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Investigation of Enteric Parasites of Zoo Animals and Zookeepers in Beni-Suef Governorate, Egypt

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In this study, the enteric parasites of zoo animals and zookeepers in Beni-Suef governorate, Egypt were investigated. Fecal samples from thirteen animal species were examined by floatation and formol ether sedimentation techniques. Zoo animals were classified into non-human primates, carnivores and herbivorous animals. The examination of non-human primates revealed the presence of *Trichuris trichura* eggs, *Giardia intestinals* and *Entamoeba histolytica* cysts. In carnivores, *Toxoascaris leonina eggs* and *Isospora felis* oocysts were the most predominant findings. In herbivore wild animals, gastrointestinal nematode (GIT) eggs and *Eimeria* species oocysts were present. Larval identification by fecal culture of (GIT) eggs demonstrated the presence of *Haemonchus contortus* and *Strongyloid papillosus* larvae. Examination of zookeepers and one lab worker revealed the presence of *Giardia intestinals* and *Entamoeba histolytica* cysts. In conclusion, infection with *Giardia intestinals* and *Entamoeba histolytica* in both of human and non-human primates suggests the zoonotic transmission in the zoo.

Indeed, wild animals take a little of attention in a comparison with the domesticated ones, especially in the research area. Moreover, their medication and examination are difficult. Inadequate information on diseases and parasites of zoo animals is a major limiting factor in zoological gardens (Rajashekaraiah *et al.,* 1971). When herds of these wild animals are kept in captivity in zoological gardens, the problem of parasitic infections can aggravate and pose a serious threat to endangered species, occasionally causing sudden and unexpected local declines in abundance (Muoria *et al.,* 2005). Furthermore, the nutritional status of captive animals can also enhance or diminish their resistance to disease (Geraghty *et al.,* 1982). Gastro-intestinal tract parasites have considerable implications on conservation and management of non-human primates (Collet *et al.,* 1986). Parasitic infections may affect animal health and ultimately the studies for which these animals are used, many of these parasites are zoonotic pathogens (Purcell and Philipp, 2005). Human and non-human primates sharing the same ecosystems may occasionally lead to sporadic outbreaks of zoonosis (Lee *et al.,* 2010). Besides, Unhygienic handling of workers with animals increases the possibility of zoonotic transmission (Adejinmi and Ayinmode, 2008).

The objective of this study was to investigate the enteric parasites of zoo animals in Beni-Suef Governorate. In addition, the zoonotic potential of these pathogens in zookeepers was estimated.

Material and methods

Study site and animal housing. This study was conducted in Beni-Suef Governorate zoo, Egypt. A total of 61 animals from 13 species were examined. Over six months from June to November 2012, zoo animals were examined regularly twice per month. Animals were classified into non-human primates, carnivores and herbivorous animals. Non-human primates were 8 Grivet monkeys housed in 4 boxes, 7 Hamadryas baboons housed in a large caged area and one Brown capuchin in a single box. Data of the examined animals were obtained from zoo labels on the cages and boxes of each species. Carnivores were 5 African lions, one Hyena and 2Egyptian a jackal, each animal was in a separate box. Herbivorous animals were 3 Llama, 2 Shetland pony, 4 Simitar-horned oryx, 1 European deer, 20Mountain goats, 5Barbary sheep and 2 Hippo. Each herbivore species was housed in a specific yard.

Sample collection.

Animal samples. Fresh fecal samples were collected by zookeepers in the morning in labeled plastic bottles. Each sample was representing to one or more animals housed in a single cage.

Human samples. Fresh stool samples were taken from six zookeepers who have been in close association with the examined animals. Nature of work, degree of animal contact and health status of each zookeeper were recorded. An additional fecal sample was obtained from one faculty lab worker who suffered from digestive troubles at the end of the work.

All samples were properly transported to the parasitological laboratory, faculty of veterinary medicine, Beni-suef University where examination was immediately commenced.

Examination techniques. All fecal samples were examined macroscopically for adult nematodes and tapeworm proglottids. Each sample was subjected for examination by fecal floatation technique using different solutions (saturated salt solution, zinc sulphate and Sheathers solution) according to Zajac and Conboy (2006). Furthermore, formol ether sedimentation technique (Lee *et al.,* 2010) was applied for each sample. Iodine solution was used to facilitate protozoan and cyst identification. Parasites were identified on the basis of egg or oocyst color, shape and contents (Soulsby, 1982).

Fecal cultures of the detected *Strongylida* eggs were performed (Eckert, 1960) followed by larval identification according to the keys mentioned by Gibbons *et al.,* (2006).

Results

Humans. Fecal examination of zookeeper samples revealed a positive result (83.3%) for each of *Entamoeba histolytica* and *Giardia intestinals* cysts. In addition, *Entamoeba coli* and *Endolimex* spp. cysts were demonstrated in a level of 33.3%, while a rate of 16.7% was recorded for *Anclystoma* spp and Strongyloides spp. eggs. It was worth mentioning that the lab worker was found to be infected with *Giardia intestinals* cysts.

Non-human primates. Microscopical fecal examination revealed the presence of *Trichuris trichura* eggs and *Giardia intestinals* cysts in Brown capuchin. *Trichuris trichura* eggs, *Entamoeba histolytica, Entamoeba coli* and *Giardia intestinals* cysts were reported in Grivet monkey. With regard to Hamadryas baboon samples, *Trichuris trichura* eggs , *Entamoeba histolytica* cysts and *Giardia intestinals* cysts were detected. Macroscopically feces from *Entamoeba histolytica* and *Giardia intestinals* infected animals were semi formed or diarrheic with offensive odor.

Table (1): Coproscopical examination of human and non-human primates for parasitic infection.

Parasite	Humans							Non-human primates		
	Zookeepers						Lab worker	Brown capuchin (*Cebus paella*)	Grivet *monkey* (*Cholorocebs aethiops*)	Hamadryas baboon (*Papio hamadryas*)
	1	2	3	4	5	6				
***Entamoeba histolytica* cyst** (2)	+	–	+	+	+	+	–	–	+	+
***Giardia intestinalis* cyst** (2)	+	–	+	+	+	+	+	+	+	+
***Entamoeba coli* cyst** (2)	–	+	+	–	–	–	–	–	+	–
***Endolimex* spp. cyst** (2)	–	+	+	–	–	–	–	–	–	–
***Anclystoma* spp. egg** (1)	–		+	–	–	–	–	–	–	–
***Trichuris* spp egg** (1)	–	–	–	–	–	–	–	+	+	+

Superscript (1): Floatation (concentration floatation technique).
Superscript (2): Sedimentation (Formol ether technique).

Table (2): Results of fecal examination of carnivorous and herbivorous animals.

Parasites	Species									
	Carnivores				Herbivores					
	African lion	Egyptian jackel	Hyena	Hippo	Barbary sheep	Mountain goat	European deer	Simitar-horned oryx	Shetland pony	Llama
Toxoascaris* eggs *leonina 1	+	-	-	-	-	-	-	-	-	-
***Isospora felis* oocyst** 1	+	-	-	-	-	-	-	-	-	-
***Strongyloides papillosus* eggs** 1	-	-	-	-	+	+	-	-	-	-
***Haemonchus contortus* eggs** 1	-	-	-	-	+	+	-	-	-	-
***Eimeria* species oocyst** 1	-	-	-	-	+	+	-	-	-	-

Superscript (1): Floatation (concentration floatation technique).

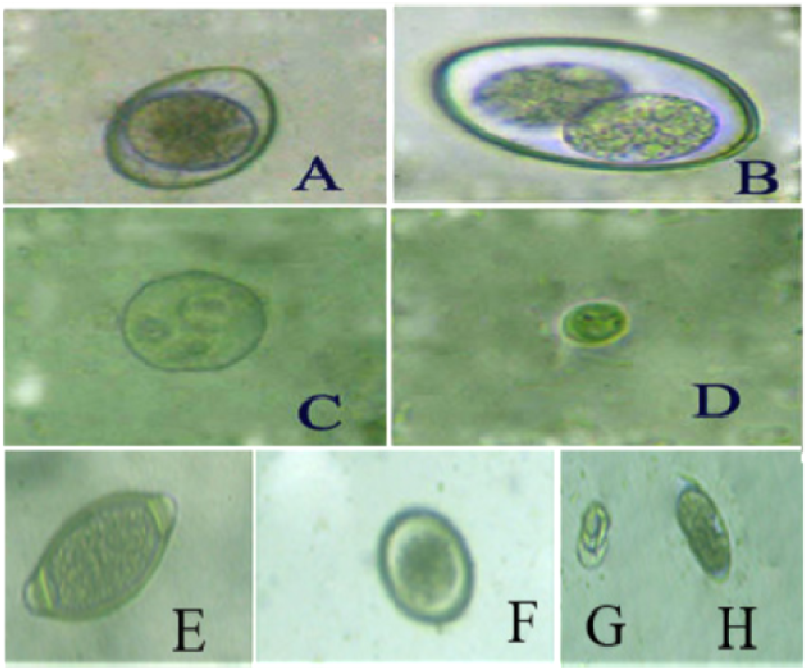

Plate (1): Different protozoa (cysts/oocysts) and helminth eggs.

A. *Isospora leonina* (unsporulated oocyst X40).
B. *Isospora leonina* (sporulated oocyst X100).
C. *Entamoeba histolytica* cyst (X 100).
D. *Giardia intestinals* cyst (X 100).
E. *Trichuris trichura* egg (X40).
F. *Toxoascaris leonina* egg (X10).
G. *Strongyloides papillosus* egg (X10).
H. Gastrointestinal egg (*Haemonchus contortus* after larval identification) (X10).

Carnivorous animals. African lions harbored *Toxoascaris leonina* eggs (80%) and *Isospora felis* oocysts (40%). Fecal examination of other carnivorous animals (Hyena and Egyptian jackal) revealed a negative result for gastrointestinal parasites.

Herbivorous animals. Gastrointestinal nematode eggs and *Eimeria* spp oocysts were found in Mountain goats and Barbary sheep. *Haemonchus contortus* and *Strongyloides papillosus* were the identified nematodes. Fecal examination of other herbivorous animals (Hippo, Europian deer, Simitar-horned oryx, Shetland pony and Llama) revealed a negative result for gastrointestinal parasites.

Discussion

Concerning non-human primates, *Trichuris trichura, Entamoeba histolytica* and *Giardia intestinalis* were highly prevalent in Brown capuchin (*Cebus paella*), Grivet monkey and Hamadryas baboon. The obtained parasites are of direct life cycle, which illustrates their existence in all of them and increases the zoonotic potential for people at risk. The obtained results are consistent with those of Munene *et al.,* (1998) who declared that *Trichuris trichuira* and *Entamoeba histolytica* were prevalent in captive and wild-trapped African non-human primates. In addition, Thomas *et al.,* (2005) in Uganda remarked that *Trichuris* spp., *Entamoeba histolytica, and Giardia lamblia* were detectable in Colobus monkey. Besides, Lee *et al.,* (2010) reported the infection with *Trichuris trichiura* and *Giardia lamblia* in two species of old world monkeys. Furthermore, Naoki *et al.,* (2012) isolated *Trichuris trichura* eggs from wild Japanese Macaques. In the present study, infection with *Giardia intestinalis* in Brown capuchin (*Cebus paella*) was associated with persistent diarrhea, abdominal distension and weight loss, an observation that is in agreement with the results published by Toft and Eberhard (1998) and Lee *et al.,* (2010) who demonstrated that ,*Giardia* spp. caused diarrhea in monkeys.

Regarding carnivore animals, fecal examination of lions showed the presence of *Toxoascaris leonina* eggs and *Isospora felis*. oocysts. Although each animal was in a separate box, 80% of zoo lions were found to be infected with *Toxoascaris leonina*. This may be attributed to the unhygienic manner of the zookeepers who might have shared in transmission of infection between lions specially when the direct life cycle of *Toxoascaris leonina* and *Isospora felis*. is taken into account. Similar findings were reported by Pande *et al.,* (1970); Fagiolini *et al.,* (2010) who demonstrated presence of *Toxoascaris* spp. in lions and Smith and Kok. (2006) who found *Isospora spp.* in African lions.

In wild herbivores, fecal examination revealed the presence of parasitic gastroenteritis eggs and *Eimeria* spp. oocysts. Further fecal culture identification of nematode eggs gave larvae of *Haemonchus contortus* and *Strongyloides papillosus*. These findings may be referred to the environment of captivity that provides favorable conditions for the occurrence of such parasites. These results are supported by Ibrahim *et al.,* (2012) who identified helminth larvae common in all wild ruminant species as *Haemonchus contortus, Trichostrogylus axei* and *Strongyloides papillosus*.

Concerning human beings included in this study, the prevalence of *Entamoeba histolytica* and *Giardia intestinalis* infection in zookeepers was as high as 83.3%. History of the examined zookeepers indicated that the same individuals reacting positively to these protozoal agents were concurrently suffering from digestive troubles. Data associated with the examined individuals have drawn the attention to the role of measures followed in the zoo in the occurrence of parasitoses, especially those of direct life cycle. Careless and unhygienic handling of workers with animals and their environment was highly obvious and has attracted our attention for the high zoonotic potential for people at risk. Close observation of the daily activity of zookeepers indicated that their feeding habits included common dealing with their food directly after removal of zoo animal excreta without application of any safety precautions. Not only zookeepers are exposed to such health hazards but also other categories may occasionally contract such infections from zoo animals and their keepers including zoo administrators and visitors ...etc. Visitor children are expected to be at high risk of infection. The discrepancy in parasitic infection among zookeepers may be correlated to the variation in degree of contact animals. One of zookeepers who had a restricted animal contact and was apparently healthy harbored only two non-pathogenic parasitic cysts (*Entamoeba coli* and *Endolimex* spp.). This explanation is well in line with the report of Adejinmi and Ayinmode (2008) who attributed the difference between the infected and uninfected zookeepers to varying levels of hygienic practices of individuals. One of the faculty lab workers harbored *Giardia intestinals* cyst that was in harmony with the clinical data indicating that he was suffering from digestive troubles.

The detection of *Trichuris trichura* eggs in non-human primates may represent a risk factor for human beings dealing with them. This view is supported by Monteiro *et al.,* (2007) who illustrated that *Trichuris* spp. were experimentally transmitted from non-human primates to humans and provided evidence for the possible zoonotic potential.

In this study, parasites recorded in humans were found to be similar to those recorded in non-human primates than other zoo animals. Adejinmi and Ayinmode. (2008) remarked that fecal samples from the non-human primates contained ova and cysts of parasites that were found in the fecal samples from the zookeepers. Ezenwa (2003); Munene *et al.,* (1998) said that some of the parasites observed in non-human primates were reported to be zoonotic in various parasitological literatures.

The possibility of animal-to-man or man-to-animal transmission of the detected parasites in the current study should be considered. Fayer *et al.,* (2004) reported the transmission of *Giardia* spp. from humans to beavers, dogs and muskrats and supported this possibility by similar gene sequences among isolates. It is also not clear whether, *Giardia* spp. or *Entamoeba histolytica* is strictly host-specific or not (Asano *et al.,* 1991; Stanley., 2003).

In conclusion, this study showed that gastrointestinal parasites were mainly predominant in non-human primates and were found to be existent in their workers suggesting the possibility of their zoonotic potential. It is worth to mention that the detected parasites were of short and direct life cycle. However, the interventions recommended for prevention and control of such parasitic infections will not only improve animal health, but also will increase the safety to zookeepers and animal zoo visitors. Nevertheless, confirmation of zoonotic risk requires further molecular identification for the isolated parasites.

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