Toxoplasmosis in Indo-Pacific humpbacked dolphins (Sousa chinensis), from Queensland

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Objective To describe the clinical signs, gross pathology, serology, bacteriology, histopathology, electron microscopy and immunohistochemistry findings associated with toxoplasmosis in four Indo-Pacific humpbacked dolphins (Sousa chinensis) that stranded in Queensland in 2000 and 2001.

Design Clinical assessment, gross necropsy, and laboratory examinations.

Procedure Necropsies were performed on four S chinensis to determine cause of death. Laboratory tests including serology, bacteriology, histopathology and transmission electron microscopy were done on the four dolphins. Immunohistochemistry was done on the brain, heart, liver, lung, spleen and adrenal gland from various dolphins to detect Toxoplasma gondii antigens.

Results Necropsies showed all of four S chinensis that stranded in Queensland in 2000 and 2001 had evidence of predatory shark attack and three were extremely emaciated. Histopathological examinations showed all four dolphins had toxoplasmosis with tissue cysts resembling T gondii in the brain. Tachyzoite stages of T gondii were detected in the lungs, heart, liver, spleen and adrenal gland, variously of all four dolphins. Electron microscopy studies and immunohistochemistry confirmed the tissues cysts were those of T gondii. All four dolphins also had intercurrent disease including pneumonia, three had peritonitis and one had pancreatitis.

Conclusion Four S chinensis necropsied in Queensland in 2000 and 2001 were found to be infected with toxoplasmosis. It is uncertain how these dolphins became infected and further studies are needed to determine how S chinensis acquire toxoplasmosis. All four dolphins stranded after periods of heavy rainfall, and coastal freshwater runoff may be a risk factor for T gondii infection in S chinensis. This disease should be of concern to wildlife managers since S chinensis is a rare species and its numbers appear to be declining.

Materials and methods

Source material In February 2000, an Indo-Pacific humpbacked dolphin stranded alive in Townsville, Australia (dolphin 1) and was transported by officers of the Queensland Parks and Wildlife Service (QPW) to a local marine aquarium for rehabilitation, but died 3 days later. In September 2000, another S chinensis was found dead on the bank of a river 40 km south of Townsville (dolphin 2). The dolphin was reported by members of the public to be rolling from side to side in the water and bumping into boats near a boat ramp, just before its death. In June 2001, a third S chinensis stranded live on the foreshores of Townsville, but died soon after stranding (dolphin 3). In July 2001, a fourth S chinensis was found dead in Gladstone harbour (dolphin 4).

Clinical pathology Clinical tests were only possible on dolphin 1 that stranded alive, because all other dolphins stranded dead, or died soon after stranding. Blood was taken from the tail vein of dolphin 1 for a complete blood count and a serum biochemical profile.

Clinical therapy Treatment of dolphin 1 included oral antimicrobial therapy with Clavulox palatable tablets (Pfizer, Animal Health Australia) administered via orogastric tubing on day 1. Baytril 150 mg antibacterial tablets (Bayer Australia Ltd) and fluids were thereafter given orally, twice daily. Despite 3 days of such therapy, the dolphin died.

Necropsy procedure All three dolphins that stranded in the Townsville region were necropsied by veterinary pathologists at Oonoonba Veterinary Laboratory, Townsville, Queensland. Necropsy of the dolphin that stranded in Gladstone harbour (dolphin 4) was done by QPW officers and tissue specimens were collected and forwarded to Rockhampton Veterinary Laboratory for histological examination.

Toxoplasma gondii is a protozoan parasite of worldwide distribution that infects homeothermic animals including primates, marsupials, birds, rodents, other mammals and humans.1,2 In Australia T gondii is widespread and has been described from numerous intermediate wildlife hosts including birds such as the southern cassowary, crimson rosella, satin bowerbird, domestic pigeon, regent parrot; marsupials such as the koala, common wombat, brown antechinus, kowari, mulgara, and agile wallaby; rodents such as the domestic house mouse and brown rat; and other mammals such as the dingo, domestic dog and cat, sheep and horse.3 Toxoplasmosis is rarely reported in marine animals but has been recorded in a west Indian manatee,8 a California sea lion,5 seals,5,7 Atlantic bottlenose dolphins and a spinner dolphin.8,9

In 2000 and 2001, five Indo-Pacific humpbacked dolphins (Sousa chinensis), stranded in Townsville, and one stranded in Gladstone. This paper describes toxoplasmosis in four of these dolphins, three from Townsville and one from Gladstone. We describe the findings from necropsy, histological, immunohistochemical and electron microscopic examinations.

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Histological preparations and serology
Tissue specimens collected from all four dolphins were fixed in 10% buffered neutral formalin for 48 h, trimmed and processed routinely for histology. Sections were cut at 5 μm and stained routinely with haematoxylin and eosin for light microscopy. Serum collected from dolphin 1 was tested for T. gondii antibody, using an indirect haemagglutination test kit (Laboratoires Fumouze Division Diagnostics, Le Malherbes, France).

Bacteriology
Various samples of organs and tissues were taken from dolphins 1, 2 and 3 for bacterial isolation. Samples included the brain, kidneys, lung, liver and pericardial fluid from dolphin 1; lung, pancreas and small intestinal contents from dolphin 2; kidney, liver, peritoneal and pleural fluids from dolphin 3.

Transmission electron microscopy
Selected sections of paraffin-embedded brain tissue from all four dolphins were examined by transmission electron microscopy after processing by standard methods.

Immunohistochemistry
Immunohistochemistry for detection of T. gondii antigens was performed on selected sections of brain (dolphins 1 and 4), liver (dolphins 2 and 4), heart (dolphins 2 and 4), lung (dolphin 4), spleen and adrenal gland (dolphin 3) using a commercial avidin-biotin immuno-peroxidase staining technique (Vectorstain Kit; Vector Laboratories Inc, Burlingame, CA). The primary antibody used was T. gondii polyclonal antiserum raised in goats (Dako Corp, Carpinteria, CA). The secondary antibody used was an anti-goat serum raised in rabbits (Vector Laboratories Inc). Brain and liver from mice experimentally infected with T. gondii were used as positive controls while brains from mice that had been experimentally infected with N. caninum were used as negative controls.

Results
The locations where the four S. chinensis stranded in northern Queensland in 2000 and 2001 are illustrated in Figure 1.

Clinical findings and gross necropsy
Lesions detected at necropsy included superficial, nonpenetrating skin lacerations and wounds consistent with predatory shark attack. All three dolphins that stranded in the Townsville region (dolphins 1 to 3) were extremely emaciated, showing prominence of the skull and spine. Dolphin 4, which stranded at Gladstone was in good bodily condition. Other common findings included wart-like skin lesions at the genital opening (dolphins 1, 2 and 4), and the presence of several well-demarcated rounded ulcers (0.5 to 1.0 cm diameter) on the dorsal surfaces of the tongue and soft palate (dolphins 1, 2 and 4), and the presence of several well-demarcated rounded ulcers (0.5 to 1.0 cm diameter) on the dorsal surfaces of the tongue and soft palate (dolphins 1, 2 and 4).

Other significant clinical and gross necropsy findings for each dolphin are presented below.

Dolphin 1
The dolphin was an adult female, 247 cm in length. At necropsy, there were fibrin tags adhering to the pleura of both lungs. The mesenteric lymph nodes were enlarged, and the mucosal surfaces of the pyloric region and bile duct were reddened. The stomach was distended and contained remnants of fish bones.

Dolphin 2
The dolphin was an adult female, 247 cm in length. At necropsy, there were fibrin tags adhering to the pleura of both lungs. The mesenteric lymph nodes were enlarged, and the mucosal surfaces of the pyloric region and bile duct were reddened. The stomach was distended and contained remnants of fish bones.

Dolphin 3
The dolphin was an adult female, 232 cm in length. There was a small straight scar 1 cm long located mid-ventrally, approximately 60 cm dorsal to the genital opening that connected by a sinus tract to the abdominal cavity. The mesenteric lymph nodes were enlarged and there was haemorrhagic fibrinous peritonitis. Fibrin adhered to the lining of the abdominal wall where the sinus tract opened into the abdominal cavity, and also to the mesentery, serosa of the stomach, pancreas, liver, right hemi-diaphragm and pleura of the right lung. A stingray barb, 15.2 cm long was found lodged in the liver, surrounded by fibrous tissue, purulent exudate and fibrin. The stingray spine was identified as belonging to Dasyatis sp (P. Last, personal communication).

Dolphin 4
This dolphin was a male, 180 cm in length in good body condition. Gross necropsy findings were reported as unremarkable by Q PWS officers.

Serology
Dolphin 1 showed significant haematological findings including neutrophilia (19.83 x 10^9/L). Serum fibrinogen ranged from 3.6 g/L to 4.0 g/L over 3 days and was within normal limits. Creatinine kinase rose from 232 U/L to 1526 U/L and lactic acid dehydrogenase from 1575 to 2365 U/L over 3 days.
Perivascular cuffs of mononuclear inflammatory cells were also evident. Multifocal areas of necrosis were commonly observed and these were similarly infiltrated with mononuclear inflammatory cells.

Organisms consistent with tachyzoite stages of *T. gondii* were also detected histologically in various tissues including the parenchyma of the liver (dolphin 2), the myocardium (dolphins 2 and 4) (Figure 3), the spleen and adrenal gland (dolphin 3).

Other histological findings included purulent interstitial and bronchopneumonia (dolphin 1), fibrinous bronchopneumonia (dolphin 2), diffuse fibrinous bronchopneumonia and choroiditis (dolphin 3), and diffuse interstitial pneumonia (dolphin 4).

**Immunohistochemistry**

Immunoperoxidase staining performed on selected sections of brain, liver, heart, lung, spleen and adrenal gland from the various dolphins all tested positive and confirmed that suspected tachyzoites present in these organs were those of *T. gondii* (Table 2). The immunoperoxidase staining performed on the brain and liver of mice experimentally infected with *T. gondii* used as positive controls tested positive, whereas brains of mice experimentally infected with *Neospora caninum* tested negative.

**Serum from dolphin 1 tested positive for *T. gondii* antibody at a dilution of 1 in 2560.**

**Bacteriology**

A pure growth of *Pseudomonas aeruginosa* was isolated from the lungs of dolphins 1 and 2. A number of bacteria were isolated, variously from the kidney, liver, peritoneal and pleural fluid of dolphin 3 including *Clostridium perfringens*, *Edwardsiella tarda*, *E. hoshinae*, *Pseudomonas sp*, *P. aeruginosa*, *Vibrio alginolyticus* and *V. harveyi*. The bacteria isolated from dolphin 3 were considered secondary invaders and will not be considered further.

**Histopathology**

Significant histopathological findings from all four dolphins are shown in Table 1. The most striking lesions were seen in the brains, where there were large numbers of protozoan cysts, ranging in size from 6 to 30 μm, scattered throughout the neuropil but mainly in the brain stem and cerebrum with few cysts found in the cerebellum (Figure 2). Many cysts appeared to be fragmenting. Intense infiltrates of mononuclear inflammatory cells, consisting mainly of macrophages and lymphocytes, were often associated with these cysts. There were numerous microglial nodules throughout the brain tissue but these were not always associated with discernible cysts.
Transmission electron microscopy
Examination of the electron micrographs of brain tissue in all cases showed numerous polyzoic cysts within the neural tissue (Figure 4). The cysts were identical in ultrastructure to those of the cyst-forming apicomplexan parasite T. gondii.1 The cysts were round in cross-section ranging from 20 to 30 μm in diameter. They were located intracellularly within enlarged host cells, but the host cell type could not be discerned. The cysts were bounded by a thin membranous wall (up to 40 nm thick) supported by a granular ground substance. The cyst wall did not contain any protrusions and was surrounded on the external surface by host cell mitochondria and endoplasmic reticulum. The cysts were aseptate and contained up to 70 bradyzoites in cross-section. The bradyzoites were elongate and did not contain any protrusions and was surrounded on the thick) supported by a granular.

Discussion
Our findings from histopathological examination of tissues and electron microscopy are similar to other reported cases of toxoplasmosis in marine mammals, in that T. gondii tissue cysts were detected in the brain, and T. gondii tachyzoites, in association with focal necrosis, were detected in various other body organs.4,5 The electron microscopical findings were consistent with tissue cysts of T. gondii and allowed differentiation from other cyst-forming coccidia including Sarcocystis, Frenkelia, Besnoitia, Hammondia and Neospora. The cysts could be differentiated from those of Sarcocystis, Frenkelia and Besnoitia variously by the presence of a thin primary cyst wall, the absence of a secondary cyst wall, the absence of septae and the small size of the zoites. They differed from those of Hammondia and Neospora in the small size of the membranous cyst wall and the reduced organellae content of the zoites, especially the number of rhoptries. In each of our four cases we observed large numbers of tissue cysts in the brain, and tachyzoites were observed in at least one other organ including the heart, adrenal gland, spleen and liver. The presence of numerous tissue cysts in the brain in all four dolphins suggested the possibility that these resulted from the reactivation of a chronic infection through the debilitating effect of intercurrent disease. All of the dolphins had intercurrent disease, including pneumonia (all four dolphins), pancreatitis (dolphin 1) and peritonitis (dolphins 1, 3 and 4).

Table 1. Significant histopathological findings from four Sousa chinensis with toxoplasmosis (dolphins 1, 2, 3 and 4) that were necropsied in Queensland in 2000 and 2001

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histopathology</th>
<th>Dolphin</th>
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<tr>
<td>Brain</td>
<td>Non-suppurative encephalitis, glosis,</td>
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<tr>
<td></td>
<td>focal necrosis &amp; T. gondii cysts present</td>
<td>+</td>
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<tr>
<td>Heart</td>
<td>Occasional foci of necrosis and myocarditis</td>
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<tr>
<td>Liver</td>
<td>Focal disseminated perivascular inflammation</td>
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<td>Peritoneal cavity</td>
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<td>Pancreas</td>
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<td>Lungs</td>
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<td>Tongue</td>
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a T. gondii tachyzoites present
+ present
- absent

Four S. chinensis that stranded in northern Queensland in 2000 and 2001 had encephalitis attributable to infection with T. gondii. Clinical examination was possible in only one of these and it showed clinical signs of ataxia prior to death. The importance of toxoplasmosis in Indo-Pacific humpbacked dolphins is uncertain. This study suggests that the disease should be of concern to wildlife managers since the species is listed as rare under the Nature Conservation Act 1994. Their numbers appear to be declining, but relatively little is known to date about population sizes, ecology and biology of S. chinensis.13,17,18

The manner in which these dolphins became infected with T. gondii is uncertain. Toxoplasmosis is a parasitic disease that infects only homeothermic animals.1,2 Cats are the only known definitive hosts,1 and usually become infected by ingestion of intermediate hosts that are infected with tissue cysts of T. gondii.1,2 Infection in intermediate hosts is by ingestion of tissue cysts or via ingestion of oocysts of T. gondii.1,4-16 Intermediate hosts can also be infected vertically by transmission of tachyzoites or by ingestion of other intermediate hosts containing T. gondii tissue cysts.1,2

Recent studies in the Townsville region have shown that S. chinensis feed on a variety of marine and freshwater fish and benthic invertebrates (G Parra, personal communication). A similar diet of common estuarine and reef fish, and occasionally cephalopods and crustaceans has been found for S. chinensis in South Africa and Hong Kong.17 There is no evidence to date
that *T. gondii* infects poikilothermic animals such as fish or invertebrates. However it is possible that molluscs or crustaceans may mechanically concentrate *T. gondii* oocysts, acting as vectors for infecting marine mammals such as *S. chinensis*. Lindsay et al recently demonstrated that *T. gondii* oocysts can be removed from seawater by eastern oysters (*Crassostrea virginica*) and retain their infectivity. Furthermore if *S. chinensis* occasionally fed upon unusual prey such as wounded or dead seabirds or rodents that were infected with *T. gondii* tissue cysts, then they may incidentally acquire toxoplasmosis.

In Australia *S. chinensis* have a broad geographic distribution yet exhibit some territoriality within inshore coastal areas and do not migrate vast distances throughout their natural range. Recent studies on the ecology and migration patterns of *S. chinensis* in the Townsville region have shown that they frequent inshore creeks, rivers and coastal areas that are highly urbanised (G Parra, personal communication). Infection with *T. gondii* may thus be acquired locally under specific circumstances. The ingestion during feeding, of water or sediment contaminated with *T. gondii* oocysts is one possible route of infection. It is possible that coastal waters become contaminated with oocysts of *T. gondii* from sewage discharge or floods that carry sediments contaminated with them into creeks and rivers. It may not be coincidental that three infected dolphins stranded in the Townsville region within a year of heavy rainfall and flooding. Miller et al recently reported that coastal freshwater runoff is a risk factor for *T. gondii* infection in southern sea otters (*Enhydra lutris nereis*) in southern California. Coastal freshwater runoff may similarly be a risk factor for *T. gondii* infection in *S. chinensis*.

*S. chinensis* may also acquire toxoplasmosis by direct ingestion of infected feline faeces discarded from recreational vessels or from feral cats. In Townsville and Gladstone, feral cats inhabit the rock walls associated with the ports and marinas and domestic cats are kept as pets on board boats and recreational yachts. Domestic cats are also commonly kept as pets in households throughout Townsville and Gladstone, and cat faeces may be discarded into sewers. Although the proportion of infected cats excreting oocysts is generally not high in a given population (less than 2% in most countries), a cat may shed millions of oocysts in its faeces and they can remain viable at 15 to 35°C for up to 1 year. Townsville has a tropical climate where the average sea surface water temperatures vary from 24.6°C in winter to 29.1°C in summer (Australian Oceanographic Data Centre). Townsville sea surface water temperatures favour the long term viability of *T. gondii* oocysts in coastal waters. One of the dolphins that had toxoplasmosis was from Gladstone harbour and was thought to be one of a pod of four to six animals regularly seen at the entrance to Auckland creek and the adjacent marina.

A pilot survey will be conducted on the eastern coast of Queensland to assess the significance of this disease in marine mammals that inhabit marinas and urban coastal areas. This could have implications for the future management of *S. chinensis*, especially pertaining to disposal of domestic cat faeces in marinas and sewage systems in Australia, and for the control of feral cat and rodent populations in coastal regions, especially in marinas and ports.

### Acknowledgments

The authors wish to thank the staff of Onoonba and Rockhampton Veterinary Laboratories and the QPWS field officers in Townsville and Gladstone, particularly Kim van Stelen, Helen Smith, David Savage, Neil Mattocks, Mark Read, Malcolm Turner and Patrick Centurio for their help in carcase retrieval and specimen collection. Dr Kirstin Dobbs (Great Barrier Reef Marine Park Authority) provided information on the conservation status of *S. chinensis*, and assisted during necropsies, Dr Wendy Blanshard assisted with clinical assessment of dolphin 1, and Bill Doherty assisted with mapping. Veterinary Pathology Services provided serum testing for *T. gondii*. Mr Guido Parra of James Cook University provided valuable information and discussion on population, feeding ecology and behaviour of *S. chinensis* in the Townsville region from his recent studies.

### References

Idiopathic mucosal lesions of the arytenoid cartilages of 21 Thoroughbred yearlings: 1997-2001

Abnormalities of the upper respiratory tract of Thoroughbred yearlings are commonly identified during pre- or post-sale endoscopic examination. Some may progress to the detriment of subsequent performance resulting in financial penalty for any purchaser. Published studies of sales examinations make no mention of mucosal lesions of the arytenoid cartilage.

This paper reports results from the post-sale, upper airway endoscopic examinations of 3312 Thoroughbred yearlings during a 5 year period and follows the discovery by the authors of mucosal ulcers and small granulomas on the axial surface of one or both arytenoid cartilages at post-sale endoscopic examination. Mucosal lesions were seen in 0.63% of the yearlings evaluated and represent the commonest documented abnormal condition of the upper portion of the respiratory tract. At subsequent examination 15 horses had healed without complications. Two horses with bilateral ulceration developed a granuloma at each arytenoid chondropathy. One horse was not available for follow up.

The authors conclude that pre-sale endoscopic examination of Thoroughbred yearlings should include careful study of the arytenoid cartilage, particularly at the rostral margin of the vocal process. Medical therapy should be considered and progress monitored. Lesions discovered post-sale warrant notification of the owner and sales company.


Medetomidine-ketamine anaesthesia induction followed by medetomidine-propofol in ponies: infusion rates and cardiopulmonary side effects

Volatile anaesthetic agents are commonly employed in equine surgical cases especially in procedures exceeding 1.5 h duration. A major deficiency of these techniques is that they are cardiopulmonary depressants and may result in a high fatality rate, particularly when anaesthesia is maintained for 3-4 h or more. While several alternatives to inhalational anaesthesia in the horse have been evaluated, the only injectable anaesthetic suitable for long-duration procedures is propofol which requires combination with various α₂ adrenoreceptor agonists to produce satisfactory depth of anaesthesia. However this regimen presents problems including hypoxaemia, hypercapnia and relatively high costs. Various propofol/medetomidine combinations of total IV anaesthesia have been used successfully, with good quality of recovery. However inductions were inconsistent and unsatisfactory in some cases.

In this study the anaesthesia of six ponies was induced with medetomidine-ketamine and then maintained with medetomidine-propofol. Induction is reported as excellent and cardiovascular function remained stable. Recovery to standing after 4 h of anaesthesia averaged 31.1 min and was achieved after one or two attempts. The authors propose this IV regimen as a possible alternative to inhalation anaesthesia with potential to reduce anaesthesia-related fatality rates in horses.