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## ***Toxoplasma gondii* AND OTHER PARASITES STUDIED IN FELINE FECES IN ZOOS IN CUBA AND BRAZIL**

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### ABSTRACT

Under certain circumstances, wild animals kept in zoos may be more exposed to infectious parasitic diseases. The purpose of this study was to determine the frequency of gastrointestinal parasites in captive wild felids in the National Zoological Park (PZN) in Cuba (Havana) and in RioZoo in Brazil (Rio de Janeiro). A total of 52 fecal samples were collected from 52 felids, as follows: 19 *Panthera leo*, two *Leopardus tigrinus*, two *Leopardus pardalis*, one *Panthera tigris altaica*, four *Panthera tigris tigris*, six *Panthera onca*, seven *Puma concolor*, one *Herpailurus yagouaroundi*, three *Acinonyx jubatus*, two *Caracal caracal* and five *Panthera pardus*. The fecal samples were processed and examined microscopically. The frequency of parasite positive animals was 17.5% (7/40) in PZN and 25% (3/12) in RioZoo. *Panthera pardus* (40%) and *Panthera onca* (20%) were most frequently infected in PZN and *Panthera leo* (100%) and *Leopardus pardalis* (50%) in RioZoo. Hookworm (12.5%) was detected in PZN as well as *Toxascaris leonina* (10%) and in RioZoo nematode larvae (9.1%), hookworm eggs (9.1%), *Toxascaris leonina* (2%) and the cestode eggs from the Diphyllbothriidae family (9.1%) were found. *Toxoplasma gondii*-like oocysts were not detected in feline feces. Although the positivity of gastrointestinal parasites detected in feline fecal samples was not very high in these zoos, both institutions need to implement and maintain sanitary measures, including routine diagnosis of parasitosis followed by specific treatment according to the infections detected.

**KEY WORDS:** Captive wild felids; gastrointestinal parasites; National Zoo Park of Cuba; Rio de Janeiro Zoo; Brazil.

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Received for publication: 10/11/2019. Reviewed: 2/1/2020. Accepted: 13/1/2020.

## INTRODUCTION

Zoological gardens are a form of *ex situ* conservation, where a wide variety of animal species are kept in small spaces, a fact that may lead to the frequent occurrence of diseases (Thawait et al., 2014). The great diversity of captive animals in these facilities, with the various parasites acquired both in the wild and during permanence in the zoo, increases the possibility of zoonotic infections (Chomel, 2008). In general, captive animals are restricted to a smaller common area than others living in the wild, thus favouring the transmission of gastro-intestinal parasites due to the concentration of evolutionary forms (Barbosa et al., 2015). In many cases, intestinal infections are asymptomatic; however newborn and young animals may present severe symptoms often leading to death (Müller et al., 2005).

Parasites and infectious diseases are a major concern in the conservation of endangered species as these can lead to mortality, dramatic population decline and might even contribute to local extinction events (Smith et al., 2009). Determining the presence of gastrointestinal parasites in wild animals in captivity permits preventive measures and minimizes the negative effects of parasitic infections, as well as improving the quality of life of these animals (Guerrero et al., 2012).

Several parasites can infect wild felids, including nematodes, such as *Toxocara cati* and *Toxascaris leonina*, which can infect felines through ingestion of water or food contaminated with parasite larval eggs or through ingestion of paratenic hosts, harbouring nematode larvae in their tissues. Trans-mammary transmission of the genus *Toxocara* may also occur (Epe, 2009). In addition to these nematodes, hookworms such as the genus *Ancylostoma* and *Uncinaria* have been linked to infected felids, also transmitted orally through ingestion of filarioid larvae or by skin penetration (Kalkofen, 1987). Among the cestodes and trematodes the Diphyllbothriidae Family (*Diphyllbothrium* and *Spirometra* genera) and the Dicrocoelidae Family (*Platynosomum fastosum* species), stand out respectively. These parasites present complex biological cycles with different intermediate hosts. Felids can be infected by ingesting the intermediate host containing immature forms of the parasite (Patton & Rabinowitz, 1994; Conboy, 2009). Protozoa, such as Coccidia (genus *Cystoisospora* sp.) must be highlighted due to its frequency, also *Toxoplasma gondii* due to its importance in public health, with felids being its only definitive hosts. These protozoa infect felids mainly by ingestion of sporulated oocysts in water, or through predation of intermediate hosts harboring tissue cysts (Dubey, 1993).

In general, gastrointestinal parasites, including protozoa and helminths, may lead to weight loss, metabolic imbalance, reproduction disorders, anemia and dehydration in felids. In the most severe cases, these parasites can also cause fetal malformation, locomotor injuries and even death (Azpiri et al., 2000; Barutzki & Schaper, 2003).

Although the literature shows a large number of studies regarding gastrointestinal parasites in zoo animals, few studies have been conducted with felids, mainly on the American continent. This study was performed to analyze the frequency of gastrointestinal parasites in feline feces kept under human care in zoos in Cuba and Brazil, given the importance of zoos to the animals and indirectly to society, regarding the conservation of animal species, scientific research, education and leisure, besides the scarcity of information on the subject, especially at national level.

## MATERIALS AND METHODS

The study was conducted between June 2016 and November 2017 after receiving the approval of the Ethics Committee for the Use of Animals (ECUA) under Fluminense Federal University license number 794 and SISBIO number 52578-1. A total of 52 fecal samples were collected from the various felines kept at the Rio de Janeiro Zoo, Brazil (RioZoo) and the National Zoo Park, Havana, Cuba (PZN). Fecal samples were collected directly from the floor where the animals were kept. In the enclosures of both zoos only one or, at most, two animals of the same species are kept per enclosure. In general, the enclosures have a covered area and an external area, where there is cement flooring, grass and a water source. However, in PZN, *Panthera leo* specimens are kept in large groups on paddocks, which contain bare ground, also in PZN, *Caracal caracal* specimens are kept in bare ground enclosures with a small covered area.

The felines in both zoos are fed strictly protein. The cleaning of the enclosures occurs daily, both in RioZoo and PZN, and the feces are removed by the keepers in the morning with the help of a broom and dustpan. The anti-parasitic routine in PZN felines occurs every other month, the drugs being given with water or food. In RioZoo, the routine is usually performed when an animal has any clinical symptomatology compatible with gastrointestinal infection.

Overall, PZN has a higher number of feline specimens than RioZoo, especially *Panthera leo*. However, not all feline species are kept in both zoos. Table 1 shows the feline species present in both zoos.

Table 1. Number of felines in the Cuban and Brazilian Zoos

Common Name (Scientific Name)	PZN	RioZoo	Total
African Lion ( <i>Panthera leo</i> )	46	2	48
Bengal Tiger ( <i>Panthera tigris tigris</i> )	2	2	4
Siberian Tiger ( <i>Panthera tigris altaica</i> )	-	1	1
Jaguar ( <i>Panthera onca</i> )	6	1	7
Tiger cat ( <i>Leopardus tigrinus</i> )	-	2	2
Cougar ( <i>Puma concolor</i> )	7	1	8
Ocelot ( <i>Leopardus pardalis</i> )	-	2	2
Caracal ( <i>Caracal caracal</i> )	2	-	2
Leopard ( <i>Panthera pardus</i> )	7	-	7
Cheetah ( <i>Acinonyx jubatus</i> )	3	-	3
Jaguarundi ( <i>Herpailurus yagouaroundi</i> )	-	1	1
Total	73	12	85

PZN: National Zoological Park; RioZoo: Rio de Janeiro Zoo

To minimize animal stress and ensure feline welfare, the fecal samples were collected directly from the floor or ground of the enclosures, where the animals were kept. The fecal samples were collected during the morning, when cleaning the enclosures, always attempting to prioritize the collection of fresh fecal samples. All samples were collected by the Zoo keepers themselves. At the time of collection the samples were stored in new plastic bags without chemical preservative. The samples were finally stored in thermal boxes and immediately sent to the laboratory. The fecal samples from RioZoo were transported to the Laboratory of Parasitology at the Biomedical Institute, Fluminense Federal University. At the laboratory the samples were processed by centrifugal sedimentation technique as described by Ritchie (1948) and modified by Young et al. (1979) and by the sucrose solution flotation technique as described by Sheather (1923) modified by Huber et al. (2003). The fecal samples from PZN were taken to the Cuban National Laboratory of Parasitology and processed using the same techniques but without the modifications applied in Brazil.

Fecal samples were considered positive when felids evidenced developmental stages of parasites, including protozoa and/or helminths. Final results were expressed descriptively at the lowest possible taxonomic level. Parasitological results were also presented according to sex and age group.

## RESULTS

Of the 52 fecal samples collected, 10 (19.2%) were found positive for either a single or mixed parasite infection. The overall prevalence of gastrointestinal parasites was 17.5% (7/40) in PZN and 25% (3/12) in RioZoo. Fecal samples with the highest positivity for parasites were those from PZN, highlighting *Panthera pardus* and *Panthera onca* stools collected in enclosures and in RIOZoo *Panthera leo* stools also collected in enclosures. Different types of parasites were identified in this study. In PZN, hookworm eggs were the main evolutionary forms evidenced, followed by *Toxascaris leonina*. In RioZoo, the main evolutionary form detected was also *Toxascaris leonina*, followed by hookworm eggs, nematode larvae and eggs of the Diphyllbothriidae Family (Table 2). In both Institutions, *Toxoplasma gondii*-like oocysts and other protozoans were not detected in feline feces.

In general, multiple intestinal parasitism was evidenced only in three fecal samples, two in PZN and one in RioZoo. The association of hookworm eggs and *Toxascaris leonina* was detected in the two fecal samples from PZN, one sample from *Panthera leo* and the other from *Panthera pardus*. In RioZoo, only one fecal sample of *Leopardus pardalis* associated hookworm eggs, nematode larva and eggs of the family Diphyllbothriidae. In PZN, animals under five years of age were the most frequently affected (18.2%). However, in RioZoo the most affected age group was over 10 years old (40%). In both Zoos, the feces collected in the enclosures with male animals corresponded to the highest number of fecal samples positive for parasite evolutionary stages (Table 3).

Table 2. Evolutionary stages of parasites detected in feline feces in zoos in Cuba and Brazil

Zoo	Animals	Fecal samples	Positive samples	Nematode larvae	Hookworm eggs	<i>Toxascaris leonina</i>	Diphylobothriidae Family
PZN	<i>Panthera leo</i>	17	3 (17.6%)	-	1 (33.3%)	3 (100%)	-
	<i>Panthera tigris tigris</i>	2	-	-	-	-	-
	<i>Panthera onca</i>	5	1 (20%)	-	1 (100%)	-	-
	<i>Puma concolor</i>	6	1 (16.7%)	-	1 (100%)	-	-
	<i>Caracal caracal</i>	2	-	-	-	-	-
	<i>Panthera pardus</i>	5	2 (40%)	-	2 (100%)	1 (50%)	-
	<i>Acinonyx jubatus</i>	3	-	-	-	-	-
	Subtotal	40	7 (17.5%)	-	5 (71.4%)	4 (57.1%)	-
	<i>Panthera leo</i>	2	2 (100%)	-	-	2 (100%)	-
	<i>Panthera tigris tigris</i>	2	-	-	-	-	-
RioZoo	<i>Panthera tigris altaica</i>	1	-	-	-	-	-
	<i>Panthera onca</i>	1	-	-	-	-	-
	<i>Leopardus tigrinus</i>	2	-	-	-	-	-
	<i>Puma concolor</i>	1	-	-	-	-	-
	<i>Leopardus pardalis</i>	2	1 (50%)	1 (100%)	1 (100%)	-	1 (50%)
	<i>Herpailurus yagouaroundi</i>	1	-	-	-	-	-
	Subtotal	12	3 (25%)	1 (33.3%)	1 (33.3%)	2 (66.6%)	1 (33.3%)
	Total	52	10 (19.2%)	1	6	6	1

PZN: National Zoological Park; RioZoo: Rio de Janeiro Zoo

Table 3. Distribution of parasitic positivity evidenced in feline feces collected in zoos according to animal age and sex.

Variables	Investigated animals (n)	Animals in PZN		Animals in RioZoo	
		Positives (n)	Investigated animals (n)	Positives (n)	
Age range (years)	< 5	22	4 (18.2%)	0	0
	5 to 10	11	2 (18.2%)	7	1 (14.3)
	> 10	7	1 (14.3%)	5	2 (40%)
	Total	40	7 (17.5%)	12	3 (25%)
Sex	Female	14	1 (7.1%)	4	1 (25%)
	Male	26	6 (23.1%)	8	2 (25%)
	Total	40	7 (17.5%)	12	3 (25%)

PZN: National Zoological Park; RioZoo: Rio de Janeiro Zoo

## DISCUSSION

General frequencies in parasite evolutionary stages, 17.5% and 25% respectively, were detected in the analysis of fecal samples from felines in the zoos in Cuba (PZN), Havana and Brazil, Rio de Janeiro (RioZoo), including in different animal specimens. The few articles found in the literature that analyze feces from felines under human care in zoos showed that parasitic frequencies in PZN and RioZoo were lower than those from felines kept in other zoos. 62.5% positivity in general developmental stages of parasites, therefore higher than in this study, was reported by Aranda et al. (2013) in four zoos in Peru including *P. onca*, *P. concolor*, *L. pardalis*, *L. wieddi* and *L. tigrinus* feces. Also higher positivity was reported by Lim et al. (2008) in Malaysia, 54.5% when analyzing feces of *P. concolor*, *P. onca* and *P. tigris* and other feline species and by Müller et al. (2005), in zoos in Santa Catarina, Brazil, with 46% in Pomerode, including *P. tigris*, *P. leo*, *P. onca*, *P. concolor*, *L. tigrinus* and *L. wiedii* feces, and 64.3% in Brusque, including *Felis serval*, *P. onca* and *L. wiedii* feces.

Importantly, although parasitic positivity was lower in PZN and RioZoo than in other zoos, the diagnosis of these potentially parasitic agents is generally expected at any zoo. Since the captive feline, in a confined environment, may be reinfected with its own parasites by touching the floor, contaminated feeders or drinkers. Both PZN and RioZoo are located in tropical cities with hot and

humid climates, which are ideal for rapid development of parasite infecting structures in the environment, especially geohelminths. This fact may have favored the frequency, even though low, of these parasites in both zoos. Feces collected from PZN felines presented an even lower parasitic frequency than those from RioZoo. This demonstrated that the lower positivity in PZN may be related to the routine administration of antiparasitic drugs, which RioZoo performed only when the animals presented any clinical symptomatology.

Thin shell eggs, similar in size and morphology to hookworm eggs were other evolutionary shapes detected in feline feces in PZN and RioZoo. In RioZoo, thin-shelled eggs and larvae nematodes were also detected in fecal material from *L. pardalis*. In this study, the nematode larvae were not analyzed taxonomically, only catalogued. These developmental forms can be found in free-living stages of nematodes, since the feces were collected from the floor of the feline enclosure. However, in spite of the collection of fresh feces in this study, the fact that these larvae might be hookworms cannot be ruled out, since the city's climate is favorable to the development of this structure from eggs. Hookworm eggs in *L. pardalis* feces were also reported in a Zoo in Peru (Aranda et al, 2013) and in feline stools in zoos in Malaysia, Italy, Mexico, Paraná - Brazil (Lim et al., 2008; Fagiolini et al., 2010; Rendón-Franco et al., 2013; Snak et al., 2017).

Environmental contamination by hookworm evolutionary stages may favor felid reinfection, passively acquired by oral route, by filarioid larvae in food and water, by larval skin penetration, or by predation of paratenic hosts, such as rodents wandering into the feline enclosure and eventually being preyed upon. In fact, rodents have been seen circulating in both zoos. Although hookworm infections are usually asymptomatic, these parasites have been reported as infectious agents causing growth retardation in wild animals (Seguel & Gottdenker, 2017). Moreover, hookworms that infect the Order Carnivora can also be causative agents of biological Larva Migrans Cutaneous in humans. This fact highlights the care that keepers should take when cleaning these animal enclosures.

Apart from hookworm eggs, *Toxascaris leonina*, was also detected in the feces of Felidae, especially in samples of *P. leo*, in more fecal samples from PZN than from RioZoo. Eggs of this nematode were also noted in lion feces in zoos in other countries, such as Italy, Bangladesh and Nigeria (Fagiolini et al., 2010; Khatun et al., 2014; Adeniyi et al., 2015). Importantly, the roundworm eggs are very resistant to environmental conditions and may remain viable for long periods, even in the enclosures of such animals. Infection with *T. leonina* occurs by ingestion of larval egg or by ingestion of paratenic hosts with L3 encysted in tissues, such as rodents. The mechanism of vertical transmission to the young has not been described or experimentally proved. The most pathogenic effects reported in the host are symptomatic diarrhea enteritis (Epe, 2009). It is important to highlight that *P. leo* captivity in PZN seems



to favor the maintenance of this helminth in the pack, since lions are kept in groups on large paddocks with soil ground, which is the most suitable for the development of the *T. leonina* infective structure. In addition, the dirt ground impairs environmental hygiene, increasing the need for greater surveillance regarding gastrointestinal parasitic infections in this group of animals.

Eggs from the cestode family of Diphylobothriidae were only detected in the feces of *L. pardalis* from RioZoo. Eggs of this cestode have been reported in different species of captive Felidae in zoos in Bangladesh, Peru, Malaysia and Paraná-Brazil (Aranda et al, 2013; Khatun et al, 2014; Lim et al, 2008; Thawait et al., 2014; Snak et al., 2017). The eggs presented narrow rather than rounded ends, predominantly compatible with *Spirometra*. It is noteworthy that *Spirometra* has been the most frequently reported helminth in wild felines, especially when they are in the wild. Eggs with morphology similar to that evidenced in this typical *Spirometra* study were most commonly detected in small and medium-sized wild felids from the Serra dos Órgãos National Park in Rio de Janeiro (Dib et al., 2018). They were also quite recently observed in carnivore feces from the Itatiaia National Park, in Rio de Janeiro and Minas Gerais States (Dib et al., 2019). The biological cycle of these parasites requires the participation of intermediate hosts, such as aquatic copepods, freshwater fish and also amphibians or snakes. The presence of the parasitic structure in the feces collected from RioZoo may have occurred through the predation of an intermediate host that entered the feline enclosure by mistake, but the fact that the feline may have already been infected on arrival at the zoo cannot be ruled out. This considering that the anti-parasitic routine in RioZoo does not occur frequently.

Although no statistical analysis was performed due to sample size, it can be seen that most of the developmental stages of parasites detected in the feces of the felids in PZN occurred in animals under five years of age. However, in RioZoo, this variable could not be analyzed descriptively, as no individuals within this age group recovered. In both institutions, positivity for parasites was more evident in male felids. By analyzing the age and sex of felids in the zoo in Peru, Aranda et al. (2013), in contrast to this study, showed a higher frequency of parasitic structures in five to ten year old felids. However, as in PZN and RioZoo, in the Zoos in Peru, most parasites were observed in male felines. The higher parasitic positivity in younger animals in PZN may be linked to higher age-related susceptibility. The higher positivity in males in both zoos may be linked to the dominance of this sex in the enclosures. Generally, dominant male individuals roam all over the area, are in close contact with the food, utensils and other individuals in the enclosure, which increases the chances of infection by eggs, larvae, cysts or oocyst parasites, similar to that already pointed out by some research groups who analyzed intestinal parasites in non-human primates (Melfi & Poyser, 2007).

It is also noteworthy that evolutionary stages of protozoa were not detected in the feline feces. Although one of the purposes of this study was to detect *Toxoplasma gondii*-like oocysts, they were not found. The non-detection of protozoan oocysts in the feces of these animals does not exclude *T. gondii* infection, emphasizing the need for further research on this topic with wild felines, as well as constant monitoring through coproparasitological diagnosis.

At present, it appears that in both zoos there is still the need to implement a health program with a view to constant monitoring of parasites on the roster of felines and other animals, including routine parasitological diagnosis followed by treatment of the infected animals. This strategy would reduce the frequency of animals with severe symptomatology, as well as avoiding the indiscriminate use of antihelmintics.

## ACKNOWLEDGMENTS

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the professionals and technicians at the National Zoological Park of Cuba and at the Rio de Janeiro zoo who helped to collect the samples. Thanks also to the Instituto Biomédico (CMB), Universidade Federal Fluminense (UFF/RJ), Oswaldo Cruz Institute (IOC/Fiocruz) and to the postgraduate course in Tropical Medicine (IOC/Fiocruz).

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