Veterinary Parasitology 192 (2013) 67–74

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

An atypical genotype of *Toxoplasma gondii* as a cause of mortality in Hector's dolphins (*Cephalorhynchus hectori*)

W.D. Roe[a,*], L. Howe[b], E.J. Baker[c], L. Burrows[b], S.A. Hunter[d]

[a] Pathobiology Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, PN4222, New Zealand
[b] Infectious Disease Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, PN4222, New Zealand
[c] University of Edinburgh, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Roslin, Midlothian, Scotland, United Kingdom
[d] New Zealand Wildlife Health Centre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, PN4222, New Zealand

ARTICLE INFO

Article history:
Received 30 August 2012
Received in revised form 29 October 2012
Accepted 1 November 2012

Keywords:
Protozoan
Toxoplasma
Oocysts
Hector's dolphin
Mortality
Cetacean

ABSTRACT

Hector's dolphins (*Cephalorhynchus hectori*) are a small endangered coastal species that are endemic to New Zealand. Anthropogenic factors, particularly accidental capture in fishing nets, are believed to be the biggest threat to survival of this species. The role of infectious disease as a cause of mortality has not previously been well investigated. This study investigates *Toxoplasma gondii* infection in Hector's dolphins, finding that 7 of 28 (25%) dolphins examined died due to disseminated toxoplasmosis, including 2 of 3 Maui's dolphins, a critically endangered sub-species. A further 10 dolphins had one or more tissues that were positive for the presence of *T. gondii* DNA using PCR. Genotyping revealed that 7 of 8 successfully amplified isolates were an atypical Type II genotype. Fatal cases had necrotising and haemorrhagic lesions in the lung ($n = 7$), lymph nodes ($n = 6$), liver ($n = 4$) and adrenals ($n = 3$). Tachyzoites and tissue cysts were present in other organs including the brain ($n = 5$), heart ($n = 1$), stomach ($n = 1$) and uterus ($n = 1$) with minimal associated inflammatory response. One dolphin had a marked suppurative metritis in the presence of numerous intra-epithelial tachyzoites. No dolphins had underlying morbillivirus infection. This study provides the first evidence that infectious agents could be important in the population decline of this species, and highlights the need for further research into the route of entry of *T. gondii* organisms into the marine environment worldwide.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Hector's dolphins (*Cephalorhynchus hectori*) are small coastal dolphins that are endemic to New Zealand and have a limited geographic range. There are four genetically and geographically distinct groups, one confined to the West Coast of the North Island, and three others found around the South Island (Pichler and Baker, 2000; Baker et al., 2002). The North Island population, known as Maui's dolphin, is classified as a separate subspecies (*C. hectori maui*) (Baker et al., 2002). Analysis of haplotype diversity suggests a recent dramatic decline in Maui's dolphin numbers (Pichler and Baker, 2000), with an estimated remaining population of 110 individuals (Slooten et al., 2006a). The small population size, restricted range and recent population decline mean that Maui's dolphins are considered to be critically endangered (IUCN, 2010). The South Island Hector's dolphin population is classified as endangered, numbering about 7300 individuals (Dawson et al., 2004; Slooten et al., 2004). The main threat to the survival of this

* Corresponding author. Tel.: +1 646 3569099; fax: +1 646 3505714.
E-mail address: w.d.roe@massey.ac.nz (W.D. Roe).

0304-4017/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2012.11.001

species is believed to be accidental drowning/asphyxiation in fishing nets, known as bycatch (Dawson, 1991; Martien et al., 1999), but to date little has been published about infectious disease as a cause of mortality in these dolphins.

The importance of protozoal disease in marine mammal species has become increasing apparent over the past decade. The southern sea otter is a threatened coastal marine species that has been slow to recover in numbers despite being legally protected since 1911 (Kreuder et al., 2003). Recently, protozoan encephalitis caused predominantly by *Toxoplasma gondii* was identified as a major cause of mortality in this population (Kreuder et al., 2003). Sporadic cases of fatal toxoplasma infections have also been reported in several cetacean and pinniped species, frequently associated with concurrent immunosuppression due to morbillivirus infection (reviewed by Miller (2008)). In addition to causing direct mortality, *T. gondii* infections can have indirect effects on population dynamics through behavioural changes, reproductive loss and increased risk of predation (Webster, 2001; Kreuder et al., 2003).

This study describes fatal disseminated toxoplasmosis in Hector's dolphins, and considers the role of this disease in population decline of the species. The involvement of possible immunosuppressive factors and pathogen genotype are investigated, and potential sources of infection are discussed.

2. Materials and methods

2.1. Sample collection

Analyses were conducted on samples collected from Hectors dolphins that were sent to Massey University, Palmerston North, between March 2007 and November 2011. These animals had been bycaught or found beachcast (stranded dead) and were recovered as part of the New Zealand Department of Conservation's marine mammal stranding investigation response. Gross necropsy reports were available for each animal, along with archived tissues preserved in 10% neutral buffered formalin (including heart, lung, brain, lymph nodes, stomach, gonads, diaphragm, skeletal muscle, adrenal, kidney, liver and brain) or stored frozen at −20 °C (including liver, lung, lymph nodes and brain).

2.2. Histopathology

Formalin-fixed tissues were processed into paraffin blocks for routine histopathological processing, and sectioned at 4 μm for microscopic examination. All sections were stained with haemotoxylin and eosin (H&E) and special stains (Gram, Young's fungal and Giemsa) were carried out where indicated.

2.3. Immunohistochemistry

Paraffin embedded tissue sections were cut at 5 μm and mounted on positively charged glass slides, then rehydrated through a series of increasing concentrations of xylene using a Leica automatic tissue processor. Antigen retrieval was performed in 1% trypsin/calcium chloride (pH 7.8) at 37 °C for 30 min. Endogenous peroxidase activity was blocked by incubating slides in 0.3% hydrogen peroxide for 30 minutes at room temperature, followed by washing in 0.05 M tris-buffered saline (TBS; BioRad Laboratories, Hercules, California, USA). Non-specific antibody was blocked using normal horse serum (Vectastain Universal Elite kit, Vector Laboratories, Burlingame, California, USA), and slides were then incubated for 60 min at room temperature in polyclonal caprine anti-*T. gondii* antibody (VMRD Inc., Pullman, Washington, USA) diluted to 1:1000 in TBS. Antigen detection was achieved using donkey anti-goat secondary antibody (Rockland, Gilbertsville, Pennsylvania, USA) at 1:2000 dilution and ABC reagent (Vectastain Universal Elite kit, Vector Laboratories, Burlingame, California, USA) and the resulting complex visualised using 3-3′-diaminobenzidine (DAB) diluted in TBS (Liquid DAB Substrate Chromogen System, Dako, Victoria, Australia). Sections were then counterstained in haematoxylin and mounted. Positive antibody controls were run with each batch of slides using known toxoplasma-positive sheep brain sections, and PCR-confirmed toxoplasma positive dolphin tissue sections. Negative antibody controls comprised known negative sheep brain sections, and omission of primary antibody on known positive sheep brain sections.

Immunohistochemistry for morbillivirus was conducted using monoclonal mouse anti-canine distemper virus primary antibody (VMRD Inc., Pullman, Washington, USA) diluted to 1:2000 in TBS, and caprine anti-mouse secondary antibody at 1:2000 (Vectastain Universal Elite kit, Vector Laboratories, Burlingame, California, USA). Positive control tissues (lung from a harbour seal with phocine distemper virus, kindly provided by Thijs Kuiken, Erasmus Medical Centre, Rotterdam, Netherlands) and negative control tissues (sheep lung, plus infected harbour seal lung with omission of primary antibody) were run with each batch of slides.

2.4. PCR

Brain and lung samples were run for all animals, with additional tissues also being processed where available. DNA was extracted from 42 frozen and 18 paraffin-fixed tissues using a Qiagen DNeasy Kit (Qiagen, Valencia, California, U.S.A.) following the manufacturer's instructions. The samples were screened for *T. gondii* using a nested polymerase chain reaction (PCR) procedure described by Aspinall et al. (2002). To confirm successful amplification 10 μl of the final PCR product was run on a 1.5% agarose gel containing ethidium bromide prior to purification and sequencing. A known *T. gondii* isolate (incomplete strain S48, Toxovax®, MSD Animal Health, New Zealand), confirmed by sequencing, was used as a positive control and water blanks were included as negative controls. One positive amplicon from each animal was purified using a PureLink PCR purification kit (Invitrogen, Carlsbad, California, USA) and subjected to automatic dye-terminator cycle sequencing with BigDye™ Terminator Version 3.1 Ready Reaction Cycle Sequencing kit and the ABI3730 Genetic Analyser (Applied Biosystems Inc, Foster

Download English Version:

https://daneshyari.com/en/article/5804440

Download Persian Version:

https://daneshyari.com/article/5804440

Daneshyari.com