J. Comp. Path. 2000, Vol. 122, 73–76
Article No. jcpa.1999.0341, available online at http://www.idealibrary.com on IDEAL®

SHORT PAPER

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

I. Mikaelian, J. Boisclair, J.P. Dubey*, S. Kennedy† and D. Martineau

*Canadian Cooperative Wildlife Health Centre and Centre Québécois sur la Santé des Animaux Sauvages, Faculté de Médecine Vétérinaire, Université de Montréal, 3200 Sicotte, C.P. 5000, Saint-Hyacinthe, Qc, Canada, J2S 7C6, * Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, Agricultural Research Service, US Department of Agriculture, Beltsville, Maryland 20705–2350, USA and † Veterinary Sciences Division, Department of Agriculture for Northern Ireland, Stoney Road, Stormont, Belfast BT4 3SD, UK*

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 $\geqslant 25$ 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

0021–9975/00/010073+04 $35·00

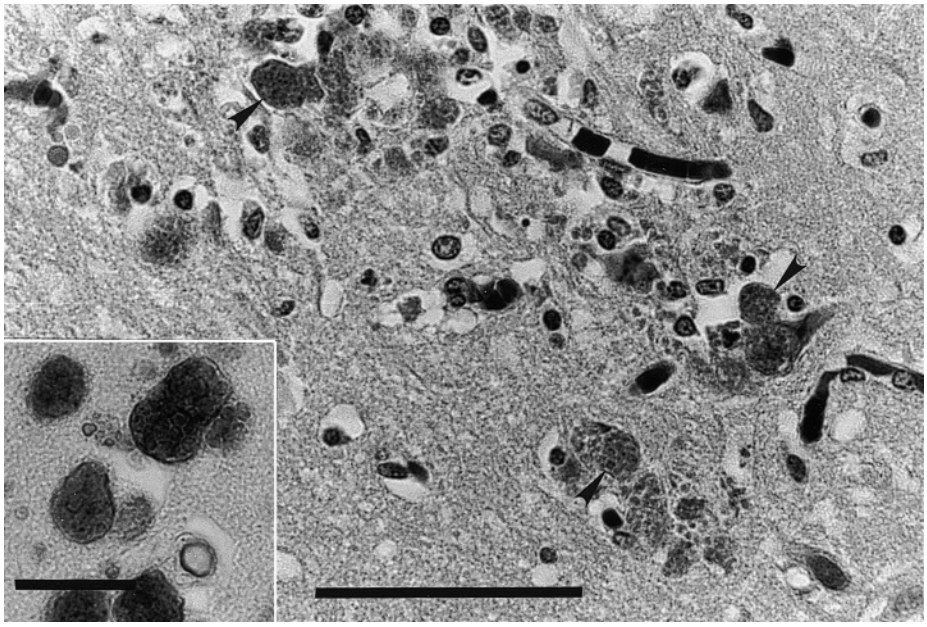

Fig. 1. Cerebral necrosis with intra-lesional *Toxoplasma gondii* cysts (arrowheads) and tachyzoites in a beluga whale. Haematoxylin and eosin. Bar, 100 μm. Inset: cysts react strongly in the avidin-biotin-complex immunohistochemical method with anti-*T. gondii* serum. Bar, 30 μm.

brain at the junction of grey and white matter. A patent urachus was noted. On histological examination, the medullary sinuses of all lymph nodes were found to be moderately distended by macrophages. Numerous oval protozoal tachyzoites (*c.* 2×4 μm) were present free in the medullary sinuses and within the cytoplasm of some macrophages. Occasionally, 10 to 20 tachyzoites were clustered within an intra-cytoplasmic parasitophorous vacuole ($\leqslant$ 15 μm in diameter). The presence of similar organisms in the brain, generally in perivascular 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 polyclonal 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 cysts and tachyzoites were detected immunohistochemically 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 protein of phocine distemper virus with techniques and reagents described by Kennedy *et al.* (1991). 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 $\geqslant$ 1 in 25 were considered to be positive (Dubey and Desmonts, 1987; Dubey *et al.*, 1995; Dubey,

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 acurostrata*) 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.*

Acknowledgments

We are grateful to J. Cardin, J. Deslandes, C. Lussier, L. Pépin and B. Pépin-Faille for skilful laboratory assistance. We also thank P. Béland and M. Kingsley, who determined the age of the whales. This study was funded by the Canadian Cooperative Wildlife Health Centre, the Centre Québécois sur la Santé des Animaux Sauvages, Parks Canada, the World Wildlife Fund (Canada), and Fisheries and Oceans Canada.

References

Borrell, A., Aguilar, A., Corsolini, S. and Focardi, S. (1996). Evaluation of toxicity and sex-related variation of PCB levels in Mediterranean striped dolphins affected by an epizootic. *Chemosphere*, **32,** 2359–2369.

Buergelt, C. D. (1983). Toxoplasmic meningoencephalitis in a West Indian manatee. *Journal of the American Veterinary Medical Association*, **183,** 1294–1296.

Cruickshank, J. J., Haines, D. M., Palmer, N. C. and St Aubin, D. J. (1990). Cysts of a *Toxoplasma*-like organism in an Atlantic bottlenose dolphin. *Canadian Veterinary Journal*, **31,** 213–215.

De Guise, S., Lagacé, A., Béland, P., Girard, C. and Higgins, R. (1995). Non-neoplastic lesions in beluga whales (*Delphinapterus leucas*) and other marine mammals from the St Lawrence Estuary. *Journal of Comparative Pathology*, **112,** 257–271.

De Swart, R. L., Ross, P. S., Vedder, L. J., Timmerman,

H. H., Heisterkamp, S., Loveren, H. V., Vos, J. G., Reijnders, P. J. H. and Osterhaus, A. D. M. E. (1994). Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. *Ambio*, **23,** 155–159.

Di Guardo, G., Corradi, A., Agrimi, U., Zizzo, N., Morelli, L., Perillo, A., Kramer, L., Cabassi, E. and Kennedy, S. (1995). Neuropathological lesions in cetaceans found stranded from 1991 to 1993 on the coasts of Italy. *European Journal of Veterinary Pathology*, **1,** 51–57.

Domingo, M., Visa, J., Pumarola, M., Marco, A. J., Ferrer, L., Rabanal, R. and Kennedy, S. (1992). Pathologic and immunocytochemical studies of morbillivirus infection in striped dolphins (*Stenella coeruloalba*). *Veterinary Pathology*, **29,** 1–10.

Dubey, J. P. (1997). Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. *Veterinary Parasitology*, **71,** 307–310.

Dubey, J. P. and Beattie, C. P. (1988). *Toxoplasmosis of Animals and Man*, CRC Press, Florida.

Dubey, J. P. and Desmonts, G. (1987). Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal*, **19,** 337–339.

Dubey, J. P., Thulliez, P., Weigel, R. M., Andrews, C. D., Lind, P. and Powell, E. C. (1995). Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. *American Journal of Veterinary Research*, **56**, 1030–1036.

Elferink, J. G., Deierkauf, M. and Van Steveninck, J. (1986). Toxicity of organotin compounds for polymorphonuclear leukocytes: the effects on phagocytosis and exocytosis. *Biochemical Pharmacology*, **35**, 3727–3732.

Holshuh, H. J., Sherrod, A. E., Taylor, C. R., Andrews, B. F. and Howard, E. B. (1985). Toxoplasmosis in a feral northern fur seal. *Journal of the American Veterinary Medical Association*, **187,** 1229–1230.

Iannuzzi, L. and Renieri, G. (1973). Indagini sulla toxoplasmosi dei pesci. Nota II: prove di resistenza, in acqua di fonte e in acqua di mare dei trofozoiti di *Toxoplasma gondii*. *Annali de Faculta de Medicina Veterinaria di Messina*, **10**, 207–212.

Inskeep, W., Gardiner, C. H., Harris, R. K., Dubey, J. P. and Goldston, R. T. (1990). Toxoplasmosis in Atlantic bottle-nose dolphins. *(Tursiops truncatus). Journal of Wildlife Diseases*, **26**, 377–382.

Kennedy, S., Smyth, J. A., Cush, F. P., McAliskey, M., McCullough, M. and Rima, B. K. (1991). Histopathologic and immunohistochemical studies of distemper in harbor porpoises. *Veterinary Pathology*, **28**, 1–7.

Lindsay, D. S. and Dubey, J. P. (1989). Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. *American Journal of Veterinary Research*, **50,** 1981–1983.

Martineau, D., Béland, P., Desjardins, C. and Lagacé, A. (1987). Levels of organochlorinated chemicals in tissues of beluga whales *(Delphinapterus leucas)* from the St Lawrence estuary, Quebec, Canada. *Archives of Environmental Contamination and Toxicology*, **16,** 137–147.

Metcalfe, C. D., Metcalfe, T., Ray, S., Paterson, G. and Koenig, B. (1999). Polychlorinated biphenyls and organochlorine compounds in brain, liver and muscle of beluga whales (*Delphinapterus leucas*) from the Arctic and St Lawrence Estuary. *Marine Environmental Research*, **47,** 1–15.

Migaki, G., Sawa, T. R. and Dubey, J. P. (1990). Fatal disseminated toxoplasmosis in a spinner dolphin (*Stenella longirostris*). *Veterinary Pathology*, **27,** 463–464.

Mikaelian, I., Tremblay, M.-P., Montpetit, C., Tessaro, S. V., Cho, H. J., House, C., Measures, L. and Martineau, D. (1999). Seroprevalence of selected viral diseases in a population of beluga whales (*Delphinapterus leucas*) in Canada. *Veterinary Record*, **144,** 50–51.

Oksanen, A., Tryland, M., Johnsen, K. and Dubey, J. P. (1998). Serosurvey of *Toxoplasma gondii* in North Atlantic marine mammals by use of agglutination test employing whole tachyzoites and dithiothreitol. *Comparative Immunology, Microbiology and Infectious Diseases*, **21,** 107–114.

Ridgway, S. H. (1972). Homeostasis in the aquatic environment. In: *Mammals of the Sea. Biology and Medicine*, S. H. Ridgway, Ed., Charles C. Thomas, Springfield, Illinois, pp. 590–747.

Smialowicz, R. J., Andrews, J. E., Riddle, M. M., Rogers, R. R., Luebke, R. W. and Copeland, C. B. (1989). Evaluation of the immunotoxicity of low level PCB exposure in the rat. *Toxicology*, **56,** 197–211.

Thomas, P. T. and Hinsdill, R. D. (1978). Effect of polychlorinated biphenyls on the immune responses of rhesus monkeys and mice. *Toxicology and Applied Pharmacology*, **44,** 41–51.

Van Pelt, R. W. and Dietrich, R. A. (1973). Staphylococcal infection and toxoplasmosis in a young harbor seal. *Journal of Wildlife Diseases*, **9,** 258–261.

Vladykov, V.-D. (1946). *Etudes sur les Mammifères Aquatiques. VI. Nourriture du Marsouin Blanc ou Béluga (Delphinapterus leucas)* du Fleuve Saint-Laurent, Québec, Canada: Contrat du Département des Pêcheries.

Yang, F., Chau, Y. K. and Maguire, R. J. (1998). Occurrence of butyltin compounds in beluga whales (*Delphinapterus leucas*). *Applied Organometallic Chemistry*, **12,** 651–656.

Received, April 15th, 1999
Accepted, August 11th, 1999