Isolation of *Giardia* sp. from an estuarine dolphin (*Sotalia guianensis*) in Ceará state, northeastern Brazil

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Protozoan parasites have rarely been reported in marine mammals, perhaps due to insufficient sampling or because these organisms are actually rare (Raga, 1997). Microorganisms of all kinds, including bacteria, fungi, protozoa and viruses, abound in the sea. Some are of types found on land and in land-dwellers (Geraci, 1991). *Giardia* is a member of the family *Hexamitidae*, a binucleate flagellated protozoan parasite that causes intestinal infection in mammals, birds, reptiles, and quick-motion amphibians.

The estuarine dolphin, *Sotalia guianensis*, inhabits coastal and estuarine waters of Central and South America, from Honduras (Silva and Best, 1996) to Santa Catarina Island, southern Brazil (Simões-Lopes, 1988). Although the common name of the species may vary geographically along the Latin American coast, we follow the usage of estuarine dolphin as proposed by Rosas and Monteiro Filho (2002). Some studies reported parasites in *S. guianensis* (Bastos and Marigo, 2004[3]; Melo et al., 2006), however, to date, the presence of *Giardia* sp. has not been reported for this dolphin species. The objective of this study is to report on the first detection of *Giardia* sp. related with the post-mortem alterations in a live estuarine dolphin stranded in Ceará state, northeastern Brazil.

On November 2006, a female estuarine dolphin calf (116cm in total length, and 16 Kg in weight) was rescued alive by the AQUASIS' crew, in Águas Belas Beach (04º03'26.9"S, 38º11'05.9"W), on the eastern coast of Ceará state. According to information reported by Rosas and Monteiro Filho (2002) and Rosas *et al.* (2002), length of birth in *Sotalia guianensis* was estimated at 92.2 cm, and animals one year old would be approximately 125 cm long.

The dolphin was severely emaciated and dehydrated, and unable to float. At the Marine Mammal Rehabilitation Center/SESC Iparana, the animal was rehydrated with oral electrolyte solution, an intramuscular broad spectrum antibiotic was administered, and a life jacket was used to keep it floating in the swimming pool (Figure 1). During the first day in rehabilitation, the animal frequently produced green, liquid and fetid faeces. On the second day defecation was not observed. The dolphin received an electrolyte solution through a nursing bottle five times daily. On the second day, the animal spontaneously accepted a milk formula (Worthy, 2003).

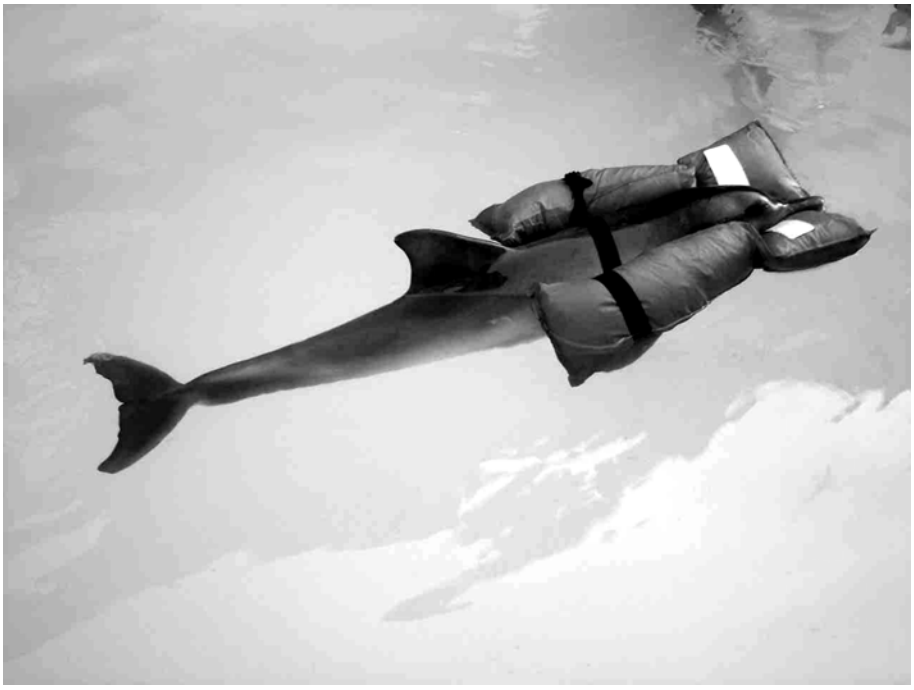

Figure 1. Rescued female estuarine dolphin calf from Águas Belas Beach, eastern Ceará, Brazil, with a lifejacket to mantain its flotability.

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[3] Bastos, L., B. and Marigo, J. (2004) *Ocorrência de parasitos do gênero* Anisakis *em mamíferos marinhos encalhados no litoral do Estado da Bahia.* Page 22 *in* Abstracts, III Encopemaq (Encontro Nacional sobre Pesquisa e Conservação de Mamíferos Aquáticos). Itaparica, BA, Brazil.

However, despite all efforts, the dolphin died on the third day. Two fecal samples were collected on the first and third days, immediately after defecation, and stored at 4° C for two hours until processed at the Labovet veterinary laboratories.

The necropsy was performed within an hour of the dolphin's death. During the *post-mortem* examination, the three stomach chambers were filled with black and viscous liquid content. The duodenum was full of black liquid content and gas, and there was increased wall thickness and a green-to-black color appearance as compared with the whole intestinal tract (Figure 2). The faeces were pasty and green-to-black in color. The liver had round edges and was yellow in color with a fatty appearance. The spleen was moderately enlarged and friable when cut. Histo pathologic analysis on the liver showed severe hydropic degeneration and discrete fat deposition on hepatic cells. The lungs were congested, with edema and diffuse petechiation distributed throughout. Mild white pulp hyperplasia was observed in the spleen. The kidneys presented discrete diffuse hemorrhagic pinpoints between the lobes. The intestines were examined by compartment (duodenum, middle portion and rectum). During the histopathologic examination, we observed mild enteritis with prominent lymphocytes, plasma cells and a severe nodular hyperplasia of the underlying lymphoid tissue with intense immune cell proliferation, mainly in the middle portion (Figure 3). A few trophozoites of *Giardia* sp. were observed between the intestinal crypts. Unfortunately, it was not possible to identify to species the *Giardia* infecting the dolphin because only trophozoites were observed in the intestinal tissue sample.

Figure 2. Green-to-black color appearance of the partial intestinal mucosae of the estuarine dolphin from this study.

During fecal sample examinations with light microscopy in 400X, by means of both direct and flotation methods, *Giardia* sp. trophozoites infection were identified (Fortes, 2004) (Figure 4).

As observed by Olson *et al.* (1997), Measures and Olson (1999) and Deng *et al.* (2000), *Giardia* spp. can be found in pinnipeds, suggesting that California sea lions could be potential reservoirs of both *Giardia* and *Cryptosporidium*. Heckmann (1987) identified hexamitid flagellates in the colons of bowhead whales (*Balaena mysticetus)*. Using molecular techniques, Hughes-Hanks (2005) observed that *Giardia* spp. are more prevalent in northern right whales (*Eubalaena glacialis)*, bowhead whales, and ringed seals (*Phoca hispida)* as compared to terrestrial mammals. These authors also showed that the greatest prevalence of the protozoan was observed in fecal samples of animals less than seven years of age. In these studies, protozoan infections were observed in all areas studied, suggesting a lack of geographical patterns.

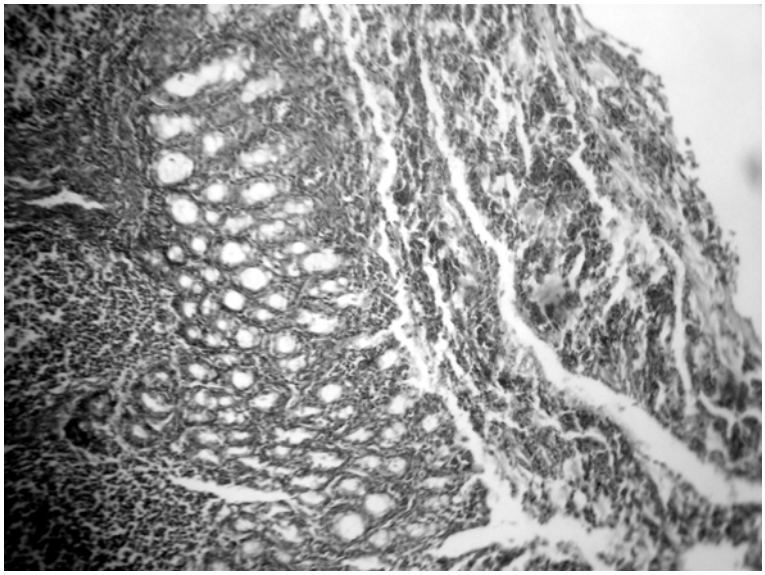

Figure 3. Intestine. Mild enteritis with a severe nodular hyperplasia of the lymphoid tissue (arrow) – HE, magnification: 400x.

Even when these parasites are detected in the intestinal tract of marine mammals, their impact on general health is unclear (Dailey, 2001; Hughes-Hanks, 2005). In this study, the alterations observed in the liver can suggest acute starvation, where body fat is mobilized for transport to the liver for metabolic use. The alterations observed in the spleen and in the lymphoid system in the middle and distal portions of the intestinal tract can be considered to be evidence of inflammation and an intense imunologic response against an invading pathogen. However, the lesions observed through gross and microscopic examination showed that the dolphin calf

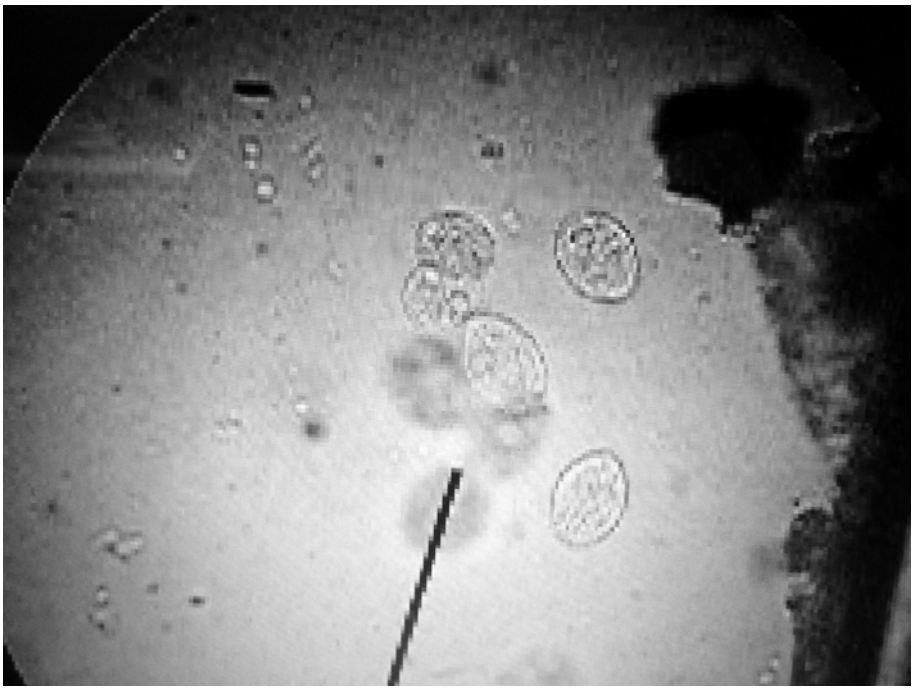

Figure 4. Oocysts of *Giardia* sp. observed in direct examination and flotation examination (Magnification: 400x).

was severely debilitated and was not able to adequately resist the infection.

Although the literature does not suggest giardiasis (*Giardia* infection) as a direct cause of an animal's death, due to the lesions observed in the liver, intestinal tract and immunologic systems, we believe that the symptoms of abnormal faeces and severe emaciation we observed can be attributed to the protozoan infection.

Giardia cysts are transmitted by the fecal-oral route between humans and animals (Fayer *et al*., 2004). Deng *et al*. (2000) described the common protozoan found in marine mammals as *Giardia duodenalis*. Hughes-Hanks (2005) observed that *Giardia intestinalis* Assemblage A can be found not only in the marine mammals but also in marine environments, indicating a direct relationship between human waste and the discharge of wastewater into the marine environment. Cysts of the protozoan can survive in water for weeks, and are not killed by concentrations of chlorine routinely used in water treatment, especially when the water being treated is cold (Wallis, 1994). Recent evidence indicates that these protozoans are also found in coastal waters, having contaminated a variety of shellfish and infected many species of marine mammals. These findings raise concern for the health of animals in coastal waters and for that of humans who eat raw shellfish or use these waters for recreation (Fayer *et al*., 2004).

Marine mammals are considered environmental sentinels because they are good indicators of mid- to long-term changes in the environment (Aguilar and Borrell, 1994). As the estuarine dolphin inhabits densely human-populated coastal areas, it is likely that such animals may become infected by protozoan agents from contact with human waste.

Since 1972, naturally occurring diseases in wild marine mammals have been studied intensively, although only recently in South America, firstly to evaluate their zoonotic potential and secondly to determine the risk of ocean-borne disease agents being introduced into and spreading among terrestrial mammals. Several agents with disease potential for land animals have been isolated from marine mammals (Smith *et al*., 1978; Dierauf and Gulland, 2001; Geraci and Lounsbury, 2005; Bogolmoni *et al*., 2006).

Attention to worldwide pollution of the coastal marine environment has focused primarily on hydrocarbonated and organochlorinated compounds as well as heavy metals and plastic debris (Karuppiah *et al*., 2005; Fair *et al*., 2007; Meirelles and Barros, 2007), while massive but unseen amounts of faeces from humans and domestic animals are discharged and carried into runoffs, bringing encysted zoonotic protozoan parasites to estuaries and coastal waters (Fayer *et al*., 2004). Proper management and disposal of human and terrestrial animal faeces are essential to keep the coastal marine environment and its species free from a number of enterically transmitted pathogens, including zoonotic protozoan agents. It is clear that *Giardia* sp. and other protozoans are present in the marine environment. However, the mere detection of parasites is not sufficient to demonstrate their impact on animal or human health. Future studies on the health of marine mammals should include an evaluation of the

parasite´s presence and its real impacts on marine mammals. More extensive parasitological studies should be performed during routine physical examination of captive animals and at necropsy to confirm the relationship between the presence of protozoans and diseases in marine mammals.

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