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Detection and molecular characterization of *Giardia* and *Cryptosporidium* in common dolphins (*Delphinus delphis*) stranded along the Galician coast (Northwest Spain)

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ABSTRACT

The ubiquitous protozoan parasites *Giardia* and *Cryptosporidium* have been detected from many species of captive and free-living wildlife, representing most mammalian orders. Twenty species of marine mammals have been reported to inhabit Galician waters and the region has one of the highest rates of stranding in Europe. Evidence from stranding, reported by-catches and sightings, suggests that the common dolphin (*Delphinus delphis*) is the most abundant cetacean on the Galician coast (Northwest Spain). The objective of this study was to detect and molecularly characterize isolates of *Giardia* and *Cryptosporidium* obtained from common dolphins stranded in this area. Between 2005 and 2012, sections of large intestine from 133 common dolphins stranded along the Galician coast were collected by the personnel of the Galician Stranding Network (*Coordinadora para o Estudo dos Mamíferos Mariños*, *CEMMA*). Using direct immunofluorescence antibody test (IFAT) and PCR amplification and sequencing of the SSU-rDNA, β-giardin genes and the ITS1-5.8S-ITS2 region, *Giardia* and *Cryptosporidium* were detected in 8 (6.0%) and 12 samples (9.0%), respectively. In two samples, co-infection by both parasites was observed. The molecular characterization revealed the presence of *Giardia duodenalis* assemblages A (genotypes A1 and A2) and B and *Cryptosporidium parvum* in these samples. This constitutes the first study in which the presence of *Giardia* and *Cryptosporidium* has been investigated in common dolphins on the European Atlantic coast, and it is also the first report of *C. parvum* in this host. Our findings indicate that these animals could act as reservoir of these waterborne parasites or could be victims of the contamination originated by anthropogenic activities.

1. Introduction

Giardia and *Cryptosporidium*, two ubiquitous protozoan parasites, have been detected from many species of captive and free-living wildlife, representing most mammalian orders. In marine environments, *Giardia* sp. cysts were first reported in intestinal contents of ringed seals

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(*Phoca hispida*) from the western arctic region of Canada using a fluorescent monoclonal antibody test (Olson et al., 1997). Although cysts were morphometrically identical to those of *Giardia* sp., no molecular characterization was undertaken to identify the species found in this host. Likewise, *Cryptosporidium* infection was first described in a dugong (*Dugong dugon*) in a coastal town of Australia by Hill et al. (1997), who observed by transmission electron microscopy numerous forms of *Cryptosporidium* intermediate life stages in small intestine sections, but oocysts were not found. This isolate was subsequently characterized as *Cryptosporidium hominis* (Morgan et al., 2000). Since then, several isolates of *Giardia* and *Cryptosporidium* obtained from different pinniped and cetacean species in a few locations of North America and in the Antarctic continent have been described and sometimes characterized at the molecular level, revealing that marine mammals can act not only as reservoirs for species that infect humans and domestic animals, but also harbour new genotypes of both parasites (Gaydos et al., 2008; Lasek-Nesselquist et al., 2010; Rengifo-Herrera et al., 2011, 2013; Bass et al., 2012).

The Galician coast (Northwest Spain) is an important area for cetaceans, not only within Spain but also at the European level. Up to 20 marine mammal species (16 cetaceans and 4 pinnipeds) have been reported to inhabit this area, which has one of the highest recorded rates of stranding in Europe (Covelo and Martínez, 2001; López et al., 2004; Pierce et al., 2010). Evidence from stranding, reported by-catches and sightings, suggests that the common dolphin (*Delphinus delphis*) is the most abundant cetacean species on the Galician coast, with an estimated population of 7,000–10,000 individuals living in Galician waters (López et al., 2002, 2004; Pierce et al., 2010).

The aim of the present work was to investigate the presence of *Giardia* and *Cryptosporidium* in common dolphins (*D. delphis*) stranded on the Galician coast.

2. Materials and methods

2.1. Sample collection

Between 2005 and 2012, a total number of 133 intestinal samples from stranded common dolphins (*D. delphis*) were collected in the Northwest of the Iberian Peninsula along the Galician coast by experienced personnel of the Galician Stranding Network (*Coordinadora para o Estudo dos Mamíferos Mariños*, *CEMMA*) (Fig. 1). Animals were identified to the level of species, measured and sexed. Among these samples, 54 corresponded to females and 78 to males. Taking into account the age of the animal, 43 samples were from adult individuals (27 females and 16 males); 79 from juvenile specimens (21 females and 58 males); 10 from calves (6 females and 4 males) and in one animal was impossible to determinate the age. After necropsy, sections of large intestine were collected and stored at −20 °C until being processed in the Laboratory of Parasitology of the Department of Microbiology and Parasitology of the University of Santiago de Compostela.

Intestinal contents (2.50 ± 0.48 g) were diluted in 10–20 ml of phosphate buffered saline (PBS) 0.04 M pH 7.2, filtered through a set of two sieves (mesh size 150 and 45 μm), shaken with diethyl ether (2:1, v/v) and centrifuged at 1000 × *g* for 15 min at 4 °C. The resulting two up layers were carefully discarded, the sediment was washed in PBS by centrifugation at 1000 × *g* for 15 min at 4 °C and the pellet was resuspended in 500 μl of PBS 0.04 M pH 7.2.

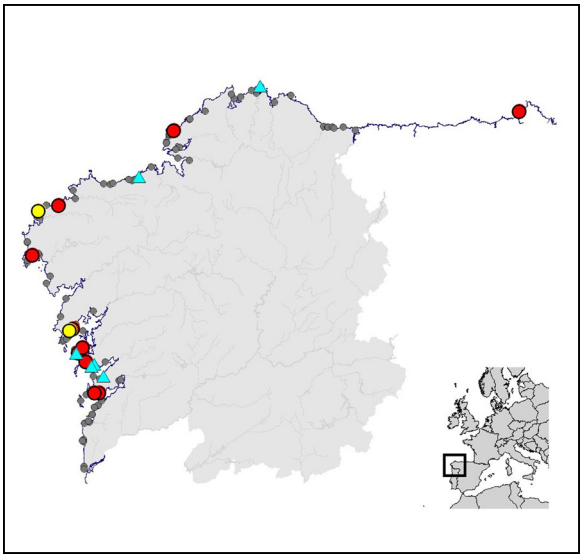

Fig. 1. Locations of the common dolphins (*D. delphis*) stranded along the Galician coast. Grey circles ( ) indicate negative samples, blue triangles ( ) indicate *Giardia*-positive samples, red circles ( ) indicate *Cryptosporidium*-positive samples and yellow circles ( ) indicate co-infection by both parasites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2.2. Detection of Giardia cysts and Cryptosporidium oocysts by epifluorescence microscopy

Direct immunofluorescence antibody test (IFAT) was performed on 50 μl of the sediments using the AquaGlo™ G/C Direct test (Waterborne, Inc., New Orleans, LA, USA), according to the manufacturer's instructions. The cysts/oocysts were identified by epifluorescence microscopy at 400× magnifications on the basis of their shape, size and the pattern and intensity of immunofluorescence staining.

2.3. Molecular characterization of Giardia spp. and Cryptosporidium spp.

Nucleic acids were extracted from the remaining 450 μl of the sediments obtained previously using the QIAamp® DNA Stool Mini Kit (QIAGEN®, Hilden, Germany) according to the manufacturer's instructions. DNA was stored at −20 °C until use.

For *Giardia*, protocols for the amplification of a ~170-bp fragment of the small subunit ribosomal DNA (SSU-rDNA) gene, of a ~315-bp fragment encompassing the ITS1-5.8S-ITS2 region in the ribosomal unit and of a 511-bp of the β-giardin (bg) gene were used as described previously (Read et al., 2002; Lalle et al., 2005; Cacciò et al., 2010). For *Cryptosporidium*, a two-step nested-PCR technique was