SHORT PAPER

Toxoplasmosis in Beluga Whales (Delphinapterus leucas) from the St Lawrence Estuary: Two Case Reports and a Serological Survey

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Summary

Toxoplasmosis was diagnosed in two free-ranging beluga whales from the St Lawrence estuary, Quebec, Canada, in 1988 and 1998. Histologically, tachyzoites and bradyzoites were present in the brain, spleen, lymph nodes, adrenals and lungs of both animals, and in the thymus of one. These organisms were readily labelled by an indirect immunohistochemical method for Toxoplasma gondii antigens. In the lymph nodes, spleen and lungs the organisms were associated with histiocytic infiltration. In the brain of one animal they were associated with mild multifocal gliosis and haemorrhages. There was no evidence of concomitant morbillivirus infection. Serum samples were collected from 22 beluga whales stranded between 1995 and 1998 on the shores of the St Lawrence Estuary and examined for antibodies to T. gondii by the modified agglutination test. Antibody titres of ≥25 were found in six (27%) of the animals. This is the first confirmed report of toxoplasmosis in beluga whales.

Toxoplasmosis is caused by Toxoplasma gondii, an obligate intracellular protozoan parasite that infects a wide variety of mammals and birds. This coccidian parasite, which has a worldwide distribution, has been reported only occasionally in marine environments, and documented cases in cetaceans are few in number (Cruickshank et al., 1990; Inskeep et al., 1990; Migaki et al., 1990; Domingo et al., 1992; Di Guardo et al., 1995). In cetaceans and other marine mammals, toxoplasmosis is often associated with morbillivirus infection (Domingo et al., 1992; Di Guardo et al., 1995) or suspected immunosuppression (van Pelt and Dietrich, 1973; Inskeep et al., 1990).

We report here a case of toxoplasmosis in a St Lawrence Estuary beluga whale (Delphinapterus leucas) and, in addition, review a case reported by De Guise et al., (1995), who described toxoplasma-like organisms in the spleen of a beluga whale without confirming their identity or characterizing the lesions. We also report the results of a survey of archival sera, designed to throw light on the relationship between T. gondii infection and toxoplasmosis in beluga whales in the St Lawrence Estuary.

A 6-month-old male beluga calf was found stranded on the shores of the St Lawrence Estuary at Saint-Denis-de-Kamouraska, Quebec, Canada (69° 52′ W, 45° 48′N) in June 1998. All lymph nodes were enlarged (2 to 5 times normal size), with a diffusely pale and wet cut surface. A few petechiae were found on serial cross sections of the
Fig. 1. Cerebral necrosis with intra-lesional *Toxoplasma gondii* cysts (arrowheads) and tachyzoites in a beluga whale. Haematoxylin and eosin. Bar, 100 μm. Inset: cysts react strongly in the avidin-biotin-complex immunohistochemical method with anti-*T. gondii* serum. Bar, 30 μm.

brain at the junction of grey and white matter. A
patent urachus was noted. On histological ex-
amination, the medullary sinuses of all lymph nodes
were found to be moderately distended by macro-
phages. Numerous oval protozoal tachyzoites (c.
2 × 4 μm) were present free in the medullary sinuses
and within the cytoplasm of some macrophages. Occasional,
10 to 20 tachyzoites were clustered
within an intra-cytoplasmic parasitophorous va-
cuole (≤ 15 μm in diameter). The presence of
similar organisms in the brain, generally in peri-
vascular areas at the junction of grey and white
matter, was associated with mild haemorrhages or
gliosis (Fig. 1). In the thymus and in the lymphoid
tissue of the anal mucosa, similar organisms were
associated with moderate histiocytic infiltration and
marked diffuse lymphoid depletion.

Beluga DL-11-88 (De Guise *et al.*, 1995), a female
aged >31 years, was stranded at Port-au-Persil
(69° 57′W, 47° 30′N) in October 1988. Major
macroscopical lesions consisted of severe unilateral
sclerosing mastitis and severe unilateral renal
haemorrhages. Microscopically, numerous intra-
and extra-histiocytic *T. gondii*-like tissue cysts and
tachyzoites were observed in the spleen, lymph
nodes and lungs. A cerebellar section, which was
the only central nervous system section available,
was apparently free of inflammation and protozoa.

Paraffin wax-embedded sections of a variety of
organs from both animals were treated with poly-
clonal rabbit anti-*T. gondii* and *Neospora caninum*
serum in an avidin-biotin immunohistochemical
examination (Lindsay and Dubey, 1989). In all
organs in which the parasite had been observed by
light microscopy, organisms strongly reacted with
anti-*T. gondii* serum (Fig. 1, inset), but not with
anti-*N. caninum* serum. In addition, *T. gondii*
tissue
cyts and tachyzoites were detected immuno-
histochemically in the liver and the adrenals of
both animals, and in the cerebellum of DL-11-88.
However, despite the presence of parasites, lesions
could not be observed in these organs due to poor
preservation.

Paraffin wax-embedded sections of the lung and
lymph nodes from both animals were treated with
a monoclonal antibody to the haemagglutinin pro-
tein of phocine distemper virus with techniques
Morbillivirus antigen was not found in any section.
Sera from 22 dead beluga whales, found stranded
on the shore of the St Lawrence Estuary, were
collected between 1995 and 1998. Serum from
each animal, diluted 1 in 25, 1 in 50, and 1 in 500,
was tested for *T. gondii* antibodies by the modified
agglutination test (MAT) (Dubey and Desmonts,
1987). Sera that caused agglutination at a dilution
of ≥ 1 in 25 were considered to be positive (Dubey
and Desmonts, 1987; Dubey *et al.*, 1995; Dubey,
Toxoplasmosis in Beluga Whales

1997). Antibody titres to *T. gondii* were 25 in three animals, and 50 in three others. Therefore, the overall seroprevalence of *T. gondii* in this beluga whale population was 27%. The antibody titre in the 6-month-old beluga calf with systemic toxoplasmosis was 25.

The presence of specific *T. gondii* antibody in animals without lesions of toxoplasmosis indicates that infection by this parasite is not invariably fatal in beluga whales. This is the first report of *T. gondii* antibodies in cetaceans. This observation is important because the prevalent hypothesis has been that marine mammals are highly susceptible to *T. gondii* infection (Migaki *et al.*, 1990; Oksanen *et al.*, 1998). Clinical infection, however, has generally been associated with immunosuppression (van Pelt *et al.*, 1973; Inskeep *et al.*, 1990; Di Guardo *et al.*, 1995).

Major potential causes of immunosuppression in marine mammals are infection by morbilliviruses (Domingo *et al.*, 1992; Di Guardo *et al.*, 1995) and high tissue concentrations of environmental contaminants such as polychlorinated biphenyls (PCBs) (Borrell *et al.*, 1996). It is unlikely that the two belugas with toxoplasmosis were affected by a morbillivirus because lesions of cetacean morbillivirus infection were not found in these animals, immunohistochemical examination for morbilliviruses was negative, and beluga whales from the St Lawrence Estuary are seronegative to dolphin and phocine morbilliviruses (Mikaelian *et al.*, 1999).

The concentration of environmental contaminants in the two beluga whales examined was not assessed; beluga whales from the St Lawrence, however, are known to accumulate high concentrations of environmental contaminants, including PCBs (Martineau *et al.*, 1987; Metcalfe *et al.*, 1999) and tributyltin (Yang *et al.*, 1998). The immunosuppressive activity of these compounds is well recognized in man (Elferink *et al.*, 1986) and laboratory animals (Thomas and Hinsdill, 1978; Smialowicz *et al.*, 1989), and has been demonstrated in marine mammals (de Swart *et al.*, 1994). However, any possible implication of environmental contaminants in the two cases reported here remains speculation.

The MAT measured only IgG antibody because the mercaptoethanol used in the test would have destroyed IgM. The MAT is highly sensitive and specific, as has been shown by extensive validation in pigs experimentally and naturally infected with *T. gondii* (Dubey *et al.*, 1995; Dubey, 1997). However, this test has not been validated in marine mammals. The finding of an antibody titre of 25 in a whale that had histologically confirmed toxoplasmosis was noteworthy, suggesting that even a low titre is indicative of *T. gondii* infection.

*T. gondii* infection is generally acquired by ingesting meat containing tissue cysts, or by ingesting food or water contaminated with oocysts excreted by Felidae. It is unlikely that beluga whales become infected through their food; the latter consists of marine fish and invertebrates (Vladykov, 1946), which seem unlikely hosts for *T. gondii* (Dubey and Beattie, 1988). Beluga whales, however, engulf large amounts of sediment while they feed (Vladykov, 1946) and, like other marine mammals, drink seawater (Ridgway, 1972). Sediments and seawater may contain *T. gondii* oocysts as a result of contamination by flood water (Holshuh *et al.*, 1985) or sewage effluent (Buergelt, 1983). Oocysts have been shown to survive at least 72 h in saline water (Iannuzzi and Renieri, 1973). Interestingly, minke whales (*Balaenoptera acutorostrata*) inhabiting the northwestern Atlantic are seronegative to this parasite (Oksanen *et al.*, 1998). Taken together, these observations suggest that proximity to human settlements and domestic cats increases the exposure of marine mammals to *T. gondii*.

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