

# Spatio-temporal variations and age effect on *Toxoplasma gondii* seroprevalence in seals from the Canadian Arctic

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## SUMMARY

Toxoplasmosis is a significant public health threat for Inuit in the Canadian Arctic. This study aimed to investigate arctic seals as a possible food-borne source of infection. Blood samples collected from 828 seals in 7 Canadian Arctic communities from 1999 to 2006 were tested for *Toxoplasma gondii* antibodies using a direct agglutination test. Polymerase chain reaction (PCR) was used to detect *T. gondii* DNA in tissues of a subsample of seals. Associations between seal age, sex, species, diet, community and year of capture, and serological test results were investigated by logistic regression. Overall seroprevalence was 10.4% (86/828). All tissues tested were negative by PCR. In ringed seals, seroprevalence was significantly higher in juveniles than in adults (odds ratio = 2.44). Overall, seroprevalence varied amongst communities ( $P=0.0119$ ) and by capture year ( $P=0.0001$ ). Our study supports the hypothesis that consumption of raw seal meat is a significant source of infection for Inuit. This work raises many questions about the mechanism of transfer of this terrestrial parasite to the marine environment, the preponderance of infection in younger animals and the natural course of infection in seals. Further studies to address these questions are essential to fully understand the health risks for Inuit communities.

Key words: *Toxoplasma gondii*, seals, Canadian Arctic, wild felids, waterborne transmission.

## INTRODUCTION

The coccidian parasite *Toxoplasma gondii* is one of the most common parasites, with a worldwide distribution in all warm-blooded animal species tested to date (Tenter *et al.* 2000). It infects nearly 1 in 3 humans in the world and can cause serious illness in immunocompromised people and unborn children if their mothers become infected while pregnant (Tenter *et al.* 2000). Wild and domestic felids are the only known definitive hosts of *T. gondii*, and play a crucial role contaminating the environment with oocysts excreted in their faeces

(Dubey, 2010). After ingesting oocysts, intermediate hosts (mammals and birds) develop tissue cysts that may persist for life. In turn, animals with tissue cysts become possible sources of infection for predators, including cats, thus completing the parasite life cycle (Dubey, 2010).

In the Canadian Arctic, there is evidence of high rates of exposure of Inuit to *T. gondii* (Gyorkos, 1980; Tanner *et al.* 1987; Messier *et al.* 2009). In particular, in Nunavik (northern Quebec above 55°N latitude), the overall seroprevalence in the Inuit population is 60% (Messier *et al.* 2009). This is surprising since felids are rare or absent in much of the Arctic and sub-Arctic. The relatively low rates of exposure (5–10%) in northern Cree (50–53°N) who share roughly the same ecosystem and water supply as the Inuit, point to differences in hunting practices and dietary habits between these two ethnic groups (Levesque *et al.* 2007; Campagna *et al.* 2011). The

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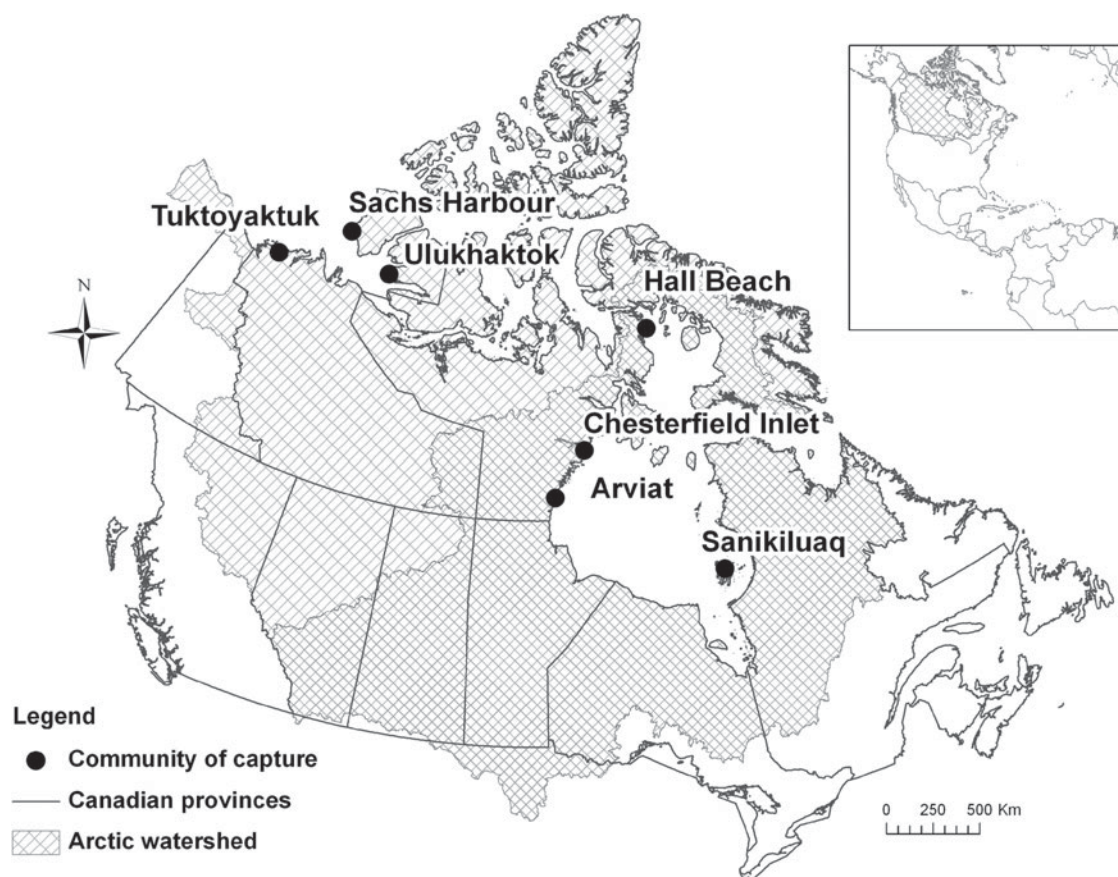


Fig. 1. Location of the seven Canadian Arctic Inuit communities where arctic seals were sampled from 1999 to 2006.

regular consumption of uncooked meat of marine mammals, particularly seals, by the Inuit has been proposed as a possible source of *T. gondii* infection in Inuit communities in previous epidemiological studies (Forbes *et al.* 2009; Messier *et al.* 2009).

How marine mammals become infected with *T. gondii*, in the absence of direct contact with oocysts from the feces of infected felids in the Arctic, is unknown. To date, there is no known definitive host for *T. gondii* in the marine environment (Measures *et al.* 2004). However, recent evidence from California suggests that the parasite can sometimes cross from terrestrial (domestic cats) to marine ecosystems (sea otters or *Enhydra lutris nereis*) via *T. gondii* oocysts that reach the coastal marine environment in surface run-off (Conrad *et al.* 2005; Miller *et al.* 2008b). Some pinnipeds from the Arctic have been found to be seropositive for *T. gondii* including walrus (*Odobenus rosmarus*), ringed seals (*Pusa hispida*), harbour seals (*Phoca vitulina*) and bearded seals (*Erignathus barbatus*) (Leclair and Doidge, 1998; Dubey *et al.* 2003; Jensen *et al.* 2010), providing further support for the hypothesis of transmission of *T. gondii* between terrestrial and marine ecosystems. In the Canadian Arctic, very little is known about the prevalence of *T. gondii* infection in pinnipeds.

In this study, we used serological and molecular methods to assess the potential role of seals as a source

of *T. gondii* infection in the Canadian Arctic. This work raises several questions as to likely transmission pathways of *T. gondii* in this region and the extent to which seal meat may pose a health risk for Canadian Inuit.

#### MATERIALS AND METHODS

##### *Samples and data used in the study*

A total of 828 blood samples were available for study; mostly from ringed seals (*Pusa hispida*) ( $n=788$ ) with smaller numbers of bearded seals (*Erignathus barbatus*) ( $n=20$ ) and harbour seals (*Phoca vitulina*) ( $n=9$ ). A small number of samples from unknown species were included in some analyses ( $n=11$ ). The seals were killed by Inuit during subsistence harvests (September to July) between 1999 and 2006 in 7 communities: Sanikiluaq, Arviat, Chesterfield Inlet and Hall Beach in Nunavut (Hudson Bay); and Sachs Harbour, Tuktoyaktuk and Ulukhaktok in the Northwest Territories (Fig. 1). Whole blood, muscle, kidney, liver, lung and lymph node were some of the tissues sampled as part of Fisheries and Oceans Canada sampling programme. All samples were stored at  $-80^{\circ}\text{C}$  following collection. Associated data included the sex, age, geographical location of capture and year of sampling of the harvested animals. Ringed and harbour seal ages were

determined by counting the annual growth layer groups in the dentine (Northwest Territories seals) or cementum (Hudson Bay seals) of tooth sections (Stewart *et al.* 1996). The diet of ringed seals from Arviat, in western Hudson Bay, was explored through the analysis of carbon (C) and nitrogen (N) stable isotopes (SI) in liver and muscle tissues, representing food ingested days and weeks before harvest, respectively, following the methods described by Vincent-Chambellant (2010).

### Serological analysis

Blood samples were analysed for anti-*T. gondii* antibodies using a direct agglutination test (Toxo screen DA<sup>®</sup>; Biomérieux S.A., Marcy-l'Étoile, France). In each well, diluted blood samples were mixed with formalin-treated toxoplasma tachyzoites that agglutinate in the presence of specific immunoglobulin G antibodies, and 2-mercaptoethanol to suppress non-specific agglutination. Blood samples were tested at 1:40 and 1:4000 dilutions. Samples that obviously agglutinated at a 1:40 dilution were considered seropositive and were further tested at dilutions of 1:60, 1:180, 1:540, and 1:1620. A single reader (A. S.) interpreted all the tests to eliminate inter-observer variability. As a consequence of freezing, whole blood samples contained haemolysed blood. To determine whether or not haemolysed samples contained inhibitors that interfere with the agglutination reaction, we added positive control serum (dilutions 1:40 to 1:4000) to 5 negative but haemolysed blood samples and re-tested. We found no change in the test results suggesting that haemolysis did not influence the agglutination reaction.

### Molecular analysis

To assess the extent to which serological results implicate seal meat as a source of infection for Inuit and to evaluate serological test sensibility (i.e. the possibility for false seronegative test results), we analysed a subsample of seal tissues sampled in Arviat. Using the QIAamp DNA Mini Kit (QIAGEN), DNA was extracted from muscle, kidney, lung and liver of 14 ringed seals (5 seropositive and 9 seronegative) and from the muscle alone of 135 seals (23 seropositive ringed seals and 2 seropositive harbour seals, 107 seronegative ringed seals and 3 seronegative harbour seals) from which suitable tissue samples were available. A real time polymerase chain reaction (PCR) assay was used to detect a 529-bp repeat element occurring up to 200 to 300 times in the *T. gondii* genome (Kasper *et al.* 2009).

### Statistical analysis

We investigated seal sex, community of harvest, age, year of sampling and seal species, as explanatory

variables for seropositivity. In a separate analysis, we assessed C and N SI ratio variables on seropositivity of ringed seals from Arviat. Seals were classified as: pups (<1 year), juveniles (1–5 years) and adults (>5 years) based on dental ages (Holst *et al.* 1999). Each explanatory variable was screened for potential unconditional association with *T. gondii* serostatus using a generalized linear model with a logit link function in SAS version 9.1 (PROC GENMOD, SAS Institute Inc., Cary, NC). Those variables significantly associated with serostatus at the  $P < 0.20$  level were included in a multivariable regression model. Linearity of the relationship between the SI ratios and the log odds of the seropositivity was assessed by categorizing the continuous SI ratios and visualizing plots of the odds ratio against mean values of the different categories. The possibility for multicollinearity was investigated by standard diagnostic methods. The possible presence of confounding bias was evaluated by adding all eliminated predictors (whether or not included in the multivariable model), one at a time, and back into the final model to avoid the exclusion of an important predictor potentially masked by another variable. We were unable to test for possible interactions in certain groups because of small sample sizes. The relative goodness of fit of the model was assessed by Pearson and deviance Chi-square tests. The level of significance was  $P < 0.05$  throughout the multivariable analysis. To reduce the risk of a Type I error when making multiple comparisons, alpha was reduced using Holm's sequential Bonferroni method.

### RESULTS

A total of 86/828 seals (10.4%, 95% confidence interval CI = 8.3–12.5) were seropositive for *T. gondii*. Positive titres were generally low (1:40 to 1:180). No tissue sample was positive by PCR.

Overall, 80/788 ringed seals (10.2%, 95% CI = 8.0–12.3), 2/20 bearded seals (10.0%, 95% CI = 1.2–31.7) and 2/9 harbour seals (22.2%, 95% CI = 2.8–60.0) were seropositive for *T. gondii*. The difference in seroprevalence according to species was not significantly different ( $P = 0.50$ ). Due to small sample size, no further analysis was performed on the bearded and harbour seal data.

There was no statistical difference in the proportion of seropositive male and female ringed seals (Table 1). Seroprevalence was greatest for juvenile ringed seals (35/218: 16.1%), followed by pups (11/128: 8.6%) and adults (29/396: 7.3%) (Table 1, Fig. 2). There was significant variation in seroprevalence amongst ringed seals sampled in the different communities. The highest seropositivity rates were observed in Hall Beach and Arviat (3/13 and 45/289, respectively) and was lowest in Chesterfield Inlet (1/41) (Table 1, Fig. 1). Due to very low sample sizes (1 and 3, respectively), the data from years 1999 and

Table 1. Prevalence of specific antibodies to *Toxoplasma gondii* among ringed seals from Nunavut and the Northwest Territories, Canada, according to sex, age class, community and year of sampling, and level of significance for unconditional association between *T. gondii* seropositivity and each explanatory variable

(Significant effects ( $P < 0.05$ ) are shown in bold.)

Variables	N <sup>a</sup>	Prevalence (%)	95% CI <sup>a</sup>	P-value <sup>b</sup>
Total	788	10.2	8.1–12.2	
Sex				0.9945
Male	442	10.0	7.2–12.7	
Female	331	10.0	6.7–13.2	
Age class				<b>0.0034</b>
Pup	128	8.6	3.7–13.4	
Juvenile	218	16.1	11.2–20.9	
Adult	396	7.3	4.8–9.9	
Community				<b>0.0029</b>
Arviat	289	15.6	11.4–19.7	
Chesterfield Inlet	41	2.4	0.1–12.9 <sup>c</sup>	
Hall Beach	13	23.1	5.0–53.8 <sup>c</sup>	
Sachs Harbour	28	7.1	0.9–23.5 <sup>c</sup>	
Sanikiluaq	229	7.9	4.4–11.3	
Tuktoyaktuk	17	5.9	0.1–28.7 <sup>c</sup>	
Uluksaktok	171	5.8	2.3–9.4	
Year of sampling				<b>0.0009<sup>d</sup></b>
1999	1	0		
2000	3	0	0–70.8 <sup>c</sup>	
2001	46	15.2	6.3–28.9 <sup>c</sup>	
2002	31	0	0–11.2 <sup>c</sup>	
2003	181	17.7	12.1–23.2	
2004	179	8.4	4.3–12.4	
2005	296	7.8	4.7–10.8	
2006	51	5.9	1.2–16.2 <sup>c</sup>	

<sup>a</sup> N, sample size; CI, confidence interval.

<sup>b</sup> Likelihood ratio test Type III effect in GENMOD Procedure.

<sup>c</sup> Exact 95% CI (Fisher's).

<sup>d</sup> Samples collected in 1999 and 2000 not included.

2000 were omitted. The model converged and the proportion of seropositive seals varied significantly between years of sampling. The proportion of seropositive samples was higher in seals harvested in 2001 (7/46) and 2003 (32/181) compared to other years (Table 1).

In ringed seals from Arviat, the carbon SI ratio was significantly lower in seropositive seals compared to seronegative seals ( $P = 0.030$ ). This last variable was not tested in a multivariate model because of the correlation with year and age variables. The nitrogen SI ratio was not associated with seroprevalence ( $P = 0.60$ ).

Age class, community and year of sampling (excluding data from ringed seals harvested in 1999 and 2000 and those harvested from Hall Beach where no age data were available) were the variables that remained significant in the most parsimonious multivariable logistic regression model for *T. gondii* seropositivity (Table 2). No evidence for collinearity was detected among these predictors. The seroprevalence was significantly higher in juveniles than

in adults and in ringed seals sampled in Arviat compared to Sanikiluaq. Samples collected in 2001 and 2003 were more likely to be seropositive than those collected in 2005.

#### DISCUSSION

To our knowledge this is the first large-scale study of *T. gondii* prevalence in pinnipeds in the Canadian Arctic and it is based on the largest collection of seal blood and tissues gathered to date from this region. Our serological data suggested that at least 10% of arctic seals were exposed to *T. gondii*. An interesting and unexpected pattern of age-seroprevalence was found in ringed seals: seroprevalence did not increase continuously with age, which is consistent with ringed seals becoming infected primarily at a young age. The seals from Arviat in western Hudson Bay had the highest rate of *T. gondii* infection amongst ringed seals sampled in the communities under investigation, and exposure to the parasite may have been particularly high in the years 2001 and 2003.

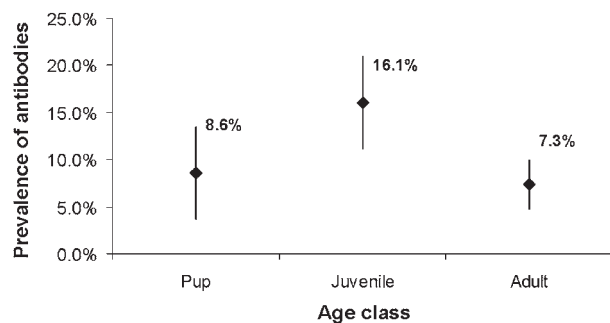


Fig. 2. Prevalence of antibodies against *Toxoplasma gondii* (%) among ringed seals from the Canadian Arctic by age class: pup (<1 year old), juvenile (1–5 years old) and adult (>5 years old). Error bars indicate the standard deviation.

In this study, *T. gondii* DNA was not detected in seal tissues despite large numbers of muscle samples analysed, emphasizing the difficulty of finding *T. gondii* cysts in tissues by PCR (Hill *et al.* 2006). Unfortunately, neither heart nor brain tissues, often considered target sites for *T. gondii* encystation (Dubey, 2010), were available for PCR testing.

The direct agglutination test that we employed in our study has been widely used to detect *T. gondii* antibodies in a variety of marine mammals (Oksanen *et al.* 1998; Dubey *et al.* 2003, 2005; Measures *et al.* 2004; Cabezon *et al.* 2004, 2011; Jensen *et al.* 2010). This test is considered to be both sensitive and specific in mammals (Dubey, 2010), and capable of detecting infection in seals (Gajadhar *et al.* 2004). In a study on sea otters, all those with demonstrable *Toxoplasma* infections had detectable MAT antibodies (Thomas *et al.* 2007). While the state of haemolysis of the samples included in this study could theoretically have affected the direct agglutination test sensitivity, we investigated this possibility and found no evidence for potential effects of haemolysis on seroprevalence results. Since all blood samples were in similar condition, we expect our inter-group comparisons to be valid. None of the positive serological reactions had a titre higher than 1:180, which is consistent with other studies using the direct agglutination test in marine mammals (Cabezon *et al.* 2004, 2011; Dubey *et al.* 2003, 2005; Jensen *et al.* 2010; Lambourn *et al.* 2001; Measures *et al.* 2004). In the present study, the samples were haemolysed and using a cut-off dilution lower than 1:40 would have resulted in the test being unreadable for some samples. However, titres even lower than the 1:40 cut-off used in our study may need to be considered since *T. gondii* cysts were detected in the tissues of a naturally infected whale which had an agglutination test titre of only 1:25 (Mikaelian *et al.* 2000). It is possible that decline in seropositivity in adult seals could be due to antibody levels declining below a level detectable at a 1:40 dilution.

The seroprevalence rate found in our study is consistent with previous studies on *T. gondii* in seals

Table 2. Statistically significant explanatory variables in the multivariable logistic regression model of the presence of specific antibodies against *Toxoplasma gondii* in 738 ringed seals from six communities in the Canadian Arctic

Factor <sup>a</sup>	Odds ratio (OR)	95% CI for OR	P-value <sup>b</sup>
Age class			0.0093
Pup	1.27	0.56–2.88	0.5731
Juvenile	2.44	1.34–4.44	0.0035
Adult	1.00		
Community			0.0119
Arviat	2.45	1.27–4.74	0.0077
Chesterfield Inlet	0.17	0.01–2.11	0.1696
Sachs Harbour	1.43	0.19–10.86	0.7263
Sanikiluaq	1.00		
Tuktoyaktuk	1.08	0.06–20.13	0.9568
Ulukhaktok	0.61	0.17–2.13	0.4360
Year			0.0001
2001	11.20	2.53–49.62	0.0015
2002 <sup>c</sup>	N/A	N/A	N/A
2003	3.37	1.80–6.33	0.0002
2004	1.65	0.76–3.57	0.2052
2005	1.00		

<sup>a</sup> Adults sampled in Sanikiluaq in 2005 were used as the reference.

<sup>b</sup> Wald chi-square test.

<sup>c</sup> OR for 2002 non-existent (prevalence = 0%).

harvested in other arctic locations (ringed seals, harbour seals and bearded seals in Alaska (Dubey *et al.* 2003); ringed seals and bearded seals in the archipelago of Svalbard, Norway (Jensen *et al.* 2010)). The discovery of a non-archetypical strain designated Type X *T. gondii* in both wild terrestrial carnivores and in a mussel in California supports the theory of a link between terrestrial and marine coastal environment via freshwater run-off (Miller *et al.* 2008b). Indeed, fecal contamination of coastal marine environments by terrestrial mammals is a well-recognized problem for several pathogenic protozoa (e.g. *Giardia* and *Cryptosporidium*: (Appelbee *et al.* 2005; Miller *et al.* 2010). We speculate that *T. gondii* infection of seals in the Canadian Arctic could similarly be due to contamination of the marine environment by *T. gondii* oocysts excreted by felids in the arctic watershed, and transported via freshwater run-off to the marine environment. *T. gondii* oocysts can sporulate in seawater and remain infectious for mice for 24 months (Lindsay and Dubey, 2009). The Canadian lynx (*Lynx canadensis*) is the only widespread and abundant wild felid present in the Canadian arctic watershed (Banfield, 1974) and may therefore play an important role in contaminating the arctic marine environment. Indeed, reported seroprevalence rates are high (44%) in lynx from Quebec (Labelle *et al.* 2001). Like other wild felids, a single infected lynx may excrete many millions of *T. gondii* oocysts into the terrestrial environment during the course of its life (Jones and Dubey, 2010).

The pattern of seroprevalence to *T. gondii* among age classes observed in the present study was unexpected. In most infected mammals including humans, both infection and seropositivity can be readily detected for life (Dubey, 2010). If exposure to a pathogen causing a persistent infection is constant, it would be expected that seroprevalence should increase with age at a more or less constant rate. In this study, seropositivity was highest in juvenile ringed seals, while adults had lower seroprevalence rates. This pattern is consistent with infection of ringed seals during their first years of life. A cessation of exposure as ringed seals age may explain natural waning of antibody titres over time in older animals despite continued infection that become difficult to detect with serological tests. There is no evidence to date that infection of seals causes significant morbidity or mortality that could explain the serological pattern observed (Gajadhar *et al.* 2004). Although spontaneous clearing of infection in the adult ringed seals with a loss of tissue cysts (Opsteegh *et al.* 2011) could plausibly explain the loss of antibodies, other authors believe in life-long *T. gondii* infection in seals like most other intermediate hosts (Dubey, 2010). It is also possible that some ringed seals are infected transplacentally as has been suggested for arctic foxes (*Vulpes lagopus*) (Prestrud *et al.* 2007), and as seen in others marine mammals such as dolphins and sea otters (Jardine and Dubey, 2002; Resendes *et al.* 2002; Miller *et al.* 2008a). However, reports of transplacental *T. gondii* transmission in marine mammals are infrequent and this route of infection may be rare (Miller *et al.* 2008a). Furthermore, congenital toxoplasmosis generally occurs only during acute infection of the mothers (Dubey, 2010) and, considering the relatively low seroprevalence in adult ringed seals, this route of transmission would likely be both inefficient and infrequent. Age-related hunting behaviour, segregation in habitat, geographical occurrence and, therefore, diet may cause young ringed seals to be more exposed to *T. gondii* than older seals (Born *et al.* 2004; Vincent-Chambellant, 2010). C and N stable isotope ratios support a difference in diet between adult and younger ringed seals, with a higher benthic/inshore component in the former (Vincent-Chambellant 2010).

*T. gondii* infection in seals might occur via ingestion of oocysts concentrated in their prey (Robertson, 2007). Molluscs, that filter water from freshwater run-off, are a possible source of infection for marine mammals (Arkush *et al.* 2003; Lindsay *et al.* 2004). However, analysis of stomach contents from ringed seals in Arviat suggests that molluscs are consumed in very small quantities compared to the other prey types (Vincent-Chambellant, 2010). Moreover, the low carbon stable isotope ratio found in young seropositive ringed seals compared to seronegative animals suggests that the former group

is not preferentially using the benthic environment to feed compared to the latter. During the open-water season, the diet of ringed seals of all age classes sampled in Arviat consists principally of fish (Vincent-Chambellant, 2010), raising the possibility that fish may be an important source of bio-concentration of oocysts originating from a terrestrial environment. Like molluscs, fish gills could plausibly filter *T. gondii* oocysts out of seawater making them an efficient *T. gondii* vector for piscivorous marine mammals (Massie *et al.* 2010).

Toxoplasmosis is a major public health issue in the Canadian Arctic (Messier *et al.* 2009). Our study suggests that seals are exposed to *T. gondii* and may serve as a significant source of infection for Inuit people who frequently consume seal meat uncooked. Broad geographical and inter-annual patterns of seropositivity were observed that could reflect geographical and inter-annual variations in contamination of the marine environment associated with the transport of oocysts from the terrestrial environment. Further studies are needed to better understand these spatio-temporal variations, as well as the differential infection of seals of different ages. In particular, we are currently conducting a risk assessment study to understand whether hydrological transport of oocysts is a sufficient mechanism to explain *T. gondii* infection in seals and Inuit in the Canadian Arctic.

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