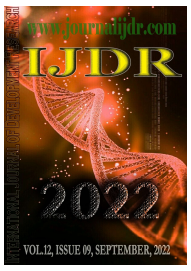
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 12, Issue, 09, pp. 58674-58678, September, 2022
https://doi.org/10.37118/ijdr.25318.09.2022



RESEARCH ARTICLE OPEN ACCESS

IDENTIFICATION OF PROTOZOA AND HELMINTHS IN FECES SAMPLES OF CAPYBARA (HYDROCHOERUS HYDROCHAERIS) THAT INHABIT THE LAGOA MAIOR IN TRES LAGOAS, BRAZIL

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ARTICLE INFO

Article History:
Received 07th July, 2022
Received in revised form
27th July, 2022
Accepted 08th August, 2022
Published online 20th September, 2022

Key Words:

Helminths, Protozoa, Zoonosis;
Parasitological Infectious.

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ABSTRACT

The capybara (*Hydrochoerus hydrochaeris*) a semi-aquatic generalist herbivores found in South American. The objective was to evaluate the presence of parasites in wild capybara species that inhabit the Lagoa Maior region in Tres Lagoas Brazil. Four stool samples were collected totaling 20 samples per collection (2 in dry periods and 2 in rainy periods). Stool samples were processed using standardized parasitological techniques and the observation and identification of the parasitic forms was carried out through optical microscopy and morphological and morphometric evaluation. Twelve gastrointestinal parasites were identified, 4 protozoa: *Neobalantidium coli* cysts, *Entamoeba* spp. cysts, *Eimeria* spp. oocysts, and cysts of *Giardia* spp.; and 8 metazoans, from the Nematoda classes: eggs of *Protozoophaga obese* (5%), eggs and larvae of *Strongyloides* spp. (51.3%), eggs of *Capillaria* spp. (*Echinocoleushydrochoeris*) (27.5%), eggs of *Trichuris* spp. (28.8%), eggs from members of the *Trichostrongyloidea* family (22.5%) and the order *Ascaridida* (27.5%); Trematoda: eggs of *Taxorchisschistocotyle* (28.8%) and Cestoda: eggs of *Monoecocestus* spp. (2.5%). The identification of these parasites is extremely important, due to the probability of infection among capybaras causing serious health problems and can be transmitted to both domestic animals and humans that frequent this environment.

Citation: ***Ketrin Ribeiro Fávaro, Mitzy Stephanny Machado, Ana Julia da Silva Rodrigues Carvalho-Leite et al.*** "Identification of protozoa and helminths in capybara (Hydrochoerus hydrochaeris) feces that inhabit Lagoa Maior in Tres Lagoas, Brazil*", International Journal of Development Research*, 12, (09), 58674-58678.

INTRODUCTION

The capybara (*Hydrochoerus hydrochaeris)* is the main representative of the *Hydrochoeridae* Family, native to South America, distributed from Panama to northern Argentina, it is considered the largest existing rodent, with up to 1.30 meters (m) in length and between 0.50 and 0.60 m in height. These animals can reach up to 100 kilograms (Kg) in weight, however, their average weight is 50 to 60 kg (Schai-Braun and Hacklander, 2016). Capybaras are generalist herbivores with a semiaquatic habit, where water is used for various purposes, from hydration, protection and as a thermal regulator; however, for food, these animals prefer to forage in non-flooded areas, with shrub cover, being also used for resting.

Therefore, these animals are characterized by living in hot regions and close to water bodies, with areas of grazing and forest cover. Capybaras are social animals that live in groups, but can also be found alone, living in isolation, outside of social groups. The number of members in the social group varies from six to sixteen members, consisting of adult males and females and their offspring (Vucetich *et al.,* 2012). Due to changes in their ecosystems, which have undergone anthropic changes in recent decades, with the development of crops and livestock, these animals have been increasingly moving to urban areas. Thus, the presence of these animals, in urban areas, allows a constant interaction with other animals, domestic or wild and also with man. In this sense, a particular interaction between humans, animals and the environment can lead to the emergence and transmission of zoonotic diseases and an increase in the transmission of pathogens of wild origin.

Thus, it becomes essential to monitor parasitic infections in synanthropic species such as capybaras within their natural habitats, to identify potential zoonotic parasites and their impact not only on human health, but also on domestic animals and endemic populations (Rahman *et al.,* 2020, Barrera-Ramirez *et al.,* 2019). Wild animals are hosts of a variety of parasites, which can act both as opportunists and as primary agents These pathogens can pose a threat to animal population management and recovery programs, especially for endangered species. The presence of intestinal parasites in wild animals affects their ability to survive, and may even be the primary cause of death, becoming an important factor to be considered in attempts to conserve free wild fauna. The main intestinal parasites found in these animals are related to the food and habits of the animals, being caused by a variety of protozoa and helminths. These parasites manifest themselves in the organism of living beings, causing several damages to the health of the infected host, and interfering in your quality of life (De Oliveria-Avelar 2015, Lin *et al.,* 2022). It is also highlighted that wild animals are reservoirs of parasites with zoonotic potential, which consequently can pose a risk to human health. In addition, because they have a high zoonotic potential, these parasites can infect humans who come into contact with their infective form (Rahman *et al*., 2020; Barrera-Ramirez *et al* 2019; Corriale *et al.,* 2011). According to the Municipality of Tres Lagoas – MS, Brazil, the Lagoa Maior, have a large population of capybaras, due to an area with great ecological diversity, formed by extensive and voluminous bodies of water, fields open and grazing areas. Therefore, due to the harm that the effects of intestinal parasites can have on the health of these animals and other animals that also live in this habitat or frequent this environment, including man, it is extremely important to carry out the mapping of the presence of protozoa and helminths existing in the group of capybaras that are in close contact with the population, generating local epidemiological information on the subject, but it is also important to build strategies for the prevention and control of parasitic infections, improving the quality of life these animals.

MATERIAL AND METHODS

The capybaras included in this study inhabit of Lagoa Maior, in Tres Lagoas, Brazil which is located in the urban area close to the downtown (Figure 1 and 2A).

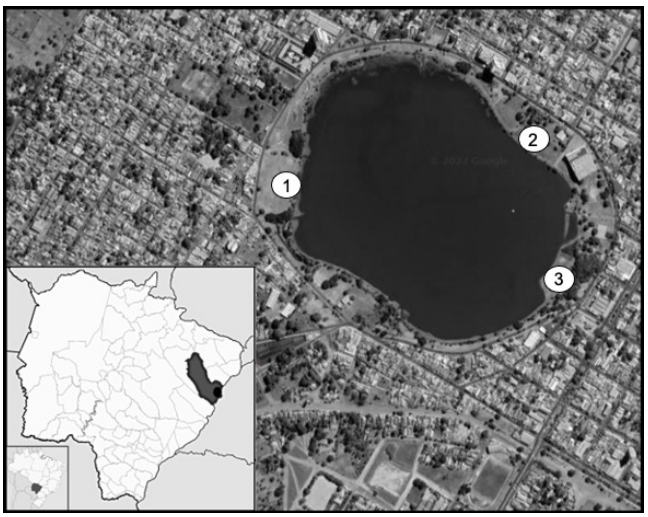

Figure 1. Location of Lagoa Maior in Tres Lagoas-MS, Brazil. The capybaras, objects of this study, inhabit the banks of the entire lagoon. Location of the 3 collection points

Four collections were carried out between April 2021 and March 2022, in an attempt to assess the presence of endoparasites at different times of the year, especially in dry periods (1st and 2nd collections) and rainy periods (3rd and 4th collection). All collections were carried out in the morning and carried out in 3 different regions around the lagoon, where the concentration of animals is visibly higher, and where they spend most of their time feeding. Region 1 is located near the coordinates of 20°46'59.3"S and 51°43'11.3"W; collection region 2 was located near the coordinates of 20°46'50.1"S and 51°42'52.9"W; and region 3 was located in an area of environmental preservation, located at coordinates 20°47'05.9"S and 51°42'48.3"W (Figure 1). Randomized collections were carried out, totaling 20 samples per collection.

Figure 2. Collection of samples: A. Some capybaras found inhabiting the Lagoa Maior region. B. Collection of fresh stool samples in sterile vials for each sample, containing preservation solution

The collections were performed using universal collection bottles, disposable and sterile material for each sample. The freshest feces were preferably chosen, which were placed in flasks containing preservative solution (M.I.F) (Figure 2B) In addition, it is worth mentioning that the collection of analysis material from these animals was carried out, without any physical or psychological aggression to the animal, following procedures standardized by International Guides for the Use of Wild Mammal Species (Sikes *et al.,* 2016). Because faeces collection is directly from the ground, after defecation, there is no need for approval by the Ethics Committee in animal research. After collection, the samples were identified and packed in thermal boxes and transported to the Laboratory of Immunology and Human Parasitology at the School of Medicine of Federal University of Mato Grosso do Sul. The samples were analyzed by standardized parasitological techniques: simple spontaneous sedimentation; flotation by centrifugation with zinc sulfate and sedimentation-float. The characteristics of the parasitological stages such as cysts, oocysts, and sporocysts, eggs and larvae were observed by optical microscopy, through the preparation of slides stained with lugol. Identification was performed through the morphology and morphometry observed according to identification guides or other articles on the same topic (Assis *et al.,* 2019; Cedrola *et al.,* 2018, Fagundes *et al.,* 2017).

RESULTS

After evaluating the 80 fecal samples, 12 gastrointestinal parasites were found, 4 protozoa belonging to the phyla: Ciliophora, Amoebozoa, Apicomplexa and Sarcomastigophora and 8 metazoans belonging to the phyla Platyhelminthes and Aschelminthes, classes Trematoda, Cestoda and Nematoda. Helminths identified in samples collected and their evolutionary forms were: *Protozoophaga obesa* (Ovo); *Strongyloides* spp. (Egg and Larva), Trichuris spp. (Egg), family: *Trichostrongyloidea* (Egg), *Capillaria* spp. (*Echinocoleus hydrochoeris*) (Egg), Order: *Ascaridida* (Eggs), *Monoecocestus* spp. (Egg) and *Taxorchis schistocotyle* (Egg). The protozoa found and their evolutionary forms were: *Neobalantidium coli* (Cist), *Entamoeba* spp. (Cysts), *Eimeria* spp. (Oocyst) and *Giardia* spp (Cyst). When analyzing the frequencies of the parasites detected, we observed that the collections carried out between October to December 2021 and January to March 2022 had the highest number of positive samples for parasites, however the collections with the lowest number of positive samples occurred between April and June 2021 and between July and September 2021. Also, analyzing the frequency of detection of parasites, throughout the period (year), the most frequent parasites were *Strongyloides* spp.

Table 1. Species of endoparasites and their evolutionary forms found in feces of capybaras (*Hydrochoerus hydrochaeris*), frequency of detection of parasites in each collection performed (number of samples) and in the total period and its percentage and parasites detected in others studies carried out in South America and their references

	Dry Period		Rainy Period				
	Apr. – Jun. 2021	Jul. – Set. 2021	Oct. – Dec. 2021	Jan. – Mar. 2022			
Parasites and forms detected	1st Collection	2nd Collection	3rd Collection	4th Collection	Total	Previous detection	Reference
Protozoophaga obesa (E)	2	0	0	2	5% (4/80)	A, Br, Bo, C, V	1-9
Strongyloides spp. (E/L)	8	9	11	13	51.3%(41/80)	A, Br, C	1-6, 8-10
Capillaria spp.(E)	4	4	6	8	27.5% (22/80)	A, Br, C	1-3, 5-7, 9-13
Trichuris spp.(E)	7	5	5	6	28.8% (23/80)	A, Br, C	2, 6, 14
Trichostrongyloidea (E)	5	3	4	6	22.5% (18/80)	A, Br, C	3, 5, 6, 9, 10
Ascaridida (E)	4	0	8	10	27.5% (22/80)	A, C	2, 5
Monoecocestus spp. (E)	0	0	0	2	2.5% (2/80)	A, Br, Bo, C, V	1-6, 9
Taxorchis schistocotyle(E)	4	3	9	7	28.8% (23/80)	A, Br, C, V	2, 4-6, 9
Neobalantidium coli (Cys)	2	1	1	3	8.8% (7/80)	A, C,	2, 5
Entamoeba spp. (Cys)	4	0	2	3	11.3% (9/80)	C	2
Eimeria spp. (Oo)	2	1	3	4	12.5% (10/80)	A, Br, Bo, C, V	1-3, 5, 10, 15-16
Giardia spp. (Cys)	0	0	0	1	1.3% (1/80)	C	16

Caption:E: eggs; L: larvae; Oo: Oocyst;Cys: Cyst; Fam: Family; Ord.Order; A: Argentina; Br: Brazil; Bo: Bolivia; C: Colombia; V: Venezuela. References:1. Alves e*t al.,* 2022,2. Uribe *et al.,* 2021,3. Castilho *et al.,* 2020,4. Souza *et al*., 2015,5. Corriale *et al.*, 2011,6. Sinkoc *et al.,* 2009,7. Wendt *et al.*, 2009, 8. Chiacchio *et al.,* 2004,9. Bonuti *et al.*, 2002, 10. Santos *et al.*, 2011,11. Sprenger *et al*., 2018,12. Del Rosário-Robles *et al.,* 2013, 13. Sinkoc*et al*., 2004,14. Esberhardt*et al.*, 2018, 15.Fagundes *et al.*, 2007, 16. Rodriguez-Durán *et al*., 2015.

(51.3%), followed by *Trichuris* spp. and *Taxorchis schistocotyle* (28.8%). Among the parasites with the lowest detection: *Giardia* spp (1.3%), *Monoecocestus* spp. (2.5%), followed by *Protozoophaga obesa* (5%) (Table 1).

DISCUSSION

Capybaras are animals with a long-life span, with high phenotypic plasticity and genetic variability, allowing them to easily and quickly adapt to environmental changes and the anthropized areas. This adaptive success in anthropized areas generates high population density, which can contribute to the infection of animals and increase transmission between them, for various pathogens, including enteroparasites. In addition, due to adaptation to anthropized areas and proximity to humans, they can pose a risk to human health, as well as to domestic animals. It is estimated that more than 60% of human infectious diseases are zoonotic, and wildlife reservoirs are the source of most emerging infectious diseases (Bovo *et al.,* 2016; Truppel, 2009; Sinkoc *et al.,* 2009; Taylor *et al.,* 2001). Many studies have shown that capybaras can be reservoirs of several zoonotic pathogens: endoparasites; ectoparasites (transmitters of spotted fever - *Rickettsia* spp.); bacteria and viruses (Farioski *et al.,* 2019; Luz *et al.,* 2019), which are of public health interest.

The literature on endoparasite infections in capybaras in South America is relatively extensive, with identifications of different species of protozoa and helminths in different periods and places, highlighting mainly studies carried out in Brazil, Argentina, Colombia, Venezuela and Bolivia, evaluating animals that inhabit urban areas or in rural areas (Table 1). The present study, revealed the presence of 12 gastrointestinal parasites: 4 of which belong to the phyla: Ciliophora, Amoebozoa, Apicomplexa and Sarcomastigophora and 8 metazoans belonging to the phyla: Ciliophora, Amoebozoa, Apicomplexa and Sarcomastigophora. to the phyla Platyhelminthes and Aschelminthes, classes Trematoda, Cestoda and Nematoda. We observed for the first time, in capybaras from urban regions of Brazil, the presence of protozoa of the species *Neobalatidium coli* and the genus *Entamoeba* spp., which had been reported only in populations of capybaras from Argentina and Colombia, which live in rural areas (Uribe et al., 2021, Corriale *et al.,* 2011,). It is important to note that within the genus *Entamoeba* spp., we have the species *Entamoeba histolytica* that causes amebiasis in humans, causing more than 100,000 deaths every year, through invasive intestinal infections or extraintestinal diseases, which can cause liver, lung and brain abscesses (Carrero *et al.,* 2020). *Neobalatidium coli* does not usually cause disease in immunocompetent humans, however, it can infect in pigs, rodents, horses, ruminants and non-human primates, which may explain the inter-transmission of this parasite between species (Ahmed *et al.,* 2020). In this study, despite having been found in a single sample, it was also possible to detect the presence of the genus *Giardia* spp. (Cyst) causing infection in capybaras in Brazil. Previously, this identification had only been observed in wild and rural capybaras in Colombia (Rodrigues-Durán *et al.,* 2015). Interestingly, an evaluation the presence of *Giardia* spp. in samples of capybara feces collected from the same locality, that this study, through *Nested*-PCR did not detect the circulation of this protozoan (Marta *et al.,* 2022). Previously, an evaluation of feces samples (n=53) of capybaras living in urban areas in Curitiba, Brazil also did not detect the genus *Giardia* spp. Thus, we suggest, that although these protozoa can be found parasitizing capybaras, these animals are not an important reservoir of this protozoan in urban areas. Similarly, as observed in others studies, the detection of this genus occurred primarily in the rainy season, which provides greater opportunity for this group of pathogens, due to the high intake of food by the animals (Rodrigues-Durán *et al.,* 2015). It was also detected protozoa *Eimeria* spp. (Oocysts) which was found in 12.5% of the analyzed samples. This protozoan belonging to the phylum Apicomplexa has been identified numerous times in capybara feces in studies carried out in Brazil, Argentina, Bolivia, Colombia and Venezuela (Alves *et al.*, 2022,Uribe *et al.*, 2021, Castilho *et al.*, 2020, Rodrigues-Durán *et al.*, 2015, Corriale*et al*., 2011; Fagundes *et al.*, 2007, Santos *et al*., 2011).

The occurrence of infection by *Eimeria* spp. is very common in animals bred in captivity, and may present clinical manifestations and even death, especially in young animals. Some studies have shown that infection by *Eimeria* spp. can affect up to 100% of animals living in wild areas, and is directly correlated with population density. Although there are no reports of human infection by the genus, this protozoan can infect other types of animals, such as birds, sheep, lambs, rabbits and rodents, can cause the death of these animals (Uribe *et al.,* 2021). Helminths were also detected in more than 95% of the capybara feces samples collected in the different periods, but with greater frequency and greater variety of parasites in the rainy periods. Some studies suggest that this high prevalence and diversity of endoparasites may be related to characteristic behaviors of these animals, such as life in society and the habit of grazing close to the ground, which can facilitate the contact and ingestion of helminth eggs and larvae from other already infected animals in the group. In addition, periods of greater rainfall led to an increase in pasture, and greater food intake, which increases the contact of these animals with endoparasites, increasing the possibility of contact with helminths (Truppel, 2009; Chiacchio *et al.,* 2004). Eggs of nematodes of the super family *Trichostrongyloidea* were the most frequently detected, where eggs and larvae of *Strongyloides* spp. were present in more

than 50% of the analyzed samples. Strongyloidiasis, infection by species of the genus *Strongyloides* spp. is still considered in many places as a neglected disease, and infected animals could contribute to the spread of the disease. It is noteworthy that although the species circulating in capybaras are different from the species of the genus that infect humans, immunosuppressed individuals could be infected by these other species (Krolewiecki and Nutman, 2019). Other genera of this family were also found, such as *Trichuris* spp. (28.8%) and *Capillaria* spp. (*Echinocoleus hydrochoeris*) (27.5%). Helminthological research has shown that the only species of the genus *Capillaria* spp. infects capybaras (*Capillaria hydrochoerid*) which can be found both in animals from natural and anthropic regions. This high rate of identification by these genera (*Strongyloides* spp., *Trichuris* spp., *Capillaria* spp.) can be explained by the high resistance of these eggs in the environment, which can remain viable for months. In addition, feeding close to the ground, as well as coprophagic habits, can facilitate ingestion and contact with these parasites (Truppel, 2009; Sinkoc *et al.,* 2009). It is noteworthy that eggs belonging to helminths of the super family *Trichostrongyloidea* have similar morphological characteristics, which often makes it impossible to accurately identify the genus or species, therefore, some eggs that had characteristics that grouped them within this family, but did not allow the identification of the genus or species, were identified only as belonging to this super family, being found in 22.5% of the analyzed samples. These parasites are often found parasitizing capybaras, being reported in animals that live in different regions of South America, in anthropized and natural areas (Alves *et al.,* 2022; Uribe *et al.*, 2021; Castilho *et al*., 2020; Eberhrdt, 2019, Sprenger *et al.*, 2018, Souza *et al.*, 2015, Del Rosário-Robles *et al.*, 2013, Corriale *et al.*, 2011; Santos *et al.,*2011; Sinkoc *et al.*, 2009,Wendt 2009, Chiacchio *et al.*, 2004, Sinkoc 2004, Bonuti *et al.*, 2002). Among the parasites of class Nematoda, it was also possible to detect eggs of the species *Protozoophaga obese* (*Oxyuridae*) in about 5% of the samples analyzed. This identification index is considered low, when compared to others studies, which found approximately 100% of the capybaras parasitized by this species (Sinkoc *et al.,* 2009). This infection can be detected in animals found living in wild populations in natural areas, in urban areas or even in captivity, and can be justified by the large number of eggs excreted in the feces during the infection, which allows many eggs to be ingested by other animals (Truppel, 2019, Chiacchio *et al.,* 2004,Sinkoc 2004). Eggs belonging to the order Ascaridida were also identified in 27.5% of the samples analyzed. Similar to the eggs of the *Trichostrongyloidea* superfamily, eggs of the order Ascaridida may have similar morphological characteristics, making it difficult to identify and accurately determine the genus or species only by morphological and morphometric characteristics. Analysis of feces samples from capybaras that inhabit the Orinoco River Basin in Colombia, showed that about 28% of the animals analyzed were infected by parasites of this order, suggesting that species belonging to this order are frequent parasites of capybaras (Uribe *et al.*, 2021). On the other hand, also observed the presence of parasites of the order Ascaridida in animals that inhabited the Esteros del Ibera in Argentina (Corriale *et al.,* 2011).

Eggs of helminths of the *Taxorchis schistocotyle* species (Class Trematoda) were also found in 28.8% of the samples analyzed. This species has a wide distribution and has already been described infecting capybaras that inhabit regions of Argentina, Brazil, Colombia and Venezuela, being found in samples of animals that inhabit wild or anthropic regions (Uribe *et al.,* 2021, Souza *et al.,* 2015, Corriale *et al.*, 2011, Bonuti *et al*., 2002). Other study has evidenced the presence of these parasites through necropsy studies and fixation of gastrointestinal material from wild capybaras from the Taim reserve, in Brazil (Taylor *et al.*, 2001). Recently, also detected the presence of *Taxorchis schistocotyle* eggs in capybaras inhabiting the Orinoco River Basin in Colombia through simple sedimentation techniques. This parasite has been associated with multifocal necrotizing colitis in capybaras, being an important cause of death among these animals (Uribe *et al.*, 2021). Cestoda class helminths were also found, belonging to the genus *Monoecocestus* spp. in 2.5% of the analyzed samples and only during the last collection, carried out in the period of greater intensity of rains. These parasites have also been described infecting capybaras that inhabit different regions of South America and several species of this genus have been reported: *M. hydrochoeri*, *M. hagmanni, M macrobursatum* and *M. jacobi* (Uribe *et al.*, 2021, Souza *et al.,* 2015, Corriale *et al.*, 2011, Sinkoc *et al*., 2009; Bonuti *et al*., 2002). Parasites of this genus use, as intermediate hosts, oribatid mites, where the development of cysticercoid larvae occurs. These mites inhabit the surface of the soil, vegetation and pastures of different environments, allowing the infection of these animals at the time of feeding. Finally, when analyzing the results, we observed an increase in the number of samples with detection of endoparasites in the third and fourth collection, which are periods with greater intensity of rainfall, which, as mentioned earlier, favors the supply of pasture and greater food intake, which increases the contact of these animals with endoparasites, increasing the infection. In addition, as these are gregarious animals, the infection of a portion of the animals will lead to the contamination of many others (Truppel, 2009, Sinkoc *et al.*, 2009).

CONCLUSION

The role of capybaras in the epidemiological chain of the parasites is relevant, as the eggs being released with the feces increase the potential risk for the transmission of diseases both among animals of the species, which can compromise the health, especially of the youngest animals, well as the infection of other animal species and humans. The Lagoa Maior is a tourist site in the city, where most residents exercise, have leisure time and even walk their dogs. It is worth mentioning that some of the helminths and protozoa identified in this study are of extreme importance, both veterinary (infection of other animals, mainly domestic) and medical (human infection), and may trigger cases of infectious diseases, such as amoebiasis, strongyloidiasis, giardiasis, trichuriasis, and others. In this context, the constant monitoring of the health of these animals through epidemiological survey of infection, not only by endoparasites, but for other zoonotic diseases is extremely important. In addition, it is important to carry out environmental education campaigns so that the population can be aware of the possible risks, but above all, respect the animals, their habitat and ensure that the competent bodies constantly monitor their health.

ACKNOWLEDGEMENTS

The authors are thankful to the Federal University of Mato Grosso do Sul, for providing the laboratory of Immunology and Parasitology facilities to work.

Declaration of interest: The authors declare no conflict of interests.

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