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Article *in* Antarctic Science · December 2007

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Antarctic Science 19 (4), 437–438 (2007) © Antarctic Science Ltd 2007 Printed in the UK DOI: 10.1017/S0954102007000429

Short Note
First report of *Cryptosporidium* spp. oocysts in stool of Adélie penguin from the Antarctic using acid-fast stain

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Received 30 June 2006, accepted 7 December 2006, first published online 29 June 2007

Introduction

Cryptosporidium is an intracellular parasite that belongs to the phylum Protozoa and subphylum Apicomplexa (Sporozoa). It was first described by from the digestive tract of laboratory mice (Garcia 2001), and has been found in several animal groups including fishes, reptiles, birds and mammals, including humans (Jellison *et al*. 2002).

Cryptosporidium is found globally and is of great importance in public health, due to the high risk that it represents for immuno-compromised individuals (especially HIV positive patients). It is also considered as an indicator of environmental contamination and of water quality (Atías 1998, Garcia 2001). At present this parasite has been recorded from all the continents, except Antarctica.

Cryptosporidium oocysts are very small, (4–6 μm), which makes them difficult to detect in routine coprological analysis. Because of this, and since these oocysts have acid-fast properties, a Ziehl-Neelsen stain technique has long been used to detect oocysts on faecal smears (Garcia 2001).

More recently PCR based methodologies have been implemented which have also enabled the description of new species for this genus (Ryan *et al*. 2003). There are named species of *Cryptosporidium* for a wide variety of hosts including cattle, birds, dogs, cats, humans, fish, rodents, lizards and snakes (Xiao *et al.* 2004).

This note reports on the presence of *Cryptosporidium* oocysts in Adélie penguins (*Pygoscelis adeliae* (Hombron & Jacquinot)) from the Antarctic, even though there are no reports of this parasite on any species that lives on the continent.

Materials and methods

The stool samples were obtained during January and February of 2005 on Ardley Island (62º13'S, 58º54'W), King George Island, South Shetland Islands, (Antarctic Specially Protected Area No. 150). All 167 stool samples (112 chicks and 55 adults) were obtained directly from the cloacae. The collection date, age (chick or adult) and weight of each animal were recorded. The samples were fixed in 10% formaldehyde, stored in individual plastic bags at 4°C and analysed at the Parasitology Laboratory, College of Veterinary Medicine of the Universidad de Chile. Samples were centrifuged at 900 g for 15 min in plastic tubes after which a small aliquot from the sediment was used for making a smear on a glass slide. After drying at room temperature fuchsin was added to the smear, left for 20 min, before washing with water and subsequently with acid alcohol for 1 min. Finally the samples were stained with methylene blue solution before microscopic analysis (Atías 1998, García 2001). *Cryptosporidium* oocysts stains clearly under this protocol making them easy to identify.

Results

Of the 167 samples analysed, only 11 (6.59%) were positive for spherical acid-fast structures of 5 μm diameter, compatible with *Cryptosporidium* spp.; of these, ten were chicks and one was an adult.

Discussion

It is interesting to find this protozoan parasite in Adélie penguins, since a survey using the same methodology on gentoo penguins (*P. papua* Forster) at Munita Peninsula, (64°49'S, 62°51'E) Paradise Bay, had found no positive results.

Although the methodology used on this survey is not the most innovative, it is the methodology of choice in our country (due to the low cost and simplicity) (Atías 1998, Cordero & Rojo 1999). It is believed that there are no other parasite structures that fulfil the acid-fast characteristic and measures 5 μm in diameter. However, its major drawback is that it does not allow the identification of the species of *Cryptosporidium* involved.

The finding of *Cryptosporidium* oocysts on the stool samples of Adélie penguins makes this the first report of *Cryptosporidium* oocysts from Antarctic penguins.

Finally we intend to use molecular biology methodologies to identify the species of *Cryptosporidium* that is affecting this penguin population in order to determine if we are in front of the introduction of the parasite, or if it is a new species naturally associated to the penguin colonies on the Antarctic continent.

Acknowledgements

We would like to thank to Marcela Herrera and Claudia Godoy for the recollection of the samples used on this survey. This study was financed by the FIV 2005 grant, Favet, U. de Chile.

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