



## CRYPTOSPORIDIUM SPP. OOCYSTS AND GIARDIA SPP. CYSTS IN FAECES OF CAPYBARAS (*HYDROCHOERUS HYDROCHAERIS*) FROM CHICO MENDES NATURAL MUNICIPAL PARK, CITY OF RIO DE JANEIRO, BRAZIL: POTENTIAL RISKS FOR ZOOLOGICAL TRANSMISSION

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### ABSTRACT

Capybaras (*Hydrochoerus hydrochaeris*) are the largest rodents in the world. These animals live in groups and inhabit areas close to rivers, wetlands and lagoons and can live near areas inhabited by humans and domestic animals. Capybaras play an important role in the transmission of etiological agents of zoonoses, such as diseases caused by *Cryptosporidium* spp. and *Giardia* spp. This research aimed to investigate the presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in capybara faeces collected in the Chico Mendes Municipal Natural Park, located in the city of Rio de Janeiro, Brazil, evaluating

the possible zoonotic risks of diseases caused by these parasitic agents. A total of 30 samples of fresh capybara faeces were collected from different locations in the park. The samples were submitted to spontaneous sedimentation and Ritchie techniques, stained by Ziehl-Neelsen's technique and observed in light microscopy with 100X magnification for oocysts. *Giardia* spp. cysts were investigated by observing the sediment in preparation between slide

and coverslip, in which a drop of Lugol's iodine was added, and observed under light-field microscopy with the 40X objective. *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts were observed in all 30 samples, corresponding to a 100% prevalence of infection of these mammals for the investigated protozoa. This extreme rate of contamination is probably related to the continuous exposure of these animals to untreated sewage contamination. Soil contamination of the Chico Mendes Municipal Natural Park with capybara faeces, as well as the persistence of these animals as reservoirs of pathogenic protozoa, points to the risk of zoonotic transmission of *Cryptosporidium* spp. and *Giardia* spp. among the local fauna and humans that frequent this recreational site.

**KEYWORDS:** *Cryptosporidium* spp., *Giardia* spp., Capybara, Zoonosis.

## INTRODUCTION

Capybaras (*Hydrochoerus hydrochaeris*) are rodents belonging to the Family Caviidae and Subfamily Hydrochoerinae. They are the largest rodents in the world and their area of occurrence covers most of the South American continent, except for arid zones.<sup>[1]</sup> Capybaras lives in groups and inhabit areas close to rivers, wetlands and lagoons. The population of capybaras is increasing in nature and also in authorized captivities in some regions of Brazil.<sup>[2]</sup> These animals can live close to areas inhabited by humans and domestic animals, playing an important role in the transmission of etiological agents of zoonoses.<sup>[3]</sup>

*Cryptosporidium* is a Genus of protozoa belonging to the Filum Apicomplexa, Class Sporozoa, Subclass Coccidia, Order Eucoccidiida, Suborder Eimerine, and Family Cryptosporididae.<sup>[4,5]</sup> *Cryptosporidium* spp. is a protozoan Genus that was first described by Ernest Tyzzer, parasitizing the stomach of rats.<sup>[6]</sup> This researcher also found a similar parasite in 1912, but smaller in size, in the small intestine of the same host and named it *Cryptosporidium parvum*. *Cryptosporidium* infects cells of the respiratory and digestive tract of humans and animals, both immunocompetent and immunocompromised, and can be transmitted between humans, between animals, and zoonotically.<sup>[4,5]</sup> Water is also an important source of infection as *Cryptosporidium* survives for a long time in humid environments and it is resistant to chlorination and ozonation.<sup>[5,7,8]</sup> Warm and humid climates are favourable for the survival of *Cryptosporidium* spp. oocysts in the environment.<sup>[9]</sup> More than twenty species are currently recognized for the Genus *Cryptosporidium*.<sup>[10]</sup> Bamaiyi and Redhuan<sup>[11]</sup> considered *Cryptosporidium* to be one of the leading diarrhoea etiological agents in the world and the disease occurs in all classes of animals, including humans. This

protozoan expands rapidly among hosts and infects mainly immunocompromised people, especially HIV+ individuals. Pisarski.<sup>[12]</sup> estimates that cryptosporidiosis is one of the five most prevalent neglected zoonotic parasitic diseases in the world, and estimates that a quarter of children with diarrhoea suffer from *Cryptosporidium* infection, and the real extent and impact of the disease is unknown at present.

Cryptosporidiosis in wild mammals has been cited by several researchers, in most cases, it is related to *Cryptosporidium parvum* infection or similar genotypes. This protozoan species has already been diagnosed in several species and orders such as Arctiodactyla, Perissodactyla, Rodentia, Chiroptera, Didelphimorphia and Primates.<sup>[13,14,15,16]</sup> According to Zahedi *et al.*<sup>[17]</sup> *C. parvum* is often reported as a pathogen in a wide range of wild animals, including rodents, cattle, camelids, horses, canines, non-human primates and sea mammals. *Cryptosporidium parvum* is the species with the highest zoonotic potential that most frequently infects humans. Recent studies based on genetic, structural and biochemical data, point to a new systematic for the Genus *Giardia*. This protozoan is currently included in the Phylum Metamonada, Class Trepanonadea, Subclass Diplozoa, Order Gardiida and Family Gardiidae<sup>[18,19]</sup>. *Giardia* causes outbreaks of diarrhoea of food and water origin worldwide. Currently, *Giardia* spp. is classified into eight distinct genotypic groups, from A to H, where groups A and B are infectious to humans. The zoonotic potential of *Giardia* species is considerably high. Durigan *et al.*<sup>[20]</sup> analysed the genomic diversity of *Giardia duodenalis* in hospitals, veterinary clinics and environmental sources in the city of São Paulo, Brazil, and indicated that, besides to the endemicity of this pathogen in all examined sites, molecular analyses showed that most of the isolated microorganisms had genetic markers for zoonotic capacity. The elucidation of *Giardia* transmission dynamics between humans and animals depends on advances to disclose how genetic characteristics, population structures, host segregation and other factors influence the interspecific infectious capacity of this protozoan.<sup>[21]</sup>

Water transmission has great prominence in the epidemiology of *Giardia* spp. and it is still one of the major public health problems in the world.<sup>[22]</sup> The species of the Genus *Giardia* represent one of the most common causes of water transmission in humans and animals. The transmission to new hosts occurs through the ingestion of cysts present in contaminated water or food with faeces of infected human or animals.<sup>[18,19,22]</sup>

*Cryptosporidium* and *Giardia* have reputed as high relevant pathogens as they were included by the World Health Organization in the Neglected Diseases Initiative.<sup>[23,24]</sup> WHO also

consider *Cryptosporidium* and *Giardia* as reference pathogens for the evaluation of water potability: microorganisms that serve as a framework for the design and implementation of sanitary measures and targets for the treatment of drinking water with acceptable quality. Among protozoa, several species can be transmitted to humans through the ingestion of satisfactorily untreated water, such as *Cryptosporidium* spp., *Giardia* spp., *Entamoeba histolytica*, *Toxoplasma gondii*, *Balantidium coli*, *Cyclospora cayetanensis*, *Isospora belli* among others.<sup>[25]</sup> Most of these microorganisms infect humans and animals, having water playing an important role in this process, notably in the potential of zoonotic transmission of several pathogenic agents between animals that frequent water ambient, as capybaras, and human populations. Rivers, streams and lakes contamination with sewage carry pathogenic elements to the hydric media, infecting the resident fauna. Cryptosporidiosis and giardiasis of rodents can spread to humans and domestic animals in rural and urban areas through rodents that course in different environments, easing the dispersion of infections, mainly in high-density areas.<sup>[26]</sup> This way, infected animals act as zoonotic agent's hosts, persevering locally as contamination sources to animals, environment and surrounding human population, completing the biologic chains of pathogenic elements. Displacement of infected animals to other places expands the contamination foci of the pathogenic agents, infecting other animals and human populations. Currently, there is scarce data on the ecologic role of capybaras on the transmission of pathogens, especially zoonotic diseases.

This research aimed to investigate the presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in capybara faeces collected at Chico Mendes Municipal Natural Park, located in the city of Rio de Janeiro, Brazil, evaluating the possible zoonotic risks of diseases caused by these parasitic agents.

### **Study field**

The Chico Mendes Municipal Natural Park occupies an area of 40 hectares in the centre of Recreio dos Bandeirantes, west zone of the city of Rio de Janeiro. The surrounding area is one of the fastest-growing urban areas in the city in the last decade and the park is almost completely surrounded by middle-class buildings of up to three floors. To the northwest, the park borders the community of Terreirão, a subnormal conurbation with poor urbanization and infrastructure and no sewage treatment. The park's soil is sandy and is home of a diverse flora and fauna of restinga (a coastal tropical moist broadleaf forest). Approximately 12 hectares of the park is occupied by the smallest lagoon of the city, known as Tacha's lagoon

(“Lagoa das Tachas”). This lagoon, which is one of the great attractions of the park, is heavily polluted by urban sewage discharged both by the slums and some residential and commercial buildings not yet connected to the local sewage system <sup>[27]</sup>. The hypereutrophication of the lagoon’s waters and the Tachas channel due to the discharge of untreated sewage favours the accelerated proliferation of aquatic plants, especially water hyacinths (*Eichhornia crassipes*), which serve as food for capybaras. Tacha's lagoon communicates with Marapendi lagoon through the Tachas channel, forming an ecological passage, which connects to Barra da Tijuca beach through the Marapendi channel, which flows into the sea. Both Marapendi lagoon and Barra da Tijuca beach are used as entertainment places in their water bodies, for the practice of water sports, fishing and recreational bathing. The numerous groups of capybaras roam freely through all the areas of the park, approaching the visitors, and the faeces of these animals can be easily found along the trails of this Conservation Unit.

## MATERIAL AND METHODS

To acquire samples from different individuals, we opted to collect 30 samples of fresh capybara faeces from different places in the Chico Mendes Municipal Natural Park. The material was packed into individual plastic bags and sent to the Parasitic Diseases Research Laboratory of the UNIABEU University Center in Belford Roxo, Rio de Janeiro, Brazil. The samples were submitted to spontaneous sedimentation and Ritchie techniques. With part of the sediment, slides were prepared and after drying were fixed with methanol and stained by the Ziehl-Neelsen’s technique for observation of *Cryptosporidium* spp. oocysts. Subsequently, the slides were observed in light microscopy with 100X magnification, where oocysts of coccidia appeared in red on a blue background. The search for *Giardia* spp. cysts was performed by observing the sediment in preparation between the slide and coverslip, in which a drop of Lugol's iodine was added, and observed under light-field microscopy with the 40X objective.

## RESULTS

In all of the 30 examined samples of capybara faeces *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts were detected, corresponding to a 100% prevalence of infection of these mammals for the investigated protozoa.

## DISCUSSION

According to Zahedi *et al.*,<sup>[17]</sup> humans, wildlife and domestic animals potentially contribute to *Cryptosporidium* discharges that contaminate surface waters. Human invasion of natural ecosystems leads to increased interactions between human and animal populations. The growing number of zoonotic diseases is a consequence of anthropogenic intervention in many habitats. Water capture areas and water reservoirs are at the forefront of this conflict as they can easily be contaminated by zoonotic water-borne pathogens. Thus, the epidemiology of species of *Cryptosporidium* and *Giardia* with zoonotic attributes and the interaction between wild animals and humans in watercourses shared by these species is becoming increasingly important for public health.

According to Ferraz *et al.*,<sup>[28]</sup> the population of capybaras is much higher in man disturbed environments. This is due to the absence of predators and the availability of natural resources. As a semi-aquatic animal, it uses water bodies to move between neighbouring areas, and the dispersal of the species is facilitated by linking habitats through these watercourses. We believe that these migrations to other areas are factors that ease the dispersal of parasite elements to other environments. Thus, the population of capybaras infected by *Giardia* spp. and *Cryptosporidium* spp. is not restricted to the focus of the Chico Mendes Municipal Natural Park, being capable to make displacements towards neighbouring areas, following the channel to the Marapendi lagoon, reaching nearby streets and more distant areas, such as the Jacarepaguá lowlands, dispersing pathogens to other environments, animals and, possibly, human populations living in adjacent neighbourhoods.

Santos<sup>[29]</sup> affirm that *Giardia* and *Cryptosporidium* are protozoa of worldwide distribution whose epidemiology is important due to their zoonotic potential considering that wild animals are often reported as hosts of human giardiasis and cryptosporidiosis. Thus, the author performed a research to evaluate the occurrence and determine the genotypes responsible for infection in neotropical mammals. A total of 452 faecal samples were examined from 7 different sites out of a total of 52 different mammalian species. These samples were evaluated using microscopic diagnostic methods complemented by molecular techniques. Results showed an apparent prevalence of 6,2% for *Giardia* spp. and 4,8% for *Cryptosporidium* spp. The study revealed that 17 different species of wild mammals presented infection with these microorganisms, 11 for *Giardia* spp., 9 for *Cryptosporidium* spp. and 3 for both protozoa. The molecular characterisation revealed the predominance of

zoonotic genotypes in captive mammals and host-specific genotypes in free-living animals. The author considered that these findings demonstrated that wild animals can be infected by zoonotic and agent-specific genotypes, indicating the importance of researches involving this approach to verify possible relationships between protozoa, human hosts, domestic and wild animals and the interaction with different environmental variables. We highlight that the difference between the genotype profile found between captive and free-living animals verified by Santos<sup>[29]</sup> reveals the impact of the proximity between animals and humans on the predominance of strains with zoonotic capacity, and the risk that this contact may cause to both populations in the exchange of these pathogens.

A comprehensive health approach implies that the health of human populations are connected to the health of animals and the environment. Rodents, which are abundant and widely distributed, have been considered as the main hosts of *Cryptosporidium* infection for humans and other animals. However, there is a lack of information on global patterns of *Cryptosporidium* occurrence in rodents. The global prevalence of *Cryptosporidium* infection in rodents has been estimated to be around 17%, highest in North America and the Caribbean (27%) and lowest in South America (5%). These results highlight the role of rodents as hosts and reservoirs of *Cryptosporidium* strains capable of infecting humans, which highlights the need for increased attention to the implementation of control measures to reduce cryptosporidiosis as a public health threat and a zoonosis of global importance.<sup>[30]</sup> The observations made by Taghipour *et al.*<sup>[30]</sup> regarding the role of rodents as hosts and zoonotic control considerations are also applicable to rodents carrying *Giardia* spp. The results of our survey, where all samples were positive for *Cryptosporidium* spp. in capybaras, point to an atypical scenario when compared to the average prevalence for this pathogen estimated by Taghipour *et al.*<sup>[30]</sup>

A research on *Cryptosporidium* in capybaras in Brazil was conducted by Meireles *et al.*<sup>[31]</sup> The study was performed examining 145 fecal samples of free-living capybaras in the Province of São Paulo. The results evinced a positivity of 5,52% for the protozoan, and the zoonotic species *Cryptosporidium parvum*, identified through the PCR technique, was confirmed to all positive samples. The authors reported that this was the first reported case of *Cryptosporidium* in capybaras, and considered that *C. parvum* has zoonotic characteristics, pointing that the protozoan present in mammals living in semi-aquatic environments can contaminate the water for human supply with *Cryptosporidium* oocysts. The analysis of

Kvačič *et al.*<sup>[32]</sup> for the results reported by Meireles *et al.*<sup>[31]</sup> is that these animals were contaminated due to exposure to water polluted by human activities. We agree with the assessment of Kvačič *et al.*<sup>[32]</sup> and reinforce that infection by *C. parvum* in capybaras comprises a strong zoonotic risk component, since this species has a high capacity to infect humans, especially in environments that allow close contact between these animals and humans, remarkable conditions in the Chico Mendes Municipal Natural Park.

Researchers Sarmiento *et al.*<sup>[33]</sup> investigated the occurrence of *Cryptosporidium* species in captive capybaras in the province of Chaco, Argentina. The authors collected faecal samples directly from the rectum of 33 specimens and the material was examined in the laboratory of the Faculty of Veterinary Sciences - UNNE. The coproparasitological examinations were performed through Ritchie's technique and the sediments were stained by Ziehl-Neelsen's technique. Considering the morphometric characteristics of the found microorganisms and the affinity for the dye for alcohol-acid-resistant, researchers affirm that the characteristics correspond to the species *Cryptosporidium parvum*. This protozoan was present in 20 samples, corresponding to 60,6% of the total. These authors cited that this protozoan is not unique to the studied rodent, and can be a source of infection for any susceptible animal species, including humans. We believe that different degrees of integration between humans, capybaras and the environment can directly interfere with the gradation of infection by *Cryptosporidium* spp. The comparison between available research reveals contrasting scenarios: the prevalence of *Cryptosporidium* infection among capybaras living at the unhealthy environment due to sanitary sewage discharge verified in our research was 100%; the infection rate verified by Sarmiento *et al.*<sup>[33]</sup> in farms – environments where there is contact between humans and capybaras but with better sanitary conditions than those of the Chico Mendes Municipal Natural Park – displayed a prevalence of 60,6%; the incidence of *Cryptosporidium* among free-living animals investigated by Meireles *et al.*<sup>[31]</sup> was 5,52%.

Reginatto *et al.*<sup>[34]</sup> examined faeces of three capybaras (*Hydrochoerus hydrochaeris*) from a conservationist breeding farm in the city of Santa Maria, Province of Rio Grande do Sul, Brazil. The faecal samples were subjected to the zinc sulphate flotation and staining technique using the Kinyoun method. In all samples of capybaras, *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts were observed. The authors highlighted that this was the first record of infection by *Giardia* spp. in capybaras. Despite the small amount of samples examined by Reginatto *et al.*<sup>[34]</sup> these researchers found 100% positivity in capybara faeces



for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts, coinciding with the results obtained among capybaras from Chico Mendes Municipal Natural Park.

Rodríguez-Durán *et al.*<sup>[35]</sup> analyzed 360 faecal samples from a natural population of capybaras in the city of Arauca, Colombia. The total of positive samples for *Giardia* spp. was only 1,66%. The difference between the incidence rates for *Giardia* spp. and those obtained in our research suggests that populations in the natural environment, although susceptible to protozoan, present low prevalence rates, while capybaras inhabiting environments contaminated by human sewage discharges are subject to very high risks for *Giardia* spp. contamination.

In our research, the positivity rate for *Cryptosporidium* spp. and *Giardia* spp. in capybara faeces samples was 100%. We believe that the high infection rate is linked to the overpopulation of capybaras for the reasons given by Ferraz *et al.*<sup>[28]</sup> and mainly to the anthropogenic environmental intervention indicated by Zahedi *et al.*<sup>[17]</sup> The population of these semi-aquatic rodents in the studied region is continuously exposed to a high load of untreated sewage from a highly populated urban area, which makes them particularly susceptible to contamination and recontamination by several pathogens. Faeces of humans living in a large geographical area around the park that are contaminated with pathogens acquired from different sources – therefore, pathogens with greater genomic variability – mix and drain in a concentrated flux through the sewage to the lagoon inhabited by the capybaras. This fact explains a series of potential risks, especially the spread of pathogens' strains with zoonotic capacity according to the predominance of genotypes found in animals in closer contact with humans verified by Santos<sup>[29]</sup> Among the investigated Genera of protozoa in our research, we highlight the high probability that *Giardia* spp. of genotypic groups A and B, as well as *Cryptosporidium parvum*, which was the species most frequently found in capybaras, are part of the list of pathogens that find in the capybaras of Chico Mendes Municipal Natural Park a viable reservoir.

The capybaras of the Chico Mendes Municipal Natural Park roam throughout all area of the Conservation Unit. According to a survey conducted by Costa *et al.*<sup>[36]</sup> 45% of the visitors use the park as a recreation area for their children. The contact of children with the faeces of the capybara or with the sand contaminated by these faeces leads to the possibility of contamination by the ingestion of particles contaminated by *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts. The contamination of the water of the Tachas lagoon and its

contiguity through the channel carries *Cryptosporidium* spp. and *Giardia* spp. to the Marapendi lagoon, where recreational activities with contact between humans and the contaminated water are frequent. Also, the displacement of capybaras from the park to adjacent areas may expand the possibility of dispersal of these pathogens to other environments, animals and possibly human populations in nearby neighbourhoods. Such circumstances may be pointed out as zoonotic risk factors in the transmission of *Cryptosporidium* spp. and *Giardia* spp.

## CONCLUSION

The presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts were verified in all 30 examined samples of capybara faeces. This extreme contamination rate is probably related to the continuous exposure of these animals to untreated sewage contamination. Soil contamination of the Chico Mendes Municipal Natural Park with capybara faeces, as well as the persistence of these animals as reservoirs of pathogenic protozoa, point to the risk of zoonotic transmission of *Cryptosporidium* spp. and *Giardia* spp. between the local fauna and visitors that frequent the park.

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