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Source: Journal of Wildlife Diseases, 40(2) : 294-300

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-40.2.294>

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Seroprevalence of *Toxoplasma gondii* in Canadian Pinnipeds

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ABSTRACT: Sera ($n=328$) collected from phocids (1995–97) from the east coast of Canada, including harp seals (*Phoca groenlandica*), hooded seals (*Cystophora cristata*), grey seals (*Halichoerus grypus*), and harbor seals (*Phoca vitulina*), were diluted 1:25, 1:50, and 1:500 and tested by a modified agglutination test for antibodies to *Toxoplasma gondii*. Titers equal to or greater than 1:25 were considered evidence of exposure. Grey seal (11/122, 9%), harbor seal (3/34, 9%), and hooded seal (1/60, 2%) had titers of 1:25 and 1:50. Harp seals ($n=112$) were seronegative. Probable maternal antibody transfer was observed in one harbor and one grey seal pup at 10 and 14 day of age, respectively. Transmission of *T. gondii* in the marine environment is not understood. The discovery of *T. gondii* in marine mammals might indicate natural infections unknown because of lack of study or might indicate recent contamination of the marine environment from the terrestrial environment by natural or anthropogenic activities.

Key words: Canada, pinnipeds, seals, seroprevalence survey, *Toxoplasma gondii*.

Toxoplasma gondii or “*T. gondii*-like protozoa” have been reported from tissues of marine mammals for over 50 yr (Ratcliffe and Worth, 1951). Most of these reports involve captive marine mammals in zoologic parks, marine mammals undergoing rehabilitation, or stranded marine mammals examined at necropsy. Toxoplasmosis has been described in pinnipeds (phocids, otariids), sea otters, manatees, and odontocetes (see Dubey et al., 2003 for review). Reports of serologically positive marine mammals include phocids, otariids, walrus, sea otters, and odontocetes (Dubey et al., 2003). Pacific harbor seals (*Phoca vitulina*) have been diagnosed with toxoplasmosis (Van Pelt and Dieterich, 1973; Miller et al., 2001b). Lambourn

et al. (2001) reported antibody titers (modified agglutination test [MAT]) to *T. gondii* in Pacific harbor seals. Dubey et al. (2003) found antibodies to *T. gondii* using MAT in four phocid species (ringed seal [*Phoca hispida*], bearded seal [*Erignathus barbatus*], spotted seal [*Phoca largha*], and Pacific harbor seals).

Presence of *T. gondii* in some captive marine mammals has been attributed to contamination of their food, water, or enclosures with oocysts from infected domestic or feral cats (Ratcliffe and Worth, 1951; Jardine and Dubey, 2002). Some researchers have hypothesized that sewage, storm, freshwater, or agricultural runoff containing oocysts passed by domestic or wild felids contaminate freshwater and marine environments, leading to infections in wild marine mammals (Bandoli and de Oliveira, 1977; Miller et al., 2002).

The St. Lawrence River and Estuary and the Gulf of St. Lawrence on the east coast of Canada have areas of dense, concentrated human populations, as well as uninhabited or sparsely inhabited areas, with various industrial, forestry, fishery, and agricultural activities (St. Lawrence Centre, 1996). There is considerable marine traffic in the St. Lawrence Seaway, and the St. Lawrence ecosystem receives industrial, agricultural, and human waste (Payment et al., 2000). The largest primary physicochemical sewage treatment plant in North America is located in Montreal on the St. Lawrence River. Posttreatment analyses of effluents discharged into the St. Lawrence River indicated that 0% human enteric viruses, 76% *Giardia* cysts, 27% *Cryptosporidium* oocysts, and 25%

fecal coliforms were removed (Payment et al., 2001). Two cases of toxoplasmosis and antibodies (27%) to *T. gondii* have been reported in stranded beluga (*Delphinapterus leucas*) from the St. Lawrence Estuary (De Guise et al., 1995; Mikaelian et al., 2000).

Within the St. Lawrence ecosystem, two phocid species are resident year-round—Atlantic harbor seal and grey seal (*Halichoerus grypus*)—and are generally coastal in behavior, frequenting the inshore. Two other phocid species—harp seal (*Phoca groenlandica*) and hooded seal (*Cystophora cristata*)—in the St. Lawrence ecosystem are winter migrants from the Arctic, are pagophilic (ice-loving), and frequent the offshore. Zoonotic diseases such as toxoplasmosis have been a concern for consumers of marine mammals on the east coast of Canada and for native peoples such as Inuit in arctic Canada (Curtis et al., 1988; McDonald et al., 1990). To understand the epizootiology of *T. gondii* in pinnipeds a serologic survey was conducted to document exposure to this protozoan parasite by species, age, and sex of four phocids in the St. Lawrence ecosystem.

Seals ($n=328$) were shot or live-captured with scientific permit issued by Fisheries and Oceans Canada from the Gulf of St. Lawrence and Estuary. Harp ($n=112$) and hooded ($n=60$) seals were collected or sampled from near the Magdalen Islands ($47^{\circ}23'N$, $61^{\circ}52'W$) in March 1996 and 1997. Harbor seals ($n=34$) were live-captured near Metis-sur-Mer ($48^{\circ}40'N$, $68^{\circ}00'W$; $n=17$) and Bic ($48^{\circ}23'N$, $68^{\circ}53'W$; $n=16$), Québec, in the St. Lawrence Estuary (one harbor seal was found dead in a net at Metis-sur-Mer) in 1995. Grey seals ($n=122$) were collected or sampled from near Metis-sur-Mer ($n=1$), Bic ($n=2$), Quebec or Hay Island ($46^{\circ}01'N$, $59^{\circ}41'W$; $n=78$), and Port Hood ($46^{\circ}01'N$, $61^{\circ}32'W$; $n=41$), Cape Breton, Nova Scotia, in 1995–97. Blood was obtained from a jugular vein, the heart, or a lumbar intravertebral extradural vein by syringe and untreated Vacutainers® (Becton Dickinson

and Company, Franklin Lakes, New Jersey, USA). Blood was kept warm until clotted then was centrifuged for 20 min at $2,500 \times G$, and sera were removed and stored at $-20^{\circ}C$ then at $-70^{\circ}C$.

Sera were analyzed by MAT (Dubey and Desmonts, 1987). The usefulness of this MAT has been recently demonstrated with experimentally infected grey seals (Gajadhar et al., 2004). Serum was diluted 1:25, 1:50, and 1:500. Sera agglutinating the antigen at a dilution of 1:25 or higher were considered positive and evidence of exposure to *T. gondii*. Positive controls were positive at 1:25, 1:50, 1:100, and 1:200 and negative at 1:400, 1:800, 1:1,600, and 1:3,200.

Seals were aged by counting the number of growth layer groups (GLGs) in the dentine or cementum of canine or incisor teeth sectioned longitudinally (where one GLG=1 yr of age; Mansfield and Fisher, 1960; Bowen et al., 1983; Mansfield, 1991; Bernt et al., 1996). Young-of-year animals or pups were in their first yr of life, or age<1 yr; juveniles were age>1 yr and sexually immature; adults were sexually mature and age=4–8 yr for male or age=4–6 yr for female harp seals (Sergeant, 1991; McLaren, 1993; Hammill et al., 1995); age=4–6 yr for male and age=3–4 yr for female hooded seals (Oritsland, 1975; McLaren, 1993; Kovacs et al., 1996); age=6 yr for male and age=4 yr for female grey seals (Hammill and Gosselin, 1995); and age=6 yr for male and age=3–4 yr for female harbor seals (Boulva and McLaren, 1979; McLaren, 1993). Differences in seroprevalence between sex, age, and species were examined by Fisher's exact test at $P<0.05$ with SASMETA® (version 6.12, SAS Institute Inc., Cary, North Carolina, USA).

Eleven of 122 grey seals were seropositive to *T. gondii* with a prevalence of 9% ($P[0.046 \leq P \leq 0.16]=95\%$). This included five adult males (age=10, 12, 13, 17, 20 yr), five adult females (age=10, 11, 12, 25 yr, and one adult of unknown age), and one male pup. Prevalence in adults only

was 13%. Titers were 1:25 (five animals) or 1:50 (six animals). The male pup, 14 days of age with a titer of 1:50, belonged to an adult female (age=25 yr) with a titer of 1:50. Two seropositive females with titers of 1:25 had pups (18–19 days of age) that were seronegative. There was no statistical difference in seroprevalence between males and females ($P=0.756$) or between juveniles and adults ($P=0.095$). Seropositive grey seals were collected at Port Hood ($n=4$) and Hay Island ($n=7$). Seronegative grey seals included 30 male and 38 female adults, one adult of unknown sex, 21 male and 19 female pups, and one male and one female juvenile.

Three of 34 harbor seals were seropositive with a prevalence of 9% ($P[0.019 \leq P \leq 0.14]=95\%$). Seropositive animals included one male pup (10 days old, titer 1:25), one adult female (age=7 yr, titer 1:50), and one adult male (age=6 yr, titer 1:25). Prevalence in adults only was 29%. Unfortunately, no serum was available from the mother for testing. Seropositive harbor seals were collected at Metis-sur-Mer ($n=2$) and Bic ($n=1$). Seronegative harbor seals included two male and three female adults, eight male and seven female pups, and nine male and two female juveniles.

Seroprevalence of *T. gondii* in hooded seals ($n=60$) was 1.7% ($P[0.0004 \leq P \leq 0.09]=95\%$), with one adult female (age=18 yr) seropositive at a titer of 1:25. Prevalence in adults only was 2.4%. Seronegative hooded seals included 20 male and 20 female adults and 10 male and nine female pups. Low sample sizes in stratified groups precluded analyses by sex or age and seroprevalence in harbor or hooded seals.

All harp seals ($n=112$) were seronegative to *T. gondii*. The sample included five male and 60 female adults and 29 male and 18 female pups. There was no statistical difference in seroprevalence of *T. gondii* among grey, harbor, or hooded seals for all ages and sexes combined ($P=0.131$).

This study reports that grey, hooded,

and Atlantic harbor seals in the Gulf of St. Lawrence River and Estuary have been exposed to *T. gondii*. In a similar serologic survey conducted in the northeast Atlantic Ocean, none of over 600 marine mammals, including harp seals, ringed seals, hooded seals, and minke whales (*Balaenoptera acutorostrata*), were seropositive to *T. gondii* (Oksanen et al., 1998). Dubey et al. (2003) examined sera from over 750 marine mammals and reported antibodies to *T. gondii* in four phocid species: California sea lion (*Zalophus californianus*), Pacific walrus (*Odobenus rosmarus*), sea otter (*Enhydra lutris*) from the Pacific coast, and bottle-nosed dolphin (*Tursiops truncatus*) from California and Florida (USA).

Felids, in which the sexual cycle of *T. gondii* occurs, are the only known definitive hosts of this coccidian parasite. Intermediate hosts, in which the asexual cycle occurs, include a wide range of warm-blooded vertebrates, including humans and some birds. Infection occurs horizontally by consumption of *T. gondii* oocysts passed by felids into the external environment, where they sporulate and contaminate water, food, and fomites, or by consumption of *T. gondii* zoites or tissue cysts in raw, undercooked meat or viscera of infected intermediate hosts (see Tenter et al., 2000, for review). *Toxoplasma gondii* also can be transmitted vertically, either transplacentally or transmammary (Dubey and Beattie, 1988). *Toxoplasma gondii* is often of little clinical significance to immunocompetent animals or humans, unless contracted during pregnancy. However, latent infections leading to severe clinical toxoplasmosis could be reactivated in hosts where immunity is declining with age; in hosts with concurrent immunosuppressive viral infections, such as AIDS patients or animals with distemper or herpesvirus; in hosts given corticosteroids or other immunosuppressive drugs; or in hosts contaminated with chemicals that depress the immune system (Dubey, 1998).

In this study, antibodies to *T. gondii*

were found in one harbor seal and one grey seal pup 10 and 14 days old, respectively. Both animals were not yet weaned. It is likely that maternal antibodies (IgG) were transferred to pups in the colostrum or milk rather than transplacentally because of pinniped endotheliochorial placentation (see Ross et al., 1994). Lambourn et al. (2001) reported only two of 143 Pacific harbor seal pups (age < 1 yr) with titers of 1:25 against *T. gondii*. An 11-mo-old St. Lawrence beluga calf (DL-05-98) was diagnosed with toxoplasmosis (De Guise et al., 1995; Mikaelian et al., 2000). Lactation in beluga lasts up to 2 yr, and during their second yr, calves might feed on invertebrates (Brodie, 1971). Fatal congenital infections have been reported in a sea lion pup and in dolphin calves (Ratcliffe and Worth, 1951; Inskeep et al., 1990; Jardine and Dubey, 2002; Resendes et al., 2002). Dubey et al. (2003) reported antibody titers against *T. gondii* in various marine mammals ranging from 1:25 to 1:51,200. Neither age nor sex was provided for most animals tested.

Understanding the epizootiology of *T. gondii* in wild, free-ranging marine mammals is problematic. Because only asexual stages, tachyzoites or bradyzoites, have been seen in histologic sections, marine mammals appear to be acting as intermediate hosts. Definitive hosts, other than felids, could be involved in the marine environment but have simply not been identified because of lack of study. Potential definitive hosts might be predators of marine mammals such as sharks, polar bears (*Ursus maritimus*), orca (*Orcinus orca*), or scavengers. As a cautionary note, some reports of *T. gondii* in marine mammals could be equivocal (see Dubey et al., 2003).

Consumption of raw or uncooked meat infected with *T. gondii* tissue cysts has been considered the major route of transmission to humans (Tenter et al., 2000); however, waterborne infections have been documented or suspected in the Americas (Benenson et al., 1982; Bowie et al., 1997;

Aramini et al., 1999; Bahia-Oliveira et al., 2003). Bandoli and de Oliveira (1977) first suggested that toxoplasmosis in a dolphin was likely by *T. gondii* oocyst contamination of the marine environment. Sewage, storm, freshwater, or agricultural runoff containing oocysts passed by domestic or wild felids have been considered sources of contamination for freshwater and coastal marine environments where many marine mammals live. Oksanen et al. (1998) reasoned that sparse human settlement and minimal sewage outflow in the northeast Atlantic, where their marine mammal sera were collected, accounted for lack of exposure to *T. gondii*. Miller et al. (2002) found no association between seropositivity to *T. gondii* in southern sea otters in California and human population density or exposure to sewage; however, the latter risk factor could not be fully evaluated and therefore requires further field study, as well as experimental work. In this study, only one hooded seal and no harp seals had evidence of exposure to *T. gondii*. Although feeding habits differ among all four phocid species examined, seroprevalence could reflect residency time in contaminated coastal areas. Arctic phocids, like harp and hooded seals, spend about 6 mo in the St. Lawrence ecosystem and 6 mo in the Arctic. Miller et al. (2002) found a threefold increased likelihood of sea otters being seropositive to *T. gondii* where freshwater runoff was high. The St. Lawrence ecosystem has abundant freshwater riverine flow, tributaries, and annual ice and snow melt, and it is noteworthy that mean annual freshwater discharge of the St. Lawrence ecosystem exceeds the sum of the freshwater discharge of the entire eastern coast of the United States (Sutcliffe et al., 1976). Dubey et al. (2003) reported generally higher seroprevalences of *T. gondii* in southern marine mammals compared with those from Alaska.

Protozoan parasites other than *T. gondii*, well known in terrestrial ecosystems, have also been reported recently in coastal marine mammals (Hill et al., 1997; Lapointe

et al., 1998; Measures and Olson, 1999; Deng et al., 2000; Morgan et al., 2000; Lindsay et al., 2001b; Miller et al., 2001a). The occurrence of these protozoan pathogens (i.e., *T. gondii*, *Sarcocystis neurona*, *Cryptosporidium hominis* [= *C. parvum* "human genotype"; see Morgan-Ryan et al., 2002], *Giardia duodenalis* assemblage A [A. Appelbee, M. Olson, and L. Measures, unpubl. data]) in marine mammals might be examples of "pathogen pollution" (see Harvell et al., 1999; Daszak et al., 2000), that is, contamination of the marine environment from the terrestrial environment by recent natural or anthropogenic activities. Alternatively, they could be natural infections unknown in marine mammals because of lack of study. Some of these protozoans have been reported in molluscs, which can filter large volumes of water and be sources of infection for invertebrate-eating sea otters (Lindsay et al., 2001a; Arkush et al., 2003) or humans. Cetaceans and pinnipeds, except walrus and bearded seals, are not known to consume molluscs to any significant degree but might become infected by drinking small quantities of fresh or salt water (see Ridgeway, 1972; Skallstad and Nordøy, 2000; Storeheier and Nordøy, 2001).

Toxoplasmosis is a serious zoonosis, and finding Canadian marine mammals exposed to *T. gondii* is of concern to native peoples, such as Inuit, to sealers on the east coast of Canada who consume marine mammals, or to domestic or international seal meat markets. Recent studies indicate that human health can be at risk (Gajadhar et al., 2004).

J. Gosselin, V. Lesage, M. Hammill, and E. Albert assisted in the field and laboratory, and we greatly appreciate their help. J. Fortin assisted with statistical analyses.

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Received for publication 25 April 2003.