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Original Article

Toxoplasma gondii in cetaceans of Brazil: a histopathological and immunohistochemical survey

Toxoplasma gondii em cetáceos do Brasil: estudo histopatológico e imuno-histoquímico

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Abstract

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii*. In cetaceans, *T. gondii* infection is a significant cause of morbidity and mortality. Despite the worldwide range and broad cetacean host record of *T. gondii* infection, there is limited information on toxoplasmosis in cetaceans from the Southern hemisphere. We investigated the occurrence of *T. gondii* by histopathology and immunohistochemistry in tissue samples of 185 animals comprising 20 different cetacean species from Brazil. Three out of 185 (1.6%) animals presented *T. gondii*-associated lesions: a captive killer whale *Orcinus orca*, a free-ranging common bottlenose dolphin *Tursiops truncatus* and a free-ranging Guiana dolphin *Sotalia guianensis*. The main lesions observed in these animals were necrotizing hepatitis, adrenalitis and lymphadenitis associated with protozoal cysts or extracellular tachyzoites presenting immunolabeling with anti-*T. gondii* antibodies. This study widens the spectrum of species and the geographic range of this agent in Brazil, and provides the first reports of *T. gondii* infection in a captive killer whale and in a free-ranging common bottlenose dolphin in South America.

Keywords: Toxoplasmosis, mortality, stranding, South America, protozoan infection, marine mammal.

Resumo

Toxoplasmose é uma doença parasitária causada pelo protozoário *Toxoplasma gondii*. A infecção por *T. gondii* é uma causa significativa de morbidade e mortalidade, nos cetáceos. Apesar da abrangência mundial e amplo registro de espécies de cetáceos infectadas por *T. gondii*, informações sobre toxoplasmose em cetáceos do hemisfério sul são limitadas. Neste estudo pesquisou-se por meio de histopatologia e imuno-histoquímica a ocorrência de *T. gondii* em amostras de tecido de 185 animais, compreendendo 20 diferentes espécies de cetáceos que ocorrem no Brasil. Três dos 185 (1,6%) animais apresentaram lesões associadas a *T. gondii*: uma orca *Orcinus orca* mantida em cativeiro, um golfinho-nariz-de-garrafa

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Tursiops truncatus e um boto-cinza *Sotalia guianensis* de vida livre. As principais lesões observadas nesses animais foram hepatite, adrenalite e linfadenite necrotizantes associadas a cistos protozoários ou taquizoítos extracelulares, marcados com anticorpos anti-*T. gondii*. O presente estudo amplia o espectro de espécies susceptíveis a esse agente e o seu alcance geográfico no Brasil, fornecendo o primeiro relato da infecção por *T. gondii* em uma orca mantida em cativeiro e em um golfinho-nariz-de-garrafa de vida livre na América do Sul.

Palavras-chave: Toxoplasmose, mortalidade, encalhe, América do Sul, infecção por protozoários, mamífero marinho.

Introduction

Toxoplasma gondii is a zoonotic intracellular coccidian protozoan of the phylum Apicomplexa (DUBEY, 2008). This protozoan is one of the most common parasites of warm-blooded animals (DUBEY et al., 2004), and was described for the first time in Brazil and in Tunisia, in 1908 (NICOLLE & MANCEAUX, 2009; SPLENDORE, 2009). The first record of toxoplasmosis in cetaceans was in a Guiana dolphin *Sotalia guianensis* from Rio de Janeiro state, Brazil, in the 1970's (BANDOLI & OLIVEIRA, 1977). Since then, the exposure and infection by this protozoan has been detected through serological, pathological and molecular techniques in a wide variety of captive and free-ranging cetaceans worldwide (INSKEEP et al., 1990; OMATA et al., 2006; MAZZARIOL et al., 2012; GONZALES-VIERA et al., 2013; IQBAL et al., 2018). Most of these studies have covered individual cases or small groups of cetaceans (DI GUARDO et al., 2010; GONZALES-VIERA et al., 2013). Toxoplasmosis is a significant cause of morbidity and may lead to stranding and death, and is considered one of the most important emerging diseases in cetaceans worldwide (VAN BRESSEM et al., 2009; DI GUARDO et al., 2010; GONZALES-VIERA et al., 2013; BIGAL et al., 2018).

The epidemiology of *T. gondii* transmission has been established in several terrestrial animals (e.g., cats, livestock), and to a lesser extent, aquatic species (e.g., shellfish), including transplacental transmission in susceptible pregnant animals (TENTER et al., 2000; CATÃO-DIAS et al., 2013; JARDINE & DUBEY, 2002; MILLER et al., 2008). In terrestrial animals, infection also occurs through ingestion of tissue cysts or contaminated food and/or water containing infecting sporulated oocysts released in feline feces. However, the transmission of *T. gondii* in cetaceans has not been fully elucidated. One major source of transmission in cetaceans could be the polluted marine environments in proximity with the coast, e.g., run-off of cat feces or soil contaminated with *T. gondii* oocysts from rivers, polluted effluents and ballast water from ships (VAN BRESSEM et al., 2009). In utero transmission of *T. gondii* has been described in Risso's dolphin *Grampus griseus* and Indo-Pacific bottlenose dolphin *Tursiops aduncus* (JARDINE & DUBEY, 2002; RESENDES et al., 2002). For offshore marine ecosystems, *T. gondii* transmission patterns are still unclear.

The occurrence of *T. gondii* in marine mammals remains a poorly understood phenomenon, particularly in Brazil, where very limited information exists. Exposure to *T. gondii* was reported in Amazon river dolphins *Inia geoffrensis* (SANTOS et al., 2011), but to this date, only one cetacean species is known to be infected by *T. gondii* in Brazilian waters: the Guiana dolphin (BANDOLI & OLIVEIRA, 1977; GONZALES-VIERA et al., 2013). We hypothesized that *T. gondii* infection could be present in a wider number of cetacean species in Brazil than previous studies have suggested. Thus, the aim of this study was to evaluate retrospectively the occurrence of *T. gondii* infection in captive and free-ranging cetaceans employing histopathological and immunohistochemical (IHC) analyses on tissue samples obtained from a large marine mammal tissue bank in Brazil.

Materials and Methods

We evaluated formalin-fixed paraffin-embedded tissue samples stored at the Marine Mammal Tissue Bank of the Laboratory of Wildlife Comparative Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil. The samples included in the study came from partial or complete standard necropsies conducted on odontocetes and mysticetes between 1988 and 2014, provided by partner institutions attending marine mammal strandings, rehabilitation and incidental captures along the Brazilian coast. Tissues from 185 individuals of 20 different cetacean species (Table 1) were evaluated. Among the evaluated samples, three were from originally wild animals kept in captivity in two different amusement parks of the São Paulo state (Brazil): a killer whale *Orcinus orca* from Iceland kept in captivity in the 1980's, an Amazon river dolphin from the Amazon Basin, and a common bottlenose dolphin *Tursiops truncatus* of unknown origin. The remaining animals were all free-ranging species that stranded along the Brazilian coast, mostly from the southern region (43.9%; 80/182), followed by the southeastern (30.2%; 55/182), and northeastern (25.8%; 47/182) regions (Figure 1). The examined tissues were: liver, kidney, lung, spleen, skeletal muscle, lymph node, adrenal gland, brain, uterus, intestines, heart and eye.

Formalin-fixed paraffin-embedded tissue sections from the aforementioned organs were sectioned at 5 µm thickness and stained with H&E. Additionally, special histochemical techniques - periodic acid-Schiff (PAS), Giemsa and Grocott's methenamine silver stains - were used in selected tissue sections to improve visualization of protozoan structures compatible with *T. gondii* (LUNA, 1992; POPPER et al., 1960).

Immunohistochemical analysis for *T. gondii* was performed on selected tissues for which *T. gondii* has demonstrated tropism in cetaceans (i.e., liver, kidney, lung, brain and lymph nodes) (INSKEEP et al., 1990; MIKAELIAN et al., 2000; RESENDES et al., 2002; VAN BRESSEM et al., 2009; GONZALES-VIERA et al., 2013). Briefly, 3 µm -thick sections were deparaffinized and rehydrated through a series of graded

Table 1. Cetacean species evaluated in Brazil for *Toxoplasma gondii* by immunohistochemistry.

Species	Number of individuals	Sex[b]		
		M	F	U
Franciscana dolphin (*Pontoporia blainvillei*)	102	57	41	4
Guiana dolphin (*Sotalia guianensis*)[a]	27	14	11	2
Dwarf sperm whale (*Kogia sima*)	7	4	3	0
Atlantic spotted dolphin (*Stenella frontalis*)	6	6	0	0
Spinner dolphin (*Stenella longirostris*)	5	4	1	0
Clymene dolphin (*Stenella clymene*)	6	3	3	0
Common bottlenose dolphin (*Tursiops truncatus*)[a]	4	2	1	1
Melon-headed whale (*Peponocephala electra*)	5	3	2	0
Rough-toothed dolphin (*Steno bredanensis*)	3	2	1	0
Short-finned pilot whale (*Globicephala macrorhynchus*)	3	3	0	0
Sperm whale (*Physeter macrocephalus*)	3	1	2	0
Pygmy sperm whale (*Kogia breviceps*)	2	2	0	0
Humpback whale (*Megaptera novaeangliae*)	2	2	0	0
Fraser's dolphin (*Lagenodelphis hosei*)	2	0	2	0
Striped dolphin (*Stenella coeruleoalba*)	2	0	2	0
Killer whale (*Orcinus orca*)[a]	2	1	1	0
Short-beaked common dolphin (*Delphinus delphis*)	1	1	0	0
Risso's dolphin (*Grampus griseus*)	1	0	1	0
Gervais' beaked whale (*Mesoplodon europaeus*)	1	0	1	0
Amazon river dolphin (*Inia geoffrensis*)	1	0	0	1
TOTAL	185	105	72	8

[a]Species with positive results; [b]M = male; F = female; U = unknown.

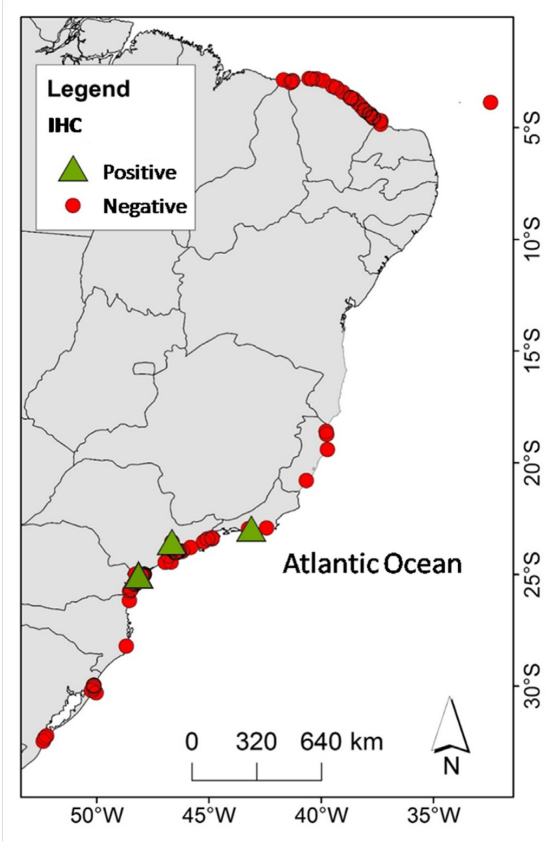

Figure 1. Geographic distribution along the coastline of Brazil of *Toxoplasma gondii*- positive (green triangle) and -negative (red circle) animals, according to immunohistochemistry.

alcohols. *Toxoplasma gondii* antigen was retrieved by heating tissue sections in citrate buffer (pH=7.0) solution for seven minutes at 90 °C. The sections were blocked with 1% normal rabbit serum in PBS for 30 minutes followed by overnight incubation with a polyclonal goat anti-*T. gondii* primary antibody (1 in 400 dilution; VMRD Inc; Pullman). This antibody does not cross-react with *Neospora caninum* (CORBELLINI et al., 2002). The sections were washed in PBS and incubated for 30 minutes with biotinylated polyclonal anti-goat secondary antibody (1 in 600 dilution; Dako) as previously described (DI GUARDO et al., 2010). Amplification of the immunologic reaction was based on an avidin-biotin-peroxidase complex method (Elite ABC kit, Vector laboratories), following manufacturer's instructions. Labeling was 'visualized' with 3-amino-9-ethyl-carbazole (Sigma) and/or diaminobenzidine (DAB D-5637; Sigma), and sections were counterstained with Mayer's haematoxylin. Tissue sections in which the primary antibodies were replaced by phosphate buffered saline or nonimmune homologous serum served as negative controls. Sections of the adrenal gland of a Guiana dolphin infected by *T. gondii* (GONZALES-VIERA et al., 2013) were used as positive control. Morbillivirus immunohistochemistry was also performed on selected tissue sections of *T. gondii* positive cases to rule out potential coinfection, following a previously published protocol (SALIKI et al., 2002; GROCH et al., 2014), using a commercial monoclonal antibody against the nucleoprotein of Canine distemper virus (VMRD Inc.), known to cross-react with *Cetacean morbillivirus*. Positive tissue samples of an infected Guiana dolphin were selected as positive control (GROCH et al., 2014).

Results

Three of 185 (1.6%) animals were IHC-positive for *T. gondii*-: a captive killer whale, a free-ranging common bottlenose dolphin, and a free-ranging Guiana dolphin. These animals presented a variety of toxoplasmosis-compatible lesions, with positively labeled protozoal cysts and free tachyzoites. The occurrence of *T. gondii* in free-ranging species was 1.1% (2/182), whereas the occurrence in captive animals was 33.3% (1/3). A summary of *T. gondii*-associated lesions in these animals follows. Additional information regarding the microscopic findings of positive animals is available in Table 2. All three *T. gondii* positive animals were IHC-negative for morbillivirus. No PCR techniques were employed in these cases because frozen tissue samples were not available.

Case No 1 (MM#452) was a 5-6 years-old juvenile (2,500 kg) male killer whale captured in Iceland in 1983, and one year later brought into an oceanarium located in the city of São Paulo, São Paulo state, Brazil. The animal remained in this facility until its

Table 2. Microscopic lesions in *Toxoplasma gondii*-positive animals in Brazil.

Organ	Microscopic lesions		
	Case 1 (killer whale)	**Case 2 (common bottlenose dolphin)**	**Case 3 (Guiana dolphin)**
Liver	Moderate randomly multifocal to coalescent necrotizing hepatitis with protozoan cysts, compatible with *T. gondii*. *T. gondii*-positive by IHC.	Moderate to marked randomly multifocal to coalescent necrotizing hepatitis with protozoan cysts, compatible with *T. gondii*, and predominantly mononuclear infiltrate, mild hepatic congestion and hemorrhage. *T. gondii*-positive by IHC.	NA
Kidney	Acute multifocal interstitial nephritis with mild to moderate mixed infiltrate, mild to moderate membranous glomerulonephritis.	Mild multifocal chronic tubular ectasia and glomerular cyst formation.	NA
Adrenal gland	NA	NA	Marked multifocal acute necrotizing adrenalitis associated with numerous round to oval protozoal cysts and extracellular tachyzoites compatible with *T. gondii*. *T. gondii*-positive by IHC.
Lung	Moderate to marked multifocal acute fibrinosuppurative and hemorrhagic bronchopneumonia with protozoan cysts (compatible with *T. gondii*) and bacteria. *T. gondii*-positive by IHC.	Moderate to marked multifocal acute fibrinosuppurative bronchopneumonia with hemorrhage.	NA
Stomach	Mild to moderate multifocal to coalescent granulocytic gastritis.	NA	NA
Intestine	Mild to moderate multifocal granulocytic lymphadenitis, mild to moderate diffuse mixed enteritis with crypt microabscesses (cryptitis).	Autolysis	NA
Muscle	Mild multifocal acute segmental myocyte degeneration and necrosis.	NA	NA
Thyroid	Mild multifocal to coalescent lymphoplasmacytic thyroiditis.	NA	NA
Lymph node	Marked necrosuppurative lymphadenitis with protozoal cysts (compatible with *T. gondii*), and lymphoid depletion with lymphocytolysis. *T. gondii*-positive by IHC.	NA	NA
Vascular	Acute fibrinoid vasculitis with mononuclear infiltrate.	NA	NA
Eye	NA	NA	No significant findings observed.

NA = not available.

death, on March 7th, 1988. No clinical data were available. Samples of lungs, liver, spleen, kidneys, skeletal muscle, glandular stomach, large intestine, thyroid glands, and lymph nodes were collected upon necropsy. The only reported macroscopic finding was a renal cyst. Microscopically, the main lesions included moderate to marked, multifocal, acute fibrinosuppurative and hemorrhagic bronchopneumonia with protozoan cysts, moderate randomly multifocal to coalescent necrotizing hepatitis with protozoan cysts (compatible with *T. gondii*), marked necrosuppurative lymphadenitis, acute multifocal interstitial nephritis with mild-moderate mixed infiltrate, mild to moderate, multifocal, chronic membranous glomerulonephritis, and acute fibrinoid vasculitis.

Case No 2 (MM#178) was a juvenile male common bottlenose dolphin of 2.43 m total body length, that stranded alive and died shortly after stranding, on October 29th, 2001, in Lagos Region, Rio de Janeiro state, Brazil (22°56'S, 42°19'W). Samples of lungs, liver, kidneys, and large intestine were collected upon necropsy. No gross findings were reported. The main microscopic findings observed were moderate to severe, multifocal random to coalescing necrotizing hepatitis with protozoan cysts compatible with *T. gondii* and moderate to marked, multifocal, acute fibrinosuppurative bronchopneumonia.

Case No 3 (MM#67) was a 1.86 m-long adult, female Guiana dolphin that stranded dead on March 3th, 1998, in Paranaguá Bay, Paraná state, Brazil (25°31'S, 48°30'W). No necropsy records were available. Samples of eye and adrenal glands were evaluated. Microscopically, the main finding was marked, multifocal, acute necrotizing adrenalitis with numerous protozoal cysts and extracellular tachyzoites compatible with *T. gondii*, and further highlighted with PAS and Giemsa stains.

Discussion

Despite the widespread geographical range of *T. gondii* infection, in Brazil, the current knowledge regarding cetacean toxoplasmosis is limited to few species and studies (BANDOLI & OLIVEIRA, 1977; SANTOS et al., 2011; GONZALES-VIERA et al., 2013). Based on microscopical and immunohistochemical examinations, we provide the first description of *T. gondii* in a common bottlenose dolphin of South America, and in a captive killer whale. Furthermore, the identification of toxoplasmosis in a Guiana dolphin corroborates with previous observations regarding this species and geographic area, the Paranaguá Bay. On the other hand, in spite of the high prevalence of *T. gondii* antibodies reported in free-ranging Amazon river dolphins (SANTOS et al., 2011), our sampled captive Amazon river dolphin was *T. gondii*- negative.

We detected protozoal cysts and free tachyzoites of *T. gondii* by IHC and observed histological changes within multiorgan necroinflammatory foci, e.g., necrotizing hepatitis, necrosuppurative lymphadenitis (killer whale), necrotizing hepatitis (common bottlenose dolphin), and necrotizing adrenalitis (Guiana dolphin). These lesions are in agreement with previous reports of *T. gondii* infection in odontocetes (CRUICKSHANK et al., 1990; RESENDES et al., 2002; DI GUARDO & MAZZARIOL, 2013) and mysticetes (MAZZARIOL et al., 2012). The main lesions observed in cetacean cases of toxoplasmosis are necrotizing hepatitis, lymphadenitis and lymphoid necrosis, interstitial pneumonia, adrenal necrosis, and non-suppurative encephalitis and meningoencephalitis with bradyzoites and free tachyzoites (INSKEEP et al., 1990; MIGAKI et al., 1990; MIKAELIAN et al., 2000; RESENDES et al., 2002; VAN BRESSEM et al., 2009; GONZALES-VIERA et al., 2013; ROE et al., 2013). In *T. gondii*-positive cases, all tested tissues were negative for morbillivirus, suggesting that *T. gondii* possibly acted as a primary agent, as previously observed (DI GUARDO et al., 2010; GONZALES-VIERA et al., 2013; ROE et al., 2013). The scarce number of brain samples available for this study (n = 4) probably prevented the identification of *T. gondii*-associated lesions in nervous tissue, which is commonly involved in cetacean toxoplasmosis cases (DI GUARDO et al., 2010; SIERRA et al., 2014).

In this study, we evaluated cetacean samples from geographic areas that correspond to a large portion of the Brazilian coastline, most of them coastal species, mainly franciscanas (*Pontoporia blainvillei*) (55.0%) and Guiana dolphins (14.5%). Coastal cetaceans, considered suitable sentinels of the marine environmental ecosystem and important indicators of marine pollution, are usually more exposed to anthropogenic activities (WELLS et al., 2004; BOSSART, 2011; MOURA et al., 2014). From these, we found histological and immunohistochemical evidences of toxoplasmosis in one Guiana dolphin. This is the third report of toxoplasmosis in this species (BANDOLI & OLIVEIRA, 1977; GONZALES-VIERA et al., 2013). This animal presented severe adrenalitis, similar to what was reported by Gonzales-Viera et al. (2013) in another Guiana dolphin from the same area (Paranaguá Bay) that died in the same year (1998). Given the marked pattern of site fidelity presented by Guiana dolphins (MOURA et al., 2014), this result suggests a common source of exposure to *T. gondii* and a possible negative impact of toxoplasmosis on this population. Although the low occurrence of *T. gondii* infection found in the present study does not allow us to infer further on this protozoan's geographic distribution, the location of the Guiana dolphin case and previous reports suggest that infection may occur at higher rates in coastal areas of Brazil. Coastal areas usually receive freshwater run-offs and terrestrial biologic pollutants, contributing to the presence of *T. gondii* (SHAPIRO et al., 2015). These results also suggest areas in which future research efforts should be concentrated.

Concerning killer whales, positive serology for *T. gondii* was reported in a killer whale calf that stranded alive in Japan, in 1988 (MURATA et al., 2004), and *T. gondii* infection was identified by molecular techniques in a stranded killer whale from the Northeastern Pacific, in California (GIBSON et al., 2011). However, these reports lacked detailed pathological examinations. In the present study, we provide the first histopathological and immunohistochemical evidences of toxoplasmosis in this species. Based on the severity and extent of characteristic multiorganic necroinflammatory foci, we believe that *T. gondii* played a major role in the death of the evaluated killer whale. In this case, potential environmental sources of *T. gondii* infectious forms may include water and/or food contamination in one or more of the following scenarios: (1) the facility's water system (e.g., used to clean fish and the enclosure, and fill the tank), especially considering that sporulated oocysts of *T. gondii* are viable in saltwater for at least

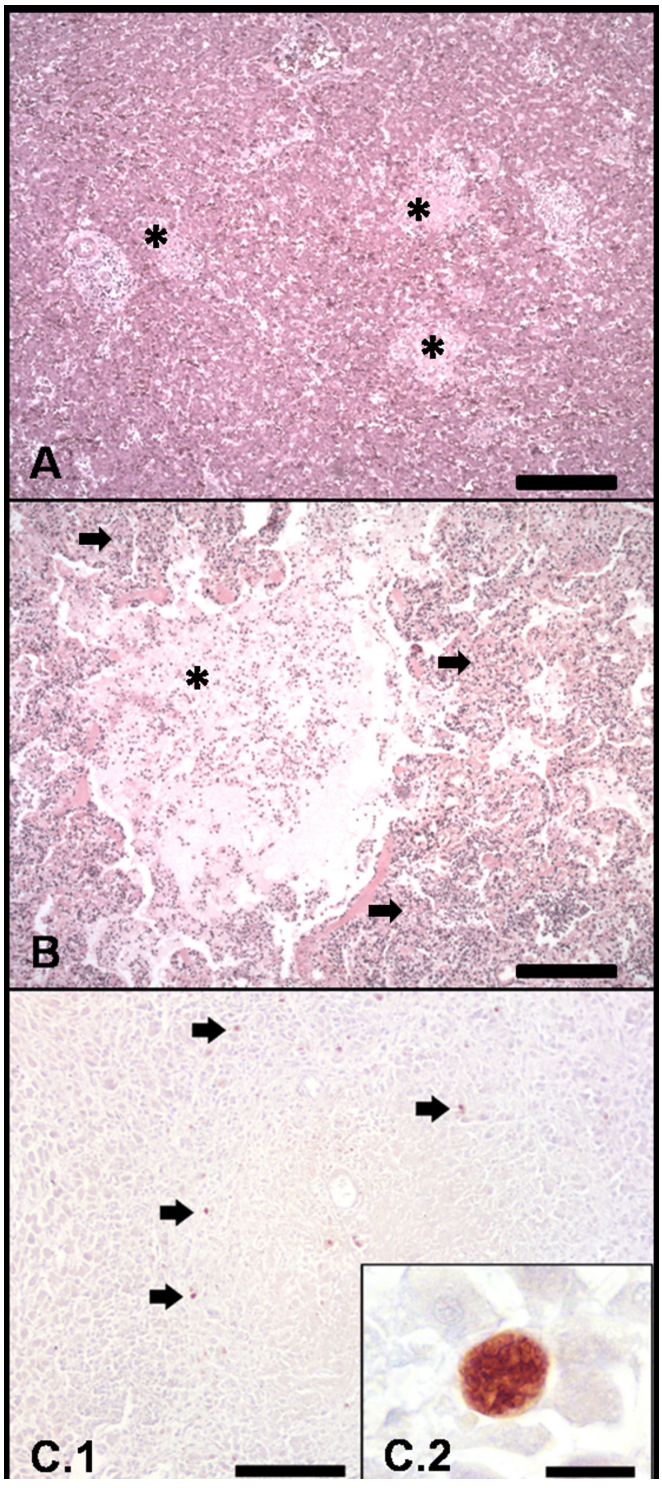

Figure 2. Microscopic lesions and immunohistochemical findings in *Toxoplasma gondii* positive animals. (A) Atlantic bottlenose dolphin (*Tursiops truncatus*). Liver, necrotizing hepatitis (asterisk), H&E, scale bar = 200 µm; (B) Killer whale (*Orcinus orca*). Lung, bronchopneumonia (arrows) with alveolar infiltrate (asterisk), H&E, scale bar = 200 µm; (C.1) Guiana dolphin (*Sotalia guianensis*). Adrenal gland, there is focally extensive lytic necrosis with multiple protozoal cysts (arrows). IHC for *T. gondii*, Mayer's hematoxylin counterstaining, scale bar = 500 µm; (C.2) Detailed view of *T. gondii* protozoal cyst containing numerous bradyzoites. IHC for *T. gondii*, Mayer's hematoxylin counterstaining, scale bar = 25 µm.

two years (AUBERT & VILLENA, 2009); (2) contaminated food (e.g., fish); and (3) contaminated enclosure (e.g., contaminated fomites, personnel and/or presence of roaming cats). These findings highlight the importance of proper hygiene and husbandry in captive cetaceans, including properly treated water and reliable food sources free of *T. gondii* sporulated oocysts. Although unlikely, considering the physiopathology of toxoplasmosis in highly susceptible or 'less adapted' hosts (CATÃO-DIAS et al., 2013) - which could be the case for the killer whale - one should also consider the possibility of infection prior to the specimen's capture in the wild (e.g., transplacental or transmammary) or initial period in captivity (1984-1988). In that case, the infection would have been quiescent until being activated by the stress of captivity and/or other predisposing factors (e.g., concomitant diseases) (LINDSAY et al., 2003; AUBERT & VILLENA, 2009; MAZZARIOL et al., 2012).

Toxoplasma gondii infections in common bottlenose dolphins have been observed worldwide; in the Mediterranean Sea and the Atlantic Ocean (CRUICKSHANK et al., 1990; INSKEEP et al., 1990; DI GUARDO et al., 1995; DUBEY et al., 2008; PRETTI et al., 2010; PROFETA et al., 2015; BIGAL et al., 2018), and also in captive specimens (DUBEY et al., 2009). Furthermore, it has also been detected in a related species from the Pacific Ocean - the Indo-Pacific bottlenose dolphin (JARDINE & DUBEY, 2002). However, this is the first report of *T. gondii* in an Atlantic bottlenose dolphin from South America (Figure 2), widening the geographic range of *T. gondii* occurrence for this species.

Because cetacean species' susceptibility to *T. gondii* infection is unknown, non-exposure and natural resistance to this agent should be considered in negative cases (DI GUARDO & MAZZARIOL, 2013). Omata et al. (2006) proposed that killer whales from Japan might be resistant to *T. gondii*; however, the evidence provided seemed slight because none of these animals presented antibodies against the agent.

Finally, this is the first report of a large-scale *T. gondii* survey based on histopathological and immunohistochemical examinations in cetaceans of Brazil. This study widens the spectrum of *T. gondii*-susceptible species and geographic range of this agent in Brazil and presents the first report of *T. gondii*-infection in a captive killer whale, and in a free-ranging Atlantic bottlenose dolphin from South America. Furthermore, it corroborates with previous observations of toxoplasmosis in Guiana dolphin and the geographic area of Paranaguá Bay. Further studies are warranted to characterize the circulating strains and clarify the distribution of this protozoan, its transmission route(s), unsolved pathogenetic mechanisms, cetacean host-specific susceptibilities and potential implications to the conservation of cetacean species of Brazil and mechanisms involved in human exposure.

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