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Short communication

Detection of a novel genotype of *Cryptosporidium* in Antarctic pinnipeds

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ABSTRACT

A study was conducted to investigate the presence of *Cryptosporidium* and *Giardia* in Antarctic marine mammals. A total of 270 faecal samples from different species of pinnipeds from different locations in the South Shetland Islands and Antarctic Peninsula were analysed by immunofluorescence microscopy and PCR. *Cryptosporidium* was detected by PCR in three samples from Southern elephant seals (*Mirounga leonina*) and 2 Weddell seals (*Leptonychotes weddellii*). However, no oocysts were observed in any of the samples by immunofluorescence microscopy. Molecular characterisation of the isolates, using the 18S rDNA, the HSP70 and the COWP loci, revealed the presence of a *Cryptosporidium* sp., previously reported from an Antarctic Southern elephant seal, in the elephant seals and a novel genotype in Weddell seals. *Giardia* could not be detected in any of the samples analysed.

1. Introduction

Cryptosporidium spp. and *Giardia duodenalis* (syn. *G. intestinalis*, *G. lamblia*) are protozoan parasites which infect a wide variety of hosts including humans and domesticated and wild animals worldwide (Xiao and Fayer, 2008). Currently, the genus *Cryptosporidium* contains up to 22 species and over 40 genotypes, while *Giardia duodenalis* includes 7 assemblages or genotypes, designated A through G (Fayer, 2010; Fayer et al., 2010; Robinson et al., 2010; Feng and Xiao, 2011; Ren et al., 2012). In addition, an assemblage H has been recently described in seals (Lasek-Nesselquist et al., 2010). Proper identification and characterisation of the species and genotypes involved in infection are needed to elucidate the routes of transmission. Traditionally, species were primarily differentiated according to host specificity, oocyst or cyst morphology and site of infection (Fayer, 2010). However, taxonomy based on these criteria has proven inadequate. Furthermore, genetic analysis has shown that these genera are complex. The advent of molecular characterisation tools has greatly contributed to establishing a correct taxonomy for both parasites setting the basis for a better understanding of the diseases they cause and their epidemiology.

In the last years increasing research has been carried out on marine mammals since they may act as indicator species for environmental contamination with these waterborne parasites (Appelbee et al., 2005). *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts have been identified in different pinniped species which include California sea lions (*Zalophus californianus*), bearded seals (*Erignathus barbatus*), ringed seals (*Phoca hispida* syn. *Pusa hispida*), harp seals (*Pagophilus groenlandica*), grey seals (*Halichoerus grypus*), hooded seals (*Cyptophora cristatai*), harbour seals (*Phoca vitulina*), mainly from different locations in North America and an Antarctic Southern elephant seal (*Mirounga leonina*) (reviewed in Rengifo-Herrera et al., 2011). Molecular analyses identified *Cryptosporidium muris* and two

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Cryptosporidium seal genotypes, seal genotypes 1 and 2, in ringed seals in Canada (Santin et al., 2005). Recently, two additional novel *Cryptosporidium* genotypes have been described in an Antarctic Southern elephant seal (*M. leonina*) and in a harp seal (*P. groenlandicus*) from the Gulf of Maine (Rengifo-Herrera et al., 2011; Bass et al., 2012). *Giardia duodenalis* Assemblage A was identified in harp and hooded seals from Canada (Appelbee et al., 2005), Assemblage B in a harbour seal in the USA as well as in ringed seals in Canada, both Assemblages A and B in a harp seal and Assemblage F-like in mixed grey/harbour seal populations from beaches in the USA (Bogomolni et al., 2008; Dixon et al., 2008; Lasek-Nesselquist et al., 2008). A further study has identified the canine genotype D and a novel genotype related to Assemblages C and D in faeces of harbour seals from Washington Stateĭs marine waters (Gaydos et al., 2008). These studies highlight the need for more research that can provide additional information on the diversity and host range of these groups of parasites.

The purpose of this study was to further investigate the presence of *Cryptosporidium* and *Giardia* in pinnipeds from different regions in the Antarctic Peninsula.

2. Materials and methods

2.1. Faecal samples

A total of 270 faecal samples from different pinniped populations from Deception Island, and other areas in the South Shetland Islands and Antarctic Peninsula were collected during the month of February in both 2010 and 2011 (Table 1). These included samples from Weddell seals (*Leptonychotes weddellii*), Southern elephant seals (*M. leonina*), and Antarctic fur seals (*Arctocephalus gazella*). Fresh samples were collected from the ground.

After sample collection, faecal slides were prepared, fixed in methanol, and stored at −20 °C until analysed. Faecal samples were kept at +4 °C without preservatives for periods up to 2 months when they were received and analysed in the laboratory.

2.2. Cryptosporidium and Giardia detection and characterisation

Immunofluorescence staining was performed using the *Crypto*/*Giardia* Cel IF Test (Cellabs Pty Ltd., Brookvale, Australia) according to the manufacturer's instructions.

Oocyst/cyst disruption and DNA purification from faecal samples were performed as described previously (McLauchlin et al., 1999).

For *Cryptosporidium* detection and characterisation, a nested PCR procedure was performed for amplification of an 827–840 bp polymorphic fragment of the 18 rDNA (Xiao et al., 1999, 2000). For further characterisation, a 446 bp fragment of the HSP70 and a 550 bp fragment of the COWP genes were amplified according to the protocols described by Morgan et al. (2001) and Pedraza-Díaz et al. (2001), respectively.

For *Giardia*, a nested procedure was performed to amplify a 511 bp fragment of the beta-giardin gene (Lalle et al., 2005).

Positive (*C. parvum* and *G. duodenalis* assemblage D) and negative controls were included for all PCRs. A 5 μl aliquot of the PCR products was examined following electrophoresis in 1% agarose/ethidium bromide gels.

Positive amplicons were purified using the GENECLEAN Turbo kit (QBiogene, CA, USA) according the manufacturer's instructions and then directly sequenced in both directions using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) and a 3730 DNA analyser (Applied Biosystems, CA, USA) at the Unidad Genómica del Parque Científico de Madrid. Sequences were analysed using BioEdit Sequence Alignment Editor v.7.0.1 (7) (Hall, 1999). Multiple alignments were performed using the ClustalW program and neighbour-joining trees were constructed from the aligned sequences using the MEGA5 software (Tamura et al., 2011). Accession numbers of Genbank *Cryptosporidium* 18S rDNA sequences used in the analysis: *C. andersoni* (AF093496), *C. baileyi* (L19068), *C. bovis* (AY120911), *C. canis* (AB210854), *C. cuniculus* (EU437413), *C. fayeri* (AF112570), *C. felis* (AF108862), *C. fragile* (EU162751), *C. galli* (HM116388), *C. hominis* (AB369994), *C. macropodum* (AF513227), *C. meleagridis* (AF112574), *C. molnari* (HM243548), *C. muris* (AB089284), *C. parvum* (L16996), *C. ryanae* (AY587166), *C. serpentis* (AF151376), *C. suis* (AF115377), *C. ubiquitum* (AF442484), *C. varanii* (AF112573), *C. wrairi* (AF115378), *C. xiaoi* (FJ896050), *Cryptosporidium* sp. 80ANT (GQ421425), *Cryptosporidium* sp. Cc444 (JN858905), *Cryptosporidium* sp. ferret genotype (GQ121022), *Cryptosporidium* sp. mink genotype (EF641015), *Cryptosporidium* sp. Pg453 (JN858909), *Cryptosporidium* sp. Pv140 (JN858906), *Cryptosporidium* sp. Pv245 (JN858907), *Cryptosporidium* sp. Pv270 (JN858908), *Cryptosporidium* sp. seal genotype 1 (AY731234), *Cryptosporidium* sp. seal genotype 2 (AY731235), *Cryptosporidium* sp. skunk genotype (AY120903).

Accession numbers of Genbank *Cryptosporidium* HSP70 sequences used in the analysis: *C. andersoni* (AJ567390), *C. baileyi* (AF221539), *C. bovis* (AY741306), *C. canis* (AY120920), *C. cuniculus* (GU967462), *C. fayeri* (AF221531), *C. felis* (AF221538), *C. galli* (AY168849), *C. hominis* (EF591788), *C. meleagridis* (AF221537), *C. muris* (AF221543), *C. parvum* (EF576953), *C. ryanae* (EU410346), *C. serpentis* (AF221541), *C. suis* (DQ833281), *C. ubiquitum* (EF362483), *C. varanii* (FJ429602), *C. wrairi* (AF221536), *C. xiaoi* (FJ896041), *Cryptosporidium* Pg453 (JN860884), *Cryptosporidium* Pv140 (JN860883), *Cryptosporidium* Pv270 (JN860882), *Cryptosporidium* sp. ferret (AF221532), *Cryptosporidium* sp. hedgehog (GQ259143), *Cryptosporidium* sp. mink (EF428201), *Cryptosporidium* sp. seal 1 (AY731236), *Cryptosporidium* sp. seal 2 (AY731237), *Cryptosporidium* sp. seal 2 (AY731238), *Cryptosporidium* sp. skunk (AY120917), *Cryptosporidium* sp. 80ANT (GQ421426).

Accession numbers of Genbank *Cryptosporidium* COWP sequences used in the analysis: *C. andersoni* (DQ989570, AY282693), *C. baileyi* (AY282698, AF266276), *C. canis* (AF266274), *C. cuniculus* (EU437411), *C. fayeri* (AY237633), *C. felis* (AY282700), *C. hominis* (AF148741, AF481960), *C. meleagridis* (AF248742, AY282694, DQ116568), *C. muris* (AF161579, AY643491), *C. parvum* (AY282696, AY282687, AY282686, AY282695, AF248743), *C. serpentis* (AF266275), *C. ubiquitum* (HM209389), *C. wrairi* (U35027),