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Cryptosporidium spp. and *Giardia* sp. in aquatic mammals in northern and northeastern Brazil

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ABSTRACT: *Cryptosporidium* and *Giardia* are protozoans that can infect humans and wild and domestic animals. Due to the growing importance of diseases caused by protozoan parasites in aquatic species, we aimed to evaluate the frequency of infection by *Cryptosporidium* spp. and *Giardia* sp. in aquatic and marine mammals in the northern and northeastern regions of Brazil. We collected 553 fecal samples from 15 species of wild-ranging and captive aquatic mammals in northern and northeastern Brazil. All samples were analyzed by the Kinyoun technique for identification of *Cryptosporidium* spp. oocysts. *Giardia* sp. cysts were identified by means of the centrifugal-flotation technique in zinc sulfate solution. Subsequently, all samples were submitted for direct immunofluorescence testing. The overall frequency of infection was 15.55% (86/553) for *Cryptosporidium* spp. and 9.04% (50/553) for *Giardia* sp. The presence of *Cryptosporidium* spp. was detected in samples from 5 species: neotropical river otter *Lontra longicaudis* (15.28%), giant otter *Pteronura brasiliensis* (41.66%), Guiana dolphin *Sotalia guianensis* (9.67%), Amazonian manatee *Trichechus inunguis* (16.03%), and Antillean manatee *T. manatus* (13.79%). *Giardia* sp. was identified in *L. longicaudis* (9.23%), *P. brasiliensis* (29.16%), pygmy sperm whale *Kogia breviceps* (100%), dwarf sperm whale *K. sima* (25%), *S. guianensis* (9.67%), *T. inunguis* (3.81%), and *T. manatus* (10.34%). This is the first report of *Cryptosporidium* spp. in *L. longicaudis*, *P. brasiliensis*, and *S. guianensis*, while the occurrence of *Giardia* sp., in addition to the 2 otter species, was also identified in manatees, thus extending the number of hosts susceptible to these parasitic agents.

KEY WORDS: Protozoa · Parasitic diseases · Zoonosis · Aquatic mammals · Conservation

INTRODUCTION

The territorial limits of Brazil contain a rich diversity of aquatic mammals, consisting of 59 species continuously or seasonally distributed in inland waters and ocean, marine, coastal, and estuarine environments (Rocha-Campos et al. 2010, ICMBio 2011).

On many occasions, these species live in the interfaces of environments where there is a significant interaction with human populations, rendering them vulnerable to changes and disturbances of anthropogenic origin (Rocha-Campos et al. 2010), such as incidental or intentional catches (Ott et al. 2002, Pontalti & Danielski 2011), watercraft collisions (Flamm

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& Braunsberger 2014), physical, chemical, and organic contamination (Yogui et al. 2003), intense degradation of habitats (Amaral & Jablonski 2005), and occurrence of pathogens of viral (Sierra et al. 2015), bacterial (Vergara-Parente et al. 2003, Delport et al. 2015), and parasitic origin (Barbosa et al. 2015).

Inherent to parasitic diseases, infections caused by *Cryptosporidium* spp. and *Giardia* sp. (Deng et al. 2000, Appelbee et al. 2010), considered as opportunistic zoonotic agents (Xiao et al. 1998, Fayer et al. 2004), have been reported in several species of cetaceans on the European Atlantic coast and in Brazil (Altieri et al. 2007, Reboredo-Fernández et al. 2015), sirenians in Australia and Brazil (Hill et al. 1997, Borges et al. 2011), and pinnipeds in the northern California (USA) coastal region (Deng et al. 2000).

Currently, 26 *Cryptosporidium* species are considered valid, and over 40 genotypes or cryptic species have been identified (Ryan et al. 2014). Several *Cryptosporidium* species present in humans and terrestrial mammals have been identified in aquatic mammals (Delport et al. 2014). Infections of *G. duodenalis* are reported in high frequency in aquatic and marine mammals (Delport et al. 2014), and the assemblages A, B, C, D, and F have been identified in pinnipeds and cetaceans (Dixon et al. 2008, Lasek-Nesselquist et al. 2008, Appelbee et al. 2010, Reboredo-Fernández et al. 2014).

The transmission of these pathogens can easily occur by ingestion of contaminated food and water, representing a risk for exposure and infection of aquatic mammals, which may present as clinical or subclinical manifestations (Deng et al. 2000, Borges et al. 2011).

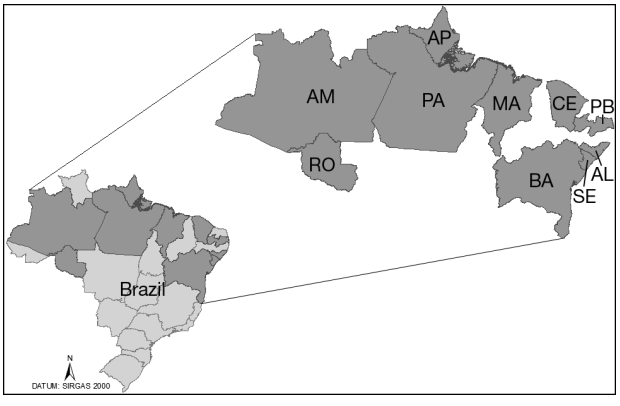

Fig. 1. Brazilian states where fecal samples were collected from 15 species of aquatic mammals: Amapá (AP), Amazonas (AM), Pará (PA), Rondônia (RO), Alagoas (AL), Bahia (BA), Ceará (CE), Maranhão (MA), Paraíba (PB), Sergipe (SE)

The possibility of occurrence of these protozoans with no clear clinical signs reinforces the need to survey and monitor the health of aquatic mammal populations. In addition to monitoring aquatic mammals in the wild, these parasites are also critical to monitor in rehabilitation and captive animals to avoid the inadvertent spread of these pathogens to the environment by way of introduction of animals intended for reintroduction programs (Kimber & Kollias 2000).

Due to a potential increased impact of these protozoans in aquatic species, we aimed to evaluate the frequency of infection by *Cryptosporidium* spp. and *Giardia* sp. in aquatic and marine mammals in northern and northeastern Brazil.

MATERIALS AND METHODS

Study areas

Sampling of aquatic mammals took place in 4 states of northern Brazil — Amapá (Jari River), Amazonas (Amanã, Mamirauá, and Tefé Lakes), Pará (Araticum and Saracá Creeks, Sapucuá Lake, and Tapajós River), and Rondônia (Madeira River) — as well as in 6 states of northeastern Brazil, along the coasts of the states of Alagoas (Pontal do Peba), Bahia (Sítio do Conde and Mangue Seco), Ceará, Maranhão (Turiaçu), Paraíba, and Sergipe (Fig. 1).

The northern region of Brazil lies within of the Amazon biome and consists of large extensions of rainforest, floodable lowland areas, igapó forests, and upland forests (Ayres et al. 2005). These environments constantly undergo changes due to rainfall, whereby flooding and overflowing of rivers usually occur between November and June, while ebb and drought occur between late June and early November (Goulding 1997, Lima 2009).

In contrast, the coastal portion of the northeastern region of Brazil experiences a hot and humid tropical climate, characterized by the absence of summer rain and a rainy season in the winter months (Golfari et al. 1978). This area consists of a great diversity of marine ecosystems, including beaches, dunes, cliffs, coral reefs, estuaries, and mangroves (Cunha 2005, Muehe & Garcez 2005).

Collection of biological samples

The collection of biological material occurred between 2011 and 2015, both in the rainy season and

the dry season, totaling 553 fecal samples from 15 species of aquatic mammals, representing Mustelidae, Cetacea, and Sirenia. The fecal samples obtained were from both marine and freshwater species, as well from wild (fecal samples found floating free on surfaces of rivers, resting places, latrines, and necropsy of stranded animals) and captive animals (during the handling procedures) (Table 1).

Fecal material was preserved in a solution composed of alcohol, formaldehyde, and glacial acetic acid (AFA), according to Ueno & Gonçalves (1994), and sent for laboratory processing at the Parasitic Diseases of Domesticated Animals Laboratory of the Federal Rural University of Pernambuco.

Research was conducted under System for Authorization and Information on Biodiversity (SISBIO) permit no. 33819-1 and approved by the Ethics Research Committee of the Federal Rural University of Pernambuco (010/2014).

Laboratory processing

All samples were submitted for formol–ether sedimentation analysis with subsequent preparation of smears and staining by the Kinyoun technique (Brasil Ministério da Saúde 1996) for identification of *Cryptosporidium* spp. oocysts. The presence of *Giardia* sp. cysts was investigated by the centrifugal-flotation technique in zinc sulfate solution (Appelbee et al. 2010, Bica et al. 2011).

Subsequently, all samples were submitted for direct immunofluorescence testing according to the Merifluor® *Cryptosporidium/Giardia* kit (Meridian Bioscience), and cysts and oocysts were identified based on their shape, size, and immunofluorescence intensity pattern (Reboredo-Fernández et al. 2015). All procedures were performed in the Parasitic Diseases of Domesticated Animals Laboratory of the Federal Rural University of Pernambuco.

Table 1. Origin and number of fecal samples from the 15 species of aquatic mammals used in this study. PA: Pará, AP: Amapá; RO: Rondônia; AM: Amazonas; BA: Bahia; SE: Sergipe; AL: Alagoas; PB: Paraíba; CE: Ceará; MA: Maranhão

Species	Habitat	No. of samples by region/state										Origin of samples	Total no. of samples
		Northern				Northeastern							
		PA	AP	RO	AM	BA	SE	AL	PB	CE	MA		
Mustelids													
Lontra longicaudis	River	33	230	38	12	–	1	–	–	–	–	Resting places, dens, latrines, rehabilitation enclosure	314
Pteronura brasiliensis	River	6	8	9	1	–	–	–	–	–	–	Resting places, dens, latrines	24
Cetaceans													
Balaenoptera acutorostrata	Sea (oceanic)	–	–	–	–	–	–	–	–	–	2	Necropsy	2
Grampus griseus	Sea (oceanic)	–	–	–	–	–	–	–	–	1	–	Necropsy	1
Inia geoffrensis	River	–	–	–	2	–	–	–	–	–	–	Free-living individuals under restraint	2
Kogia breviceps	Sea (oceanic)	–	–	–	–	–	1	–	–	–	–	Necropsy	1
Kogia sima	Sea (oceanic)	–	–	–	–	–	2	–	–	2	–	Necropsy	4
Peponocephala electra	Sea (oceanic)	–	–	–	–	–	3	2	–	3	–	Necropsy	8
Physeter macrocephalus	Sea (oceanic)	–	–	–	–	–	2	–	–	–	–	Necropsy	2
Sotalia guianensis	Sea (coastal)	–	–	–	–	5	21	4	1	–	–	Necropsy	31
Stenella attenuata	Sea (oceanic)	–	–	–	–	–	1	–	–	–	–	Necropsy	1
Stenella clymene	Sea (oceanic)	–	–	–	–	1	1	–	–	–	–	Necropsy	2
Ziphius cavirostris	Sea (oceanic)	–	–	–	–	–	1	–	–	–	–	Necropsy	1
Sirenians													
Trichechus inunguis	River	88	–	–	43	–	–	–	–	–	–	Floating samples collected in feeding areas, captive animals	131[a]
Trichechus manatus	Sea (coastal)	–	8	–	–	–	2	–	18	1	–	Captive animals, reintroduced animals, necropsy	29[b]

[a]131 samples (43 from captive animals; 88 wild animals); [b]29 samples (9 from captive animals; 20 wild animals)

Samples were considered positive when 1 of the methods used allowed for the identification of *Cryptosporidium* spp. oocysts or *Giardia* sp. cysts (Borges et al. 2011).

Data analysis

To investigate the association of protozoa, i.e. *Cryptosporidium* spp. and *Giardia* sp., with other factors such as climatic period (dry or rainy), environment (river or sea), animal maintenance condition (captive or wild), and sampling sites, Pearson's chi-squared test was used for each variable pair. In cases where the expected chi-squared values were lower than 5 in more than 20% of frequencies, Fisher's exact test was performed (Quinn & Keough 2002). All analyses were performed using the R program (R Core Team 2015).

RESULTS

Among the 15 species examined, the frequency of infection was 15.55% (86/553) with *Cryptosporidium* spp. and 9.04% (50/553) with *Giardia* sp. *Cryptosporidium* spp. were detected in 5 species and *Giardia* sp. cysts were identified in 7 sampled species. Co-infection of *Cryptosporidium* spp. and *Giardia* sp. was observed in 4 species (Table 2).

In the assessment performed for the different habitats, the percentage of *Cryptosporidium* spp. infection was higher in species found in river environments (17.40%) compared to those found in both coastal and marine environments, but this difference was not significant (8.53%; $\chi^2 = 3.7603$, df = 1, p = 0.05248). Conversely, the number of cases with *Giardia* sp. was higher in animals found in marine environments (9.75%), compared to those found in river environments (8.91%), but also with no significant statistical difference ($\chi^2 = 0.3535$, df = 1, p = 0.5522).

Among marine mammals, infection with *Cryptosporidium* spp. was higher in coastal (11.66%) than oceanic species (0%). On the other hand, the presence of *Giardia* sp. was more frequent in oceanic (40%) compared to coastal species (11.66%). Infections caused by *Cryptosporidium* spp. ($\chi^2 = 0.0138$, df = 1, p = 0.9066) and *Giardia* sp. ($\chi^2 = 0.4217$, df = 1, p = 0.5161) occurred independent of season (dry or rainy period). Similarly, there was no relationship between the presence of *Cryptosporidium* spp. (p = 0.3831) and *Giardia* sp. (p = 0.4783) and sample collection sites in different states of the northern and northeastern regions.

Infection of these protozoans was higher in wild animals (*Cryptosporidium* spp. = 16.20%; *Giardia* sp. = 9.60%) than in captive animals (*Cryptosporidium* spp. = 9.43%; *Giardia* sp. = 3.77%). However, despite this difference, the association of both *Cryptosporidium* spp. ($\chi^2 = 1.754$, df = 1, p = 0.1854), and *Giardia* sp. ($\chi^2 = 1.978$, df = 1, p = 0.1596) and the place in which the animals were kept was independent.

Table 2. Frequency of infection by *Cryptosporidium* spp. and *Giardia* sp. in aquatic mammals sampled in northern and northeastern Brazil

Species	Frequency of infection (%)		
	Cryptosporidium spp.	*Giardia* sp.	Co-infection
Lontra longicaudis	15.28	9.23	4.45
Pteronura brasiliensis	41.66	29.16	20.83
Kogia breviceps	–	100	–
Kogia sima	–	25	–
Sotalia guianensis	9.67	9.67	3.22
Trichechus inunguis	16.03	3.81	0.76
Trichechus manatus	13.79	10.34	–

DISCUSSION

The frequency of occurrence of *Cryptosporidium* spp. in *Pteronura brasiliensis* and *Lontra longicaudis* exceeded those observed in other mustelids, such as in *Lutra lutra* (Méndez-Hermida et al. 2007) and *Lontra canadensis* (Gaydos et al. 2007). Regarding the presence of *Giardia* sp., the rates of infection in this study were higher than those found in *L. lutra* (Méndez-Hermida et al. 2007) and below values observed in *L. canadensis* (Gaydos et al. 2007).

According to Méndez-Hermida et al. (2007), little is known about the prevalence of cryptosporidiosis and giardiasis in wild mammals. However, environmental pollution with human and domestic animal feces is a recognized pathway for exposure and infection of wildlife by zoonotic parasites (Fayer et al. 2004). In this study, none of the cities or riverside communities of the region have sewage treatment systems. Urban discharge or agricultural runoff may therefore have resulted in environmental contamination and pathogen exposure (Santos et al. 2011).

In the order Cetartiodactyla, the occurrence of *Cryptosporidium* spp. and *Giardia* sp. reported here was

similar to frequencies observed in Europe (Reboredo-Fernández et al. 2015) and North America (Hughes-Hanks et al. 2005).

Infection caused by *Cryptosporidium* spp. among Sirenia was higher than that observed by Borges et al. (2011) in *Trichechus inunguis* and lower in *T. manatus*. It is noteworthy that the occurrence of *Giardia* sp. in this order of aquatic mammals had not yet been reported. According to Borges et al. (2011), the high frequency of infection in manatees was associated with animals in captivity, suggesting the water transmission of *Cryptosporidium* spp. oocysts, or factors related to management, such as water source, food supply, and contact with handlers. In that study, the authors speculated that the good environmental quality of water resources of the Amazon could have contributed to a lower rate of animals identified with this protozoan.

Although the difference was not statistically significant in the present study, the frequency of infection by *Cryptosporidium* spp. and *Giardia* sp. was higher in wild animals than in captives. These results differed from those observed in other studies with manatees (Borges et al. 2011) and Australian sea lion *Neophoca cinerea* (Delport et al. 2014), where captive animals were more affected.

In this study, infection with *Cryptosporidium* spp. was higher in species that use river resources, while the occurrence of *Giardia* sp. was relatively similar between marine and freshwater mammals. According to Hughes-Hanks et al. (2005), the high prevalence of *Giardia* sp. in bowhead whale *Balaena mysticetus* and North Atlantic right whale *Eubalaena glacialis*, both oceanic species, was explained by the numerous potential routes of exposure, transmitted through the ingestion of contaminated water (Fayer et al. 2004). Additionally, certain prey, such as shrimp and zooplankton, may be capable of concentrating the infective stages in much the same manner as clams, oysters, and mussels (Gomez-Bautista et al. 2000, Fayer et al. 2004), thereby making large numbers of organisms readily available to the mammalian host (Hughes-Hanks et al. 2005). Finally, behavior that brings groups of animals together increases the likelihood of direct transmission between susceptible animals (Hughes-Hanks et al. 2005).

Contamination of Amazon water resources by these protozoans has been previously reported by Borges et al. (2007b), in studies with *T. inunguis*. These results attributed the transmission of *Cryptosporidium* spp. oocysts to compromised water resources associated with a lack of domestic wastewater treatment systems, release of human fecal waste by boats, and waste of agricultural origin that are carried into local rivers and streams (Borges et al. 2007b). In addition, the dissemination of coccidia can occur through the feces of domestic and wild animals (Dixon et al. 2008), as well as those maintained in captivity, and transmission can occur indirectly through contaminated water or food (Kimber & Kollias 2000, Borges et al. 2007c, Delport et al. 2014).

While there was no significant seasonal relationship with the occurrence of protozoa, the large variation in the amplitude of Amazon River (northern region) resources due to rain directly influences associated aquatic ecosystems (Junk et al. 1989). Pastures used for cattle and horses during the dry season are flood areas exploited by aquatic mammals during the rainfall period. Therefore, it is possible that seasonal rainfall could impact the transfer and availability of land-based protozoa to the aquatic and estuarine environment.

The high probability of the spread of *Cryptosporidium* spp. oocysts and *Giardia* sp. cysts by infected domestic species, as well as the ability of these protozoa to remain infective under ambient conditions for prolonged periods of time (Fayer et al. 2004) increase the possibility of local exposure and infection of aquatic mammals. In addition, in northeastern Brazil during the rainy season, there is a greater amount of fecal waste deposited into river resources, which are then discharged into coastal and marine environments (Silva-Cavalcanti et al. 2013).

Concerns regarding transport of contaminated feces have been described in studies with pinnipeds, where the high prevalence of *Cryptosporidium* and *Giardia* was associated with habitat condition and human use. In Nunavik (Quebec, Canada) during the summer months, the coastal environment became more contaminated with oocysts and cysts from human and terrestrial animal waste than in marine areas used by populations of *Phoca hispida* and *Erignathus barbatus* (Dixon et al. 2008).

This study allowed us to evaluate the occurrence of *Cryptosporidium* spp. and *Giardia* sp. in species of mustelids, cetaceans, and sirenians. This methodological approach is an important strategy to identify the transmission of these protozoa to aquatic and potentially marine mammals, being also indicative of the quality of habitats used by these animals (Bonde et al. 2004, Bossart 2011).

The high occurrence of these parasites in different habitats reinforces the need for additional studies to genotype these protozoans, which is vital to understanding the zoonotic potential of isolated forms

and identify reservoirs for human or wildlife infection (Appelbee et al. 2010). In Brazil, the molecular characterization of *Cryptosporidium* and *Giardia* in aquatic mammals has not been performed yet, but both pathogens were detected. The use of molecular tools can facilitate a greater understanding of the environmental sources of protozoa and host specificity (Lasek-Nesselquist et al. 2008, Delport et al. 2014, Reboredo-Fernández et al. 2014).

Even when these parasites are detected in aquatic mammals, their impact on general health is unclear (Hughes-Hanks et al. 2005). Clinical disease has been reported in Brazil with both pathogens. In studies with Antillean manatees, clinical signs were observed with *Cryptosporidium* spp. infections (Borges et al. 2011), and abnormal feces and severe emaciation in estuarine dolphins were associated with *Giardia* infection (Altieri et al. 2007). Infection by these protozoans can potentially impact the conservation of aquatic mammals.

Results from this study include the occurrence of *Cryptosporidium* spp. in *L. longicaudis, P. brasiliensis*, and *Sotalia guianensis*, whereas infection with *Giardia* sp. was confirmed in *L. longicaudis* and *P. brasiliensis* as well as *T. manatus* and *T. inunguis*, thus extending the number of recognized susceptible hosts to these parasitic agents.

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