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Short communication

Toxoplasmosis in a Guiana dolphin (*Sotalia guianensis*) from Paraná, Brazil

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ABSTRACT

This study describes toxoplasmosis in a by caught Guiana dolphin (*Sotalia guinensis*) from Paranaguá Bay, Paraná, Brazil. Interstitial pneumonia, multisystemic arteritis, multifocal adrenalitis and hepatitis were the primary lesions observed. These tissues had moderate to severe necrosis and mononuclear cells infiltration usually surrounded by tachyzoites and tissue cysts. Moderate lymphoid depletion was evident in the spleen. *Toxoplasma gondii* was positive by immunohistochemical and ultrastructural evaluation. Furthermore, the animal was negative for Morbillivirus by immunohistochemistry and had low levels of persistent organochlorines. There is evidence of environmental changes in the Paranaguá Bay that could justify the occurrence of toxoplasmosis in Guiana dolphin. The sewage run-off from main urban areas and the presence of domestic and wild felids in areas surrounding the bay could be a source of *T. gondii* oocysts from land to sea. Based on its habitat, the authors recommend this dolphin species as sentinels for the health of bays and estuaries where they occur.

1. Introduction

Toxoplasma gondii is a protozoan parasite that commonly affects a wide range of birds and mammals, including humans (Dubey and Beattie, 1988). Toxoplasmosis has been identified in many species of free-ranging and captive marine mammals such as sea lions, seals, walruses and manatees (Dubey et al., 2003, 2009), southern sea otters (*Enhydra lutris nereis*) (Conrad et al., 2005), whales (Mazzariol et al., 2012) and several species of dolphins (Inskeep et al., 1990; Migaki et al., 1990; Resendes et al., 2002; Dubey et al., 2003, 2009).

Reports of *T. gondii* infection in aquatic mammals from Brazil are restricted to few studies such as a Guiana dolphin (*Sotalia guianensis*) stranded in the state of Rio de Janeiro (Bandoli and Oliveira, 1977), and positive antibodies were found in free-living Amazon river dolphins (*Inia geoffrensis*) (Santos et al., 2011) and captive Amazonian manatees (*Trichechus inunguis*) from the Brazilian Amazon (Mathews et al., 2012).

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Guiana dolphin is a coastal species distributed from Honduras (15°58′N) in Central America down to the state of Santa Catarina (27°35′S) in Southern Brazil (Flores and da Silva, 2009). This dolphin inhabits estuaries, bays and shallow coastal waters and its conservation status is "data deficient" (IUCN, 2012). This study describes a case of toxoplasmosis in a Guiana dolphin and discusses the pathological process and the possible routes of transmission.

2. Materials and methods

A mature female Guiana dolphin (length = 173 cm, weight = 63 kg) aged 15 years, estimated by counting dentinal growth layer groups (Rosas et al., 2003), was by caught at Pontal do Sul (25°40′24″S, 48°30′39″W), Paranaguá Bay, Paraná, Brazil, in August, 1998. The carcass was necropsied and tissue samples were collected from lungs, adrenal glands, liver, kidneys, spleen, small intestine and eye and preserved in 10% buffered formalin and deposited in the Marine Mammal Tissue Bank (Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo). Subsequently, tissues were processed by conventional histological techniques and 5 μm sections were stained with Hematoxylin and Eosin and Periodic Acid-Shiff (PAS) and then examined by light microscopy. Immunohistochemistry (IHC) for *T. gondii* was performed in tissues using a noncommercial polyclonal antibody produced in rabbits, with the dilution of 1/3000. Monkey encephalitis due to *T. gondii* was used as positive control. In order to screen for Dolphin Morbillivirus (DMV) antigen in paraffin embedded tissues, immunohistochemistry technique (Fernández et al., 2008) was performed on all above-mentioned tissues. Electron microscopic of paraffin-embedded liver and kidney were reprocessed and fixed in 3% glutaraldehyde and embedded in Poly/Bed 812-Araldite 502 resin. Ultra-thin 80 nm sections were stained with uranyl acetate and lead citrate and examined using a JEOL, JEM-1011 electronic microscope.

3. Results

Gross lesions were not recorded by field researchers; thus, this study focused on microscopic, immunohistochemical and ultrastructural findings. Microscopically, lungs showed severe sub-acute interstitial pneumonia with mononuclear leucocytes invading the alveolar septa and fibrin exudation with degenerated neutrophils and macrophages filling the alveolar lumens; scattered pulmonary necrosis were surrounded by sparse numbers of multinucleated giant cells. Bronchitis consisting of few mononuclear cells as well as intracellular and free tachyzoites were observed inside and around the bronchial lumen in both histology (Fig. 1) and IHC (Fig. 3A). Fibrinous pleuritis with occasional multinucleated giant cells and tachyzoites was also observed. The liver showed severe acute multifocal hepatitis composed by multifocal necrosis and mononuclear leucocytes associated with tachyzoites and tissue cysts. Moderate to severe arteritis accompanied by groups of tachyzoites embedded in the smooth muscle layer were observed in small arteries in lung, liver and kidney. The adrenal cortex showed moderate

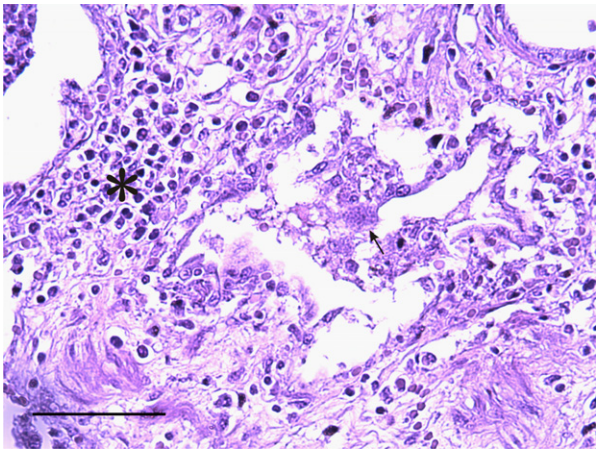

Fig. 1. Lung, Guiana dolphin. There are interalveolar septa thickening due to the presence of many mononuclear cells (asterisk) and groups of tachyzoites inside the lumen (arrow). HE stain, bar = 100 μm.

to severe acute multifocal necrotizing adrenalitis composed of mononuclear leucocytes frequently surrounded by groups of tachyzoites forming, in few cases, parasitophorous vacuoles (Fig. 2) and bradyzoites in tissue cysts (IHC) (Fig. 3B). There was extensive severe subacute enteritis with tachyzoites in the mucosa, intestinal crypts and sub-mucosa, accompanied by degenerative mononuclear cells. Moderate lymphoid depletion was observed in the spleen with few scattered tachyzoites, reveled by IHC. The eye had mild focally extensive acute retinochoroiditis with scant mononuclear cells; IHC revealed a focal group of tachyzoites. Groups of tachyzoites were evident by IHC in the skeletal muscle and optic nerve without inducing inflammation or necrosis. PAS positive bradyzoites were observed within tissue cysts in lungs, liver and small arteries of the kidney. All tissues immunohistochemically analyzed for Morbillivirus antigens were negative. Electronic microscopy evaluation, bradyzoites within a thin

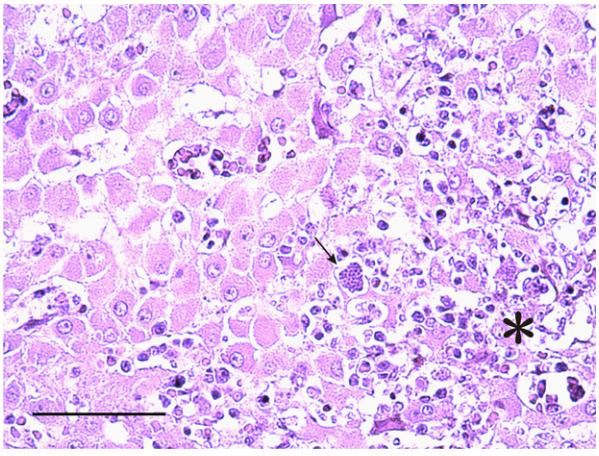

Fig. 2. Adrenal gland, Guiana dolphin. There is a focally extensive area of necrosis and mononuclear cells infiltration (asterisk) associated with a group of tachyzoites surrounded by a parasitophorous vacuole (arrow). HE stain, bar = 100 μm.

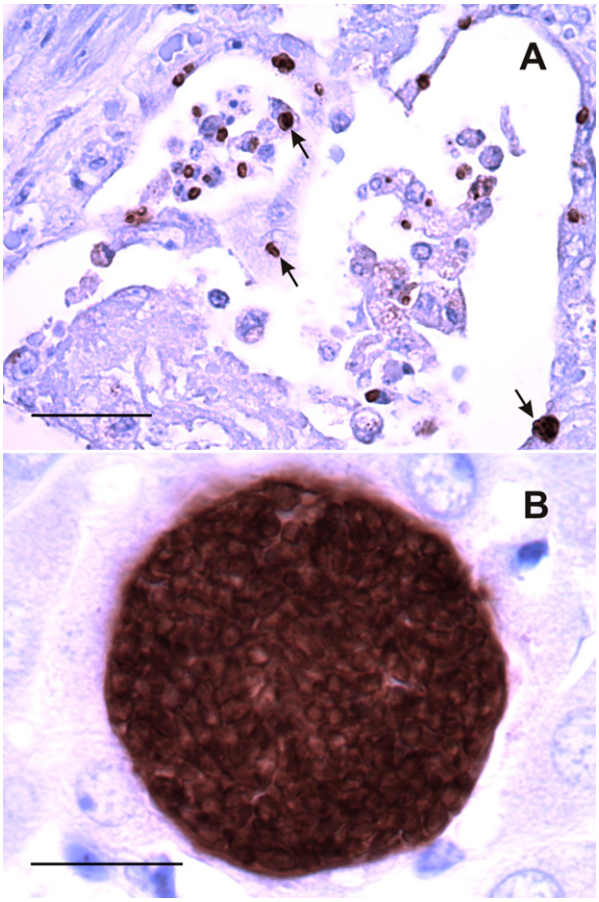

Fig. 3. (A) Lung, Guiana dolphin. Many tachyzoites surrounded by mononuclear cells within the bronchiolar lumen (arrows), bar = 50 μm. (B) Adrenal gland, Guiana dolphin. A tissue cyst filled by bradyzoites, bar = 25 μm. Immunohistochemistry stain with *T. gondii* antibodies.

cyst wall were observed among hepatocytes and tachyzoites of approximately 2 μm in diameter with a double membrane were observed within glomeruli and Kupffer cells (Fig. 4).

4. Discussion

Based on histological characteristics, Bandoli and Oliveira (1977) described the first report of toxoplasmosis in a cetacean species in the world, involving a Guiana dolphin stranded in the Guanabara Bay, state of Rio de Janeiro, southeastern Brazil. This case of toxoplasmosis was also a Guiana dolphin from the state of Parana, southern Brazil. In addition to histological characteristics, immunohistochemistry and ultrastructural techniques were used to confirm the presence of the protozoa.

In this case, the histopathological findings consisted of multifocal necrosis accompanied by mononuclear cells in close association with tachyzoites and tissue cysts. These findings are in accordance to those described in other dolphin species (Inskeep et al., 1990; Migaki et al., 1990; Resendes et al., 2002). There was also a moderate splenic lymphoid depletion, similar to that described for Atlantic

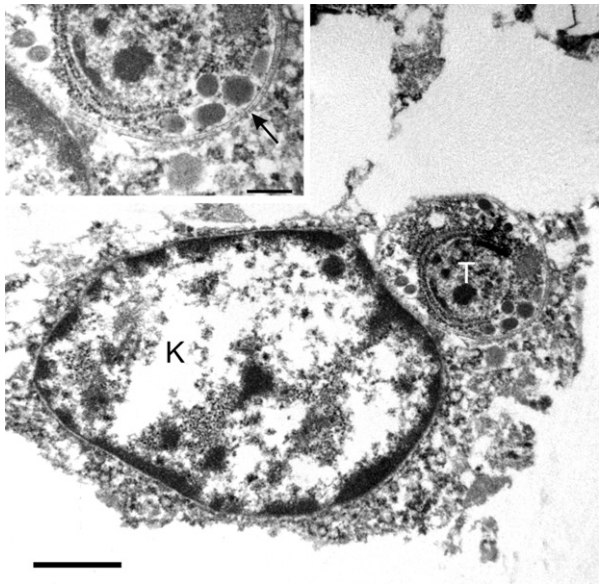

Fig. 4. Liver, Guiana dolphin. There is a Kupffer cell (K) engulfing a tachyzoite (T), bar = 1 μm. Inset: Detail of the tachyzoite with a double membrane separated by a lucent space (arrow), bar = 300 nm.

bottlenose dolphins (Inskeep et al., 1990), and compatible with immunosuppression, which may be caused by multiple factors, including pollution and viral diseases.

Bandoli and Oliveira (1977) concluded that the pollution of the marine environment could have influenced the occurrence of the disease observed in the Guiana dolphin stranded in the Guanabara Bay. The animal in the present case came from a less impacted bay (Paranaguá Bay) (Lailson-Brito et al., 2010). In fact, levels of persistent organochlorines were measured in the Guiana dolphin studied resulting that they were within the average in comparison to dolphin species worldwide and were lower than in dolphin populations inhabiting more industrialized areas (Kajiwara et al., 2004). It seems that the immunosuppression caused by these substances is unlikely.

Van Bressem et al. (2009a) have argued that the environmental conditions of the Paranaguá Bay could be rapidly changing. They observed a high percentage of skin diseases in dolphins, which are frequently associated with populations that use polluted areas. Paranaguá Bay is influenced by different factors, for instance, an important source of water pollution might be the sewage run-off from the main urban areas (Marone et al., 2005). One of the risk factors associated with toxoplasmosis is the land-based surface run-off, which has been indicated in Southern sea otters (Miller et al., 2002), black sea dolphin (*Tursiops truncatus ponticus*) and beluga whales (*Delphinapterus leucas*) (Alekseev et al., 2009). A similar situation enhanced with the introduction of domestic cats (Pereira, 2009) and the presence of wild felids in areas surrounding the Paranaguá Bay (Leite and Galvão, 2002) may increase the possible sources of *T. gondii* oocysts. *T. gondii* oocysts are highly environmentally resistant and could be transported from land to the marine environment (Miller et al., 2002; Conrad et al., 2005). Recently, Massie et al. (2010) demonstrated, under experimental conditions, that filter-feeding fish could hold

infective *T. gondii* oocysts. Additionally, oysters (*Crassostrea rhizophorae*), a common bivalve shellfish from the Brazilian southern coastal area can filter and retain *T. gondii* oocysts from the marine environment (Esmerini et al., 2010). None of the species used in these studies are known to be consumed by Guiana dolphins (Rosas et al., 2010); however, it is another possible route of infection that should be better evaluated.

In relation to immunosuppressive viral agents, Morbillivirus is particularly important for different groups of vertebrates and has been associated with cases of toxoplasmosis affecting dolphins (Soto et al., 2011) and whales (Mazzariol et al., 2012). However, IHC for Morbillivirus antigens in this case was negative and should be ruled out as a possible cause of immunosuppression.

Although *T. gondii* is considered as an opportunistic agent in aquatic mammals (Migaki et al., 1990; Domingo et al., 1992), studies suggest that this protozoan might be a primary agent on these species (Dubey et al., 2009; Di Guardo et al., 2010); considering the point mentioned and that the two main immunosuppressant factors (Morbillivirus and organochlorines) were ruled out, we believe that *T. gondii* was the primary agent of chronic morbidity in the Guiana dolphin studied and as a sick animal, this disease possibly contributed to its by catch.

Unfortunately, the brain was not examined because this animal was part of studies on cranial morphology and the skull was deposited at the collection of the *Instituto de Pesquisas Cananéia* (IPeC) (Rosas et al., 2003). Nevertheless, tachyzoites observed in the optic nerve allow us to suspect that the central nervous system could also have been affected. Animal protozoan retinochoroiditis was only described in a sea otter infected with *Sarcocystis neurona* (Dubey and Thomas, 2011), which had similar characteristics as the present case in the Guiana dolphin. This fact emphasizes the importance of improving biological sampling from stranded marine mammals along the Brazilian coast.

Further investigations should be carried out to find out the real impact of toxoplasmosis on the population of Guiana dolphins and other aquatic mammals from the Brazilian coast, since toxoplasmosis is considered an emerging infectious disease in cetacean species (Van Bressem et al., 2009b; Di Guardo et al., 2011). Additionally, based on the Guiana dolphin's habitat, this species could serve as a good sentinel to access the health of the bays and estuaries where they occur.

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