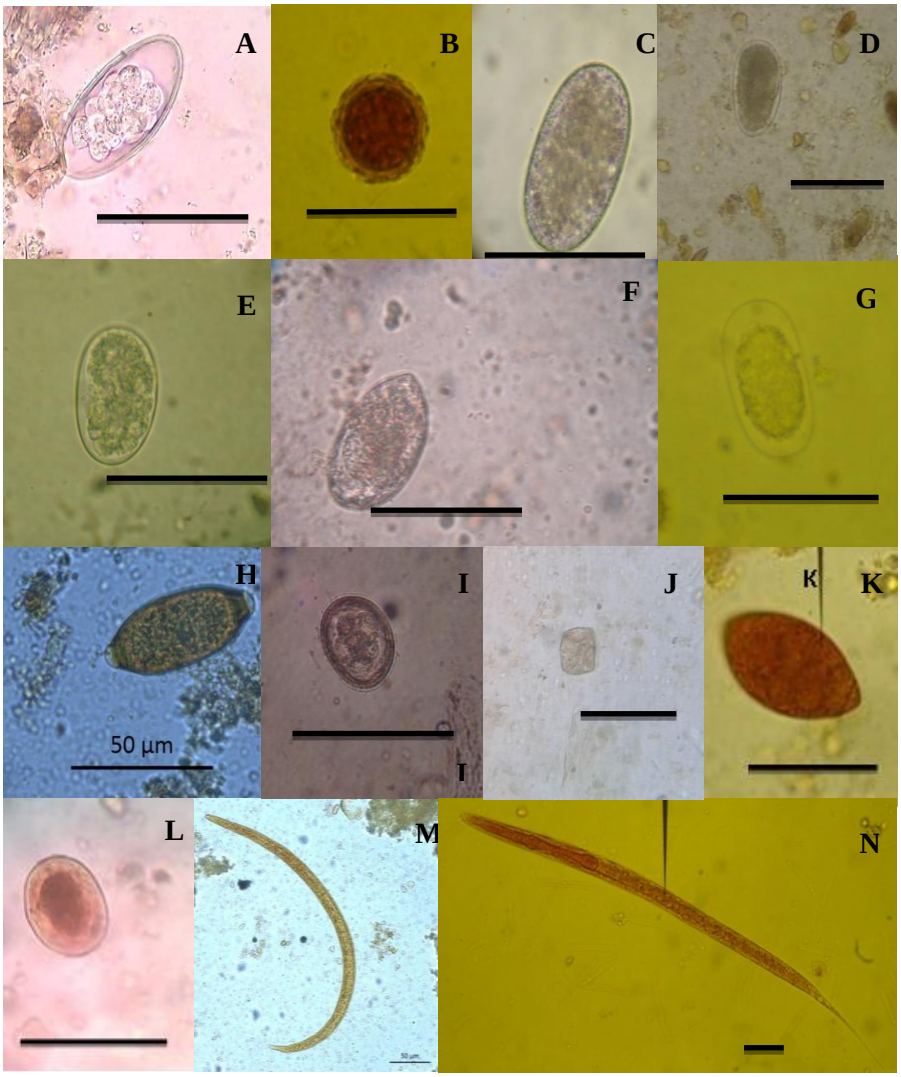

Figure 1: Helminth eggs and larvae in the faecal samples analysed from mammals in Dehiwela Zoo (A) Egg of *Nematodirus* sp. (B) Egg of *Toxocara* (C, D, E, F) Strongyle type eggs, (G) Egg of *Strongyloides* sp. (H) Egg of *Trichuris* sp. (I) Egg of *Toxocara* sp (J) Egg of *Moniezia* sp. (K) Egg of *Spirometra* sp. (L) Hookworm egg (M) Unidentified nematode larva in black rhino and (N) Unidentified rhabditiform larva in flying squirrel (Scale bar = 50 µm)

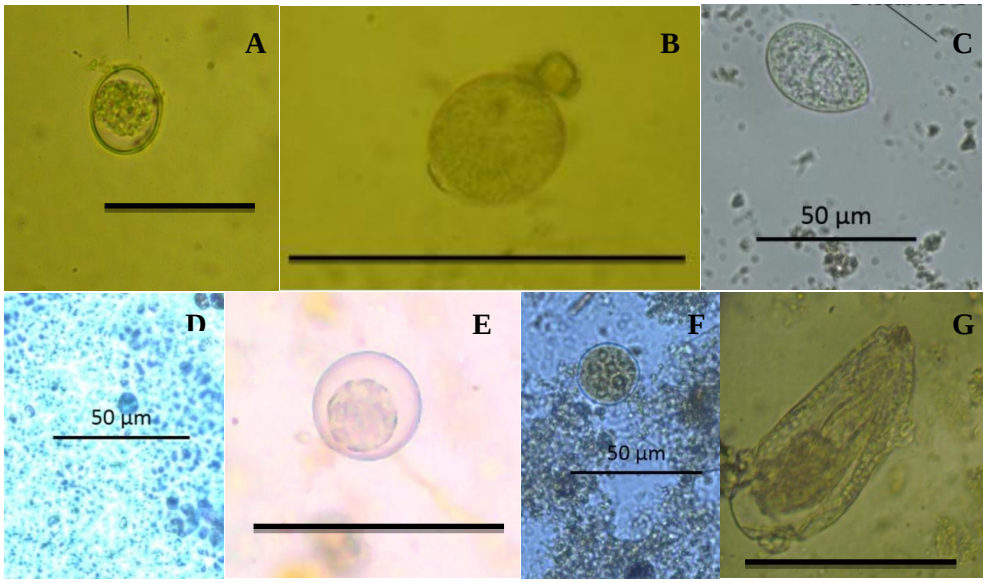

Figure 2: Protozoan cysts in the faecal samples analysed from mammals in Dehiwela Zoo (A, B) coccidian oocysts (C) Cyst of *Balantidium* sp. (D) Cyst of *Giardia* sp. (E & F) Cysts of *Entamoeba* sp. (G) Unidentified protozoan cyst. (Scale bar = 50 µm)

Present study examined mammals belong to nine orders, of which individuals of seven orders were infected with one or more GI parasites. None of the individuals examined in Orders Lagomorpha (Rabbit, *Lepus* sp.) and Diprotodontia (Wallaby, *Dorcopsis* sp.) had any GI infections. Gurler *et al*., (2010) also reported the absence of GI parasites in Orders Lagomorpha and Diprotodontia in the Samsun Zoological gardens in Turkey.

All the individuals in three orders: Perisodactyla (n=6), Erinaceomorpha (n=1) and Proboscidea (n=2) were infected with GI parasites. However, the number of individuals sampled in Erinaceomorpha and Proboscidea was low and the findings therefore cannot be generalized uncritically. Carnivores, artiodactyles and rodents had the highest number (five each) of parasites in each order. Among the carnivores five were infected with *Toxocara* sp. (26.3%), which is a common parasite in felines and canines (Lim *et al*., 2008). Among the carnivores infected with *Toxocara* sp. Bengal tiger *(Panthera tigris tigris*), African lion (*Panthera leo*) and a white tiger (*P. tigris tigris*) were imported from other zoos while the leopard (*Panthera pardus*) and the fishing cat (*Prionailurus viverrinus*) were wild caught. It could be that these animals have all been infected from birth as *Toxocara* can be transmitted in the milk from mother to offspring and already had the infection before they were brought to the Zoo. However, the major route of transmission is through ingesting embryonated eggs in the environment via faecal oral route cannot be ruled out as they may have got the infections from other carnivores in the zoo as well. The close proximity of the cages of the infected animals: Bengali tiger, African lion and a white tiger could be a main reason for spreading the infection. Carnivores were also infected with *Entamoeba* spp. and *Strongyloides* sp. with a high prevalence of 26.3 % and 10.5%, respectively. The presence of synanthropic rodents such as the house mouse and the rat, which live in or near human dwellings and in the zoological gardens, is known to serve as a reservoir of several types of infections. Factors such as urbanization, overcrowded cities and inadequate sanitation have led to increasing number of these animals and consequently becoming increasingly common in transmitting diseases to humans and other animals (Brasil, 2002).

Among the 18 species of Primates investigated, high prevalence GI parasite (61.1%) was recorded. *Trichuris* sp. was the most prevalent (38.9%) parasite. Similar findings were obtained in the study of Dawet *et al*. (2013) where infection of *Trichuris* sp. was the most

frequent parasite (58.0%) in Primates at the Jobs Zoological Gardens, Nigeria. However, Gunasekara *et al*. (2012) recorded *Trichuris* only in two Primates (toque monkey and Hamadryas baboon) out of 15 Primates examined in Dehiwala Zoo. The World Health organization (WHO) established infection intensity categories for *T. trichiura* for humans in order to inform the management of large-scale deworming programs (WHO 1987). According to this *T. trichiura* infection was defined as light (1–999 EPG) or heavy (>10,000 EPG, WHO 1987). A further category of 'moderate' (i.e. for EPG counts between 1,000 and 9,999 EPG) was subsequently added by WHO (Montressor *et al.*, 1998). *Trichuris* infection in primates therefore can be considered light which was less than 999 EPG except in Hamadryas baboon (5100 EPG). *Trichuris* is a soil transmitted nematode where infections can easily spread through oral faecal route. Among the Primates at Dehiwala Zoo, Gunasekara *et al*. (2012) identified six species of protozoa: *Cryptosporidum* sp., *Balantidium* sp., *Blastocyst* sp., *Entamoeba* sp., *Giardia* sp., and coccidian in the chimpanzee, orang-utan, hamadryas baboon, Japanese macaque, siamang gibbon, toque monkey, grey langur, silvered leaf monkey, sooty mangabey and Formosan monkey. The helminthes they reported were hookworm larvae and eggs of *Ascaris*, strongyle and *Trichuris* . They found that toque monkey was positive for five species of GI parasites: *Trichuris*, hookworm, *Ascaris, Balantidium, Blastocycstis*. However, in the present survey toque monkey was not infected with a single parasite. Out of 15 Primates studied by Gunasekara *et al.* (2012) 11 were included in the present study while Siamang gibbon (*Symphalangus syndactylus),* Squirrel monkey (*Saimiris ciureus*) Sooty mangabey (*Cercocebus aterrimus*) and hooded capuchin (*Cebus paella*) were not present in the Zoo at the time of sampling but seven other species were present, some of them were imported, captive bred or caught from the wild (Table 1).

Of the herbivores, all the perisodactyles were infected with at least a single parasite species but only eight individuals out of 18 artiodactyles (44.4%) were infected. Both these orders had *Moneizia* infections which is a common tapeworm in herbivores. In the life cycle of *Moneizia*, an oribatid mite is involved as the intermediate host. Eggs are passed out with faeces from the ruminant host along with the gravid proglottids into the soil and these eggs are eaten by oribatid mites. Grazing ruminant may ingest the mites in pastures and the eggs hatch out and develop into tapeworms in the small intestine. The black rhino (*Diceros bicornis*) who was pregnant during the time of sampling had a high infection of strongyle type eggs (2300 EPG) and may had been vulnerable to parasitic infections. In the case of proboscideans only two individuals: African and Asian elephants were sampled and both were found infected with three different types of parasites. Strongyles and *Entamoeba* sp. were found in the African elephant and unidentified protozoan cysts were found in the Asian elephant. If the food given (mostly jack leaves) has got contaminated before they were brought to the zoo, animals can be infected with the parasites after eating them. Damp and unhygienic conditions maintained in the enclosure can also increase susceptibility to the infections (Ortiz *et al.*, 2006; Vanitha *et al.*, 2011).

Of the four rodents examined, three were infected with GI parasites (75.0%). These rodents were not treated for worms (personal communication with a veterinarian at the Dehiwala Zoo) as they usually do not show symptoms of worm infections. But animals are more prone to parasite diseases when they are in captivity than in their natural environment. Such situations can also be dangerous to the visitors, workers and veterinarians. Lagomorphs and diprotodonts in the Dehiwala zoo did not have any GI parasites. Similarly, a study conducted in the Samsun Zoological Gardens in Turkey, lagomorphs and diprotodonts were free of parasitic infection (Gurler *et al*., 2010).

Mixed infections of GI parasites among the mammals at Dehiwala zoo were not very common (25.0%). Mixed infections of *Trichuris* sp. and *Giardia* sp. and *Enatmoeb*a sp. in primates and strongyle type together with various protozoans in coati, black rhino and spotted deer were observed.

This study shows, more than half of the mammals at Dehiwala Zoo were infected with GI parasites, and some with high intensities. The presence of parasite eggs, oocysts or cysts in the faecal sample does not mean the animal is sick or will be sick nor does it mean that the animal should be treated (Wood *et al.*, 1995). All gastrointestinal parasites are not equal; some are

highly pathogenic and some are incidental. The current practice at Dehiwala Zoo is to treat every animal routinely giving anthelmintics every three months. A better control strategy should involve a more targeted approach to manage parasites effectively minimizing the pathogenic species by regular faecal examination along with administration of desired worm treatment at regular intervals.

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