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Short communication

Helicobacter spp. from captive bottlenose dolphins (*Tursiops* spp.) and polar bears (*Ursus maritimus*)☆

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Abstract

The gastric fluid of six bottlenose dolphins and the faeces of four polar bears from the same oceanarium were examined for the presence of *Helicobacter*. As detected by PCR, all dolphins and 8/12 samples collected from polar bears were positive for *Helicobacter*. Novel sequence types were identified in samples collected from these animals of which several were unique to either the dolphins or the polar bears. At least one sequence type was, however, detected in both animal taxa. In addition, a sequence type from a dolphin shared a 98.2–100% identity to sequences from other *Helicobacter* species from harp seals, sea otters and sea lions. This study reports on the occurrence of novel *Helicobacter* sequence types in polar bears and dolphins and demonstrates the broad-host range of some species within these animals.

Keywords: *Helicobacter*; Captive marine mammals; Prevalence; Host-range

The genus *Helicobacter* occurs in the digestive system of both humans and a broad range of animal taxa (for a review, see Solnick and Schauer, 2001). Recently, this host range has also been reported to include various marine mammals such as dolphins, whales and seals (Harper et al., 2002, 2003b). Whilst only one formally recognised species (namely *H. cetorum*) has been described in these animals to date (Harper et al., 2002), other putative species have been detected (Harper et al., 2003a). Several species (including *H. cetorum*) have been reported to occur in association with conditions of gastrointestinal disease and, in some instances, may infect more than one host type (for a review see Fox, 2002). This is of particular importance in the captive setting where the detection of such agents is essential for the diagnosis and treatment of disease (Al-Soud et al., 2003; Fowler, 1996).

The gastric fluid from six captive bottlenose dolphins (*Tursiops* spp.) and the faeces from two separate polar bear (*Ursus maritimus*) enclosures (one containing two adults and the other two juveniles) from Sea World (Queensland, Australia) were examined for the presence of *Helicobacter* using 16S rRNA PCR and sequence analyses. Although all six dolphins maintained a clinical history of gastritis, all animals were asymptomatic at the time of sampling.

Approximately 5–10 mL of gastric fluid was collected from each dolphin, dispensed into sterile vials and placed immediately on ice. In addition, six faecal samples of approximately 20 g in weight were collected from each of the polar bear enclosures, placed into sterile collection vials containing 40 mL of sterile phosphate buffered saline (PBS) supplemented with 20% glycerol, mixed, and placed on ice. All samples were homogenised

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for 1 min and a 200 μL aliquot (or approximately 200 mg) removed and placed into a sterile 2 mL microcentrifuge tube. Total DNA was extracted from faeces using a QIAamp DNA Stool Mini Kit and from gastric fluid using a QIAamp DNA Mini Kit (QIAGEN). As a control for the extraction process (SF), 10 μL of a 10^8 CFU/mL culture of *H. pylori* strain 26695 (School of Microbiology and Immunology, University of New South Wales, Australia) was added to a 200 μL aliquot of the sample homogenate and processed in conjunction with all other samples.

Total DNA extracts were subjected to PCR using genus-specific primers (Germani et al., 1997) to produce amplicons of approximately 400 bp in length as described previously (Oxley et al., 2004). Two positive and two negative controls were included with each series of reactions. One positive control (+VE) was used to detect PCR inhibition and contained 1 μL of the sample DNA extract and 1 μL of *H. pylori* genomic DNA. The other positive control (HP) was used to verify the success of the PCR reaction and contained 2 μL of *H. pylori* genomic DNA. The negative controls were used to detect the presence of false products and contained either 2 μL of *Escherichia coli* genomic DNA (EC) or 2 μL of water (W).

Amplicons were cloned using the pGEM-T Easy Vector System (Promega) in accordance with the manufacturer's instructions and clones selected using ampicillin plates containing IPTG and X-Gal. Plasmid DNA was isolated using Wizard Plus SV Minipreps (Promega) and submitted to the Australian Genome Research Facility (AGRF) for sequencing.

Sequences were entered into BioEdit (version 5.0.9) (Hall, 1999) and aligned using ClustalW (version 1.4) (Thompson et al., 1994). Sequences from other organisms contained within the GenBank database were also included in the alignment. Differences in nucleotide sequence of approximately 3% or greater between any two rDNAs were used to distinguish a separate sequence type (Stackebrandt and Goebel, 1994). Tree diagrams representing these alignments were constructed with the Molecular Evolutionary Genetics Analysis program (MEGA) (version 2.1) (Kumar et al., 2001) using the neighbour-joining method. All sequences were deposited within the GenBank database.

Amplicons of the expected size of 400 bp were observed for all six dolphins and four of the six samples collected from each of the adult and juvenile polar bear enclosures (Fig. 1). A tree diagram demonstrating the alignment of nucleotide sequences from several animals as well as other organisms contained within the GenBank database is presented in Fig. 2. All sequences clustered with members of the genus *Helicobacter*. Four groups of sequences differing by at least 10 bp (97.5% identity) were observed of which two appeared to be unique to polar bears (Groups 1 and 3). Whilst a difference of no more than 6 bp (98.6% identity) was observed within these two groups of sequences, a difference of more than 11 bp (97.3% identity) was observed between these and all other *Helicobacter* species. Based on a 3% difference between any two rDNAs used to distinguish a separate species (Stackebrandt and Goebel, 1994), these two groups may thus represent two distinct and possibly novel sequence types in polar bears.

Sequences obtained from the dolphins in this study did not cluster with those previously identified from other dolphins and whales (namely *H. cetorum* and *H. tursiopsae*). Instead, these sequences formed two separate groups (Groups 2 and 4). Group 2 consisted of a single sequence (dolphin 5) and differing by no more than 7 bp (98.2% identity), clustered with *Helicobacter*

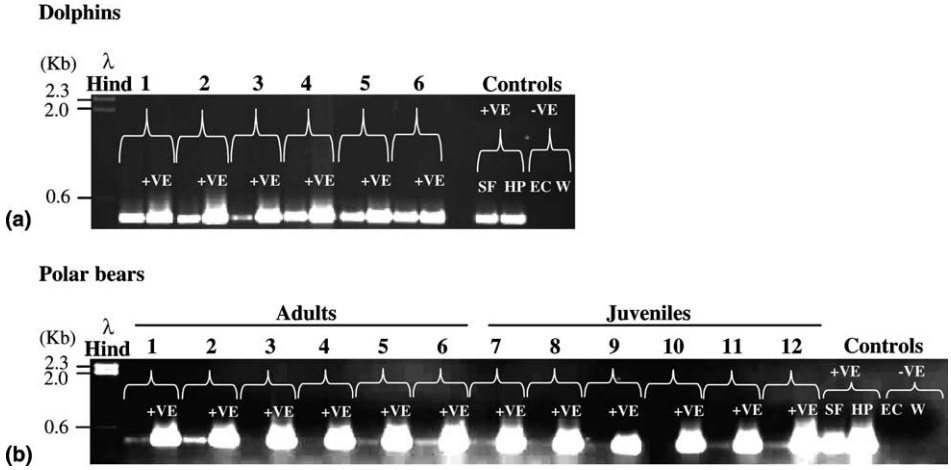

Fig. 1. Agarose gel electrophoresis of PCR products produced from total DNA extracted from (a) the gastric fluid of six captive dolphins and (b) the faecal material from adult and juvenile polar bear enclosures. The second lane of each sample is a positive control (+VE) containing total sample and *Helicobacter pylori* DNA. Additional experimental controls subjected to PCR include: SF – *H. pylori* cells added to a faecal sample prior to total DNA extraction; HP – *H. pylori* genomic DNA; EC – *Escherichia coli* genomic DNA; and W – Water (no template control). λ Hind – molecular weight marker (λ phage DNA cut with *Hin*dIII).