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ARTICLE

Qualitative risk assessment of impact of *Toxoplasma gondii* on health of beluga whales, *Delphinapterus leucas*, from the Eastern Beaufort Sea, Northwest Territories[1]

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Abstract: In recent years, the protozoan parasite *Toxoplasma gondii* has increasingly been recognized in Arctic fauna, including beluga whales (*Delphinapterus leucas*) in the Eastern Beaufort Sea (EBS), Northwest Territories. We qualitatively assessed the risks of *T. gondii* to the health of EBS beluga because of their importance in livelihood of Arctic communities as well as their potential role as sentinels. This risk assessment used a standard framework including hazard identification, hazard characterization, exposure assessment, and risk characterization. We conclude that currently, the EBS beluga are at moderate risk of exposure to *T. gondii*, and low risk of developing disease associated with toxoplasmosis, based on the small amount of data available (only healthy, hunter-harvested animals have been examined). Although there was a high level of uncertainty due to limited published data and the challenges in determining prevalence and significance of disease in wild marine mammal populations, overall the EBS population was currently considered to be at a low risk for population level impacts of toxoplasmosis. Finally, we identify knowledge gaps that can guide future research, provide better evidence for future risk assessments, and

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ultimately better detect and mitigate changing risks for health of Arctic beluga, as well as the communities that rely on sustainable, healthy populations for harvest.

Key words: qualitative risk assessment, *Toxoplasma gondii*, beluga whales, *Delphinapterus leucas*, Beaufort Sea.

Résumé : Au cours des dernières années, le parasite protozoaire *Toxoplasma gondii* a de plus en plus été reconnu dans la faune arctique, y compris chez les bélugas (*Delphinapterus leucas*) dans l'est de la mer de Beaufort (EMB), dans les Territoires du Nord-Ouest. Nous avons qualitativement évalué les risques de *T. gondii* pour la santé des bélugas dans l'EMB à cause de leur importance au niveau de la subsistance des communautés dans l'Arctique aussi bien que leur rôle potentiel comme sentinelles. Dans cette évaluation du risque, on a utilisé un cadre standard incluant l'identification des dangers, la caractérisation des dangers, l'évaluation de l'exposition et la caractérisation des risques. Nous concluons, fondé sur la faible quantité de données disponibles (seulement les animaux en bonne santé capturés par des chasseurs ont été examinés), qu'actuellement les bélugas de l'EMB sont à risque modéré d'exposition à *T. gondii* et à faible risque de développer une maladie associée à la toxoplasmose. Bien qu'il y ait un haut niveau d'incertitude en raison de la quantité limitée de données publiées et des défis quant à la détermination de la prévalence et de l'importance des maladies touchant les populations de mammifères marins sauvages, en général la population de l'EMB est considérée être à faible risque de subir des effets de toxoplasmose. Finalement, nous identifions les lacunes dans les connaissances qui peuvent guider les recherches futures, fournir de meilleures données pour l'évaluation des risques futurs et ultimement, mieux détecter et atténuer les risques changeants pour la santé des bélugas de l'Arctique, ainsi que les communautés qui dépendent de populations durables et saines pour la récolte. [Traduit par la Rédaction]

Mots-clés : évaluation qualitative du risque, *Toxoplasma gondii*, bélugas, *Delphinapterus leucas*, Mer de Beaufort.

Introduction

Toxoplasma gondii, an important food- and water-borne parasite, can infect a wide range of domestic and wild animals as well as people. *Toxoplasma gondii* can be associated with reproductive loss and mortalities, as well as population reduction in free-ranging animals (Jardine and Dubey 2002; Resendes et al. 2002; Kreuder et al. 2003; Roe et al. 2013; Herder et al. 2015; Carlson-Bremer et al. 2015; Barbieri et al. 2016). Cats and wild felids shed oocysts of *Toxoplasma* in feces, and contaminate the environment, food, and water. Intermediate hosts become infected after consuming oocysts, which develop into tissue cysts; in turn, consumption of these infected tissues leads to infection in felid definitive hosts as well as carnivorous intermediate hosts (Dubey 2010).

In recent years, *Toxoplasma* has increasingly been recognized in aquatic fauna (Shapiro et al. 2016; VanWormer et al. 2016), including northern fur seal (*Callorhinus ursinus*), Atlantic bottlenose dolphin (*Tursiops truncatus*), beluga whales (*Delphinapterus leucas*), and sea otters (*Enhydra lutris*) (Holshuh et al. 1985; Inskeep et al. 1990; Mikaelian et al. 2000; Miller et al. 2002; Kreuder et al. 2003). In addition, mortalities due to *Toxoplasma* infection in marine animals have been reported across the world (Kreuder et al. 2003; Roe et al. 2013; Carlson-Bremer et al. 2015; Barbieri et al. 2016). Clinical disease due to toxoplasmosis has been documented in a range of captive and free-ranging marine mammals (see Dubey et al. 2003).

In northern Canada and Alaska, various studies have reported exposure to *T. gondii* in terrestrial and marine wildlife, including pinnipeds and polar bears (see Measures et al. 2004; Simon et al. 2011; Jenkins et al. 2013), although these reports indicated exposure to *Toxoplasma* (i.e., seroprevalence of antibody), but not true infection (i.e., detection of parasite or DNA in tissues). Recently, DNA of *T. gondii* has been detected in tissues of beluga whales from the Beaufort Sea (Haman et al. 2013). Transmission dynamics of the parasite

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between terrestrial and marine mammals of northern Canada is not well understood, nor is the impact of *Toxoplasma* on the health of marine mammals. In combination with other stressors, pathogens, and contaminants, *T. gondii* has the potential to have population-level effects in marine mammals.

Beluga whales are an important species for northern communities, both culturally and as a food resource (Loseto et al. 2009; Sheikh et al. 2011). While the Eastern Beaufort Sea (EBS) population of beluga was considered Not at Risk when assessed in May 2004 (COSEWIC 2016), other populations of beluga are of conservation concern. Long life span, high trophic levels, and large fat stores make marine mammals suitable as sentinel species for ocean and human health (Carignan and Villard 2002; Bossart 2011). Beluga whales can act as sentinels for environmental contaminants in marine ecosystems (Massé et al. 1986; De Guise et al. 1995*b*; Lebeuf et al. 2007); *T. gondii* has also been considered an environmental contaminant, washed into the ocean from terrestrial ecosystems. In this study, we qualitatively assess the risks of *T. gondii* for the health of beluga whales from the EBS, Northwest Territories because of their cultural and subsistence importance to Arctic communities as well as their potential role as sentinels for transmission of this important parasite in ocean ecosystems. Food safety risks of this zoonotic parasite were not considered in this risk assessment.

Methods

Given the limited data on *T. gondii* in beluga whales, we selected a qualitative, versus quantitative, and risk assessment approach. We utilized a framework already developed by various agencies viz. Food and Agriculture Organization–World Health Organization (FAO–WHO), Agency for Toxic Substances and Disease Registry (ATSDR), Canadian Food Inspection Agency (FAO–WHO and FAO/WHO 2011), FAO/WHO (2002) and CFIA and Council of Canadian Academies (2011). The major components of this framework include (1) hazard identification and (2) risk assessment, consisting of hazard characterization, exposure assessment, and risk characterization (Fig. 1)

We extrapolated health effects and exposure levels from other marine mammals, especially species sympatric with belugas and sharing similar food resources (Bauer et al. 2016). We searched key words (Table 1) in search engine "PubMed" in June 2017. Titles and/or abstracts were screened manually for relevance to pathology, transmission, clinical manifestations, mortalities, and serology related to toxoplasmosis in marine mammals from North America. We also considered publications cited in the selected publications for further analysis.

Results

Hazard identification

Toxoplasma gondii *and life cycle*

There are three infective stages of *T. gondii*: (1) sporozoites (contained in sporulated oocysts), (2) bradyzoites (in tissue cysts of intermediate hosts), and (3) tachyzoites. Domestic and wild cats are the only definitive hosts, shedding unsporulated oocysts for up to 20 days after initial infection. After sporulation (within 1–2 days), oocysts can be infectious to susceptible intermediate hosts, which include most vertebrate species (Dubey 2010). Rapidly dividing tachyzoites disseminate throughout acutely infected intermediate hosts and establish in tissues, where they slowly divide as bradyzoites within tissue cysts. Carnivorous intermediate hosts can also become infected by consuming tissue cysts within other intermediate hosts. Cats become infected by consuming oocysts or tissue cysts in infected intermediate hosts (Dubey 2010). In female mammals infected for the first time during pregnancy, transplacental transmission of tachyzoites from mother to fetus can occur (Dubey et al. 2008*b*).

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Fig. 1. Qualitative risk assessment framework for impact of *T. gondii* on health of beluga [Agency for Toxic Substances and Disease Registry (ATSDR), CFIA and Council of Canadian Academies 2011, FAO/WHO 2002, and FAO-WHO and FAO/WHO 2011].

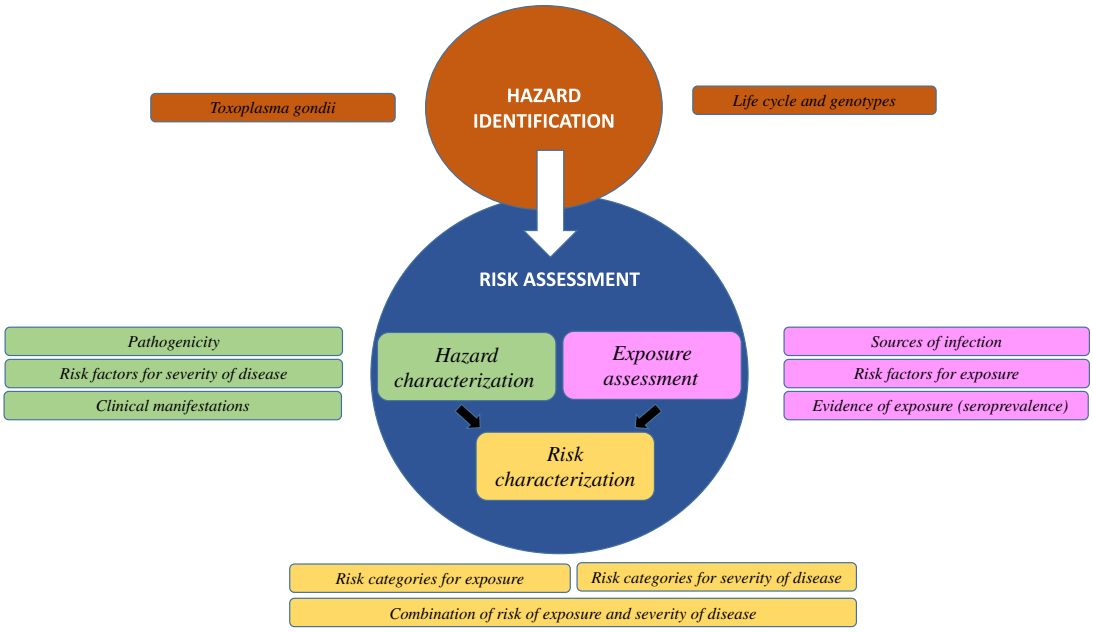

Table 1. Key words used in search engine PubMed.

Key words		Results	Key words		Results
Toxoplasma gondii	Marine	152	Toxoplasmosis	Marine	117
	Otter	63		Otter	48
	Whale	53		Whale	44
	Cetacean	51		Cetacean	43
	Pinniped	46		Pinniped	39
	Dolphin	45		Dolphin	38
	Seal	37		Seal	31
	Aquatic	37		Aquatic	25
	Polar bear	32		Polar bear	26
	Porpoise	8		Porpoise	7
	Walrus	5		Walrus	5
	Beluga	2		Beluga	1

Genotypes

Genus *Toxoplasma* consists of only one species with three dominant genotypes (I, II, and III) based on variability in genetic markers (i.e., presence of alleles at multiple loci in *Toxoplasma* isolates) and pathogenicity in mice (Howe and Sibley 1995). Studies based primarily on samples from humans and domestic animals indicated that Type II strains represent more than 60% of infections in warm-blooded vertebrates, followed by Type I or III (Howe and Sibley 1995; Howe et al. 1997). The three clonal lineages are sporadically reported in marine mammals but other genotypes (atypical, Type A, Type X, and Type 12) dominate (Miller et al. 2004*b*; Conrad et al. 2005; Honnold et al. 2005; Dubey et al. 2007, 2008*a*, 2009, 2011). Multiple genotypes have been reported in marine invertebrates

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(Shapiro et al. 2015). Very little is known about the genotypes present in Arctic marine mammals, although it is likely that a mixture of atypical and clonal lineages is present, especially in species that migrate into subarctic and temperate waters. In EBS beluga, two new atypical genotypes of *T. gondii* have been described (Haman et al. 2013), suggesting that toxoplasmosis in EBS beluga does not represent a recent northward expansion of clonal lineages established in the south.

Risk assessment

Hazard characterization

In this section, we summarize the effects of the hazard (*T. gondii*) in marine mammals based on published literature. The bodies of dead marine wildlife are difficult to locate and therefore, analyses into the cause of death or co-occurrence with other factors is problematic. Most pathological examinations in wildlife are on naturally occurring mortalities where *T. gondii* is the only one of many other potential causes of or contributors to mortality. This has the potential to confound results and lead to uncertainties when attributing specific pathologies to *T. gondii*.

Clinical manifestations

Clinical toxoplasmosis has been reported in marine mammals (Dubey et al. 2003; Carlson-Bremer et al. 2015; Herder et al. 2015). Neither clinical toxoplasmosis nor pathological lesions associated with *T. gondii* have been reported in beluga in the EBS, and the parasite has thus far only been detected in healthy animals harvested by hunters.

Toxoplasmosis has been documented as a cause of mortality in various marine mammals (Kreuder et al. 2003; Roe et al. 2013; Carlson-Bremer et al. 2015; Barbieri et al. 2016). Gross pathology included ascites, hepatomegaly, pleural and pericardial effusions, and pulmonary edema (Gibson et al. 2011). Microscopic lesions of encephalitis and myocarditis are commonly observed and potentially fatal (Carlson-Bremer et al. 2015). Histological lesions associated with *T. gondii* have been reported in two stranded beluga from the St. Lawrence Estuary (SLE), Québec, Canada (De Guise et al. 1995*a*; Mikaelian et al. 2000), as well as California sea otters (*Enhydra lutris nereis*; Kreuder et al. 2003), elephant seal (*Mirounga angustirostris*; Dubey et al. 2004), and striped dolphin (*Stenella coeruleoalba*; Dubey et al. 2007). Clinical signs observed in stranded harbor seals include seizures, apparent blindness (bilateral mydriasis and poor pupillary light responses), and depression (Miller et al. 2001). Behavioral changes have been reported in experimentally infected grey seals (*Halichoerus grypus*) (Gajadhar et al. 2004). Otters with protozoal encephalitis may present with dull mentation, abnormal motor function and seizures (Kreuder et al. 2003). Such neurological presentations can make marine mammals more susceptible to predation, boat strikes, or to stranding (Kreuder et al. 2003; Miller et al. 2004*a*). Thus, behavior changes due to *Toxoplasma* infection could be an indirect cause of mortality in marine wildlife.

Transplacental transmission of *T. gondii* has been linked to abortion, still birth, neonatal death, or congenital cerebral malformations in marine mammals such as sea otters, dolphins, and Hawaiian monk seals (Jardine and Dubey 2002; Resendes et al. 2002; Miller et al. 2008*b*; Shapiro et al. 2016). Such reproductive failure suggests that toxoplasmosis can be a risk to wildlife populations, especially those under pressure from other sources of mortality and reproductive stressors (Barbieri et al. 2016; Shapiro et al. 2016).

Risk factors for severity of disease

Immunosuppression by environmental contaminants or morbillivirus has been found to increase the risk of opportunistic *T. gondii* infections in marine mammals, as well as the reactivation of latent infections. This is especially important to consider in the Canadian

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Arctic, where migration and sequestration of contaminants is known to occur (Mikaelian et al. 2000; Mazzariol et al. 2012). Environmental contaminants routinely detected in Arctic regions have the ability to adversely affect both cellular and humoral immune function in exposed individuals and increase susceptibility to disease (Gibson et al. 2011). Previous studies revealed high levels of methylmercury in tissues of EBS beluga (Lockhart et al. 2005) and more recently, Loseto et al. (2015) revealed declines. Because mercury suppresses immune function (Krey et al. 2015), beluga with high levels of methylmercury may be at an elevated risk of developing disease associated with *T. gondii* (Frouin et al. 2012).

Morbillivirus could make marine mammals more susceptible to *T. gondii* infection (Mazzariol et al. 2012). Morbilliviruses have not been documented in EBS beluga, but have been reported from other marine mammals from the Beaufort Sea (Kirk et al. 2010) and in ringed seals in the northwestern Canadian Arctic (Duignan et al. 1997). In two stranded beluga whales from the SLE with pathology associated with *T. gondii*, concomitant morbillivirus infection was not detected (Mikaelian et al. 2000).

Concurrent infection of *T. gondii* and *Sarcocystis neurona* is common in some marine mammals and may be more likely to result in mortality and severe encephalitis (Lindsay et al. 2001*b*; Gibson et al. 2011). Three of 23 beluga whales from the Beaufort Sea were infected with *T. gondii* (13%) and 20 (87%) were infected with a novel *Sarcocystis* species (Haman et al. 2013). *Sarcocystis* is generally considered an incidental finding in free-ranging wildlife and infection is often asymptomatic in the usual reservoir species. Presently, relatively little is known about the significance of this *Sarcocystis* sp. for beluga health, the prevalence of co-infection between these two parasites in this population, and if there is any association with severity of disease.

There is some evidence of association between genotype of *T. gondii* and pathogenicity in mammalian species; Type I is pathogenic in mice and Type II can be associated with high cyst intensity and significant meningoencephalitis (Suzuki et al. 1989; Sibley and Boothroyd 1992; Howe and Sibley 1995; Howe et al. 1996, 1997). In sea otters, strain X caused moderate to severe meningoencephalitis more frequently than Type II strains (Miller et al. 2004*b*). In contrast, no association was found between genotype and disease severity in marine mammals from North American Pacific Coast (Gibson et al. 2011). More work is needed to determine the genotype(s) of *T. gondii* present in beluga in the EBS, and any links to clinical toxoplasmosis. In people, clinical disease associated with *Toxoplasma* infection was more severe when acquired through consumption of oocysts rather than from tissue cysts (Dubey 2004). Therefore, if the major route of transmission of *Toxoplasma* to beluga is via oocysts, there could be possibility of more severe disease.

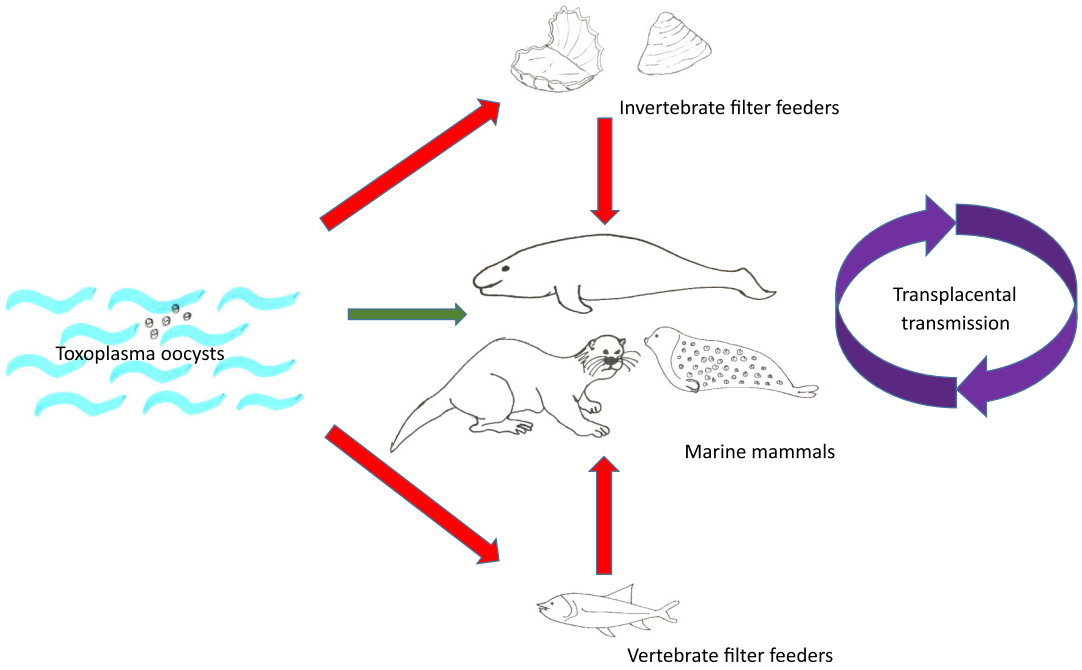
Exposure assessment

This part of the assessment examines the potential routes, sources, and risk factors for exposure of Arctic beluga to *T. gondii* (Fig. 2). As the infective dose of *T. gondii* oocysts is not known for beluga, we assumed that one oocyst is sufficient to induce infection, based on experimental studies on other susceptible intermediate hosts (pigs and mice) (Dubey 1996; Dubey et al. 1996).

Sources of infection

Sources of infection with *T. gondii* in marine mammals are not well understood. Introduction of the parasite into marine ecosystems is considered to be from land as no marine host is known to shed oocysts (VanWormer et al. 2016), although some authors have suggested an unknown marine definitive host (Measures et al. 2004; Conrad et al. 2005). Contamination of coastal marine environments with oocysts of *T. gondii* in surface runoff from the boreal region is a potential source of infection for wildlife in the Canadian

Fig. 2. Potential sources of *Toxoplasma gondii* in marine mammals.



Arctic (Simon et al. 2013). Due to their hardy nature, oocysts can survive in sea water for more than a year (Lindsay and Dubey 2009). Beluga whales could be exposed to oocysts via ingestion of sediment during feeding, or by drinking seawater (Vladykov 1946; Ridgway 1972). The Canadian lynx (*Lynx canadensis*), bobcat (*Lynx rufus*), and cougar (*Felis concolor*) are the only potential wild felid definitive hosts for *Toxoplasma* in Canada (Bowie et al. 1997; Aramini et al. 1998; Labelle et al. 2001). To the best of our knowledge, there is no report of *T. gondii* in wild felids from northwestern Canada (Yukon and Northwest Territories), and both wild and domestic felids are rare above the tree-line.

Filter feeding fish can serve as a mechanical vector transporting *T. gondii* oocysts in their alimentary canal (Massie et al. 2010) and invertebrate hosts (mussel, bivalve molluscs, and oysters) can concentrate *T. gondii* oocysts which remain infectious and viable in their tissues for days to months (Lindsay et al. 2001*a*, 2004; Arkush et al. 2003; Miller et al. 2008*a*). Beluga from the EBS primarily eat Arctic Cod (*Boreogadus saida*) (Loseto et al. 2009) as well as coastal fish such as Arctic Cisco (*Coregonus autumnalis*) and Pacific Herring (*Clupea pallasi*) (Quakenbush et al. 2015). Other populations of beluga eat a wide range of invertebrate hosts including shrimp, squid, clams, crabs, etc. However, the role that these and other prey species for beluga play as a source of oocysts in Arctic marine ecosystems remains unknown.

Ingestion of cysts of *T. gondii* in the tissues of other intermediate hosts is another possible mode of transmission for carnivorous marine mammals, especially those that hunt or scavenge carcasses from the terrestrial ecosystem (such as polar bear). This may include ingestion of animal carcasses and offal dispersed by seafaring vessels, and ingestion of sea birds (Oksanen et al. 1998). This route of transmission is less likely in marine mammals such as beluga that feed on invertebrates/cold blooded animals, or are exclusively herbivores (Dubey et al. 2003). True infection with tissue cysts (as occurs in mammalian and avian

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intermediate hosts) is not thought to occur in cold-blooded vertebrate hosts (Omata et al. 2005).

Transplacental transmission of *T. gondii* has been described in Risso's dolphin, *Grampus griseus* (Resendes et al. 2002), Indo-Pacific bottlenose dolphin, *Tursiops aduncus* (Jardine and Dubey 2002), southern sea otter (Miller et al. 2008*b*), Hawaiian monk seal (Barbieri et al. 2016), and California sea lion (*Zalophus californianus*; Carlson-Bremer et al. 2015). Transmammary transmission has only been documented in domestic and laboratory animals (Tenter et al. 2000). Vertical transmission may help maintain the transmission of *T. gondii* in marine mammals, especially in regions like the Arctic tundra where felids are rare (Shapiro et al. 2016). Based on the available information on feeding habits of beluga, ingestion of water/food contaminated with oocysts seems the most plausible route, with maintenance in the population via transplacental transmission. As beluga whales are a potential true intermediate host, they may serve as amplifiers of infection, as ingestion of one oocyst can lead to numerous tissue cysts.

Evidence of exposure to T. gondii *in marine mammals*

Seroprevalence of antibodies to *Toxoplasma* in marine mammals of northern North America and reports of *T. gondii* in beluga in this and other areas of the world are presented in Tables 2 and 3, respectively. It is important to note that serology only indicates exposure to *T. gondii* over the course of a lifetime, rather than acute infection. There are relatively few studies in which viable *Toxoplasma* has been isolated from marine mammals [sea otters; Cole et al. 2000, Pacific harbor seals (*Phoca vitulina richardsi*); Miller et al. 2001, striped dolphin; Dubey et al. 2007, and bottlenose dolphins (*T. truncatus*); Dubey et al. 2008*a*], and one report of detection of DNA of *T. gondii* in tissues of EBS beluga (Haman et al. 2013). *Toxoplasma* seropositivity was not invariably associated with lesions in beluga in the SLE (Mikaelian et al. 2000). *Toxoplasma gondii* has been reported in other marine mammals in the Beaufort Sea, including bearded seals, harbor seals and ringed seals, and polar bears, since 1986 (Rah et al. 2005; Table 2).

Risk factors for exposure

None of the reports included in this research studied risk factors for exposure to *T. gondii* in beluga whales; thus, we extrapolated from risk factors identified in other marine mammals. In general, older animals are more likely to be exposed to *T. gondii* over the course of their lifetime, including California sea lions (Carlson-Bremer et al. 2015) and southern sea otters (Miller et al. 2002; Kreuder et al. 2003). In contrast, higher seropositivity was observed in young versus old seals (Simon et al. 2011), which may be consistent with vertical transmission.

Close human proximity, high human density, and high levels of coastal runoff are significantly associated with *Toxoplasma* status in marine wildlife, as supported by observations that seroprevalence was higher in southern populations of marine mammals (Miller et al. 2002; Hanni et al. 2003; Gaydos et al. 2007). Therefore, EBS beluga would seem to be at lower risk of exposure than many marine mammal populations, due to the low density of human habitation (and presumably ownership of outdoor cats) in the region. However, it is important to note that coastal runoff is changing in the western Arctic of North America (Stuefer et al. 2017).

Risk characterization

In this part of the assessment, we categorized severity of disease and exposure, and combined these values to generate an overall estimate of risk associated with *T. gondii* in the EBS and SLE beluga populations (for comparison).

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Table 2. Serostatus of *Toxoplasma gondii* in marine animals in northern North America.

Host species	Location	Method	Cut-off	Seroprevalence (%)	Number positive/total	Reference
Bearded seals (*Erignathus barbatus*)	Alaska	MAT	1:25	50	4/8	Dubey et al. 2003
	Beaufort Sea, Canada and Hudson Bay	DAT	1:40	10	2/20	Simon et al. 2011
Bowhead whale (*Balaena mysticetus*)	Canada[a]	MAT	1:25	50	1/2	Al-Adhami et al. 2016
Grey seals (*Halichoerus grypus*)	St. Lawrence Estuary, Quebec	MAT	1:25	9	11/122	Measures et al. 2004
Harbor seals (*Phoca vitulina*)	Alaska	MAT	1:25	16.4	51/311	Dubey et al. 2003
	St. Lawrence Estuary, Quebec	MAT	1:25	9	3/34	Measures et al. 2004
	Beaufort Sea, Canada and Hudson Bay	DAT	1:40	22.2	2/9	Simon et al. 2011
Harp seals (*Phoca groenlandica*)	St. Lawrence Estuary, Quebec	MAT	1:25	0	0/112	Measures et al. 2004
Hooded seals (*Cystophora cristata*)	St. Lawrence Estuary, Quebec	MAT	1:25	2	1/60	Measures et al. 2004
Polar bears (*Ursus maritimus*)	Resolute Bay, Nunavut	ELISA		0	0/60	Philippa et al. 2004
	Beaufort Sea, Canada	LAT	1:16	13	18/136	Kirk et al. 2010
	Beaufort Sea, Alaska	LAT	1:32	6.3	16/253	Rah et al. 2005
	Beaufort Sea, Canada	LAT	1:32	0	0/9	Rah et al. 2005
Ribbon seals (*Phoca fasciata*)	Alaska	MAT	1:25	0	0/14	Dubey et al. 2003
Ringed seal (*Phoca hispida*)	Alaska	MAT	1:25	15.6	5/32	Dubey et al. 2003
	Beaufort Sea, Canada and Hudson Bay	DAT	1:40	10.2	80/788	Simon et al. 2011
	Canada[a]	MAT	1:25	14.81	16/108	Al-Adhami et al. 2016
Sea lions (*Zalophus californianus*)	Alaska	MAT	1:25	29.6	8/27	Dubey et al. 2003
Spotted seals (*Phoca largha*)	Alaska	MAT	1:25	11.1	1/9	Dubey et al. 2003
Walruses (*Odobenus rosmarus*)	Alaska	MAT	1:25	5.6	3/53	Dubey et al. 2003
	Canada[a]	MAT	1:25	14.74	5/34	Al-Adhami et al. 2016

Note: DAT: direct agglutination test; ELISA: enzyme linked immunosorbent assay; MAT: modified agglutination test; LAT: latex agglutination test. Orange and blue colors indicate prevalence of 0%–10% and 11%–50%, respectively (out of all 21 reports). Purple and yellow colors indicate prevalence of 0%–10%, and more than 10%, respectively (for 10 reports with a sample size >50). Green color indicates reports with a sample size <50.

[a]Exact location not provided in the publication.

Table 3. Reports of *Toxoplasma gondii* in beluga whales.

Location	Specimen tested	Method	Number positive	Total number	% positive	Reference
California, USA	Sera	MAT[a]	0	3	0	Dubey et al. 2003
Svalbard	Plasma	DAT	0	12	0	Jensen et al. 2010
St. Lawrence Estuary, Quebec, Canada	Tissue	IHC	2[b]	2	100	Mikaelian et al. 2000
St. Lawrence Estuary, Quebec, Canada	Sera	MAT	6	22	27	Mikaelian et al. 2000
Beaufort Sea, Canada	Tissue	Multilocus PCR-DNA sequencing	3	23	13	Haman et al. 2013
Canada[c]	Sera	MAT	10	46	22	Al-Adhami et al. 2016

Note: DAT: direct agglutination test; MAT: modified agglutination test; IHC: immunohistochemistry; PCR: polymerase chain reaction.

[a]MAT cut-off value was 1:25 in all reports in the table above.

[b]One beluga whale has 1:25 titre, other was not tested.

[c]Exact location not provided in the publication.

Table 4. Risk categories for severity of disease and probability of exposure.

Risk categories	Severity of disease	Disease score	Prevalence	Exposure Score	Overall risk category
Low	No clinical signs or pathology	1	0–10	1	1–3
Moderate	No clinical signs but pathological lesions	2	11–50	2	4–6
High	Clinical disease including reproductive effects, neurological disease, behavioral changes, and mortality	3	50–100	3	7–9

Classification of risk categories for severity of disease (hazard characterization)

We categorized severity of disease associated with *T. gondii* as low (1), moderate (2), or high (3) on the basis of clinical signs and pathology (Table 4). No clinical disease or pathology has been reported in the EBS beluga population, hence, a risk category of low (1). Microscopic lesions associated with *T. gondii* have been reported in stranded beluga in the SLE, hence, a risk category of moderate (2) (Mikaelian et al. 2000).

Classification of risk categories for exposure (exposure assessment)

We classified low, moderate, and high risk categories for exposure based on the prevalence of *T. gondii* in marine mammals of northern Canada and Alaska (Tables 2 and 3). Out of 21 reports mentioned in Table 2, 11 had prevalence of 0%–10% (orange) and 10 had prevalence of 11%–50% (blue). Out of 10 reports with a sample size >50, seven had prevalence of 0%–10% (purple) and three had prevalence of more than 10% (yellow). Therefore, we categorized prevalence of 0%–10%, 11%–50%, and more than 50% as low (1), moderate (2), or high (3) exposure to *T. gondii*, respectively. Thus, we considered exposure risk to be moderate in the beluga whale population from SLE (score 2, seroprevalence 27%) as well as the EBS population (score 2, tissue prevalence 13%).

Overall risk characterization (combination of risk of exposure and severity of disease)

Overall risk characterization score was calculated by multiplying the risk scores for disease severity and exposure, with a range of 1–9 (with 9 being the worst possible outcome). For EBS beluga, we calculated a final risk score of 2 (Table 5), or low risk. For SLE beluga, we calculated a final risk score of 4 (Table 5), or moderate risk. There was insufficient data available to stratify the beluga populations into different risk groups; however, based on

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Table 5. Risk characterization score for beluga whales from Eastern Beaufort Sea, NT, Canada and the St. Lawrence Estuary, QC, Canada (for comparison).

	Hazard characterization		Exposure assessment		Risk characterization
Beluga population	Clinical manifestations	Risk score for severity	Prevalence	Risk score for exposure	Overall risk score (risk score for severity × risk score for exposure)
Eastern Beaufort Sea, Canada	No clinical signs or pathology	1	Moderate (13%)	2	2 (low)
St. Lawrence Estuary	No clinical signs but pathological lesions	2	Moderate (27%)	2	4 (moderate)

what is known in other animals and people, beluga that are immunocompromised, have high levels of contaminants or concomitant disease, and transplacentally infected fetuses and neonates, are at higher risk of developing severe disease due to toxoplasmosis. Finally, we identify many knowledge gaps that give this risk assessment a high level of uncertainty.

Discussion and conclusion

Although the first case of marine toxoplasmosis was reported more than 60 years ago (Ratcliffe and Worth 1951), the first evidence of toxoplasmosis in Canadian marine mammals (beluga whales) was not reported until 2000 (Mikaelian et al. 2000), and the first report of *T. gondii* in Arctic beluga came in 2013 (Haman et al. 2013). It is likely that increasing recognition of *Toxoplasma* infection in marine mammals indicates a combination of increasing detection effort as well as increasing contamination of ocean environment with oocysts from domestic feline populations (Dubey et al. 2008*a*). Assessing the risks of toxoplasmosis in marine mammals is important not only for health and conservation of wildlife but also to ensure sustainability of wildlife populations important for human harvest, such as arctic beluga. We conducted a qualitative risk assessment of impact of *T. gondii* on health of beluga whales from the EBS, including Alaska, the Yukon, and Northwest Territories, Canada. Our findings indicated that this beluga whale population is at low risk at the current time; however, there is a very limited amount of published data available. Throughout the risk assessment, we identified risk factors (environmental contaminants, co-infection with viruses, *Sarcocystis* spp., etc.) that should be considered for future monitoring. Baseline data on prevalence and impact of disease caused by pathogens and contaminants in Canadian marine mammals will be helpful for detecting changes in health status that may be linked to environmental and anthropogenic change.

Throughout the risk assessment, we also identified many knowledge gaps which need to be addressed (Box 1), including major route/s of transmission of *T. gondii* to marine mammals, risk factors associated with different infective stages, role of domestic and wild cats in contamination of sea water, transmission patterns within target populations, and variation among parasite genotypes from animal hosts within the Arctic and between the Arctic and more temperate regions. Spatial mapping of *Toxoplasma* genotypes is also needed to understand the pathogenicity and transmission patterns in marine and terrestrial animals in the Arctic, where there may be regular seasonal introduction of clonal lineages with animals that migrate into subarctic and temperate regions, as well as atypical genotypes originating in lynx in the boreal forest.

Lack of specific clinical manifestations or postmortem features make diagnosis of toxoplasmosis difficult. Serological examination is one of few noninvasive tools for screening *T. gondii* exposure in wild animals. These tests are not only helpful in screening endangered

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Box 1. Knowledge gaps identified in this risk assessment that need to be addressed by future research and monitoring for toxoplasmosis in the Eastern Beaufort Sea beluga population.

Hazard identification

- What definitive hosts are the source of oocysts present in Arctic marine ecosystems?
- What strains of *T. gondii* are present in the Arctic?
- What strains of *T. gondii* are present in beluga whales and their terrestrial predators (polar bears)?

Hazard characterization

- What is the burden of disease for toxoplasmosis in beluga?
- What inherent risk factors increase susceptibility to disease caused by *T. gondii*?
- What is the intensity and tissue distribution of *T. gondii* in infected animals?
- Are the currently available tests reliable for detection of *T. gondii* in beluga whales?
- What are (histo) pathological changes associated with *Toxoplasma* in beluga whales?
- Does clinical toxoplasmosis exist in beluga population? How would we detect it?
- Does *T. gondii* affect reproductive potential of beluga?

Exposure assessment

- What is the prevalence of toxoplasmosis in beluga (detection of antibodies as well as parasites and/or DNA in tissues)?
- What are the sources of oocysts for beluga whales?
- What prey species of beluga may serve as mechanical vectors?
- Are these prey species true intermediate hosts?
- How long do oocysts survive in Arctic waters?
- What is the environmental burden of oocysts in Arctic waters and sediments?
- Is transplacental transmission an important mode of persistence of *T. gondii* in marine and High Arctic ecosystems where felids are absent or rare?

Risk characterization

- Which groups of animals are at highest risk? (Animals with high mercury exposure?)
- Where are the uncertainties in our information, technical assays, and system level understanding?

or declining species, but they may also aid in epidemiological studies to investigate risk factors for the infection. There are many challenges with serological screening in wild animals, including accessing live animals for sample collection, collecting high quality sera from carcasses at postmortem, adapting and validating serological tests designed for domestic animals to wildlife, and interpreting results in the absence of gold standards that assists in the establishment of cut-off values. Therefore, necropsy of stranded marine mammals as well as healthy animals harvested by hunters is key to determining if toxoplasmosis, among other stressors, is contributing to clinical disease or mortality. The Arctic poses particular challenges in accessing and transporting tissue samples from remote regions to the laboratories, making it important to get the most information possible out of available samples. Thus, there is a need to apply more sensitive molecular techniques for detecting DNA of *T. gondii*, such as sequence specific magnetic capture DNA isolation and real-time

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polymerase chain reaction (PCR), which has more sensitivity than conventional tissue-based PCR methods (Opsteegh et al. 2010).

Risk assessments are a helpful tool to summarize the best available knowledge and to identify knowledge gaps, thus highlighting future research priorities. In turn, data generated by researchers can serve to improve future risk assessments, to develop appropriate risk communication materials, and ultimately to manage risks, if deemed necessary. Risk assessments have been used by health agencies, conservation organizations, and policy makers to develop policies for prevention and control of disease(s), even in high uncertainty and also in rapid response situations. Based on the information and data analyzed in this study, *Toxoplasma* does not currently constitute a high risk for beluga from the Beaufort Sea; however, further investigations are required to increase certainty and assess temporal trends for this parasite in beluga and in other wildlife species important for ecosystem health and human harvest in the Canadian Arctic.

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