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Prevalence of enteroparasites in primates kept at the Brasília Zoo, Brazil

Prevalência de enteroparasitos em primatas mantidos no Zoológico de Brasília, Brasil

Prevalencia de enteroparásitos en primates mantenidos en el Zoológico de Brasília, Brasil

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Lana Cristina Evangelista Ferreira Sá
ORCID: https://orcid.org/0000-0002-9887-4034
University of Brasília, Brazil
E-mail: lanaa.c@hotmail.com
Emanuel Messias Lima da Silva
ORCID: https://orcid.org/0000-0003-2609-6197
University of Brasília, Brazil
E-mail: emanueleco997@gmail.com
Cícera Vanessa Feitosa Morais
ORCID: https://orcid.org/0009-0005-7628-4964
Anhanguera College of Brasília, Brazil
E-mail: vanessa.12morais@gmail.com
Luiz Fernando de Castro
ORCID: https://orcid.org/0009-0004-5724-1099
Anhanguera College of Brasília, Brazil
E-mail: oluizfernando163@gmail.com
Crícia Rogéria Ribeiro Rocha
ORCID: https://orcid.org/0009-0000-9538-2926
Anhanguera College of Brasília, Brazil
E-mail: cricia.ribeiro@gmail.com
Iriani Rodrigues Maldonade
ORCID: https://orcid.org/0000-0003-2283-1270
Embrapa Hortaliças, Brazil
E-mail: iriani.maldonade@embrapa.br
Eleuza Rodrigues Machado
ORCID: https://orcid.org/0000-0002-2491-3862
JK College, Brazil
E-mail: eleuzarodriguesmachado498@gmail.com

Abstract
Parasitic infections are a primary disease affecting wild animals in captivity, which can cause morbidity and even mortality. The study aimed to identify the prevalence of enteroparasites at the Brasília Zoo, Brazil, and their possible risk factors. The research was conducted in 2019, and 37 primates from 19 species participated. Three stool samples were collected from the enclosures and were processed using three parasitological methods: (i) Spontaneous sedimentation, (ii) Rugai, and (iii) Kato-Katz method. Soil samples were collected from five points in each enclosure and processed using the Spontaneous sedimentation method. Water samples were collected from drinking fountains and analyzed for the presence of enteroparasites, using the Spontaneous sedimentation method, and for thermotolerant coliforms and *Escherichia coli* using the COLILERT microbiological method. The results showed that 54% of the stool samples were positive, mostly for the commensal parasite *Entamoeba coli*. All soil samples were contaminated, mainly by the pathogenic protozoan *Giardia* sp. The presence of thermotolerant coliforms was detected in all water samples. This was the first study carried out on the prevalence of intestinal parasites in primates kept at the Brasília Zoo. It is essential for the health and well-being of animals kept in captivity, parasitological monitoring, as the identification of these parasites helps in the treatment and monitoring of infections, in addition to avoiding infections of people who work directly with them.
Keywords: Primates; Parasitology; Microbiology; Enteroparasites; Intestinal parasites.

Resumo
As infecções parasitárias são uma das principais doenças que acometem animais silvestres em cativeiro, podendo gerar morbidade e até mortalidade. O objetivo do estudo foi identificar a prevalência de enteroparasitos em primatas mantidos no Zoológico de Brasília, Brasil e seus possíveis fatores de riscos. A pesquisa foi realizada no ano de 2019, participaram 37 primatas de 19 espécies. Três amostras de fezes foram coletadas dos recintos e foram processadas usando três métodos parasitológicos, (i) o de sedimentação espontânea, (ii) o de Rugai e (iii) o de Kato-Katz. As amostras de solo foram coletadas de cinco pontos distintos de cada recinto e processadas usando o método de sedimentação espontânea. As amostras de água foram coletadas dos bebedouros e analisadas para a presença de enteroparasitos, pelo método de sedimentação espontânea, e para coliformes termotolerantes e *Escherichia coli*

usando o método microbiológico COLILERT. Os resultados mostraram que 54% das amostras de fezes estavam positivas, sendo a maioria pelo parasito comensal *Entamoeba coli*. Todas as amostras de solos estavam contaminadas, principalmente pelo protozoário patogênico *Giardia* sp. A presença de coliformes termotolerantes foi detectada em todas as amostras de água. Esse foi o primeiro estudo realizado com prevalência de parasitos intestinais em primatas mantidos no Zoológico de Brasília. É essencial para a saúde e bem-estar dos animais mantidos em cativeiro, o monitoramento parasitológico, pois a identificação desses parasitos auxilia no tratamento e acompanhamento das infecções, além de evitar a infecções de pessoas que trabalham diretamente com eles.
Palavras-chave: Primatas; Parasitologia; Microbiologia; Enteroparasitos; Parasitos intestinais.

Resumen
Las infecciones parasitarias son una de las principales enfermedades que afectan a los animales salvajes en cautiverio. El objetivo del estudio fue identificar la prevalencia de enteroparásitos en primates mantenidos en el Zoológico de Brasilia, Brasil y sus posibles factores de riesgo. La investigación se realizó en 2019, participaron 37 primates de 19 especies. Se recolectaron tres muestras de heces de los recintos y se procesaron utilizando tres métodos parasitológicos, (i) el método de sedimentación espontánea, (ii) de Rugai y (iii) de Kato-Katz. Se recogieron muestras de suelo en cinco puntos diferentes de cada recinto y se tomaron muestras de agua de bebederos y se analizó la presencia de enteroparásitos por el método de sedimentación espontánea y de coliformes fecales y *Escherichia coli* por el método microbiológico COLILERT. Los resultados mostraron que el 54% de las muestras de heces fueron positivas, siendo la mayoría para el parásito comensal *Entamoeba coli*. Todas las muestras de suelo estaban contaminadas, principalmente por el protozoo patógeno *Giardia* sp. Se detectó la presencia de coliformes termotolerantes en todas las muestras de agua. Este fue el primer estudio realizado con la prevalencia de parásitos intestinales en primates mantenidos en el Zoológico de Brasilia. Es fundamental para la salud y el bienestar de los animales mantenidos en cautiverio, el seguimiento parasitológico, ya que la identificación de estos parásitos ayuda en el tratamiento y seguimiento de infecciones, además de evitar contagios de las personas que trabajan directamente con ellos.
Palabras clave: Primates; Parasitología; Microbiología; enteroparásitos; Parásitos intestinalis.

1. Introduction

The relationship between parasite-host is a gradual adaptation by matching a set of ecological, ethological, physiological, and biochemical characteristics, making the association balance. Unbalanced relationships can occur when the parasite proliferates unreasonably. This proliferation can lead to high parasite loads in the host, resulting in disease, which can affect reproduction, the quality of life of the host and, in many cases, lead to death (Stoner & González Di Pierro, 2006; Agostini et al., 2017; Becker et al., 2018).

The infection by enteroparasites in non-human primates can be linked to a series of factors such as (i) the way of feeding, (ii) the quality of water ingested, (iii) contact with feces from other animals, and (iv) contact with wild animals, domestic animals, and the man himself (Diniz 1997; Johnston et al., 2010; Zhou et al., 2019). Another very important factor related to parasitic infections in animals kept indoors is the conditions of the environment in which these animals are found, such as the humidity, temperature, and type of soil. These environments are usually more humid and warmer, an ideal climate for developing cysts and oocysts of protozoa, eggs, and larvae of helminths (Stuart et al.,1993; Adrus et al., 2018; Zhou et al., 2019; Shehani et al., 2022).

In wildlife, animals might have a natural resistance against parasitic infections or live in a balanced system with their parasites. Factors such as a change in environment and living conditions in captivity potentially influence an increase in the sensitivity to parasitic infections (Goossens et al., 2005), which can threaten wildlife population management and recovery programs and pose a health problem for species that are kept in zoos (Zhou et al., 2019; Cavallero et al., 2019). Despite that parasitic infections are one of the primary diseases that affect wild animals in captivity and, when left untreated can cause morbidity and even mortality, few studies have been dedicated to understanding the epidemiology of different parasitic diseases in wild animals in zoos (e.g., Andrade, 2002; Souza et al., 2019; Fia et al., 2014; Shen et al.,2019). Once in the zoo, enclosure animals are more prone to parasitic infections despite proper attention to controlling feeding, water, and hygiene maintenance (Kashid et al., 2002).

Studies on enteroparasites and ectoparasites in primates kept in zoos are scarce, however, understanding the prevalence of infections caused by intestinal parasites is of great importance for the study of animal health (Robertson et al., 2019; Schurer et al., 2019). In systems parasite-host relationships in primates, some studies report that species of parasites that infect non-human primates can cause infections in humans (e.g., Daszak et al., 2000; Robertson et al., 2019; Shen et al., 2019). For example, as protozoa: *Giardia* sp. (Robertson et al., 2019), *Balantidium coli* (Barbosa et al., 2015; Shen et al., 2019), *Entamoeba* spp. (Shen et al., 2019), and that some helminths: *Ascari*s spp. (Schurer, et al., 2019), *Oesophagostomum* sp., *Trichuris* sp., and *Strongyloides stercoralis* (Shen et al., 2019). Thus, there may be the possibility of sharing etiological agents between these individuals with humans.

Until this point, no research on enteroparasites in primates kept at the Zoo has been done; therefore, knowing which species of intestinal parasites infect these animals allows us to administer the appropriate dosage and dewormer for each pathogen, improve the quality of food and water offered to them, allowing these animals to have a better quality of life. In this context, the objective of this study was to verify the prevalence of enteroparasites in primates kept at the Brasilia Zoo, Brazil, and their possible risk factors.

2. Methodology

2.1 Study area and population

The research was conducted at Fundação Jardim Zoológico de Brasília (FJZB) in the year 2019. The project was submitted, evaluated, approved, and released by the Ethics Committee on Animal Use (CEUA/ FJZB), of the Fundação Jardim Zoológico de Brasília, on February 21, 2019 (Protocol No. 00196.000.02258/2018-47). Samples were collected from non-human primates kept in enclosures at the Brasilia Zoo. A total of 37 animals from 19 species were examined (Table 1).

Table 1 - Non-human primates were kept at the Brasília Zoo, DF, Brazil, in June 2019.

Scientific name	Popular names in Brazil	Enclosure	No. of individuals
Alouatta caraya	Bugio-preto	38	2
Alouatta belzebul	Bugio-de-mãos-ruivas	46	3
Alouatta puruensis	Bugio ruivo	44	2
Cebus albifrons	Caiarara-de-testa-branca	42	1
Chiropotes sagulatus	Cuxiú-marrom	75	1
Chiropotes satanas	Cuxiú-preto	75	1
Ateles marginatus	Macaco-aranha-de-testa-branca	70	1
Ateles chamek	Macaco-aranha-de-cara-preta	69	3
Ateles paniscus	Macaco-aranha-de-cara-vermelha	73	2
Lagothrix cana	Macaco-barrigudo	67	2
Aotus nigriceps	Macaco-da-noite	1	3
Sapajus libidinosus	Macaco-prego-do-nordeste	68	4
Saimiri boliviensis	Macaco-de-cheiro	43	1
Callicebus cupreus	Zogue-zogue	41	1
Saguinus imperator	Sagui-imperador	40	1
Saguinus bicolor	Sagui-de-coleira	2	3
Callithrix geoffroyi	Sagui-da-cara-branca	40	1
Leontopithecus rosalia	Mico-leão-dourado	1	2
Macaca fuscata	Macaco japonês	3	3

Source: Authors.

2.2 Collection of stool samples

A total of three fecal samples were collected from the enclosures where the animals lived. The samples were stored in individual, preservative-free, sterile plastic bottles (universal collectors) and identified with the collection date and the primate species. The samples were sent to Laboratório de Parasitologia Médica e Biologia de Vetores (UnB) and to Laboratório de Parasitologia da Faculdade de Anhanguera – Unidade Taguatinga (FAB), where they were processed and analyzed.

The samples were processed using three parasitological methods: the Spontaneous Sedimentation method (Hoffmann, Pons, and Janer, 1934), with this method it was possible to identify protozoan cysts and oocysts, and helminth eggs and larvae (Rey, 2008; Neves et al., 2018), the Rugai method (Neves et al., 2018), used to search for helminth larvae present in fresh feces, and the Kato-Katz method that was used to identify and quantify helminth eggs (Kato-Katz, 1972; Rivero-Rodríguez et al., 2000; Rey, 2008; Neves et al., 2018).

2.3 Collection of soil samples

The soil samples were collected from five different points in each enclosure. From each collection point, 50 g of soil was obtained and placed in clean, dry, and correctly identified plastic bags. They were then sent to Laboratório de Parasitologia of Faculdade Anhanguera de Brasília, where they were processed and analyzed using the spontaneous sedimentation parasitological method. The five samples from each enclosure were homogenized, obtaining 250 g of soil analyzed per enclosure. From each piece per enclosure, 20 slides were prepared, stained with Lugol's iodine, and examined by two different examiners using an optical microscope with objectives of 10x and 40x.

The degree of positivity of the evolutionary forms (cysts, protozoan oocysts, and helminth eggs or larvae) of the ectoparasites species found per enclosure was given as positive (+) or negative (-). The number of evolutionary forms found is represented by the number of + signs, being from one to five (+), from six to ten (++), and above 11 (+++).

2.4 Collection of water samples

The water samples were collected from the drinking fountains distributed in the animal enclosures. Three water samples were collected from each trough, with 2 liters each, and five days between collections. The water samples were stored in sterile glass bottles, adequately identified and sent to Laboratório de Parasitologia Médica e Biologia de Vetores (UnB), where they were processed and analyzed for the presence of enteroparasites, coliforms thermotolerants, and *Escherichia coli*.

The three liters of water collected were used separately to detect thermotolerant coliforms and *E. coli* using the Colilert microbiological method (Colilert Test Kit, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092, USA). For this, the samples were subdivided into 100 mL and placed in sterile, clear glass vials, then, a packet of the kit was added where it was homogenized with the water samples. Subsequently were incubated at 35±0.5 °C for 24 hours. As a negative control, 100 mL of milli-Q water was homogenized with a Colilert kit package. After 24 hours, the samples were observed in an Ultraviolet Light emitting source (Ultraviolet Led 390Nm For Hygialux - Kld).

3. Results

3.1 Prevalence of enteroparasites in non-human primates

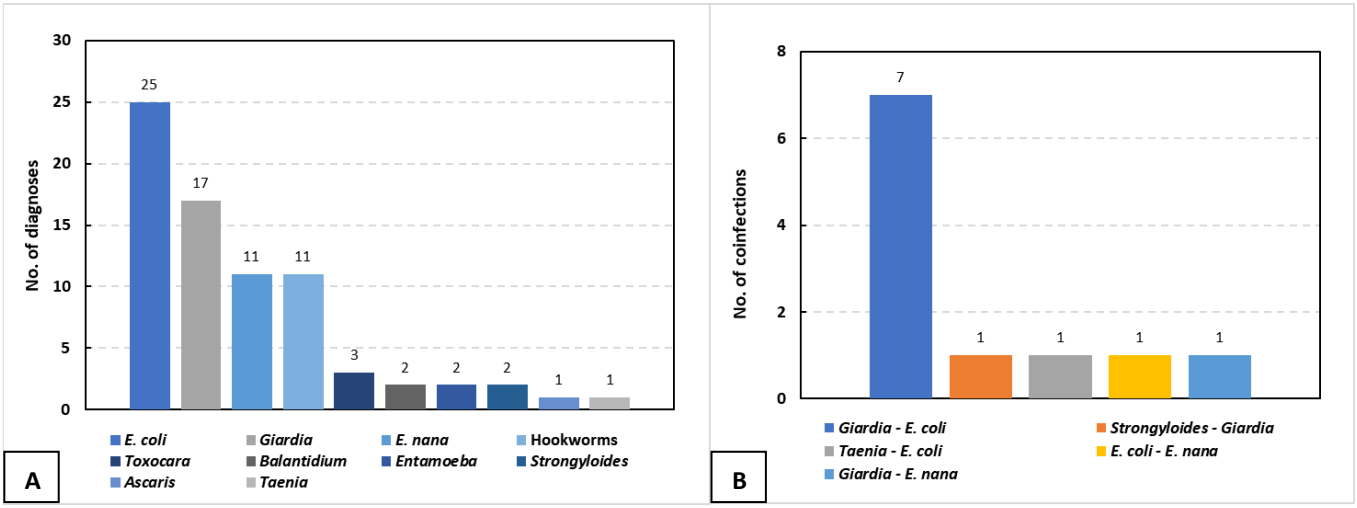
A total of 37 adult primates kept at the FJZB were included in the study, as shown in Table 1. All animals that participated in the study were dewormed with Ivermectin, Levamisole and Mebendazole according to the animal's weight four months before the beginning of the experiments.

A total of 114 samples were analyzed, 54% of which were positive for some intestinal parasite or commensals. The diversity of pathogenic and commensal intestinal parasites and their respective occurrences, in descending order were:

Entamoeba coli (25), *Giardia* sp. (17), *Endolimax nana* (11), Hookworms (11), *Toxocara* sp. (3), *Entamoeba* sp. (2), *Balantidium coli* (2), *Strongyloides* sp. (2), *Ascaris* sp. (1), *Taenia* sp. (1) (Figure 1A and 2).

Of the stool samples evaluated, 64.9% were found to be mono-parasitic and 14.8% biparasitic, with most coinfections being *Giardia* sp. and *Entamoeba coli* (Figure 1B).

Figure 1 - Pathogenic and commensal intestinal parasites that were diagnosed in primate stool samples at the Brasilia Zoo in June 2019.

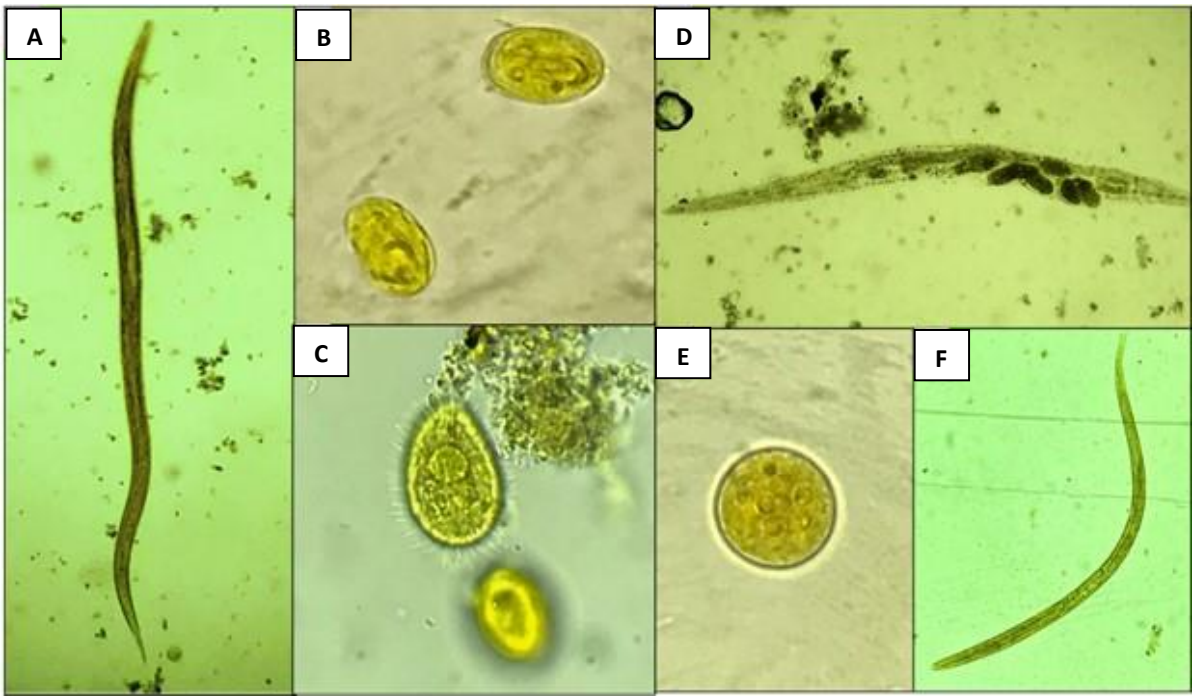


Source: Authors.

Of the diagnosed pathogenic intestinal parasites, the protozoan *Giardia* sp. stands out, with greater frequency, followed by helminths belonging to the Hookworms family. On the other hand, the animals showed a high level of commensal protozoa such as *E. coli* and *E. nana*.

As observed, there was an association between two pathogenic parasites: Giardia sp. with Strongyloides sp., two other *Giardia* sp. with the commensals: *E. coli* and *E. nana*, and an important association between Taenia sp. with the commensal E. coli, and finally an association between two commensal protozoa. As shown (Figure 1B) *Giardia* sp. was the intestinal parasite with the highest bi associations.

Figure 2 - Parasites detected in stool samples were recorded by light microscopy. (**A**) *Strongyloides* sp. larva, (**B**) *Giardia* sp. cysts, (**C**) *Balanditidum* sp. trophozoite (**D**) Hookworm female, (**E**) *Entamoeba coli* cyst, (**F**) Hookworm larva.

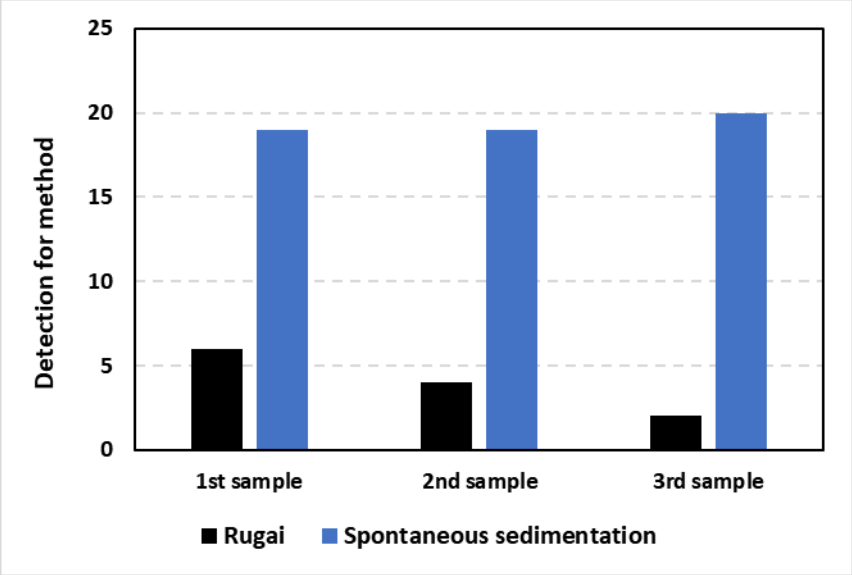


Source: Authors.

As shown in the figures (Figure 2), in (A) *Strongyloides* sp. filarioid larvae, due to the presence of a carved tail and filarioides esophagus, (B) *Giardia* sp. cysts, in which it is possible to visualize the morphological characteristics: oval shape, double membrane and two nuclei and axoneme, (C) *Balanditidum* sp. trophozoite with piriform shape, double membrane, presence of cilia and large nucleus, (D) Hookworm female, characterized by the presence of uterus with eggs, presence of sheath, (E) *Entamoeba coli* cyst, with presence of double membrane and five nuclei , (F) Hookworm filarioid larva, presenting filarioid esophagus, pointed tail, double membrane. Although other intestinal parasites have been diagnosed, their evolutionary forms are not shown.

Regarding the number of parasite cases diagnosed, considering the parasitological methods used in the research, it was found that the Spontaneous sedimentation method was the one that detected more evolutionary forms of intestinal parasites (59 cases), using the Rugai method 15 cases were detected and no cases were visualized using the Kato-Katz method, there were no differences between the different samples evaluated (Figure 3).

Figure 3 - Distribution of cases of intestinal parasites diagnosed in primate stool samples using Rugai and spontaneous sedimentation methods.



Source: Authors.

As pointed out in Figure 3, in both methods used evolutionary forms of intestinal parasites and commentaries were diagnosed, although the Spontaneous sedimentation method was significantly the most effective for detection of intestinal parasites.

3.2 Presence of enteroparasites in the soil in the enclosures

Of the 19 soil samples evaluated, all were found to be contaminated with some species of intestinal parasite mainly by the pathogenic protozoan *Giardia* and the commensal protozoans *E. coli* and *E. nana* (Table 2).

Table 2 - Distribution of the degree of infection by species of enteroparasites in the soils of the evaluated enclosures, in the Brasília Zoo, DF, in the year 2019.

		Soil Samples	
Area	**Enclosures**	**Protozoa**	**Helminths**
Iguanarium	Two (2)	*Entamoeba coli* (+++)	*Ascaris* sp. (+)
Feline	One (1)	*E. coli* e *Giardia* sp. (+++)	*Ascaris* sp. (+) Hookworms (+)
Micarium	Nine (9)	*E. coli*, *Endolimax nana*, *Giardia* sp., *Balantidium* sp. (+++)	*Ascaris* sp. (+) Hookworms (+) *Strongyloides* sp. (+)
Island 1	Three (3)	*E. coli*, *E. nana* e *Giardia* sp. (+++)	*Ascaris* sp. (+) *Toxocara* sp. (+)
Island 2	Four (4)	*E. coli*, *E. nana* e *Giardia* sp. (+++)	*Toxocara* sp. (+)

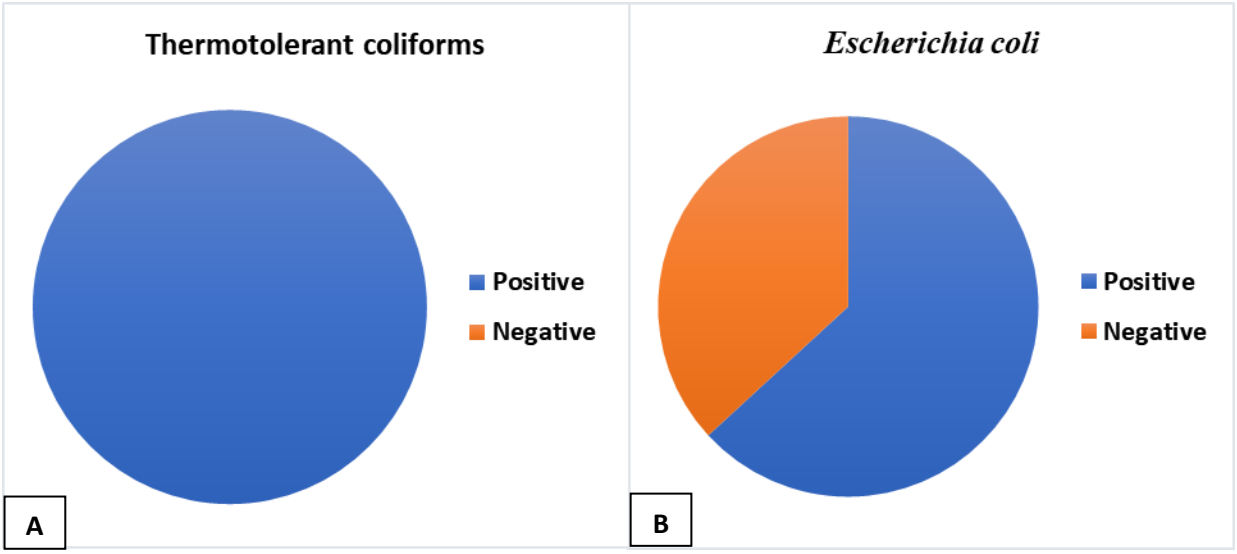
+ = Positive; sp. = Species. Source: Authors.

Regarding the areas where the animals were found (Table 2) and which had the highest number of species and load of enteroparasites and commensals diagnosed, they were Micarium and Island 1, when compared to the other three: Iguanarium, Feline and Island 2.

3.3 Water quality

Regarding the presence of thermotolerant coliforms and *Escherichia coli* bacteria, all samples examined were found to be contaminated with thermotolerants coliforms (Figure 4A), and 12 samples were positive for *Escherichia coli* (Figure 4B).

Figure 4 - Analysis of the water from the drinking fountains in the animal enclosures of the Brasília Zoo, as to the presence of thermotolerant coliforms (A) and *Escherichia coli* bacteria (B), in June 2019.



Source: Authors.

All analyzed water samples were positive for thermotolerant bacteria (Figure 4A), however, only 12 of them were positive for E. coli, the other samples were positive for other bacteria, which were not identified in this research.

4. Discussion

The non-human primates that participated in the study were diagnosed with eight species of intestinal parasites (four protozoa and four helminths). These results are similar to those described in the literature (Milozzi et al., 2012; Barbosa et al., 2019), which recorded six and seven parasite species, respectively. *Entamoeba coli* and *Giardia intestinalis* species were predominant in the samples analyzed, and these results corroborate data already found in the literature (Silva et al., 2009), and differ from another study (Santos, 2006), in which the highest prevalence was nematodes. *Ancylostoma* sp. and *Strongyloides* sp. are the most common helminths in primates (Stuart et al., 1993). Thus, the low prevalence of helminths in the samples of the present study may be related to the antiparasitic treatment that the zoo animals received four months before the collection of the fecal samples of the present study.

The most abundant pathogenic protozoan in the samples, which can be transmitted by contact with fecal waste from non-human primates, was the parasite *Giardia* sp., with detection in 15% of the positive samples. *Giardia* cysts are excreted in the feces and become infective in the environment, commonly transmitted by consumption of contaminated food or water or direct contact. In the case of symptomatic infections, the host may have severe giardiasis with severe diarrhea, potentially leading to weight loss, dehydration, cognitive impairment, and in more severe cases, death (Cogswell, 2007; Parr et al., 2013). Of the *Giardia intestinalis* strains or sub-strains at least two (Assemblies A and B) infect humans and non-human primates, among other mammals (Hunter & Thompson, 2005). Although only characteristic *Giardia* cysts were detected in the present study, we did not perform tests to verify which strains were infecting the animals.

In the present study, a low prevalence of *Balantidium coli* was also found in the individuals examined. These results corroborate findings in the literature, which argue that *B. coli* is the only pathogenic ciliate widely distributed in primates, however, the risk of human infection by this protozoan originating from non-human primates has not yet been demonstrated (Gillespie et al., 2010). However, there is a high possibility that these animals are a source of infection for humans, especially for those who work directly with them (Belova & Krylov, 1998; Parkar et al., 2010; Stensvold et al., 2009).

The methods used in the study are a rapid and inexpensive means of detecting parasite cysts, oocysts, eggs, and larvae and are noninvasive and valuable for studies in wild animals. A combination of methods was used to obtain more accurate results. The use of combined methods to detect, identify and quantify infections by helminths or gastrointestinal protozoa, increases the accuracy of laboratory diagnosis. Therefore, the association of techniques generates a better result (Menezes et al., 2013; Rodrigues et al., 2021). The spontaneous sedimentation technique, when compared to the Rugai and Kato-Katz techniques, proved to be more efficient in the diagnosis of evolutive forms of enteroparasites.

In most bacteriological analyses of water, bacteria from the coliform group act as indicators of fecal pollution, since they are always present in the intestinal tract of homeothermic animals (Fernandes et al., 2021). The detection of *Escherichia coli* is essential in microbiological analyses because it indicates fecal contamination, and because it is exclusive of fecal samples, thus reflecting on the hygienic-sanitary quality of the water (APHA, 2017). In the present study, all samples examined were contaminated by thermotolerant coliforms and 63% were positive for *Escherichia coli*. These results corroborate the data presented in the literature (Oliveira et al., 2022), in which all the enclosures evaluated showed positivity for total coliforms and *E. coli*.

Probably, these high values are due to contamination by the feces of the animals living there, in addition to the visit of other free-living animals to the enclosures. These feces, which may contain agents that cause diseases, as is the case of enteroparasitosis, are usually deposited on the ground and, from there, spread by rain, wind, and the very action of animals, which favors the contamination of the environment and the troughs used by them. Thus, these events also explain the high detection of parasites in the soil. Therefore, part of the soil contamination source comes from the housed animals' feces. Similar results were found in Guarulhos Zoo, São Paulo, Brazil (Souza et al., 2019; Fia et al., 2014).

Zoos are usually green areas, which helps to reduce the stress of animals, but access and complete sanitation and disinfection of the enclosures have become more complex. The possible sources of infection in these places are related to native rodents, wild birds that have access to captivity, and from residues present in the keepers' shoes, conditions that imply the maintenance of latent parasitic infections in the animals (Lasprilla et al., 2009; Snak et al., 2014). Results show that intestinal parasites are widely found in animals confined without proper hygiene measures, i.e., the low frequency of helminths recorded in non-human primates kept in captivity is due to the adoption of hygiene measures in the enclosures and proper management of these animals (Fagiolini, et al., 2010; Carmo & Salgado, 2003; Bichi et al., 2016).

Given the above, there should be frequent hygienization and disinfection of the enclosures and drinking fountains where the animals live, feed and drink water, in addition to the constant renewal of water in the lakes and installation of filters. It is also essential to incorporate routine monitoring to determine the risks to animal health and thus intervene and improve the management of the park and the quality of life of these and other animals that live in captivity.

5. Final Considerations

This was the first study on the prevalence of intestinal parasites carried out in non-human primates kept at the Brasília Zoo, Distrito Federal, Brazil.

The prevalence of infection in the non-human primates was high, considering that the individuals were treated four months before collecting the stool samples with the anthelmintics: Levamisole, Mebendazole and Ivermectin. These

anthelmintics administered had little or no effect on the protozoa: *Giardia* sp. and *Entamoeba* sp., 48.2% of the animals were infected with at least one of these intestinal parasites. The spontaneous sedimentation technique was the most efficient in detecting enteroparasite cysts, oocysts, eggs and larvae.

We suggest that stool examinations are routine for monitoring is essential for the health of animals kept in captivity since identifying these parasites helps in the treatment and follow-up of infections, improving the quality of life of these animals in zoo.

The study's main limitation was that in the enclosures containing more than one animal, we could not individualize the stool samples, which were analyzed according to the enclosure and not the individual. Therefore, we plan to conduct a more in-depth study with qualitative and quantitative molecular techniques and robust statistical tests to obtain a more accurate diagnosis.

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