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## Occurrence of *Cryptosporidium* spp. in wild animals living in the Cascavel city park, Paraná, Brazil

## Ocorrência de *Cryptosporidium* spp. em animais silvestres do Parque municipal de Cascavel, Paraná, Brasil

Alessandra Snak<sup>1\*</sup>; Felipe Gustavo Garcia<sup>2</sup>; Luis Eduardo da Silveira Delgado<sup>3</sup>; Silvia Cristina Osaki<sup>4</sup>

### Abstract

Digestive and respiratory tracts parasite's protozoan, *Cryptosporidium* spp. now-a-days is a major zoonotic agent, it causes self-limiting diarrhea, remaining in the body passively until the moment immune system decreases, leading to an increase in its multiplication in the mucosa and appearance of clinical signs. As there are few studies on cryptosporidiosis in wild free-living and captive animals, especially in Brazil, this study aimed to identify the presence of *Cryptosporidium* spp. in feces of captive animals in Cascavel, PR Municipal Zoo. Between 2011 and 2012 there have been four collections of bird feces and five mammalian feces totaling 65 and 118 samples respectively. Samples were sent to the laboratory of Veterinary Parasitology of Federal University of Parana. The feces were diluted, centrifuged and the pellets were used to make blades which were stained by the Ziehl-Neelsen modified method and observed under a microscope with 1000X magnification. Then the blades containing the oocysts were observed under a capture microscope, where they were measured. Mammals showed 49.15% and birds 44.61% of positivity. Oocysts' sizes ranged from 3,54µm to 5,81µm with an average of 4,32µm for birds and 3,11µm to 5,60µm averaging 4,63µm to mammals. As of yet, there isn't effective treatment against this parasite and considering that it's a zoonotic disease, preventive measures should be taken to prevent transmission to humans.

**Key words:** *Cryptosporidium*, wild animals, Ziehl-Neelsen, zoonosis

### Resumo

Protozoário que parasita o trato digestório e respiratório dos animais, o *Cryptosporidium* spp. é um importante agente zoonótico da atualidade, responsável por causar diarreia auto limitante, permanecendo no organismo de forma passiva até o momento em que há uma queda da imunidade, levando a um aumento na sua multiplicação na mucosa e aparecimento de sinais clínicos. Como há poucos estudos sobre a criptosporidiose em animais silvestres de vida livre e de cativeiro, especialmente no Brasil, esse trabalho teve como objetivo realizar uma pesquisa para identificar a presença de oocistos de *Cryptosporidium* spp. em fezes dos animais cativos no Zoológico Municipal de Cascavel, Paraná. No período de 2011 e 2012 foram realizadas quatro coletas de fezes de aves e cinco de fezes de mamíferos totalizando 65 e 118 amostras respectivamente. As amostras foram enviadas ao laboratório de parasitologia veterinária da Universidade Federal do Paraná, Setor Palotina. As fezes foram diluídas, centrifugadas e os sedimentos foram utilizados para a confecção de lâminas, que foram coradas pelo

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método de Ziehl-Neelsen modificado e observadas em microscópio com aumento 1000X. Em seguida as lâminas contendo os oocistos foram observadas em microscópio de captura, onde os mesmos foram mensurados. Os mamíferos apresentaram 49,15% de positividade e as aves 44,61% das amostras positivas. O tamanho dos oocistos variou de 3,54µm a 5,81µm com uma média de 4,32µm para aves e de 3,11µm a 5,60µm com média de 4,63µm para mamíferos. Como ainda não há tratamento eficaz para esse parasito e considerando que se trata de uma zoonose, medidas preventivas devem ser tomadas para evitar a transmissão para humanos.

**Palavras-chave:** *Cryptosporidium*, animais silvestres, Ziehl-Neelsen, zoonoses

## Introduction

*Cryptosporidium* spp. are protozoa that parasitize humans, domestic and wild animals, mainly inhabiting the gastrointestinal and respiratory tract (SAVIOLI et al., 2006; FAYER, 2010; PEREIRA et al., 2010; SANTIN, 2013). Currently, there are about 26 species of *Cryptosporidium* spp. accepted (ICZN) and 40 described genotypes infecting humans and / or animals (RYAN et al., 2014).

The main species with zoonotic characteristics are: *C. hominis*, *C. parvum*, *C. canis*, *C. felis*, *C. meleagridis* and more recently species *C. ubiquitum*, wherein the latter two species affect wild and domestic birds and deer respectively (LI et al., 2014).

Currently a large number of zoos, conservation parks and centers of wildlife rehabilitation have shown concern regarding the presence and degree of animals' contamination by parasites. The zoo environment, due to the concentration of different animal species in restricted spaces, associated with the stress of captivity and direct contact with the human being, becomes susceptible to infection by *Cryptosporidium* spp. (LUDWIG; MARQUES, 2011).

This disease may occur without the animal or person exhibit clinical signs, which enables to occur the environmental contamination without preventive measures (SHI et al., 2010), but in immunodeficient individuals, *Cryptosporidium* spp. is causing enterocolitis, diarrhea and cholangiopathy and can lead to death (XIAO et al., 2004; ARROWOOD; STERLIN, 1989; ABRAHAMSEN et al., 2004;

LALLO; BONDAN, 2009). The disease's diagnosis in environments such as refuges and zoos, where there are large numbers of animals in constant stress and contact with humans periodically, it is extremely important for animal health, and it is also especially important to human health.

## Material and Methods

This work was carried out in the Municipal Park Danilo Galafassi, known as Cascavel Municipal Zoo, which has an area of 17,91ha, and is part of the Municipal Park Paulo Gorski (Municipal Lake and Zoo Complex) with 111,26ha (Figure 1).

The Zoo currently has about 340 animals of about 68 species, which were acquired by barter of animals with other institutions, acquisitions of other zoos, donations overseen by Brazilian Institute of Environment and Renewable Natural Resource (BIERNR), births at the zoo itself and seizure of animals held by BIERNR and Forest Police. The zoo has about 37 species of birds, 23 mammals' species and eight species of reptiles (PORTAL DO MUNICÍPIO DE CASCAVEL, 2015).

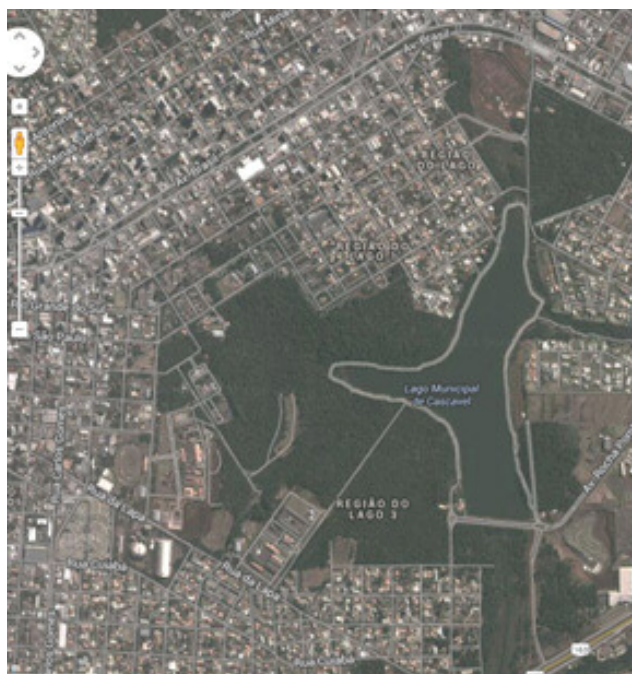
During the period of July 2011 to June 2012, five collections of mammalian feces were made, totaling 118 samples in 33 enclosures. Most of the carnivores were in individual enclosures, but the omnivores' was collective. In case of the birds, four collects were made, totalizing 65 samples in 20 enclosures, being all of these collectives.

Samples were collected from the ground avoiding contamination straw, sawdust, mud or other foreign material, being placed in clean containers and kept

under cooling, after that, about 5 g of feces were diluted in 40 ml of water and then passed through a tea strainer with gauze into another container, to the solid particles be removed. Then a small sample of the liquid was centrifuged for two minutes at 2.500 rpm, discarding the supernatant. Slides were prepared using the pellet.

After the blades were dried, the modified Ziehl-Neelsen staining method was performed (ORTOLANI, 2000). The blades were examined under a light microscope with magnification of 1000 times using the immersion objective and later on the oocysts were measured in capture microscope.

**Figure 1.** Municipal Park Paulo Gorski in Cascavel, Paraná.



Source: Google maps (2015).

## Results and Discussion

Table 1 summarizes results found for mammals. Out of the 118 analyzed samples from enclosures, 58 (49.15%) were positive for *Cryptosporidium* spp., that makes a high percentage when compared to other studies that investigated the presence of this parasite in several species of wild mammals, such as primates, sika deer, panda, wild boar, which found a positivity percentage of 3; 1.6; 15.6; 5.4 respectively (DU et al., 2015; WANG et al., 2008, WANG et al., 2015; ATWILL et al., 1997).

Three species of mammals had all negative

results: bush dog, lion and jaguar, since the animal with the highest rate of infection was collared peccary with 88.88%, followed by cambuta deer and brocket deer with 75%. These results may be due to feeding type, once that the bush dog, lion and jaguar are carnivores and the collared peccary, cambuta deer and brocket deer are herbivores, facilitating the ingestion of oocysts in the soil and the vegetation.

The oocysts measured between 3.11µm and 5.60µm, with an average size of 4.63µm (Figure 2A), similar results found by Xiao et al. (2002) in which the oocysts varied from 3µm to 8µm in size.

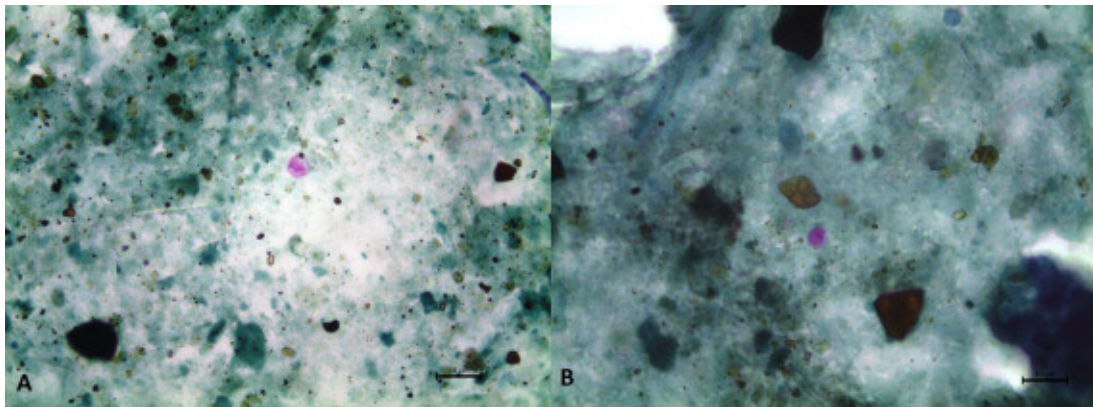
Primates showed high positivity. Out of the total of 31 analyzed stool samples, 18 (58.06%) were positive, results much higher than those found by other authors in different parts of the world, ranging from 0.5 to 27% (SAK et al., 2013;.

SALYER et al., 2012; PARSONS et al., 2015; EKANAYAKE et al., 2006). These authors claim that environmental changes caused by man and the use of these means by humans and animals provide a transmission of parasitic infection between wild and domestic animals and man.

**Table 1.** Results of mammals' samples of Municipal Park Danilo Galafassi processed by the Ziehl-Neelsen method.

Scientific name	Commom name	Number of enclosures	Samples	Pos.	% Pos.
<i>Panthera onca</i>	Jaguar	1	2	0	0
<i>Panthera leo</i>	Lion	5	14	0	0
<i>Puma concolor</i>	Cougar	3	9	6	66.7
<i>Puma yagouaroundi</i>	Jaguarundi	2	8	3	37.5
<i>Leopardus tigrinus</i>	Oncilla	2	6	3	50
<i>Leopardus pardalis</i>	Ocelot	1	3	1	33.3
<i>Panthera tigris</i>	Tiger	1	3	1	33.3
<i>Cerdocyon thous</i>	Crab-eating fox	1	2	0	0
<i>Eyra barbara</i>	Tayra	1	5	2	40.0
<i>Alouatta caraya</i>	Howler monkey	1	5	1	20.0
<i>Ateles paniscus</i>	Spider monkey	1	5	3	60.0
<i>Cebus sp.</i>	Capuchin monkey	4	17	12	70.6
<i>Callithrix sp.</i>	Marmoset	1	4	2	50.0
<i>Nasua nasua</i>	Coati	2	7	3	42.9
<i>Mazama nana</i>	Pygmy brocket	1	4	3	75.0
<i>Mazama gouazoubira</i>	Gray brocket	1	4	3	75.0
<i>Tayassu tajacu</i>	Collared peccary	3	9	8	88.9
<i>Tayassu pecari</i>	Lipped peccary	2	11	7	63.6
<b>TOTAL</b>		33	118	58	49.15

**Figure 2.** Oocysts of *Cryptosporidium* spp. stained by modified Ziehl-Neelsen method observed in increased 1000X. A) Positive stool sample of *Ateles paniscus* for *Cryptosporidium* spp.; B) Positive stool sample of *Amazona aestiva* for *Cryptosporidium* spp.



One howler stool sample was positive (20%), much lower than the percentage found by Silva et al. (2009) found that 100% positivity for this species in a research conducted using eight howlers of Santa Maria (RS). Ludwig and Marques (2011) analyzed the feces of some animals of Porto Alegre (RS) and also found *Cryptosporidium* spp. in howlers' feces. Yet Kowalewski et al. (2011) analyzed 90 feces samples of free-living howlers in Argentina and all were negative for *Cryptosporidium* spp.

Fifty percent of *Callithrix* sp. (marmoset) analyzed feces were positive, positive results right above those found by Lallo and Bondan (2009) who analyzed tamarins feces of deforested areas of Sao Paulo and all samples were negative.

Six out of nine cougar's feces samples analyzed (66.66%) found the parasite. Other authors also reported the presence of the parasite in cougar, as Fanfa et al. (2011), Cabral et al. (2001) and Ludwig and Marques (2011) in Santa Maria (RS), Uberlandia (MG) and Porto Alegre (RS) respectively.

The lipped peccaries showed high positivity in this study, similar to those found by Farret et al. (2010) in lipped peccaries in Rio Grande do Sul and Gómez et al. (2000) who analyzed feces of primates and herbivores of Barcelona Zoo, Spain, and found *Cryptosporidium* spp. oocytes in six primate species

and 12 herbivores, among them *Tayassu tajacu*, these data are similar to our work, which also was found oocysts in these animals.

Oncillas showed a positivity of 50%. Holsback et al. (2013) also found the protozoa in the same animal species in Mato Grosso do Sul and Southern Sao Paulo region.

The coati samples analyzed revealed a positivity of 42.85%, Farret et al. (2008) analyzed the feces of carnivores on Rio Grande do Sul and also found *Cryptosporidium* spp. in coatis. The authors report that the high frequency of parasitic diseases in captive animals may be due to the constant stress which these animals are subjected, because they live outside their natural habitat.

Regarding to captive birds, out of the 65 samples collected from enclosures, 29 were positive, corresponding to 44.61% of positivity (Table 2). Sevá et al. (2011) analyzed feces of 242 wild birds in São Paulo, Brazil and found 6.6% of positive samples for *Cryptosporidium* sp., a number lower than what was found in this study. The highest positivity frequencies found in our study may be due to the form of collection, which was made in pool of collective places, wherein the ideal, according to Qi et al. (2011) would make the individual collection to know actual prevalence.

**Table 2.** Results of birds' samples of Municipal Park Danilo Galafassi processed by the Ziehl-Neelsen method.

Scientific name	Common name	Samples	Pos.	Continue ...	
					%
<i>Cyanoliseus patagonus</i>	Burrowing parrot	3	3		100
<i>Baillonius bailloni</i>	Saffron toucanet	1	1		100
<i>Amazona aestiva</i>	Parrot	2	2		100
<i>Rhea americana</i>	Greater rhea	4	3		75
<i>Penelope ochrogaster</i>	Hestnut-belliend guan	4	3		75
<i>Pavo cristatus</i>	Indian peafowl	4	2		50
<i>Jabiru mycteria/ Cariama cristata</i>	Jabiru/ Red-legged seriema	4	2		50
<i>Polyborus plancus</i>	Caracara	4	2		50
<i>Ara ararauna</i>	Blue-and-yellow macaw	4	2		50
<i>Pionus maximiliani</i>	Scaly-headed parrot	6	3		50
<i>Pteroglossus castanotis</i>	Chestnut-eared aracari	3	1		33.3

... Continuation				
<i>Tyto alba</i>	Barn owl	4	1	25
<i>Ramphastos dicolorus</i>	Green-billed toucan	4	1	25
<i>Ciconia maguari</i>	Maguari stork	4	1	25
<i>Rhinoptynx clamator</i>	Striped owl	2	0	0
<i>Milvago chimachima</i>	Yellow-headed caracara	2	0	0
<i>Heterospiziasmeridionalis</i>	Savanna hawk	2	0	0
<i>Ara chloroptera</i>	Red-and-green macaw	2	0	0
<b>TOTAL</b>		65	29	44.61

After measuring the oocysts with a capture microscopy, it was seen that the size ranged from 3.54µm to 5.81µm, with an average size of 4.32µm (Fig 2B), which is consistent with the work of Xiao et al. (2002) in which the oocysts ranged from 3µm to 8µm in size.

The results showed a wide variation of positivity, from 100% for parrots, Saffron toucanet and Burrowing parrot, to 0% for Striped owl, Yellow-headed caracara, Savanna hawk and Red-and-green macaw. Qi et al. (2011) also conducted a work of identifying *Cryptosporidium* in birds and found a variation of positive results from 0 to 100%, depending on the species of the bird. These authors conducted species identification and found *C. baileyi*, *C. meleagridis*, *C. avium* and bird genotype.

The frequency of positivity for parrots was 73.33%, a number much higher than those found by Silva et al. (2008) who conducted a survey of captive parrots in Cachoeira do Sul-RS region and found 59.17% of positivity. But when we analyze the frequency of species, we found a higher prevalence for *Amazon Aesti* (100%) and lower for *Ara ararraunae* (50%) when compared to the results from our work. In a study by Rohela et al. (2005) with birds of a Malaysian, zoo it found *Cryptosporidium* in *Ara ararauna* and *Pavo cristatus*, as well as in our work. Different results to those found in this study were reported by Rohela et al. (2005) found oocysts in stool of *Ara chloroptera*, which was not found in Cascavel Zoo.

When we analyzed feces of parrots, we found a positivity of 50%, similar to Holsback et al. (2013) results, who also found *Cryptosporidium* spp. in samples of maritaca's feces in the State of Mato Grosso do Sul and São Paulo.

Regarding to greater rhea, *Cryptosporidium* spp. oocytes were found in 75% of samples analyzed. Results with lower positivity to those found in greater rheas were observed by Ludwig and Marques (2008) published the first report of *Cryptosporidium* sp. in these animals in the metropolitan region of Porto Alegre, with a positivity of 33.33%.

The oscillation of positive results can be due the elimination of oocysts that occurs in an intermittent way, periods only when the disease is active (immunosuppression) and usually when there is diarrhea.

This work has shown that there is a high prevalence of *Cryptosporidium* spp. in wild animals and how there is still no effective treatment, disease control is by preventive measures, aimed at breaking the chain of transmission, such as enclosures cleaning preventing access of other animals, oocysts research in water used for drinking, either animals as employees and visitors to the park, since water is the main route of transmission of this important zoonosis. Keepers, veterinarians and biologists should avoid direct contact with the feces of animals, use gloves to handle them and wash their hands thoroughly after the procedures done with animals.

## Conclusion

Oocysts of *Cryptosporidium* spp. were found in mammals, representing a 49.15% positivity. The species with the highest frequency of positivity were: *Mazama nana*, *Mazama gouazoubira*, *Tayassuta guan* and *Cebus* sp. The species *Panthera onca*, *Panthera leo* and *Cerdo cyonhous* were negative for the presence of the parasite.

Regarding the birds, the frequency of positive samples found was 44.61%. The species showing greater occurrence were *Amazona aestiva*, *Cyanoliseus patagonus*, *Bailloni* *bailloni*, *Penelope ochrogaster* and *Rhea americana* while the species *Ara chloroptera*, *clamator Rhinoptynx*, *chimachima Milvago* and *Heterospizias meridionalis* were negative.

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