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Foxes (*Vulpes vulpes*) as sentinels for parasitic zoonoses, *Toxoplasma gondii* and *Trichinella nativa,* in the northeastern Canadian Arctic

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ABSTRACT

Outbreaks of *Toxoplasma gondii* and *Trichinella* spp. have been recurring for decades among Inuit of Nunavik, northeastern Canada. Contact with wildlife has been identified as a risk factor for Inuit exposure to *T. gondii,* but reservoirs have yet to be confirmed based on direct detection of DNA or organism. Similarly, little is known about the occurrence of *Trichinella* spp. in wildlife species of Nunavik other than walrus (*Odobenus rosmarus*) and bears *(Ursus americanus, Ursus maritimus)*. Foxes (*Vulpes vulpes*) were targeted as possible sentinels for *T. gondii* and *Trichinella* spp. because of their high trophic position within the Arctic food chain as carnivorous scavengers. A total of 39 red foxes were sampled from four communities in southern and western Nunavik between November 2015 and September 2016. For the first time in wildlife, a novel magnetic capture DNA extraction and real-time PCR technique was used to isolate and detect *T. gondii* DNA from the heart and brain of foxes. A double separatory funnel digestion method followed by multiplex PCR was used to recover and genotype larvae of *Trichinella* spp. from tongues of foxes. Seroprevalence based on detection of antibodies to *T. gondii* was 41% (95% CI: 27–57%) using a commercially available modified agglutination test (MAT). Detection of DNA of *T. gondii* and larvae of *Trichinella nativa* (T2) occurred in 44% (95% CI: 28–60%) and 36% (95% CI: 21–51%) of foxes, respectively. Coinfection with both *T. nativa* and *T. gondii* occurred among 23% (95%CI: 13–38%) of foxes which can be attributed to co-transmission from prey and scavenged species in their diet. There was only moderate agreement between *T. gondii* serology and direct detection of *T. gondii* DNA using the MC-PCR technique (Kappa test statistic: 0.321), suggesting that using both methods in tandem can increase the sensitivity of detection for this parasite. These findings show that foxes are good sentinels for circulation of parasitic zoonoses in terrestrial northern ecosystems since they are highly exposed, show measurable indicators of infection and do not serve as exposure sources for humans.

1. Introduction

Toxoplasma gondii (*T. gondii)*, an obligate intracellular protozoan parasite, can infect people and animals worldwide (Tenter et al., 2000). The multi-stage life cycle involves felid definitive hosts and intermediate hosts including many bird and mammal species (Cenci-Goga et al., 2011). Transmission of *T. gondii* can occur through the ingestion of food or water contaminated with sporulated oocysts, ingestion of raw or undercooked animal tissues infected with cysts, trans-placental migration of tachyzoites during pregnancy and, more rarely in people, transfusion of blood-contaminated with tachyzoites (Robert-Gangneux et al., 2012). The *T. gondii* life cycle can be maintained without felids through carnivory and vertical transmission among intermediate hosts. Although infection with *T. gondii* is usually asymptomatic in healthy humans, clinical toxoplasmosis can occur in fetuses of seronegative pregnant women and immunocompromised individuals (Dubey, 2010). Recently, latent infection with *T. gondii* has been linked to the development of epilepsy and schizophrenia (Palmer, 2007; Torrey et al., 2012).

Trichinella spp. are nematode parasites which can only be

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Fig. 1. Map of Nunavik (© Lemire et al., 2015).

transmitted through the foodborne route in carnivores, omnivores and scavengers. Adult nematodes in the intestine produce larvae which establish within the skeletal and cardiac muscles of the same host. Acute *Trichinella* infection in people can produce diarrhea, vomiting, fatigue and fever within 1–2 days of consuming meat from infected animals (Houzé et al., 2007). In the Canadian North, two freeze-tolerant taxa of *Trichinella*, type T2 (*T. nativa*) and type T6, have previously caused human disease outbreaks linked to the consumption of uncooked walrus *(Odobenus rosmarus*) and black bear *(Ursus americanus*) meat (Serhir et al., 2001; McIntyre et al., 2007).

Nunavik, Canada, is home to over 12,000 Inuit living among 14 remote communities along Ungava Bay, Hudson Strait and Hudson Bay (Fig. 1). Seroprevalence of *T. gondii* was 62.8% in Inuit from Hudson Bay communities, and 58.4% in Inuit from Ungava Bay communities (Messier et al., 2009). These values are high compared to a seroprevalence of 22.5% reported in the remainder of North America, but corroborate similar results from a previous study where *T. gondii* seroprevalence was 61% and 69% among Inuit from Kuujjuaq and Salluit, respectively (Tanner et al., 1987; Jones et al., 2001). Moreover, the reported incidence of congenital toxoplasmosis of 1.7% in Nunavik exceeds the 0.2–0.8% reported incidence in the remainder of Canada (Lavoie et al., 2008). Risk factors for Inuit exposure to *T. gondii* in Nunavik include skinning wildlife, frequent consumption of caribou (*Rangifer tarandus*), as well as eating raw caribou and seal liver (Tanner et al., 1987; Curtis et al., 1988; McDonald et al., 1990). In a separate study, other risk factors included drinking reservoir water, as well as consuming seal meat and feathered game (Messier et al., 2009). Contrary to *T. gondii,* the seroprevalence based on detection of antibodies to *Trichinella* spp. in Inuit of Nunavik was low at less than 1% (Messier et al., 2012). However, recurring outbreaks of human trichinellosis linked to the consumption of walrus meat have raised health concerns by local Inuit (Proulx et al., 2002). Therefore, there is a need to determine whether terrestrial wildlife species in the Canadian North are important reservoirs for *T. gondii* and *Trichinella* spp.

Most wildlife studies rely on serology which provides evidence of exposure to, rather than infection with, a pathogen (Gilbert et al., 2013; Ryser-Degiorgis, 2013). In the case of *T. gondii*, direct detection of the parasite or its DNA is important in order to distinguish tissue infection

from life time exposure. Bioassay and conventional PCR methods have been important methods for the isolation of *T. gondii* parasite or DNA, respectively (Tenter et al., 2000). Bioassays can assess parasite presence and viability using large amounts of tissue, but are costly in terms of animal use and not all genotypes of *T. gondii* are virulent in mice (Guo et al., 2015). Conventional DNA extraction methods are poorly sensitive as they use small amounts of tissue (25–50 mg) (Opsteegh et al., 2010). A novel sequence-specific magnetic capture DNA extraction technique followed by real-time PCR technique has been successfully used to detect *T. gondii* from several domestic food animal species (Opsteegh et al., 2010; Jurankova et al., 2014; Aroussi et al., 2015; Koethe et al., 2015; Gomez-Samblas et al., 2015). The technique allows the isolation of small amounts of parasite DNA from large amounts of host tissue using magnetic beads labelled with sequence-specific parasite DNA probes that capture target DNA which is then concentrated using a magnet (Opsteegh et al., 2010). Detection of *T. gondii* DNA using the MC-PCR technique has not yet been attempted in wildlife.

Because *T. gondii* and *Trichinella* spp. have a broad range of possible hosts, preliminary investigations using a key sentinel species can be a cost-effective way of identifying their presence in the local environment before attempting detection in other target animals or humans (Pei Shan Neo and Huan Tan, 2017). An ideal sentinel species for *T. gondii* is one that can be exposed to both oocysts in the environment and tissue cysts in foods of animal origin. Other important characteristics of a key sentinel species include having a widespread distribution, a restricted home range, the ability to bioaccumulate a hazard, susceptibility to the hazard, and continuous residency in the local environment (Basu et al., 2006; Landres et al., 1988). An ideal sentinel species should also have increased exposure (higher trophic position in the food chain) compared to the target population, yet not pose a risk for pathogen transmission to the target population (Bowser and Anderson, 2018). Foxes can be exposed to both oocysts and tissue cysts of *T. gondii* and have a high probability of being infected with *T. gondii* if it occurs in the local environment. Lynx (*Lynx canadensis*) are a potential source of oocysts in southern Nunavik, but are absent in western and northern Nunavik (Naughton, 2012). Exposure of Arctic wildlife could occur through consumption of water contaminated with oocysts shed by felids from remote boreal and temperate regions and transported north in freshwater or marine currents (Simon et al., 2013). It is also possible that foxes are exposed through the consumption of migratory birds exposed to oocysts in the South (Prestrud et al., 2007; Sandstrom et al., 2013). Wildlife exposure to *T. gondii* in Nunavik is also supported by a seroprevalence of 4.2% in Canada geese, 2.5% in ptarmigan (*Lagopus lagopus*) and 14% in ringed seals (Leclair and Doidge, 2001).

Foxes are likely infected with *Trichinella* spp. due to their scavenging behavior and a diet that covers a broad range of prey species which provide opportunities for cumulative exposure to the parasite (De Craeye et al., 2011). With an average lifespan of 2–5 years, infection or exposure status in foxes reflects recent parasite presence in the food web. Their home range is stable year-round, typically 4–8 km^2, serving as a proxy for the hazard's geographic distribution (Banfield, 1974). Despite this, *Trichinella* spp. has not been reported in foxes in Nunavik (Jenkins et al., 2013). Our specific objectives were 1) to determine whether foxes are a good sentinel species for *T. gondii* and *Trichinella* spp. in Nunavik, Canada, based on direct detection techniques, and 2) to determine agreement between a direct detection method and a serological assay for *T. gondii.*commonly used for wildlife disease surveillance.

2. Materials and methods

2.1. Samples

Because serological data suggest that higher human exposure to *T. gondii* occurs in the southern and western coasts of Nunavik, red foxes (*Vulpes vulpes*) were collected from two communities in southern (Kuujjuaraapik, Kuujjuaq) and northwestern (Puvirnituq, Inukjuak) Nunavik, Québec, Canada (Fig. 1). No instructions on carcass submission criteria were specified and foxes were trapped by Inuit as part of locally-regulated fur harvesting activities. The target sample size was set at 55 carcasses based on an expected *T. gondii* seroprevalence of 18%, a 10% precision level, and a 95% confidence interval (De Craeye et al., 2011; Dohoo et al., 2010). Recording sheets were provided to collect data (species, sex, hunting location, date) and local coordinators were hired to ensure that samples were stored at −20 °C until shipped for necropsy.

2.2. Detection of T. gondii antibodies

As per Villena et al. (2012), whole hearts kept frozen in individual plastic bags were thawed at room temperature within two months of sampling for the collection of tissue fluid using a sterile disposable plastic pipette for each sample. Serological testing for antibodies against *T. gondii* was performed using the modified agglutination test (MAT) as per manufacturer instructions with a sample considered positive above a cut-off value of 1:25 (New Life Diagnostic LLC, Carlsbad, CA, United States).

2.3. Tachyzoite source and plasmid DNA for standard curves

A stock of 2.0×10^8 cultured VEG type III *T. gondii* tachyzoites stored at - 20 °C was supplied by the Canadian Food Inspection Agency's Centre for Foodborne and Animal Parasitology (CFAP) in Saskatoon, Saskatchewan, Canada (Al-Adhami et al., 2016). A serial dilution was performed to obtain solutions of 2.5×10^6, 2.5×10^5 and 2.5×10^4 tachyzoites per ml. One hundred microliters (100 µL) of each dilution was added to separate 100 g beef muscle samples as positive controls for the MC-PCR (Opsteegh et al., 2010).

To construct *T. gondii* plasmid DNA, a conventional polymerase chain reaction (PCR) was performed in an Eppendorff thermocycler (Mastercycler pro, Eppendorf AG, Germany) using the Tox 9F (5′-aggagagata tcaggactgtag-3′) and Tox 11R (5′-gcgtcgtctc gtctagatcg-3′) primers to generate a 188 bp PCR product within the 529 bp repeat-element of the *T. gondii* genome (Reischl et al., 2003) with slight modifications. Each PCR reaction contained 2 µL of *T. gondii* genomic DNA in a 50 µl reaction with 2.5 U Taq DNA polymerase (Quanta Bio Science, USA) Q), 2.5 mM $MgCl_2$, 100 µM dNTP (Invitrogen, Pharmacia Biotech), 20 mM each of primers Tox 9F and Tox 11R, 50 mM KCl, 10 mM Tris/HCl, pH 8.3. PCR reactions were amplified as per Homan et al. (2000). PCR products were then purified using the QIAquick Gel Extraction Kit (Qiagen, Toronto, Canada) and cloned to pGEM-T Easy vector (Promega) according to the manufacturer's protocol. Finally, the plasmid DNA was extracted using EZ-10 spin columns (Bio Basic Inc −ON) and was diluted in 10 fold in TE buffer (Promega) to construct the standard curve. Quantitative real-time PCR was performed in a Bio-Rad CFX 96 DNA thermal cycler (Biorad, Hercules, California, USA) with each reaction containing 1 × SYBR Green Supermix (Bio-Rad Laboratories Canada Ltd., Mississauga, ON), 400 nM each primer Tox 9F and Tox 11, and 2 µL of plasmid DNA, in a total volume of 25 µL All reactions were performed in duplicate and a no-template control (NTC). Reactions were incubated at 95 °C for 10 min followed by 40 cycles each of 15 s at 95 °C, 1 min at 60 °C.

2.4. Lowest detection limit of genomic DNA and tachyzoites for the MC-PCR technique

To determine the minimum number of DNA copies detected by the PCR assay, a minimum of 8 replicates were amplified for each ten-fold dilution of *T. gondii* genomic DNA (gDNA) ranging between 2 and 2 million femtograms (fg) per PCR reaction. To evaluate the lowest detectable number of tachyzoites for the MC-PCR method, 100 g beef muscle samples were spiked with 10-fold serial dilutions of tachyzoite

Table 1
Proportions of foxes positive for *T. gondii* and *Trichinella* in Nunavik between April 2015 and September 2016.

Community	n	*T. gondii* Proportion (± 95% CI) (# positive)		*Trichinella* Proportion (± 95% CI) (# positive)
		MAT	MC-PCR	
Kuujjuaq	7	71% (29–96%) (5)	86% (49–97%) (6)	43% (16–75%) (3)
Kuujjuaraapik	13	31% (13–58%) (4)	15% (4–42%) (2)	8% (2–33%) (1)
Inukjuak/ Puvirnituq	19	37% (13–54%) (7)	53% (32–73%) (10)	53% (31–73%) (10)
Total	39	41%[a] (27–57%) (16)	46%[1] (32–61%) (18)	36% (21–51%) (14)

* MAT: Modified Agglutination Test.
** MC-PCR: Magnetic Capture.
[a] *T. gondii* prevalence based on foxes positive on serology or tissue detection was 59% (95%CI: 43–73%).

concentrations ranging between 12.5 and 2.5 million tachyzoites per 100 g of tissue. Analysis of the lowest detection limits was determined by factoring the number of positive reactions for each concentration into a probit regression model (IBM SPSS Statistics 24, SPSS Statistics, Chicago, IL) for both the genomic DNA and tachyzoite spiking experiments.

2.5. Extraction and detection of DNA

DNA extraction from a maximum weight of 60 g each of half the brain (mean: 43 g; standard deviation: 6 g) and the entire heart (mean: 36 g; standard deviation: 10 g) from each fox was performed as per Opsteegh et al. (2010), except that the 5′ end of the competitive internal amplification control (CIAC) probe (5′ agcgtaccaa-caagtaattctgtatcgatg 3′) was labelled with HEX rather than with JOE. Moreover, real-time PCR amplification was done using the Bio-Rad CFX 96 DNA thermal cycler (Biorad, Hercules, California, USA) based on a published protocol for the detection of the 188 bp *Toxoplasma* sequence (Omar et al., 2015). The final PCR assay reaction included 0.5 M of Itaq Supermix, 20 µM of TP1 probe (5′ ccggcttggctgcttttcct 3′), 10 µM of Tox 9F, 10 µM of Tox 11R, 2 fg of CIAC, 5 µM of CIAC probe, 6.75 µL of PCR-grade water and 8 µL of template DNA. A positive PCR reaction was defined as any reaction with: 1) a Ct-value smaller or equal to 35, 2) a no-template control with a Ct-value of zero, 3) a negative MC-PCR control, and 4) a positive MC-PCR control (Opsteegh et al., 2016). All reactions where only one of two duplicates amplified, or where CIAC amplification failed to occur, were repeated.

2.6. Detection and genetic characterization of Trichinella spp

Tongues were collected from each fox and frozen at −20 °C for < 2 months until *Trichinella* larvae were recovered using a double centrifugation and enzymatic digestion assay described by Forbes and Gajadhar (1999). Briefly, approximately 10 g from each tongue was homogenized in a blender, digested in 1% HCl/Pepsin solution at 37 °C for 1 h, and concentrated by sequential sedimentation through two different separatory funnels. Sediment was examined under a stereomicroscope at 10-16× magnification for the presence of larvae. Recovered larvae were washed in PBS and frozen at minus 20 °C until further analysis. For each positive fox, DNA was extracted from 15 larvae (5 individually and 10 pooled) for further characterization as per Scandrett et al. (2018). The extracted DNA was amplified by a conventional multiplex PCR using primer sets that generate unique banding patterns on a 2.5% agarose gel for each known species and genotype of *Trichinella* (Zarlenga et al., 1999).

2.7. Statistical analyses

For both serological and molecular results, prevalence was described as proportions with their 95% confidence intervals estimated for each community. Proportions of positive and negative results were then compared between the MAT and MC-PCR methods using the McNemar's χ^2 test for paired data. Then, the kappa coefficient was calculated to determine the level of agreement between the two tests (Dohoo et al., 2010). To determine whether a difference occurs between proportions of *T. gondii* positive foxes in southern communities (Kuujjuaraapik, Kuujuak) and northwestern communities (Inukjuak, Puvirnituq), a χ^2 test of independence for small sample sizes was performed in R Studio version 1.1.442.

3. Results

3.1. Samples

Samples (heart, brain and tongue) from a total of 39 red foxes were received between April 2015 and September 2016 from Kuujuaq, Kuujjuaraapik, Inukjuak, and Puvirnituq (Table 1; Fig. 1).

3.2. Detection limit of genomic Toxoplasma DNA and tachyzoites

The estimated 95% lowest detection limit of the PCR assay was 73 fg of *Toxoplasma* genomic DNA per PCR reaction (95% CI: 23–5847) using a probit regression model that fitted the data adequately (Pearson $\chi^2 = 0.212$, $p = 0.99$). The estimated 95% lowest detection limit for the MC-PCR method was 445 tachyzoites per 100 g (95% CI: 86–742,000) of beef sample. Model fit was adequate (Pearson $\chi^2 = 9.494$, $p = 0.22$).

3.3. Detection of T. gondii antibodies

Of the 39 foxes, heart fluid from sixteen (41%) were positive to *T. gondii* antibodies with the Modified Agglutination Test (MAT): 5 out of 7 collected from Kuujuaq (75%), 4 out of 13 from Kuujjuaraapik (31%) and 6 out of 19 from Inukjuak/Puvirnituq (37%). Of the sixteen seropositive foxes, no DNA was detected in 5 foxes (Table 2). Of the 23 seronegative foxes, DNA was detected in the brain and/or heart of 7 foxes.

3.4. Detection of T. gondii DNA and tachyzoites

DNA of *T. gondii* was detected in 10 fox brains (26%; CI: 14–41%) and 17 fox hearts (44%; CI: 29–59%) for an overall PCR prevalence of 46% among all 39 foxes. A total of 8 out of 18 PCR-positive foxes were positive for both brain and heart (Table 1). There was no significant difference in the proportion of PCR-positive foxes between western (53%) and southern (40%) Nunavik ($\chi^{2=}0.22$, $p = 0.85$).

3.5. Trichinella spp.

Larvae of *T. nativa* (T2) were recovered from 14 of 39 foxes with an estimated prevalence of 36%; 1 of 13 (8%) in Kuujjuaraapik, 10 of 19 (53%) from the Inukjuak/Puvirnituq region, and 3 of 7 (43%) from

Table 2
Comparison of the MAT and MC-PCR outcomes for the detection of *T. gondii* in foxes collected in Nunavik.

	MC-PCR pos	MC-PCR neg	Total
MAT-pos	11	5	16
MAT-neg	7	16	23
Total	18	21	39

Kuujjuaq.

3.6. Co-infection with T. gondii and T. nativa

Among 14 T. *nativa* positive foxes, 9 (64%) were also PCR-positive for *T. gondii* (four were positive for both brain and heart; five were positive for heart only) with an estimated co-infection proportion of 23% out of 39 foxes (95% CI: 13–38%).

3.7. Agreement between MC-PCR and serology for T. gondii

Using either method of detection (serology or molecular), 23 of 39 foxes (59%) were exposed to and/or infected with *T. gondii*. Five foxes were positive on serology and negative on tissue DNA, 7 were serologically negative but tissue positive, 11 were positive on both, and 16 were negative on both (Table 1). There was no statistical difference between serological and molecular results using the McNemar chi square test ($p = 0.581$), and the Kappa test statistic ($k = 0.321$) showed only moderate agreement between the two tests (Dohoo et al., 2010).

4. Discussion

To our knowledge, this is the first report of the isolation of *Toxoplasma gondii* DNA in tissues of a naturally-infected wildlife species based on the magnetic capture and real time PCR technique. In this study, the PCR prevalence was 46% among 39 foxes trapped from several communities in southern and northwestern Nunavik. Both brain and heart were used in this study rather than reliance on a single tissue. The analysis of hearts allowed for the detection of 8 additional foxes positive for *T. gondii* which would have otherwise been classified as negative if prevalence had been defined *a priori* as the detection of DNA in brain only. Moreover, a competitive internal amplification control was used in our PCR protocol to avoid underestimating prevalence by allowing the distinction of false-negatives (e.g. failure of DNA to amplify) from true negatives (Opsteegh et al., 2010). Lastly, amplification of the 529 bp repeat-element is known to be more sensitive than the B1 and ITS-1 genes since there are 200–300 copies, rather than 35 and 110 copies per *T. gondii* genome, respectively (Homan et al., 2000; Farhadi et al., 2017). Our study design, therefore, maximised the overall study sensitivity. Despite this, it is still possible that *T. gondii* prevalence was underestimated in our study since DNA analyses were limited to two tissues and half of each fox brain since the other half was used for rabies detection in a separate study. These represent potential limits since many tissues other than brain and heart can be infected by *T. gondii* and since the parasite is not uniformly distributed among and within all tissues (Dubey, 2010). Regardless, this study shows that the MC-PCR technique can successfully be used to isolate and detect *T. gondii* DNA in naturally-infected wild foxes.

Although foxes in southern Nunavik could be exposed to *T. gondii* oocysts shed by lynx into the local environment, prevalence was not significantly higher in foxes from southern vs western Nunavik in the current study. In northwestern Nunavik, felid hosts are rare to absent and this is where almost 50% of the *T. gondii* -positive foxes from this study were trapped. Therefore, it is more likely that transmission of *T. gondii* in foxes in the North is maintained through the consumption of oocysts transported from temperate regions in freshwater and/or through consumption of tissue cysts in meat and organs from migratory wildlife infected in temperate regions (Prestrud et al., 2007). Seroprevalence of *T.gondii* was reported as 26% and 25% in lesser snow geese (*Chen caerulescens*) and Ross's geese (*Chen rossii*), respectively, in Nunavut, Canada (Elmore et al., 2014). DNA of *T. gondii* has been detected the heart of a hunter-harvested mallard duck in France, the brain of one hunter-harvested goose in Mississipi, USA, and among 8% ($n = 156$) of Canada geese hunted in Maryland, USA (Dubey et al., 2004; Aubert et al., 2010; Verma et al., 2016). Because foxes consume several types of migratory birds also consumed by people, further work is needed to determine if wild avian species are infected with *T. gondii*. Foxes also rely heavily on rodents (such as lemmings and voles) in their diet; however, rodents were negative for *T. gondii* on PCR of brains in central Nunavut (Elmore et al., 2015). In British Columbia, wild deer mice were identified as a potential reservoir for *T. gondii* (Aramini et al., 1999). Another study reported a PCR prevalence of *T. gondii* in the brain of naturally-infected feral rodents in the Netherlands at 4% ($n = 250$), although another study in Germany failed to detect DNA from several wild rodent species (Hermann et al., 2012). No information exists for *T. gondii* in rodents in Nunavik, which could be of interest for future research.

There was only moderate agreement between the MAT serology and MC-PCR classification of positive versus negative foxes. It is therefore ideal to use both tests in parallel to maximise detection of *T. gondii* in this species for a better understanding of overall *T. gondii* prevalence in foxes. Many studies have reported on the disagreement between serological and molecular results for detecting the same pathogen, with seroprevalence often reported to be higher than DNA-based prevalence (DeCraye et al., 2011; Hermann et al., 2012). However, prevalence was higher in our study using the MC-PCR technique (46%) versus serology (41%). In seronegative foxes that were tissue-positive, antibodies could have waned over time despite a persistent latent infection. It is also possible that antibody levels were below the 1:25 cut-off value used in this study to classify individuals as serologically positive in some tissue positive foxes based on the MAT serological test (Dubey, 1995). Conversely, foxes that were seropositive and tissue-negative could be explained by the presence of *T. gondii* in tissues other than brain and heart, or that tissue cyst formation had not yet occurred despite the occurrence of detectable antibodies following acute exposure (Robert-Gangneux, 2012). An immune response may also have conferred resistance to infection in some foxes (Gilbert et al., 2013).

We also report for the first time the occurrence of co-infection with *T. gondii* and *T. nativa* in red foxes of Nunavik. The prevalence of *T. nativa* was estimated at 36% in this study compared to a prevalence of 11% ($n = 28$) reported in Arctic foxes of Nunavut and the Yukon Territory (Gajadhar and Forbes, 2010). Because *Trichinella* spp. are only transmitted through ingestion of infected meat, red foxes in Nunavik are most likely exposed to the parasite by scavenging on the carcasses of other carnivores including black bears, polar bears, wolves and other foxes, or even marine mammals in coastal communities (Gajadhar and Forbes, 2010). Larvae of *T. nativa* were viable even following freezing of tongues, compatible with freeze-resistance as demonstrated through the recovery of viable larvae in the frozen muscle of naturally-infected walruses (Leclair et al., 2004). Our study shows that *T. nativa* occurs in foxes trapped from several communities located remotely from one another, implying that *Trichinella nativa* is widely distributed throughout southern and northwestern parts of Nunavik. Further work is indicated to determine if other species and genotypes (such as T6) of *Trichinella* are circulating in terrestrial and marine ecosystems in Nunavik.

Both *T. gondii* and *Trichinella nativa* are foodborne pathogens of high importance in human and veterinary medicine worldwide. Foxes are unlikely to be a health risk for Inuit in Nunavik from a foodborne perspective since Inuit do not generally consume foxes and anecdotal evidence suggests that the few who do cook their meat. Foxes are, however, excellent sentinels for circulation of these food-borne parasites in terrestrial northern ecosystems as they are widespread, year-round residents, highly exposed, show detectable indicators of infection, and do not serve as direct sources of human exposure. Exposure to *T. gondii* has historically been high among Inuit of Nunavik, and risk factors for Inuit exposure to *T. gondii* include contact with several wildlife species. These observations, in combination with results from this study, substantiate the need for determining prevalence of *T. gondii* in other wildlife species such as migratory birds that are consumed by Inuit in Nunavik. Future studies are also needed to determine whether terrestrial and marine cycles of *T. gondii* and *T. nativa* are linked; for

example, if foxes scavenge on infected marine mammal species. This study provides useful baseline data for monitoring changes in parasite prevalence. To document the full extent of each parasite's geographic distribution within Nunavik, additional studies should include foxes and other wildlife from other parts of Nunavik and the circumpolar North.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2018.10.003.

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