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Giardia and *Cryptosporidium* in harp and hooded seals from the Gulf of St. Lawrence, Canada

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ABSTRACT

Giardia and *Cryptosporidium* are protozoan parasites known to cause enteric disease in terrestrial wildlife species (mammals, reptiles and birds). Few surveys for *Giardia* and *Cryptosporidium* in marine wildlife species, such as pinnipeds, have been reported. The objective of this study was to determine the prevalence and genotype of *Giardia* and *Cryptosporidium* in two species of pinnipeds, harp seal (*Phoca groenlandica*) and hooded seal (*Cystophora cristata*), from the Gulf of St. Lawrence, Canada. Faecal samples were collected from pup and adult seals and examined for the presence of cysts of *Giardia* and oocysts of *Cryptosporidium* using microscopy and immunofluorescent staining. Tissues from the small intestine of adult seals were also collected and examined for infections using the polymerase chain reaction (PCR) technique. *Giardia* cysts were found in the faeces of 42% (16/38) of adult harp seals, but in none of the harp seal pups (0/20). Although *Giardia* cysts were not detected in faeces of adult hooded seals (0/10) using microscopy, 80% tested positive for *Giardia* using PCR of intestinal tissue indicative of a true replicating infection. Both harp and hooded seals harboured infections with the zoonotic strain, *Giardia duodenalis* Assemblage A, as determined using a nested-PCR technique to amplify a small subunit ribosomal (SSU-rRNA) gene of *Giardia*. *Cryptosporidium* was not detected by microscopy, nor using the PCR technique on intestinal tissues from any of the 68 seals examined.

1. Introduction

Inadequate treatment and disposal of sewage, other effluents and terrestrial runoff into the marine environment from municipal, industrial, agricultural and shipping activities have resulted in contamination of the marine environment and, in some cases, have resulted in direct infection of some marine animals with various pathogens including parasites such as *Giardia*, *Cryptosporidium* and *Toxoplasma gondii* (see Fayer et al., 2004; Appelbee et al., 2005; Dixon et al., 2008, for reviews).

The Gulf of St. Lawrence in Atlantic Canada is an ideal area to study *Giardia* and *Cryptosporidium* in the marine environment as many species of marine mammals frequent the Gulf and *Giardia* and *Cryptosporidium* have been detected in the St. Lawrence ecosystem which includes the St. Lawrence River, the St. Lawrence Estuary and Gulf of St. Lawrence (Measures and Olson, 1999; Payment et al., 2000, 2001; Graczyk et al., 2001). Both parasites have a direct life cycle, producing environmentally resistant infective stages that initiate infection following ingestion.

Measures and Olson (1999) observed cysts of *Giardia* in the rectal contents of adult harp seals from the Gulf of St. Lawrence, with a prevalence of 50% (15/30). Oocysts of *Cryptosporidium* were not detected in the same samples from that study, which included faeces from harp ($N = 47$),

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grey ($N = 19$) and harbour ($N = 8$) seals, St. Lawrence beluga, *Delphinapterus leucas* ($N = 11$) and one bottlenose whale (*Hyperoodon ampullatus*) from the Gulf of St. Lawrence and St. Lawrence Estuary (Measures and Olson, unpublished data, see Measures and Olson, 1999 for host details). It is unknown whether seals in the St. Lawrence ecosystem are parasitized with *Giardia* that are replicating in seals, or whether seals are pseudo-parasitized, i.e. ingesting cysts from the environment and passing them through the intestine without excystation and replication.

Not only is the infection status of these marine mammals unclear, but also the species and genotypes of parasites that may be present in this population are unknown. Measures and Olson (1999) used microscopy with immunofluorescent staining and morphological comparison to identify the cysts as *G. duodenalis*. No molecular characterization was performed to confirm this observation nor to determine whether the strain of *G. duodenalis* was a zoonotic strain (Assemblage A or B) or a host-adapted strain, such as those identified in dogs, cats and livestock. Molecular characterization is essential in identifying the parasite in infections, as well as aiding in the elucidation of possible sources of contamination and routes of transmission.

The objective of this study is to establish if *Giardia* cysts found in the faeces of harp and hooded seals indicates parasitic infection. To this end, a study was conducted to confirm parasitic infection with *Giardia* and *Cryptosporidium* and to determine the prevalence of these parasites in harp and hooded seals. To determine whether *Giardia* was undergoing excystation and replication in the intestine of harp and hooded seals, histological sections of the small intestine were analysed using light microscopy in order to detect trophozoites.

2. Materials and methods

Harp ($N = 58$) and hooded seals ($N = 10$) were live captured or shot under a scientific permit issued by Fisheries and Oceans Canada and sampled during the winter of 2001 from breeding ice floes located west of the Magdalen Islands (47°23′N, 61°52′W) in the Gulf of St. Lawrence, Québec. Data from animals were stratified by species, sex and age class (adult, pup). All adults were sexually mature based on their presence on the breeding patch and all females had nursing pups (i.e. mother–pup pairs). Fresh faecal samples (1–5 g) were collected directly from the rectum of live-captured seals, placed in phosphate buffered saline (PBS) and stored at 4 °C until analysed. Faeces were not collected from hooded seal pups. In addition to faeces, tissue from the small intestine (duodenum, jejunum and ileum) of dead harp (38) and hooded (10) seals was collected from all adult seals for histology and PCR analysis. Approximately 2 cm sections of small intestine were excised and fixed in 10% buffered formalin for histological analysis, or PBS and stored at -20 °C for PCR.

Faecal samples were purified by centrifugation over a 1 M sucrose cushion, then examined for the presence of *Giardia* cysts and *Cryptosporidium* oocysts utilizing fluorescein labelled monoclonal antibodies and microscopic examination as described previously (Olson et al., 1997a), with the exception that Aqua-Glo™ G/C Direct (Waterborne Inc., New Orleans) was used enabling the simultaneous detection of *Giardia* and *Cryptosporidium*.

To determine the species and genotype of *Giardia* cysts detected in the sucrose-purified faecal samples, genomic DNA was isolated following a slightly modified protocol using cetyltrimethylammoniumbromide (CTAB) (Appelbee et al., 2003) prior to PCR analysis as described below.

Genomic DNA was also isolated from the jejunum, duodenum and ileum from all seals that were negative for *Giardia* or *Cryptosporidium* by microscopic examination of faeces (Table 1). A piece of small intestine (approximately 5 cm long) was opened longitudinally then vigorously vortexed in PBS for 1 min before large pieces of tissue were removed with sterile tweezers. The remaining solution was then centrifuged at $900 \times g$ for 10 min at 4 °C, the supernatant removed and the pellet re-suspended in approximately 1 mL of tissue lysis buffer (50 mM Tris pH 8.0, 500 mM NaCl, 1% SDS). Genomic DNA was extracted from a 500 μL aliquot of this suspension using the CTAB method described previously (Appelbee et al., 2003).

A two-step nested-PCR technique was utilized to amplify a 292 bp fragment of the small subunit ribosomal (SSU-rRNA) gene of *Giardia* (Appelbee et al., 2003) or a 448 bp fragment of the 70 kDa heat shock protein (HSP70) of *Cryptosporidium* (Morgan et al., 2001). To eliminate the possibility of PCR inhibition, duplicate PCR reactions were run for each sample at each locus, one mixture containing the test DNA and a second mixture containing the test DNA spiked with *Giardia* or *Cryptosporidium* DNA.

To demonstrate parasitic infection in animals shown to be positive for *Giardia* by examination of faeces or PCR analysis of tissues from the small intestine, histological examination of tissues was conducted. Following dehydration in a graded series of ethanol, tissues from the small intestine were infiltrated and embedded using the JB-4 Embedding Kit® according to the manufacturer's instructions (Polysciences Inc., Germany). Sections of approximately 1.5 μm thick were cut, stained with Lee's methylene blue and trophozoites of *Giardia* were observed at 400× magnification and photographed.

3. Results

The prevalence of *Giardia* and *Cryptosporidium* infections in harp and hooded seals was determined through microscopic analysis of faeces stained with fluorescein labelled monoclonal antibodies (Table 1). Of the 68 faecal samples analysed, *Giardia* cysts were present in 39% (14/36) of adult female harp seals and two adult male harp seals, and cysts in all positive samples were identified as *G. duodenalis* Assemblage A by the described PCR technique. *Giardia* cysts were not detected in any of the faecal samples collected from harp seal pups (0/20) or adult hooded seals (0/10). *Cryptosporidium* oocysts were not observed in any faecal samples examined by microscopy.

As no *Giardia* cysts were found in faeces from the 10 adult hooded seals examined, the PCR technique was used on their intestinal tissues, 38 from harp seals and 10 from hooded seals. Amplicons of *Giardia* were obtained from at