FOXES AND LYNX AS SENTINELS FOR *TOXOPLASMA GONDII* ACROSS THE CANADIAN NORTH

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Saskatoon

By

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ABSTRACT

Toxoplasma gondii is one of the most successful parasites in the world and can have serious adverse effects on immunocompromised individuals and foetuses. It is thus a parasite of public health concern, especially in the Canadian North where seroprevalence in some Inuit communities is much higher than in other parts of North America. Food-borne transmission is likely a significant route of transmission among northern residents as well as Arctic carnivores, where transmission may be altered by the rapid demographic and environmental changes affecting Arctic and subarctic regions. I focused on lynx as the only definitive host that can produce oocysts in subarctic regions, and foxes as intermediate hosts at high risk of exposure. The aim of this research was first to assess *T. gondii* exposure, prevalence and genetic diversity in foxes and lynx in northern Canada. I hypothesized that foxes would act as good sentinel species for *T. gondii* in the North since they occupy a high trophic position in the Arctic and are widespread in northern Canada. I also sought to determine if lynx are serving as definitive hosts for *T. gondii* using DNA based methods (real time PCR with melting-curve analysis) which are more sensitive than efforts to detect oocysts in feces. I used a sequence specific magnetic capture DNA extraction and real-time PCR to test whole hearts and brains of foxes and lynx. I tested samples serologically by enzyme-linked immunosorbent assay (ELISA) using fluids from thawed hearts. I targeted foxes from all four Inuit regions of Canada: Inuvialuit Settlement Region in Canada's Western Arctic, Nunavut, Nunavik in northern Québec, and Nunatsiavut in Labrador. Exposure and tissue infection were lower in fox and lynx in Canada's Western Arctic, and higher in the Eastern Arctic, especially the Hudson Bay region of Nunavik. These results are compatible with recent serological findings in people from Inuit Health Surveys, supporting the hypothesis that fox are indeed suitable sentinels of environmental transmission and human exposure to *T. gondii*. DNA consistent with that of *T. gondii* was detected in feces of one lynx from boreal regions of Québec, but was detected far more commonly in lynx tissues. While lynx are a potential source of oocysts, they may also contribute to transmission of this parasite through consumption of their tissues by other carnivores and harvesters. This supports my hypothesis that fox are primarily exposed to *T. gondii* through carnivory rather than consumption of oocysts produced locally by lynx. In order to determine the role prey species play in *T. gondii* prevalence, I used stable isotopes on muscle and hair of Nunavik foxes to reconstitute their diet and link diet with their infection status. I concluded that marine food sources and migratory birds are likely a major source of exposure in foxes to *T. gondii* in Nunavik,

again similar to recent findings on consumption trends in people in Nunavik. This work reveals a better understanding of the distribution and introduction of *T. gondii* in fragile Arctic ecosystems. Ultimately, these findings inform future risk assessments to determine the potential human and animal health risks associated with *T. gondii* in northern ecosystems.

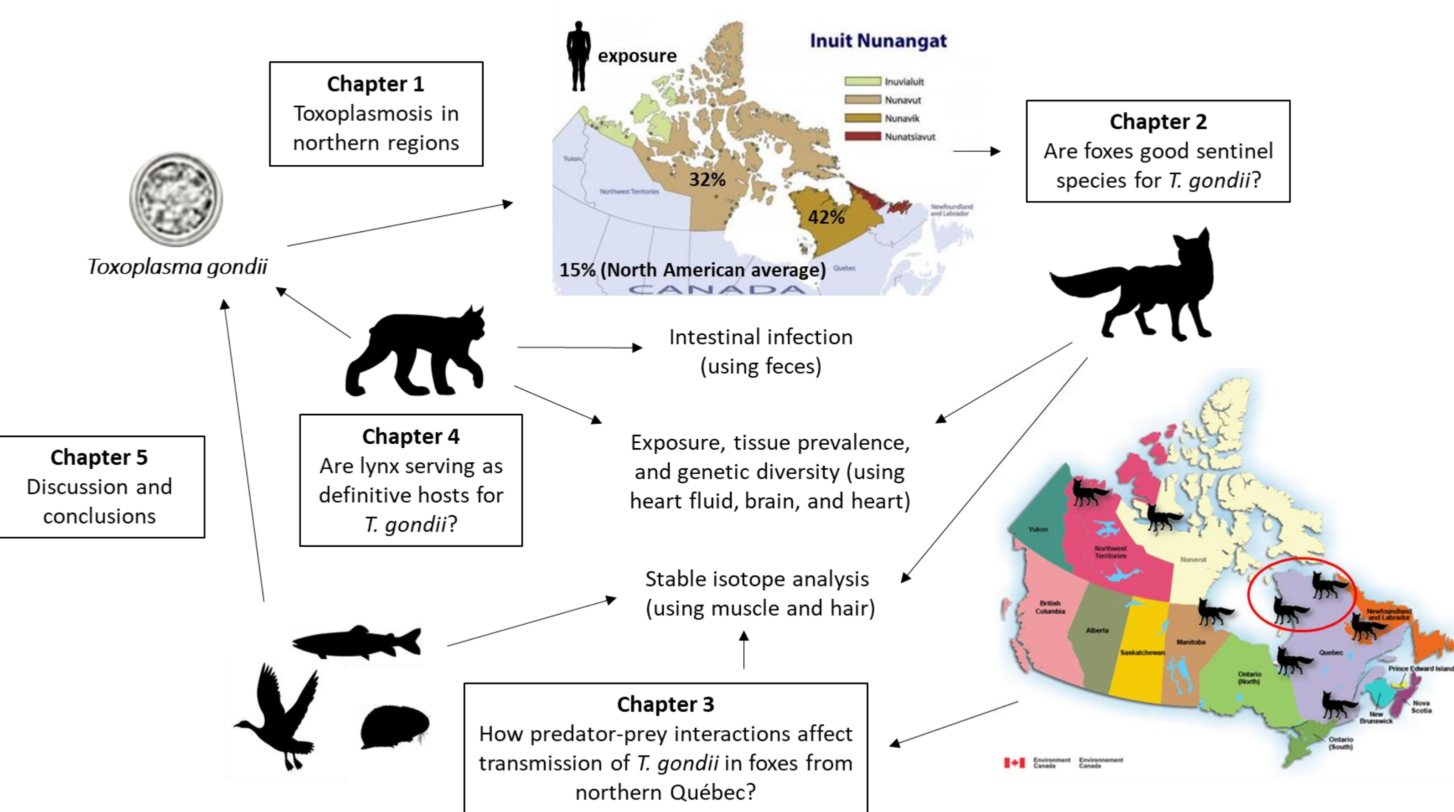

Figure 1.0: Overview of the thesis.

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DEDICATION

In memory of Estelle,

Our little shooting star.

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LIST OF ABBREVIATIONS

ACIA: Agence canadienne d'inspection des aliments
AIC: Akaike's Information Criterion
ATCC: American Type Culture Collection
BCI: body condition index
BLAST: basic local alignment search tool
CDC: Center for Disease Control and Prevention
CFAP: Centre for Foodborne and Animal Parasitology
CI: confidence interval
CIAC: competitive internal amplification control
DNA: Deoxyribonucleic acid
Df: degrees of freedom
DHSS: Department of Health and Social Services
DNA: deoxyribonucleic acid
ELISA: enzyme-linked immunosorbent assay
FAO: Food and Agriculture Organization
FITC: fluorescein isothiocyanate
g: gram
HF: heart fluid
HP: high positive
IFAT: indirect fluorescent antibody test
IgG: immunoglobulin G
IgM: immunoglobulin M
ISR: Inuvialuit Settlement Region
k: kappa value
Km: kilometer
LNUK: Local Nunavimmi Umajulivijiit Katujaqatigininga
log10: logarithm with base 10
LP: low positive
Max.: maximum
MAT: modified agglutination test
MC-qPCR: magnetic capture and real-time PCR
Mg: milligram
Min.: minimum
n: sample size
neg: negative
nm: nanometer
ND: no dilution
NTC: no template control
OD: optical density
OR: odds ratio
PCR: polymerase chain reaction
pos: positive
qPCR: quantitative PCR
RFLP : restriction fragment length polymorphism

RNUK: Regional Nunavimmi Umajulivijiit Katujaqatigininga
RPM: Rotation per minute
SE: standard error
Simmr: Stable isotope mixing model in R
S/P: sample to positive
µl: microliter
USA: United States of America
VIF: Variance inflation factor
WHO: World Health Organization

CHAPTER 1: LITERATURE REVIEW AND INTRODUCTION

The first part of the present Chapter (section 1.1) was written for the book Arctic One Health - Challenges for Northern Animals and People, as part of a multidisciplinary effort to review major health threats affecting Arctic marine and terrestrial wildlife, ecosystems, and people in the circumpolar North. As first author for the toxoplasmosis chapter, I was the primary writer and coordinator. Along with coauthors, I documented the significance of toxoplasmosis in humans and animals from northern regions, and what impact this parasite could have in a future of climate and environmental changes. This book chapter is therefore used as literature review for my thesis that focuses on understanding better the epidemiology of *T. gondii* in fragile Arctic ecosystems by using carnivores as sentinels. The second part of the introduction was written entirely by myself and provides the necessary background for my thesis, including the study species, definition of sentinel species, thesis goals and objectives, and research questions that I address.

1.1 TOXOPLASMOSIS IN NORTHERN REGIONS

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1.1. Toxoplasmosis in northern regions

1.1.1. Abstract

The apicomplexan parasite, *Toxoplasma gondii,* has a worldwide distribution, and it can infect virtually all warm-blooded animals, including wildlife, domestic animals, and humans. *Toxoplasma gondii* can cause disease, toxoplasmosis, in both humans and animals. It is a parasite of importance for public health, veterinary medicine (food safety, animal production, and welfare), and wildlife conservation, with transmission influenced by environment and host ecology: in short, a poster parasite for One Health. Here, we review what is known about transmission, genetic diversity, prevalence and health impact of *T. gondii* in its human and animal hosts, and discuss its significance in the Arctic. Since the definitive felid hosts of the parasite are rare above the treeline, transmission cycle of *T. gondii* remains enigmatic in this specific environment, while its impact on wildlife health and northern food security need attention. With global climate change and anthropogenic factors affecting the Arctic at higher rates than anywhere else on the planet, research taking a One Health approach is critically needed on the determinants of prevalence and impact of *T. gondii*, as well as the sources, methods of transmission, and environmental tolerance of *T. gondii* in the circumpolar North.

1.1.2. Introduction

Toxoplasma gondii, one of the most successful parasites in the world, is capable of infecting nearly all warm-blooded animals, including over 350 mammalian and avian species (Dubey, 2010; Lindsay & Dubey, 2007; Tenter et al., 2000). It is estimated that a third of the world human population has been exposed to and may be chronically infected with *T. gondii* (Tenter et al., 2000). *Toxoplasma gondii* is ranked in the second highest category of biological agents that could cause serious epidemics in both human and animal populations (Gajadhar & Allen, 2004), and the fourth most important foodborne parasite globally after *Taenia solium*, *Echinococcus granulosus*, and *E. multilocularis* (FAO/WHO, 2014). *Toxoplasma gondii* can cause disease, toxoplasmosis, in both humans and animals. Economic losses due to abortions that the parasite causes in domestic animals can be substantial (Dubey, 2009b). While the infection may cause only mild symptoms and clinical signs, it can also cause severe, even life-threatening manifestations, especially in immunocompromised people and developing foetuses (Desmonts & Couvreur, 1974;

Dubey, 2010; Montoya & Liesenfeld, 2004). Therefore, *T. gondii* has both public health and veterinary importance, and a One Health approach is needed in risk mitigation.

Toxoplasma gondii successfully transmits worldwide, including cold climates and northern latitudes (defined here as the Arctic and sub-Arctic regions), where it survives inside its hosts as tissue cysts and in the environment as sporulated oocysts (Dubey, 2010). Serological studies in animals and humans in the northern hemisphere have revealed an increasing exposure to the parasite along a north-to-south gradient (Jokelainen et al., 2010; Malmsten et al., 2011; Messier et al., 2009; Suvisaari et al., 2017). As the world changes, the ecology of many diseases is expected to shift (Deksne et al., 2020; Patz et al., 2000). With changing ecological factors and expansion of the geographical range of parasitic diseases due to natural and man-made causes, exposure rates to *T. gondii* in the circumpolar North are likely to increase (Pilfold et al., 2021).

1.1.3. **Life Cycle and Transmission**

The life cycle of *T. gondii* is divided into two phases: a sexual phase that occurs naturally only within felids (Martorelli Di Genova et al., 2019), and an asexual phase that can occur within all warm-blooded animals acting as intermediate hosts (Cleary et al., 2002). To the best of our current knowledge, only felids act as definitive hosts (Dubey et al., 2004). The three infective stages of *T. gondii* are: (1) a rapidly dividing, invasive tachyzoite systemically distributed in hosts, including across the placenta, in the acute phase of the infection (2) a slowly dividing bradyzoite forming tissue cysts, especially within muscle and nervous tissue (Fig. 1), and (3) an environmental stage, the sporozoite, which develops in the environment inside an oocyst produced by sexual reproduction in the intestinal epithelium of the definitive host (Robert-Gangneux & Darde, 2012). Tachyzoites and bradyzoites result from asexual reproduction in all hosts, while oocysts are the result of sexual reproduction and are naturally produced in the intestinal epithelium of the definitive hosts only (Dubey, 2010). If infected, over the course of few weeks, a cat may shed millions of unsporulated oocysts in feces into the environment, and the shedding of oocysts can occur more than once in the lifetime of the cat following re-infection (Dubey, 1995; Zulpo et al., 2018). The unsporulated oocysts sporulate and become infective within 1-5 days, faster in humid conditions and warmer temperature (Dubey et al., 1970). Three major routes of transmission are faecal-oral transmission (environmental route, through soil, fresh produce, or water contaminated

with oocysts), transmission by carnivorism (through raw/undercooked meat or other tissues containing bradyzoites), and transplacental transmission (through tachyzoites via vertical route) (Dubey, 2009a). The consequences of transplacental transmission for the foetus are usually more severe if infection occurs in early pregnancy and may lead to stillbirth or severe neurological complications. Most human infections are acquired later in life, via the environmental or the meat-borne routes (Tenter et al., 2000). The relative importance of these two transmission routes is largely unknown.

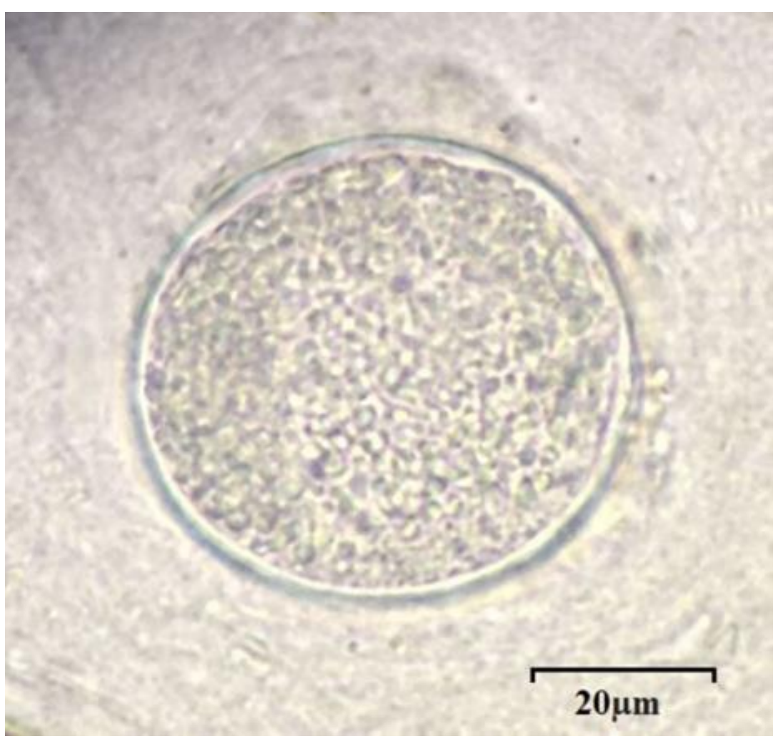

Figure 1.1: *Toxoplasma gondii* tissue cyst in the brain of an experimentally infected reindeer (*Rangifer tarandus*), visualized on compound microscopy (60X). (Reproduced from Bouchard et al. 2017)

Herbivores acquire *T. gondii* infection typically by ingesting oocysts from the environment, while carnivores and omnivores can become infected either by ingesting oocysts from the environment or by ingesting tissue cysts containing bradyzoites in the tissues of infected animals. Once introduced into a food web, *T. gondii* can be maintained by hosts ingesting tissue cysts from infected hosts, via carnivorism, cannibalism, and scavenging (Dubey, 2010; Wilson et al., 2020). In addition to humans, vertical transmission has also been reported in several domestic and free-ranging animal hosts (Calero-Bernal et al., 2013; Vargas-Villavicencio et al., 2016; Verma et al., 2016), and could play a role in transmission in a population (Hide et al., 2009). The importance of the vertical transmission route in the Arctic and sub-Arctic ecosystems remains to be better elucidated.

In the North, environmental contamination with *T. gondii* oocysts could occur in boreal and sub-Arctic regions where domestic cats and free-ranging wild felids are present (Simon et al., 2013a). However, free-ranging wild and domestic felids are largely absent above the treeline (Baker et al., 2018), which makes the transmission of *T. gondii* enigmatic in this environment. Migratory hosts, such as Arctic nesting birds, ungulates, and marine mammals can become infected through consumption of oocysts in sub-Arctic environments (Elmore et al., 2014). In Arctic regions, resident carnivores and humans could become infected through consumption of tissue cysts in migratory animals (Fig. 2) (Bachand et al., 2019).

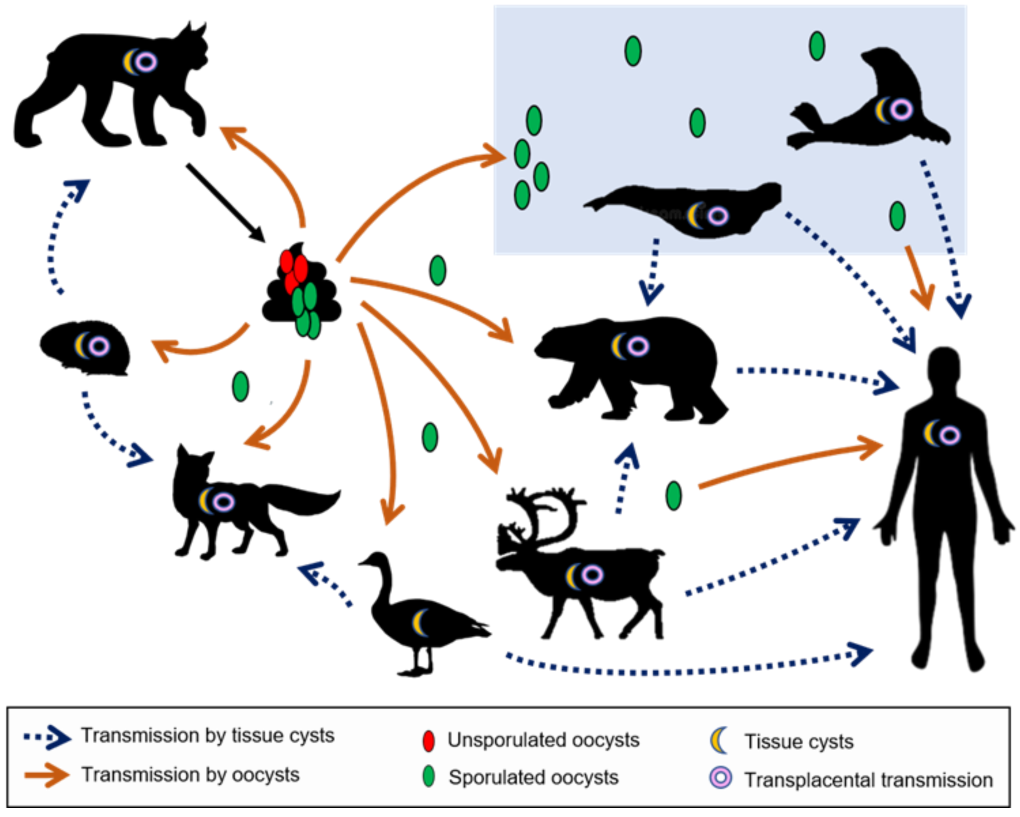

Figure 1.2: Potential routes of transmission of *Toxoplasma gondii* in the North, with focus on free-ranging hosts and the shared environment.

Food-borne transmission is likely a significant route of *T. gondii* infection among northern peoples, given their close contact with the land and wildlife, and particularly due to dietary preferences for raw, fermented or dried meat (Jenkins et al., 2013; Messier et al., 2009). Around the world, cultural habits affect the acquisition of *T. gondii* via ingestion of tissue cysts in undercooked meat (Hill & Dubey, 2002). In the North, seal, ptarmigan, and caribou are often consumed without cooking (McDonald et al., 1990). Consumption of marine mammals and seafood has been found to be a risk factor for *T. gondii* seropositivity in epidemiological studies in the Canadian North (Goyette et al., 2014a). Filter feeding invertebrates and fish may filter and concentrate the oocysts from the marine environment (Massie et al., 2010). Moreover, skinning of animals for fur has been identified as a risk factor for *T. gondii* seropositivity (McDonald et al., 1990). An epidemiological investigation of an outbreak of toxoplasmosis in Nunavik, northern Québec (Canada), identified skinning of animals and consumption of raw caribou as potential risk factors (McDonald et al., 1990). In response to this outbreak, guidelines and a screening program to prevent *T. gondii* infection in pregnant women were developed in the region (Lavoie et al., 2008). Outbreaks of acute toxoplasmosis are rare in North America, but both food- and waterborne outbreaks have been reported, and outbreaks might occur again in the future (Aramini et al., 1999; Gaulin et al., 2020). According to the Nunavik Inuit Health Survey in 2004, frequent cleaning of water reservoirs and consuming untreated surface water are risk factors for *T. gondii* seropositivity in northern Canada (Martin et al., 2007; Messier et al., 2007). Overall, the relative contributions of the different transmission routes, including food-borne and water-borne routes, are still unknown, and source attribution is challenging for this ubiquitous parasite (Koutsoumanis et al., 2018).

1.1.4. **Genetic diversity**

Toxoplasma gondii is a single species of the genus *Toxoplasma*, with multiple genotypes (Galal et al., 2019). Studies in wildlife have demonstrated a wide host and geographic range as well as wider than previously known genetic diversity of *T. gondii* (Grigg & Sundar, 2009; Shwab et al., 2014; Wendte et al., 2011). Knowledge of genotypes and strains circulating in a particular geographical region is important to better understand the epidemiology, population structure, and phylogeny. In laboratory mice, virulence varies greatly depending on the strain of the parasite (Dubremetz & Lebrun, 2012; Saeij et al., 2005). Disease-producing strains have also been found

in sylvatic cycles, with parasite genotype associated with disease severity (Boothroyd & Grigg, 2002). Highly virulent strains can potentially pose a threat to wildlife health, and a higher risk of severe toxoplasmosis if transmitted to humans (Gerhold et al., 2017). Several methods are used to identify *T. gondii* genotypes, including multilocus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), microsatellite analysis, and multilocus sequence typing (Su et al., 2006).

A limited number of studies have identified genotypes of *T. gondii* in the circumpolar regions. In North American wildlife, Type 12 strains dominate, followed by strains of types II and III. In Alaska (USA), type II and atypical genotypes were described in red fox (Vulpes vulpes) and black bear (Ursus americanus) (Dubey et al., 2010; Dubey et al., 2011). In northern Québec (Canada), type II was described in Canada geese (Branta canadensis) and lesser snow geese (Chen caerulescens), and black bear (Bachand et al., 2019; Dubey et al., 2008). Type II was also described in beluga (Delphinapterus leucas) from the St. Lawrence Estuary in Québec (Canada) (Iqbal et al., 2018). Two new atypical genotypes of *T. gondii* have been detected (but not fully described) in beluga in the Eastern Beaufort Sea (Haman et al., 2013).

In Finland, type II strains have caused mortality in wildlife (Jokelainen et al., 2011; Jokelainen & Nylund, 2012), as well as in domestic hosts (Jokelainen et al., 2012). In Denmark, type II was detected in a captive harbour porpoise (Phocoena phocoena) (Herder et al., 2015). In clinical samples from humans in Denmark, type II was predominant, while a substantial proportion of other genotypes were also described (Jokelainen et al., 2018). In Norway, type II, III, and atypical genotypes have been described in wildlife from the high Arctic archipelago of Svalbard (Prestrud et al., 2008a; Prestrud et al., 2008b).

Type II *T. gondii* strains, which are common in Europe, appear widespread in wildlife in the circumpolar region, which may reflect spillover from domestic cycles to wildlife as well as transport from sub-Arctic regions via migratory birds or in freshwater run-off. However, other genotypes are also circulating in northern latitudes, and some may reflect more localized circulation in wild felids and their prey. The genotyping data from some Arctic regions are sparse and scattered – in particular, no genotype information is available from Russia.

To determine strain-associated pathogenicity in wild animals is quite challenging due to the lack of post-mortem data from large samples of infected animals. By shaping the population structure of *T. gondii*, sexual recombination seems to play an important role in the emergence of virulent strains in wildlife. For example, the recombinant type X strains of *T. gondii* is an important cause of morbidity and mortality in southern sea otters (Miller et al., 2004; Shapiro et al., 2019b). New host species moving northwards may bring new strains of *T. gondii*, creating novel epidemiological risks and uncertainties regarding *T. gondii* evolution (Proesmans et al., 2021). Further studies are required to understand the molecular epidemiology and biological significance of genetic diversity of this zoonotic parasite in the Arctic and connected ecosystems.

1.1.5. **Hosts**

1.1.5.1. ***Toxoplasma gondii* and toxoplasmosis in humans**

Human toxoplasmosis is generally thought to be under-reported due to lacking or limited formal surveillance, and under-diagnosed due to often mild or non-specific symptoms (Barry et al., 2013). Toxoplasmosis can be life-threatening, in particular in immunocompromised individuals, and is a leading food-borne cause of hospitalization and death even in developed countries (Montoya & Liesenfeld, 2004; Scallan et al., 2011). Congenital infection can cause devastating manifestations, ranging from ocular disease to severe hydrocephalus (Desmonts & Couvreur, 1974; Hill & Dubey, 2002), and there may be sequelae later in life (Wilson et al., 1980). More recently, *T. gondii* infection has been found associated with schizophrenia and other psychiatric and mental health issues, but further studies are needed regarding causality (Weiss & Dubey, 2009; Yolken & Torrey, 2008).

Toxoplasma gondii infection has been named as the most important parasitic infection in the North American Arctic in terms of public health impact (Hotez, 2010). Cultural practices such as food preparation and dietary traditions further influence the risk of acquiring the infection in northern regions (Jenkins et al., 2013). For instance, *T. gondii* seroprevalence in Inuit communities in Nunavik in 2004 was approximately 60%, which is significantly higher than the North American average of 10-20% and twice the global average of approximately 30% (Jones et al., 2018; Molan et al., 2019; Robert-Gangneux & Darde, 2012; Tenter et al., 2000). Higher seroprevalences in Inuit than in Cree in shared communities in northern Québec have been reported, possibly due to Inuit

preferences for uncooked meat since other risk factors such as water sources were considered shared (Sampasa-Kanyinga et al., 2012). A lower seroprevalence of 8% was found in other Inuit regions, such as the Inuvialuit Settlement Region (NT), 11% in Nunatsiavut (NL), and 32% in Nunavut (NU) (Goyette et al., 2014a), which could reflect variation in dietary preferences (harvested wildlife vs store-bought, raw vs cooked, terrestrial vs marine wildlife) and differences in environmental transmission across the Canadian Arctic. In Alaska, a serosurvey in the 1960s and 1970s showed that 16% of Alaska Natives had antibodies to *T. gondii*, very similar to the North American average (Peterson et al., 1974); a more recent study showed substantially lower seroprevalence in Alaskans (3% of 887) (Miernyk et al., 2019), similar to declining trends observed in the contiguous USA. In Finland, the seroprevalence was lower in the north (11%) than in other parts of the country (18-23%) (Suvisaari et al., 2017). A similar gradient was reported in Nunavik, Canada (Messier et al., 2009), and also in pregnant women from Sweden (Ljungstrom et al., 1995). *Toxoplasma gondii* seroprevalence may be higher in more southern communities due to higher levels of environmental contamination with oocysts, increased survival and development rate of oocysts at warmer temperatures, and/or higher prevalence in animal hosts locally raised or hunted for consumption.

1.1.5.2. ***Toxoplasma gondii* and toxoplasmosis in wildlife**

In the circumpolar Arctic, a substantial proportion of terrestrial and marine mammals, as well as some avian species, have antibodies to *T. gondii*, with seroprevalences ranging from 10-60% in carnivores and 1-40% in herbivores (Jenkins et al., 2013). In Alaska, antibodies against *T. gondii* have been detected in a wide variety of species, including lynx (*Felis lynx*), black bears, grizzly bears (*Ursus arctos horribilis*), wolves (*Canis lupus*), and herbivores (Zarnke et al., 1997; Zarnke et al., 2000; Zarnke et al., 2001). Seropositivity in polar bears (*Ursus maritimus*) has been documented in Eastern Greenland, Russia, Alaska, Canada, and Norway (Oksanen et al., 2009; Rah et al., 2005), with a seroprevalence as high as 70% in western Hudson Bay, Canada (Pilfold et al., 2021). Antibodies have also been detected in Antarctic pinnipeds, demonstrating how well-established *T. gondii* is in marine ecosystems (Rengifo-Herrera et al., 2012). In Alaska, among marine mammals, antibodies have been detected in walrus (*Odobenus rosmarus*), Steller sea lions (*Eumetopias jubatus*), harbor seal (*Phoca vitulina*), ringed seal (*Pusa hispida*), spotted seal (*Phoca largha*), and bearded seal (*Erignathus barbatus*) (Dubey et al., 2003). In the high Arctic of

Svalbard, ringed seals and bearded seals showed high seroprevalence of 19% and 67%, respectively (Jensen et al., 2010), however another study did not detect any seropositive pinnipeds and whales from the North Atlantic (Oksanen et al., 1998). It should be emphasized that serology results of marine mammals may need to be interpreted with some caution due to high content in lipids in sample that may lead to false positives (Blanchet et al., 2014) .

Transmission of oocysts via freshwater runoff could contribute to the contamination of marine ecosystems and has been viewed as the most likely route of infection in sea otters off the coast of California (Conrad et al., 2005). As well, oocysts may travel via ocean currents from southern latitudes and infect northern marine wildlife.

It has also been suggested that *T. gondii* may enter the terrestrial Arctic ecosystem of northern Norway via migratory birds (Sandström et al., 2013), since no wild or domestic felids are present and an estimated 7% of barnacle geese (*Branta leucopsis*) on Svalbard are exposed to the parasite (Prestrud et al., 2007). Similarly, seroprevalence was 11% in migratory geese (primarily *Branta canadensis*) in Nunavik, eastern Canadian Arctic (Bachand et al., 2019) and over 30% in Ross's geese (*Anser rossii*) and lesser snow geese in Nunavut, central Canadian Arctic (Elmore et al., 2014). As sentinel host species (i.e. indicators of the presence of a pathogen within an ecosystem), high seroprevalence for antibodies to *T. gondii* was observed in wolverines (*Gulo gulo)* from the Northwest Territories (62%) in the western Canadian Arctic (Sharma et al., 2019b), and in arctic foxes (*Vulpes lagopus*) from Nunavut (39-64%), Nunavik (41%), and Norway (43%) (Bachand et al., 2018; Bouchard et al., 2019; Elmore et al., 2016; Prestrud et al., 2007).

While prevalence is generally higher in carnivores than herbivores, muskoxen (*Ovibos moschatus)* and mainland caribou (*Rangifer tarandus groenlandicus*) in the Northwest Territories and Nunavut, Canada, had an overall seroprevalence of 6.4% and 37% respectively (Kutz et al., 2000; Kutz et al., 2001). There are several reported seroprevalence estimates for moose (*Alces alces)*: 15% in Canada/Nova Scotia (Siepierski et al., 1990), 1-23% in USA/Alaska (Kocan et al., 1986; Zarnke et al., 2000) and 10% in USA/Minnesota (Verma et al., 2016), 20% in Sweden (Malmsten et al., 2011), 13% in Norway (Vikoren et al., 2004), and 10% in Finland (Jokelainen et al., 2010). Antibodies for *T. gondii* have also been reported in caribou in Alaska (USA) and

northern Québec (Canada) (McDonald et al., 1990; Zarnke et al., 2000), and reindeer in Fennoscandia (Oksanen et al., 1997).

Experimental infection with oocysts delivered by intrarumenal inoculation in reindeer led to fatal enteritis (Oksanen et al., 1996), while exposure to oocysts delivered by stomach tube led to largely subclinical infection with minor behavioral and respiratory changes (Bouchard et al., 2017). The dose of oocysts given, strain virulence, and host age could also explain the differences in pathology observed. No report of clinical toxoplasmosis has been described in naturally infected reindeer or caribou in the wild. However, encephalitis and placentitis associated with *T. gondii* was diagnosed in a full term, stillborn, reindeer fetus from the Houston Zoo, Texas (Dubey et al., 2002).

Although clinical disease associated with *T. gondii* is rarely reported in free-ranging wildlife, acute disseminated toxoplasmosis in arctic foxes has been reported in Svalbard (Sorensen et al., 2005). Susceptibility to severe toxoplasmosis has been reported in Australian marsupials, Hawaiian birds, European brown hare (*Lepus europaeus*), and Eurasian red squirrels (*Sciurus vulgaris)* (Jokelainen et al., 2011; Jokelainen & Nylund, 2012; Parameswaran et al., 2009; Sedlak et al., 2000; Work et al., 2016). Reasons for this remain largely unknown, but may involve genetics, immune response, and a lack of co-evolution with domestic cats (Ketz-Riley et al., 2003; Maubon et al., 2008; Shapiro et al., 2019b; Shwab et al., 2018).

Little is known about the population-level impact of *T. gondii* on free-ranging wildlife in the circumpolar Arctic; however, *T. gondii* is known to have an impact on free-ranging wildlife populations in Australia and the Pacific Northwest, and can cause abortion and congenital disease in domestic livestock. Since *T. gondii* is capable of negatively affecting reproductive success, it is considered to be of greater concern in declining populations, such as caribou and reindeer herds worldwide (Festa-Bianchet et al., 2011; Vors & Boyce, 2009). Parasitic diseases have been identified as a threat within recovery strategies and management plans for different herds in Canada (Environment Canada, 2012). For conservation as well as food safety, it is crucial to monitor the distribution and prevalence of *T. gondii* in Arctic wildlife.

Monitoring requires effective diagnostics. *Toxoplasma gondii* has developmental stages that live in tissues, and definitive diagnosis requires invasive biopsy or post-mortem techniques. Therefore, antemortem diagnosis frequently relies on collection of blood and serological techniques to determine host response to exposure. Serology is suitable for screening to obtain population-level estimates of exposure to *T. gondii*, but definitive diagnosis of clinical toxoplasmosis should not rely on a single serological test, but ideally look at rising titres, IgM and IgG levels, and the clinical picture (Li et al., 2016). Post-mortem diagnosis can involve detection of antibodies in heart blood, chest fluid, or filter paper (i.e. fluid dried on filter paper strips), or direct detection methods: polymerase chain reaction (PCR) on tissues, histological demonstration of the parasite and/or antigens via immunohistochemistry, or isolation of the organism itself using mouse bioassay or cell cultures (Dubey, 2010). Serological studies, especially in wildlife for which these tests are rarely optimized or validated (by comparing with reference tests, such as in Sharma et al, 2019a), are inherently limited; even a positive result indicates life-time exposure, not necessarily a recent infection. False-positive results in sera from marine mammals, which often are rich in lipids, have been reported (Blanchet et al., 2014). Cross-reaction with other coccidian species might also occur with serological methods (Dubey & Lindsay, 1996). Ideally, using a combination of serological and molecular methods can provide complementary information; serology overestimates the levels of active infection, while molecular methods frequently underestimate true infection status. This information is vital to develop a balanced approach to risks to human and animal health posed by this zoonotic parasite in the Arctic ecosystem.

1.1.5.3. ***Toxoplasma gondii* in the environment**

Toxoplasma gondii is widely prevalent around the world, in part due to the survival of sporulated oocysts in the environment (Dubey et al., 1998), broad intermediate host range, and multiple methods of transmission. This environmental reservoir of oocysts constitutes a considerable source of *T. gondii* infection, especially for herbivores (Dubey, 2010). Herbivores can therefore be used as sentinels or indicators of environmental contamination with oocysts of *T. gondii*, and seroepidemiological studies can be helpful in detecting geographical patterns (Elmore et al., 2015). For example, similarly to humans, there is an increasing north-to-south gradient in *T. gondii* seroprevalence in moose in Finland and Sweden, indicating higher environmental oocyst

contamination in the southern parts of these countries (Jokelainen et al., 2010; Malmsten et al., 2011).

Sporulated oocysts of *T. gondii* can survive for months in the soil in moist conditions (Frenkel et al., 1975; Lindsay & Dubey, 2009). Environmental contamination with infective sporulated oocysts from felid faeces has been determined to be the source of large water-borne outbreaks of toxoplasmosis in the Panama Canal and in southwestern Canada (Benenson et al., 1982; Bowie et al., 1997). Fewer oocysts survive in the soil under dry conditions (Lelu et al., 2012). In northern climates, snow cover likely increases oocyst survival as it reduces exposure to UV sunlight, insulates against extreme temperature, and maintains high humidity levels (Simon et al., 2013b). Oocysts can be transported from terrestrial to aquatic environments via snowmelt and runoff, and thereby be disseminated in fresh or sea water (Simon et al., 2013c). The circumpolar North is undergoing rapid warming, with a projected temperature increase of 4 to 7 °C over the next 100 years (ACIA, 2004). As a result, warmer and wetter conditions could affect the transmission of *T. gondii* in animals and people through enhanced survival and transport of oocysts from water sources flowing from south to north, as well as northward movement of felid definitive hosts. Although currently not common, domestic cats are present in some northern regions (Baker et al., 2018), and could play a role in environmental contamination, especially if immigration of people and their pet cats to the North increase along with resource extraction (such as oil, gas, and minerals) and employment opportunities due to shipping in the increasingly open waters of the Arctic.

1.1.6. **Management**

To minimise the risk of human exposure to *T. gondii*, recommendations include cooking meat, washing fruits and vegetables prior to consumption, washing kitchen utensils thoroughly with soap and water, and washing hands after contact with soil, carcasses, raw meat, and cat litter. Freezing meat for at least 3 days at -12°C (or colder) may kill the parasite and could be considered as an additional step to other processing, such as drying or fermenting (Djurkovik-Djakovik & Milenkovic, 2000). However, *T. gondii* survived in reindeer meat for up to 28 days at -11 to -56°C (Kolychev, 1969); more work is needed to determine if genotypes of *T. gondii* present in the Arctic have higher freeze tolerance than genotypes present in more temperate regions, as has been

observed in other food-borne parasites in the genus *Trichinella* (Pozio, 2016). Wearing gloves, as well as thorough cleaning and disinfection of traps, knives and other tools utilized in dressing, skinning, and processing hides and furs from animals that may harbour *T. gondii* are also recommended (Hueffer et al., 2013). To decrease risk of transmission, carcasses should be disposed of in a way that scavengers have no access to them, by burning or scavenger-proof fencing at landfills, which can be challenging in remote communities and when many animal carcasses are processed. Lynx may be a potential source of oocysts, although there are as yet no reports of oocyst shedding by Eurasian or North American lynx (Jokelainen et al., 2013; Ryser-Degiorgis et al., 2006; Simon et al., 2013a). A potential source of infection for people is drinking contaminated surface water or eating unwashed produce in areas that could be contaminated with sporulated oocysts from older faeces or carcasses of felids. Litter boxes of domestic cats should be cleaned daily, because oocysts require 1-5 days to become infective, and litter disposed of in a way that would not contaminate watersheds. Mature cats not fed raw meat in their diet and with no access to the outdoors are considered low risk for shedding oocysts of *T. gondii* (Jokelainen et al., 2012; Must et al., 2017; Shapiro et al., 2019a).

Some of these recommendations may not be achievable or culturally appropriate in remote areas of the circumpolar north, and some cultural traditions – such as consumption of dry or fermented meat and drinking untreated water – may be potential risks. Other challenges include the microscopic size of the parasite, often subclinical nature of the infection, lack of formal screening of harvested wildlife for *T. gondii*, and, potentially, lack of awareness of risks posed by undercooked meat and organs from harvested wildlife (Fig. 3). These can be addressed through developing northern capacity for veterinary public health measures. There is a need for more public education, particularly among high-risk populations such as pregnant women and immunocompromised individuals in Arctic regions. Targeting outreach efforts first to at-risk groups (e.g. pregnant women, immunocompromised) may more effectively mitigate risk while respecting traditional practices (DHSS, 2005). Conversely, risk communication should not deter the general population from consuming harvested wildlife, which are highly nutritious sources of food and contribute to the cultural well-being of northern communities (FAO, 2013; Pufall et al., 2011). Any specific public health recommendations for hunters, trappers, gatherers, and food preparers should be done in consultation and coordination with local and traditional knowledge

holders, and should respect the traditional cultural practices. Developing a better understanding of the impact of zoonotic diseases on Indigenous health would assist in determining the need for community-based monitoring programs and public health recommendations.

Figure 1.3: Caribou meat in a community freezer in Nunavik, Canada (Credit: Nicholas Bachand).

1.1.7. **Future Challenges**

Despite the absence of definitive hosts, *T. gondii* can thrive even in remote Arctic regions (Reiling & Dixon, 2019). While felines play a crucial role in the ecology of *T. gondii*, the parasite can persist in their absence, and be transported with wildlife and water even into the farthest reaches of the circumpolar north. Climate change is leading to more frequent and extreme rainfall events which in turn can lead to increased risk for freshwater contamination with *T. gondii* via runoff (Charron et al., 2004). The North is undergoing rapid climate change and anthropogenic disturbance (ACIA, 2004), making it vulnerable to emergence of infectious diseases at the interface between people and wildlife. Arctic wildlife are an inherent part of the North, and a cultural and socioeconomic cornerstone for northern peoples. Ongoing monitoring, particularly if there is community interest, would assist in better understanding the ecology of the pathogen and methods to mitigate risks to both animals and people. While *T. gondii* is rarely mentioned specifically in Species at Risk recovery strategies and management plans, parasites and disease have been listed as threats to a number of species. As access to medical and veterinary care can be limited in remote regions, monitoring for pathogens of public health significance is challenging. More information is needed to better understand the ecology, epidemiology, and impact of *T. gondii* in Arctic regions, in order to put into practice culturally appropriate and effective measures to protect human, animal, and ecosystem health – One Health.

1.2. **Thesis introduction: Study species**

1.2.1. **Foxes** (*Vulpes vulpes, Vulpes lagopus*)

1.2.1.1. ***Vulpes vulpes***

The red fox (*Vulpes vulpes*) has the largest geographic range of any living carnivore and is found in almost every habitat in the northern hemisphere, including tundra, forest clearings, coastal marshes, meadows, bushy fence lines, low shrub cover, farming areas, and around human habitation (Chester, 2016). It is one of Canada's most widespread mammals, found in all provinces and territories. Typically rusty red with a white belly and chin, and black legs, they can also harbour different color morphs such as black, silver or a cross between both (Wilson & Ruff, 1999). The male is usually slightly bigger than the female, and moves considerably further as well (Iossa et al., 2008); their territory can stretch as far as 3400 hectares in the northern part of their

range. They will form monogamous couples and mate between December and March, producing around five pups (Wilson & Ruff, 1999). Their average life expectancy is three to four years, with a maximum of nine years (Chester, 2016). Mostly nocturnal, the red fox feeds mainly on mice and small rodents. This opportunistic predator also forages on a variety of other prey species, such as muskrats, squirrels, hares, grouse, birds eggs and chicks, beetles, grass, berries, garbage, and carrion (Chester, 2016), with dietary patterns influenced by season and availability (Larivière & Pasitschniak-Arts, 1996).

1.2.1.2. ***Vulpes lagopus***

Arctic foxes (*Vulpes lagopus*) have a circumpolar distribution in all Arctic tundra regions. In Canada, they are found along Arctic coastal areas and north of the tree line, extending from Yukon to Newfoundland (Wilson & Ruff, 1999). Most individuals are white in winter and turn grayish to dark brown in summer. However, typically in coastal environments, a small proportion have a pale bluish-grey coat in winter that becomes darker in summer and are known as blue fox (Berteaux et al., 2017). They are the smallest wild canid in Canada and typically weigh between three and four kilograms, with males slightly bigger. During winter, Arctic foxes cover very large distances over both land and sea ice. Travel rates peaking at 155 km/day have been recorded previously, with 4415 km being the longest dispersal event ever recorded for an Arctic fox (Fuglei & Tarroux, 2019). However, they tend to stay in the same area once settled (Anthony, 1997). Mainly solitary, they will form pairs during the breeding season in March or April, producing a litter of usually six to ten pups per year. Arctic foxes live for three to six years. They are opportunistic predators and scavengers that generally rely on the abundance of small mammals for food, especially lemmings and voles. They therefore undergo dramatic population fluctuations in relation to the abundance of their prey. Arctic foxes will also scavenge or prey on other species like ptarmigan, arctic hare, muskoxen, and barren-ground caribou (Chester, 2016). Birds and eggs can also constitute a major component of their diets, depending on the region and year (Bantle & Alisauskas, 1998; Samelius et al., 2007).

1.2.2. **Lynx** (*Lynx canadensis*)

The Canada lynx has a Holarctic distribution, occupying predominantly the dense boreal forest of North America, and taiga of northern Canada and Alaska. It is a medium-sized cat, with

a short tail, long legs, large padded feet to traverse deep snow, prominent black ear tufts, and a light grey winter coat turning reddish-brown in the summer (Chester, 2016). Males are slightly bigger than females, typically solitary, with mating occurring during February or March. Kits (usually four) are born 60 to 65 days later, and female offspring can remain in contact with the mother for life, while male offspring will move far from their mother's range (Breitenmoser et al., 1993; Mowat et al., 2000). In the wild, lynx can live up to 14 years. Snowshoe hare (*Lepus americanus*) is their dominant prey item, with lynx populations tied closely to hare cyclic fluctuations with numbers moving in synchrony over vast areas (Poole, 2003). Lynx will only reach high population densities when hare are at peak levels. During periods of hare scarcity, female lynx may not ovulate, and no litter is produced. When hares are scarce and the availability of other prey high, lynx can hunt red squirrels (*Tamiasciurus hudsonicus*), an important alternative prey species, as well as mice, voles, and birds (Donoghue et al., 2010; Ray, 2000). They will also scavenge herbivore carcasses when encountered and might demonstrate cannibalism behavior in years of food shortage (Mowat et al., 2000; Peers et al., 2021).

1.3. Sentinel species for *T. gondii*

The term sentinel species, often described as health indicator species, is a cost-effective, timely, and sensitive way to identify a threat before it is detected in other animals or humans (Pei Shan Neo & Huan Tan, 2017). They can also be selected for rapid risk assessments on the environmental conditions of an area and reflect environmental perturbations on different spatial, temporal and trophic scales (Tabor & Aguirre, 2004). They are therefore an important component of the One Health initiative, defined here as a transdisciplinary approach recognizing the interconnection between people, animals, plants, and their shared environment. However, sentinel species are often used narrowly as proxy indicators of health threats to humans, rather than of the health threats in a particular ecosystem or habitat, or as sensitive indicators of changes in a particular environmental condition (Aguirre & Tabor, 2008).

A good sentinel species for *T. gondii* should ideally answer many criteria. They should be easy to collect and handle, as well as widely distributed in the studied area. They should be broadly exposed through multiple route(s) of transmission, and/or similar to those of the target host if being used as a proxy. For food borne hazards, a high trophic position is also desirable as it will allow

the organism to bio-accumulate and reach a measurable response, such as antibodies in blood and DNA in tissue, ideally without being detrimental for their health (Bowser & Anderson, 2018; Halliday et al., 2007). Based on some of these criteria, many animals have been proposed as good sentinel species for *T. gondii*, such as wolverines (Sharma et al., 2019b), marine mammals (Conrad et al., 2005; Gibson et al., 2011), birds of prey (Gazzonis et al., 2018), domestic free-ranging chickens (Dubey et al., 2020), white-tailed deer (Schaefer et al., 2013), and wild rabbits (McKenny et al., 2020). Nevertheless, not many species can fit the description of a good sentinel for *T. gondii* in the Canadian North, especially based on ease of access to samples.

1.3.1. **Foxes as sentinels for *T. gondii***

Foxes have been described as good sentinel species for *T. gondii* in Canada (Bachand et al., 2018) (Figure 1.4). First, they have high prevalence to *T. gondii* (compared to many herbivores), meaning that they are highly exposed to the parasite, likely through tissue cysts in prey items (Bouchard et al., 2019; Elmore et al., 2016). They are also widely distributed across northern Canada and live in close proximity to human settlements, thus sharing the same risk through similar environment and food. As well, foxes are not of conservation concern in Canada at moment, and carcasses are easily accessible. As fur-bearer animals, red and arctic foxes have been extensively trapped for their pelts across northern Canada (Slough et al., 1987). Despite the decline in fur prices, fox trapping remains a source of income in many northern communities, as well as being used for warm clothing.

Figure 1.4: Foxes as sentinels for *Toxoplasma gondii* in northern Canada.

1.3.2. **Lynx as sentinels for and potential reservoirs of *T. gondii***

Lynx are the most abundant felids in northern boreal forests, and the only possible sylvatic definitive host of *T. gondii* in most of boreal and subarctic Canada. In southern Québec, a seroprevalence of 44% (Labelle et al., 2001) and 14% (Simon et al., 2013a) was found in lynx. However, no oocysts have been detected so far in feces, nor has DNA in tissues. As a dietary specialist, lynx are not exposed to a vast array of prey. The ingestion of infected tissue from hares would be the most efficient means of transmission of *T. gondii* in Canada lynx, although transplacental or lactational infections may also be possible (Dubey et al., 1989; Dubey et al., 2009; Powell et al., 2001), and possibly ingestion of sporulated oocysts through contaminated sources of water.

Contrary to foxes, lynx have not yet been proposed as good sentinels for *T. gondii*, but they do possess some characteristics that could make them useful indicator for potential human and animal exposure. Lynx are also widely distributed and trapped across boreal regions of Canada for their pelt, occupy a high trophic position and are not of conservation concern. By potentially shedding the parasite and harboring it in tissues, they could also be seen as useful sentinel species for monitoring environmental circulation of *T. gondii* in northern boreal regions. Even though their distribution is limited to below the treeline in the Canadian Arctic, lynx can indirectly serve as a potential source of oocyst contamination in the north (Jenkins et al., 2013). First, oocysts in freshwater and snowmelt run-off can be transported north through marine currents (Simon et al., 2013c). Accumulation of oocysts in invertebrates such as mollusks, acting as transport hosts, could infect marine mammals and humans (Jones et al., 2009; Shapiro et al., 2015). Spillover to terrestrial wildlife and humans through consumption of marine wildlife in the Arctic is a possible hypothesis for transmission (Messier et al., 2009). Second, migratory herbivorous animals such as caribou or arctic nesting geese can get infected with oocysts shed by lynx into the environment when they seasonally migrate south. They will then develop tissue cysts in muscles and organs that can be consumed by arctic carnivores upon their return in northern regions (Prestrud et al., 2007).

As a consequence of temperature and precipitation change, with increased climate variability and extreme weather events, the ecology of *T. gondii* is expected to shift (Patz et al., 2000). The melting of ice and permafrost, in addition to increased precipitation, will likely boost

the transport of oocysts in northern ecosystems. A warmer and wetter temperature will also favor oocyst survival and development (Lindsay et al., 2002). As well, the tree line is predicted to move northward, in regions that were too cold before (Lenoir et al., 2008); the habitat range of lynx and their prey species will likely follow.

THESIS OVERARCHING GOALS

1. To assess *T. gondii* exposure, prevalence and genetic diversity in foxes and lynx in Arctic and subarctic regions of northern Canada (Chapter 2 and 4).
    a. To determine seroprevalence in foxes and lynx.
    b. To determine prevalence based on detection of DNA in fox and lynx carcasses (brain/heart).

        Hypothesis: Animals are increasingly exposed to *T. gondii* with age.

        Prediction: Older animals are more likely to be positive for *T. gondii* than younger animals.

        Hypothesis: Transmission of *T. gondii* is higher in subarctic than Arctic regions (due to higher levels of oocyst contamination)

        Prediction: Foxes from subarctic regions are more likely to be positive than Arctic regions.

    c. To identify genotype(s) of *T. gondii* present in foxes and lynx in northern Canada.

        Hypothesis: Carnivory on migratory wildlife is the source of infection in foxes rather than oocysts shed by lynx.

        Prediction: If true, genotypes of *T. gondii* in fox should match those in migratory prey species infected in temperate regions, rather than genotypes in lynx in subarctic regions.

2. To compare microscopy, molecular and serological techniques used to detect and characterize *T. gondii* (Chapter 2 and 4).
    a. To compare serological techniques (ELISA vs IFAT) in foxes and lynx using heart fluid.
    b. To determine presence of *T. gondii* DNA in foxes and lynx by comparing molecular and serological methods.

        Hypothesis: More animals are exposed than actively infected with *T. gondii*.

        Prediction: Serological techniques will show a higher detection than molecular.

c. To determine presence of *T. gondii* in lynx feces using microscopy for oocysts (fecal flotation) and molecular techniques for DNA (melting-curve analysis qPCR).

Hypothesis: Lynx act as definitive hosts by shedding oocysts.

Hypothesis: Melting-curve analysis qPCR is more sensitive than fecal flotation.

Prediction: Oocysts and/or DNA of *T. gondii* will be detected in feces of lynx.

3. To investigate how trophic relationships (predator-prey interactions) can affect transmission of *T. gondii* in foxes from northern Québec (Chapter 3).
   a. To use mixing models in stable isotope analyses of carbon and nitrogen to reconstitute the diet of Nunavik foxes.
   b. To investigate seasonal and geographical variation in Nunavik foxes' diet to determine risk factors for exposure.

Hypothesis: Foxes largely get *T. gondii* from their prey species, which differ in exposure between resident and migratory species.

Prediction: Exposed foxes eat more migratory prey than unexposed animals.

Hypothesis: Marine food sources contribute to *T. gondii* infection.

Prediction: Foxes that eat more marine prey will have higher exposure to *T. gondii.*

RESEARCH QUESTIONS

Chapter 2

Are foxes good sentinel species for *T. gondii*? (based on evidence of exposure, prevalence and genetic diversity of *T. gondii* in foxes of Northern Canada)

Chapter 3

How do predator-prey interactions affect transmission of *T. gondii* in foxes from northern Québec?

Chapter 4

Are lynx serving as definitive hosts for *T. gondii*? (based on evidence of exposure, prevalence and genetic diversity of *T. gondii* in lynx of northern Canada)

TRANSITION STATEMENT CHAPTER 2

In Chapter 2, I address the first aim of my research: to investigate *T. gondii* exposure, prevalence and genetic diversity in foxes in Arctic and subarctic regions of northern Canada, and assess their role as sentinel species. As first author on this manuscript published in Parasites and Vectors, I wrote the paper, conducted field, laboratory, and data analyses, and coordinated information and comments from coauthors who were key to collecting samples from across the Canadian North. Foxes occupy a high trophic position in the Arctic and are widespread in northern Canada. They can also be exposed to both oocysts through water and consumption of berries and vegetation, and tissue cysts through carnivory and scavenging. Determining prevalence in red and Arctic foxes in northern Canada gives us a better idea of the parasite's distribution and help us understand how this parasite persists in this ecosystem, while genetic diversity can help us understand where the parasite comes from and how it circulates. Finally, comparing seroprevalence in foxes to that of humans from recent serosurveys will help determine whether foxes act as good sentinels for *T. gondii* in northern regions of Canada. This Chapter provides much needed baseline data on the circulation and geographic distribution of *T. gondii* in free-ranging wildlife populations and environments where humans co-exist.

CHAPTER 2: ARE FOXES (*VULPES* SPP.) GOOD SENTINEL SPECIES FOR *TOXOPLASMA GONDII* IN NORTHERN CANADA?

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2.1. **Abstract**

In changing northern ecosystems, understanding the mechanisms of transmission of zoonotic pathogens, including the coccidian parasite *Toxoplasma gondii*, is essential to protect the health of vulnerable animals and humans. As high-level predators and scavengers, foxes represent a potentially sensitive indicator of the circulation of *T. gondii* in environments where humans co-exist. The objectives of our research were to compare serological and molecular assays to detect *T. gondii*, generate baseline data on *T. gondii* antibody and tissue prevalence in foxes in northern Canada, and compare regional seroprevalence in foxes with that in people from recently published surveys across northern Canada. Fox carcasses (*Vulpes vulpes*/*Vulpes lagopus*, n=749) were collected by local trappers from the eastern (Labrador and Québec) and western Canadian Arctic (northern Manitoba, Nunavut, and the Northwest Territories) during the winters of 2015-2019. Antibodies in heart fluid were detected using a commercial enzyme-linked immunosorbent assay. *Toxoplasma gondii* DNA was detected in hearts and brains using a magnetic capture DNA extraction and real-time PCR assay. Antibodies against *T. gondii* and DNA were detected in 36% and 27% of foxes, respectively. Detection of antibodies was higher in older (64%) compared to younger foxes (22%). More males (36%) than females (31%) were positive for antibodies to *T. gondii*. Tissue prevalence in foxes from western Nunavik (51%) was higher than in eastern Nunavik (19%). At the Canadian scale, *T. gondii* exposure was lower in western Inuit regions (13%) compared to eastern Inuit regions (39%), possibly due to regional differences in fox diet and/or environment. Exposure to *T. gondii* decreased at higher latitude, and in foxes having moderate to little fat. Higher mean infection intensity was observed in Arctic foxes compared to red foxes. Fox and human seroprevalence showed similar trends across Inuit regions of Canada, but were less correlated in the eastern sub-Arctic, which may reflect regional differences in human dietary preferences. Our study sheds new light on the current status of *T. gondii* in foxes in northern Canada, and supports the hypothesis that foxes serve as a good sentinel species for environmental circulation and, in some regions, human exposure, to this parasite in the Arctic.

Keywords: *Toxoplasma gondii*, ELISA, MC-qPCR, foxes, sentinel species, Canada

2.2. **Introduction**

The apicomplexan parasite *Toxoplasma gondii* is found in a vast array of ecosystems and can infect virtually all warm-blooded vertebrates. As approximately one third of the global human population has been exposed, *T. gondii* is one of the most successful parasites in the world (Dubey, 2010; Tenter et al., 2000). This parasite has multiple routes of transmission: environmental (contamination of soil, fresh produce, or water with sporulated oocysts shed in the feces of feline definitive hosts), meat-borne (through raw or undercooked meat containing bradyzoites), and vertical (mother to foetus via tachyzoites) (Dubey, 2009a). While the infection may cause only mild symptoms and clinical signs, neurological, ocular, and reproductive problems may occur, especially if the immune system is compromised or in developing foetuses.

In humans, a higher seroprevalence (ranging from 40% to 78%) is found in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia, and Africa (Pappas et al., 2009). This prevalence is probably due to greater levels of oocysts in the environment, where the definitive felid hosts are abundant and the climate is favorable, but could also be explained by cultural, hygienic, and nutritional habits that can influence levels of human exposure (Pappas et al., 2009). Lower seroprevalence has been observed in many European countries (<40%) as well as the USA (10-20%) (Pappas et al., 2009). In Canada's Arctic and subarctic regions, antibodies to *T. gondii* have been reported in people, with seroprevalence ranging from 8% in the western Arctic (Goyette et al., 2014b) to 40-60% in the eastern Arctic (Ducrocq, 2021; Jenkins et al., 2013; Messier et al., 2009).

Globally, exposure to the parasite in human populations increases along a north-to-south gradient, but seroprevalence in some Inuit communities in Canada's North is much higher than in other parts of North America. In Nunavik, northern QC, exposure to *T. gondii* varies regionally from 27 to 56% (Ducrocq et al., 2021), significantly higher than the North American average of 10-20% (Jones et al., 2018; Molan et al., 2019). Wild felids are rarely seen above the treeline, and domestic cats are not a traditional companion animal in northern communities, although they have been brought from the south into communities (Baker et al., 2018). Nevertheless, Inuit and wild carnivores (such as foxes) are likely exposed through handling and consumption of Arctic wildlife, or from environmental sources, such as untreated freshwater (Messier et al., 2009). Congenital

toxoplasmosis has been reported in Nunavik, and seroconversion in pregnant women was significantly related to carcass processing and consumption of wild game (caribou meat) (McDonald et al., 1990). Game meat plays an important role in the cultural and traditional values of communities in the Canadian North and is an essential part in their well-being and food security. Seroprevalence of *T. gondii* has been reported in various northern wildlife species, including birds, ungulates, and carnivores (Jenkins et al., 2013; Reiling & Dixon, 2019); wild carnivores have thus the potential to act as sentinel animal hosts for food-borne parasites like *T. gondii,* sharing transmission dynamics with humans.

Animals could act as ideal sentinel hosts for select pathogens if they possess the following features: adequate availability (population stability), measurable response (e.g. parasites in tissues, antibodies in blood), earlier response than sympatric wild species or humans, high levels of exposure, and ideally not serve a direct source of human exposure (Halliday et al., 2007; NRCCAMEH, 1991). Arctic and red foxes are widespread across northern Canada and can be exposed to both oocysts shed into the environment by felids and tissue cysts in ingested meat and organs. Skinned, intact fox carcasses are easily accessible through local hunter and trapper organizations. Fox are not currently of conservation concern in Canada, and despite risks through handling of carcasses, they do not represent a risk of food-borne pathogens to people. In addition, high exposure to *T. gondii* has also been documented in foxes in some parts of the Canadian Arctic. For example, Bachand et al. (Bachand et al., 2018) reported an exposure of 41% ($CI_{95\%}$: 27-57) in 39 Nunavik foxes, quite similar to the regional prevalence of 42% ($CI_{95\%}$: 40-44) in people from this region (Ducrocq, 2021). Similarly, Bouchard et al. (Bouchard et al., 2019) found a seroprevalence of 39% ($CI_{95\%}$: 28-50) in Nunavut foxes, compared to an exposure of 32% ($CI_{95\%}$: 29-36) in Inuit populations from Nunavut (Goyette et al., 2014b). All these criteria make foxes good candidates to serve as sentinels for *T. gondii* circulation in Arctic ecosystems (Bachand et al., 2018).

The current study has a much broader geographic scale and larger sample size than these previous surveillance studies, allowing, for the first time, a high level view of the suitability of foxes as indicators of environmental transmission (Bachand et al., 2018; Bouchard et al., 2019). In addition, we capitalize on the relatively recent publications of human seroprevalence data from

all Canadian Inuit regions from the International Polar Year Inuit Health Survey in 2007-08 (Goyette et al., 2014b) and the Nunavik Health Survey in 2017 (Ducrocq et al., 2021), as well as a study in Cree communities of James Bay (Sampasa-Kanyinga et al., 2012), allowing comparison of trends in fox and human seroprevalence.

Although the fox population in Canada appears to be stable, elsewhere in the world, Arctic foxes (*Vulpes lagopus*) are affected by environmental change, such as increased competition with other carnivores like the red fox (*Vulpes vulpes*) moving onto the tundra (Hersteinsson & MacDonald, 1992; Pamperin et al., 2009). With climatic changes and anthropogenic activities affecting the Arctic at higher rates than anywhere else on the planet (Rinke & Dethloff, 2008), this will alter the distribution and abundance of predators, prey, and parasites, including *T. gondii* and its hosts. Warmer, wetter, and more extreme climatic events (with the largest increases in temperature occurring in the western Canadian Arctic) will likely increase exposure rates to *T. gondii* in the Canadian North (Bush & Lemmen, 2019; Jenkins et al., 2013). Systematic surveillance for climate sensitive pathogens like *T. gondii* in suitable sentinel species, like foxes, will also allow us to detect changing risks due to climate change.

The methods used to screen for *T. gondii* can be indirect or direct. Serological tests are indirect, non-invasive methods commonly used in wildlife studies. They are rapid and simple to perform, but only give evidence of previous exposure through detection of antibodies, which for *T. gondii*, appear to be long-lived (Macri et al., 2009). Less commonly, infection can be detected by using direct methods such as bioassays, detection of the parasite in tissues using immunohistochemical methods, or detection of parasite DNA by PCR (Bastien, 2002). Bioassays are gold standard tests to detect *T. gondii*, but they are expensive, time-consuming, and sacrifice many animals (mice or cats) (Gomez-Samblas et al., 2015). Commercially available kits extract DNA from only a small quantity of tissue (25 to 100 mg), and thus could easily miss tissue cysts that are randomly distributed and may occur at low densities (Hill et al., 2006). Magnetic capture-qPCR (MC-qPCR) has higher sensitivity than DNA extraction kits due to its capacity to analyze up to 100 g of tissue (Opsteegh et al., 2010) and has been successfully used in wildlife (Bachand et al., 2018; Sharma et al., 2019a). Moreover, molecular methods are useful to further analyze the genotypes of *T. gondii* circulating in wild mammals. In North American wildlife, type 12 strain is

considered to be a common genotype (Dubey et al., 2021; Khan et al., 2011), followed by Type II and III, which are clonal lineages more commonly detected in livestock and people. As well, type II appears widespread in wildlife in the circumpolar region, which may reflect spillover from domestic cycles to wildlife and transport from sub-Arctic regions via migratory birds or in freshwater run-off (Bachand et al., 2019). Molecular epidemiology of *T. gondii* can reveal regional transmission dynamics, spatial patterns of *T. gondii* in relation to the genetic structure of hosts, and differences in virulence in animals and people (Dubey et al., 2021; Galal et al., 2020; Gerhold et al., 2017; Shapiro et al., 2019b; Su et al., 2010).

As serological tests are rarely optimized for wildlife, our first objective was to determine cut-off values for two serological tests (ELISA and IFAT), followed by comparison of these serological results with detection of DNA of the parasite in tissues using MC-qPCR. Our second objective was to determine antibody and tissue prevalence of *T. gondii* and associated risk factors in harvested foxes across northern Canada. Finally, our third objective was to compare regional seroprevalence for antibodies to *T. gondii* in harvested foxes (current study) and people (other studies) from different regions in northern Canada, to determine if foxes are good sentinels of environmental transmission and potential human exposure to this zoonotic parasite.

2.3. Materials and Methods

2.3.1. Sample locations

Harvested foxes were sampled from across northern Canada (>4000 km), including all four Inuit regions of Canada: Inuvialuit Settlement Region (ISR) (Inuvik, Sachs Harbour, and Ulukhaktok, NT), Nunavut (Cambridge Bay, NU), Nunavik (QC), and Nunatsiavut (NL) (Fig. 1). These four regions and sampling sites are part of Canada's North Coast region, and are characterized by long, cold winters interrupted by short, cool summers. The western and northern parts of the Canadian Arctic coastlines, including regions of ISR and Nunavut, receive limited precipitation (<300 mm annually) and experience relatively few storms. In contrast, the eastern Arctic, including Labrador/Nunatsiavut and Nunavik, experience much higher annual precipitation (up to 1000 mm) due to more frequent storms (Ford et al., 2016). Samples were also collected in subarctic regions including northern Manitoba (Churchill, MB), James Bay and Côte-Nord in QC, southern QC, and western and central Labrador (Fig. 2.1).

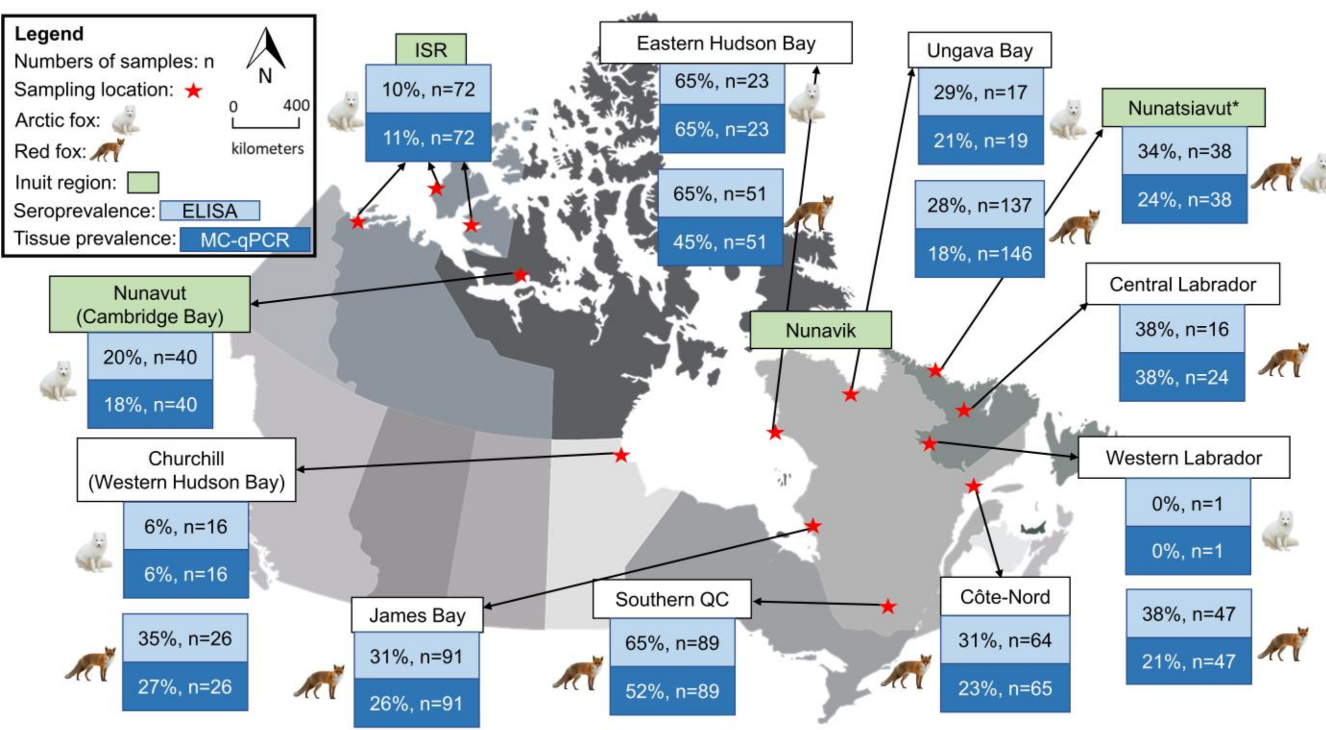

Figure 2.1 Prevalence of *T. gondii* and locations of foxes (*Vulpes vulpes*/*V. lagopus*, n=749) harvested by local trappers in Canada: ISR (NT), Cambridge Bay (NU), Churchill (MB), Nunavik (QC), James Bay (QC), Côte-Nord (QC), Southern QC, Nunatsiavut (NL), Western Labrador (NL), and Central Labrador (NL). The asterisk (*) indicates that both *Vulpes* species were combined due to high number of unknowns.

2.3.2. **Fox sampling**

Foxes (red: n=548, Arctic: n=171, unknown: n=30) were harvested by local trappers and collaborators during regular, licensed fur-trapping activities during the winters of 2015-2019. Carcasses were kept frozen at -20°C (in a freezer) or left outside in an enclosed shelter during winter (generally -20°C or colder), until shipped for necropsy at the Department of Environment and Natural Resources in Inuvik (NT), the Western College of Veterinary Medicine in Saskatoon (SK), the Churchill Northern Studies Centre (MB), he Faculté de Médecine Vétérinaire in Saint-Hyacinthe (QC), the Nunavik Research Centre in Kuujjuaq (QC), and the wildlife division office at the Department of Fisheries, Forestry and Agriculture in Goose Bay (NL). As predilection sites for *T. gondii* in animals (Gisbert et al., 2018; Juránková et al., 2014; Koethe et al., 2015), whole hearts and brains were placed individually in identified plastic bags for each fox. Samples from each individual were then double-bagged together to avoid cross-contamination between foxes.

Species, sex, age, body condition, and location were recorded for most individuals. If the exact harvest coordinates were missing, coordinates from the closest community were used. Animals were classified as an "unknown *Vulpes* species" if they were from a location with known overlap in red and arctic fox distribution and the species of the skinned carcass was not recorded. Age was determined based on teeth condition as per Chevallier et al. (Chevallier et al., 2017) or by counting cementum annuli (Matson's laboratory, Manhattan, Montana, USA). Foxes were classified as young (≤1 year old), mature (2-4 years old), and old (≥5 years old). An approximate body condition index (BCI) of fat deposits was rated visually using a scale of 1 to 3, as follows: 1, no to very little visceral fat deposits in abdominal and peritoneal cavity; 2, moderate visceral fat deposits; 3, abundant visceral fat deposits.

2.3.3. **Serological analysis**

2.3.3.1. **Collection of heart fluid (HF)**

As serum samples are difficult to obtain from harvested wildlife, we used fluid from thawed hearts which has proven to be a good serum surrogate in which to detect antibodies to *T. gondii* (Bachand et al., 2018; Sharma et al., 2019a). Despite long and suboptimal storage in remote regions, freezing and thawing should not have compromised the detection of antibodies in meat juice, as demonstrated by Mecca et al. (2011) showing no degradation of antibodies in meat juice

using ELISA when rabbit muscles were subjected to two freeze-thaw cycles. To avoid cross-contamination when thawing and processing samples, gloves and the working area were thoroughly disinfected with 70% alcohol between samples. Hearts were thawed, and tissue fluid from each bag was transferred to a 15 mL centrifuge tube using a sterile disposable plastic pipette and centrifuged at 3500*g* for 5 min. One ml was transferred in a labelled 1.5 mL Eppendorf tube, centrifuged again at 1000 g for 5 min at 4°C, and stored at 4°C for use within the next 3 days or at -20°C for longer storage.

2.3.3.2. **Determination of the optimal cut-off values for serological tests**

As commercially available ELISA and IFAT assays were not validated in foxes, we evaluated sample dilutions for ELISA and IFAT. Samples from nine foxes known to be positive and negative on magnetic capture were used to determine cut-off values: three negative animals on MC-qPCR (Group N), three low positives (Cq value >30; Group LP), and three high positives (Cq value ≤30; Group HP). The ELISA was performed at four dilutions: no dilution (ND), 1:2 (recommended as per manufacturer's instruction), 1:4, and 1:8. The IFAT was performed at four dilutions (1:2, 1:10, 1:50, and 1:100) and the results were graded from 1+ to 3+ based on fluorescence intensity. The slides were screened by two blinded readers, and the results were compared. The between-reader repeatability of the IFAT was assessed using a weighted kappa value (k). Kappa values of ≤0.40, 0.40–0.60, 0.61–0.80 and ≥0.81 represented poor to fair, fair to moderate, moderate to substantial, and substantial to almost perfect agreement, respectively (Viera & Garrett, 2005). All samples were tested in duplicate. The selected optimal cut-off value for IFAT was at 1:10 dilution, versus 1:2 for the ELISA.

2.3.3.3. **Enzyme-linked immunosorbent assay (ELISA)**

Antibodies to *T. gondii* were detected in heart fluid (1:2 dilution) using the commercially available ID Screen® Toxoplasmosis Indirect Multi-species kit (IDvet, Grabels, France). The ELISA was performed as per manufacturer's instructions. The optical density (OD) was recorded at 450 nm using an ELISA microplate reader (Varioskan LUX multimode, ThermoFisher Scientific). Samples presenting an S/P % less than or equal to 40% were considered negative. Samples greater than or equal to 70% were considered positive. If the S/P % was between 40% and 70%, the test result was considered doubtful and repeated. All samples were tested in duplicate.

Positive and negative controls provided by the manufacturers were used in each batch. In addition, positive and negative serum samples from naturally-exposed foxes were used as reference controls (Bouchard et al., 2019). To check for cross-reactivity with other coccidian species, reference positive serum samples for *Neospora caninum* (bovine) and *Hamondia hamondii* (feline) were provided by the Centre for Food-borne and Animal Parasitology (CFAP) in Saskatoon. The ELISA relative sensitivity and specificity using magnetic capture PCR as a reference test was 94% and 100%, respectively (Sharma et al., 2019a).

2.3.3.4. **Indirect fluorescent antibody test (IFAT)**

The IFAT was performed on heart fluid (1:50 dilution) using the commercially available antigen-coated Teflon-masked slides from VMRD following manufacturer's instructions (VMRD, Pullman, WA, USA). Anti-canine IgG antibodies conjugated to fluorescein isothiocyanate (FITC; rabbit origin) were applied on slides. Slides were viewed under an Olympus DP70 fluorescence microscope, at 40X objective. A complete staining around the tachyzoites was considered positive for *T. gondii* antibodies. Tachyzoites with only apical, or no staining, were recorded as negative. If discontinuous peripheral staining on tachyzoites was observed, the result was considered doubtful and repeated. All samples were tested in duplicate. Positive and negative serum samples from naturally-exposed foxes were used as reference controls, previously confirmed by IFAT (Bouchard et al., 2019). To check for cross-reactivity with other coccidian species, reference positive serum samples for *Neospora caninum* (bovine) and *Hamondia hamondii* (feline) were provided by the Centre for Food-borne and Animal Parasitology (CFAP) in Saskatoon. The IFAT relative sensitivity and specificity using magnetic capture PCR as a reference test was 94% and 100%, respectively (Sharma et al., 2019a).

2.3.3.5. **Comparison of ELISA and IFAT**

To determine the best-performing serological test, heart fluid from 158 randomly chosen foxes (117 red foxes, 29 Arctic foxes, 9 unknown) were tested (in duplicate) by ELISA and IFAT, and results were compared using magnetic capture PCR as a reference test.

2.3.3.6. **Comparison of human and fox exposure**

We used data from the International Polar Year Inuit Health Survey in 2007-08, the Nunavik Health Survey in 2017, and Sampasa-Kanyinga et al. (Sampasa-Kanyinga et al., 2012) to compare exposure of *T. gondii* in Inuit from Inuvialuit (NT), Nunavut (NU), Nunavik (QC), Nunatsiavut (NL), and Cree from James Bay (QC) to overall exposure in harvested foxes from the same regions. The human surveys used commercial (AxSYM, Abbott Diagnostics, Abbott Park, IL) and homemade immunoenzymatic assays (ELISAs) to detect IgG antibodies against *T. gondii* at the National Reference Centre for Parasitology (Montréal, QC) for Inuit communities and the National Microbiology Laboratory (Winnipeg, MB) for Cree communities.

2.3.4. **Molecular analyses**

2.3.4.1. **Magnetic capture - DNA extraction**

Magnetic capture (MC)-qPCR was used to determine infection status and quantify infection intensity (parasite burden) based on detection of DNA of *T. gondii* (Bachand et al., 2019; Sharma et al., 2019a). DNA was extracted from whole heart and brain combined as per Opsteegh et al. (Opsteegh et al., 2010). Each run included two spiked beef samples (positive controls) and one beef sample without spiking (negative control). The concentration of the undiluted *T. gondii* tachyzoite-stock (VEG type III) used for spiking was 2.5 x 10^6/mL. A 10-times dilution series was made in ultrapure water to obtain 2.5 x 10^5 and 2.5 x 10^4/mL. For positive controls, 100µL of these dilutions was added to 50*g* of beef samples, resulting in samples spiked with 2500 and 25,000 tachyzoites. Cell-cultured tachyzoites of *T. gondii* were obtained from CFAP, Saskatoon. For each sample, a back-up of tissue lysate (50 ml) was kept at -20°C. The extracted DNA was stored at 4°C if used in the following 3 days, or at -20°C until further use.

2.3.4.2. **Real-time PCR - DNA amplification and quantification**

A real-time PCR using the Tox 9F (5´-aggagagata tcaggactgtag-3´) and Tox 11R (5´-gcgtcgtctc gtctagatcg-3´) primers for the detection of the 188 bp *T. gondii* sequence within the 529 repeat-element was performed using the Bio-Rad CFX 96 DNA thermal cycler (Biorad, Hercules, California, USA), as per Bachand et al. (Bachand et al., 2018). A reaction was considered positive if 1) the Cq value was less than or equal to 35, 2) the two positive extraction controls were positive, and 3) the negative and two no-template controls were negative. Reactions with Cq values between

35 and 40 were considered positive if a 188 bp band was identified on gel electrophoresis. If only one of the two duplicates amplified, or if the CIAC amplification failed to occur, the PCR was repeated. Parasites were quantified using the following formula: log10 (tachyzoites) = (43.3–Cq) /3.07), and expressed as number of tachyzoite-equivalents (TE) (Bachand et al., 2019). The intensity of infection was calculated by dividing TE by weight of the tissue processed and expressed as tachyzoite-equivalents per gram (TEG).

2.3.4.3. Genotyping – DNA extraction and multiplex multilocus nested PCR-RFLP (Mn-PCR-RFLP)

Retained frozen lysate (50ml) from strongly positive samples on MC-qPCR (n=113, Cq value < 30) was thawed at room temperature, centrifuged at 3500 rpm for 15 min, and 250 µL transferred into a 1.5 mL Eppendeorf tube. DNA was extracted using the High Pure PCR Template Preparation kit (Roche, Mannheim, Germany). Protocol followed manufacturer's instructions except the first step was skipped, as the lysate already contained proteinase K, and 75 µL was used for elution in the final step to increase DNA amount. All samples were extracted in duplicate, and treated with 1 µL of RNAse A Solution (4mg/ml, Promega, Madison, Wisconsin, USA). Frozen aliquots of template DNA (50µL) were sent within 24h to the Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA for further genetic characterization. A Mn-PCR-RFLP method employing ten genetic markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico (Dubey et al., 2007)) was used as per Su et al. (Su et al., 2010).

2.3.5. Statistical analyses

2.3.5.1. Serological and molecular test agreement

Proportion of positive results was compared between the ELISA and IFAT, IFAT and MC-qPCR, and ELISA and MC-qPCR, using McNemar's Chi-square tests for paired data. If not significantly different, the kappa coefficient (k) was used to determine the level of agreement between the two tests. After being retested, all doubtful results from serological tests were considered negative. Analyses were performed using IBM SPSS (ver. 26; Armonk, New York, USA).

2.3.5.2. **Association between quantitative MC-qPCR and ELISA results**

A Spearman's rank-order correlation coefficient was performed to determine the covariation between antibody concentration (S/P%) and Cq values obtained from ELISA and MC-qPCR, respectively.

2.3.5.3. **Prevalence and risk factors**

Seroprevalence, tissue prevalence, and their 95% confidence intervals (CI) were calculated from the proportion of positive results using EpiTools epidemiological calculators (Sergeant, 2018). We used a logistic regression with package lme4 v.1.1-26 (Bates et al., 2015) in R v.3.6.3 (R-Core-Team, 2019) to evaluate the effect of species, sex, age, latitude, and BCI on *T. gondii* prevalence (exposure and infection). Foxes with missing data were not included in the regression, as well as individuals of unknown species. We checked that no group was under-represented and that groups were distributed similarly in each category. We checked for multicollinearity between explanatory variables by using the variance inflation factor (VIF) (see Additional file 2.1: Text S1, Table S1, Figure S1). Conditions affecting the prevalence of *T. gondii* in the fox population (e.g. climate-related factors, fox density, lemming density) may vary yearly. However, we could not include year as a fixed effect in our model as it was dependent on region (and thus latitude) (Table 2.1). We first produced a mixed-effect model including year as a random effect (Oberpriller et al., 2021), and compared it to a corresponding fixed-effect model simply ignoring year. The outputs were very similar, did not change the main conclusion, and the intra-class correlation (ICC) for year as a random effect was low (ICC=0.05). We thus selected the simpler fixed-effect model.

Table 2.1 Number of foxes (*Vulpes* spp.) per region included in the logistic regression for each sampling year in Canada.

Region	Year				Total
	2015-2016	2016-2017	2017-2018	2018-2019	
Northern Labrador, NL	0	0	0	38	38
Central Labrador, NL	0	0	0	24	24
Western Labrador, NL	0	0	0	48	48
Ungava Bay, QC	7	7	109	42	165
Eastern Hudson Bay, QC	32	21	2	20	75
Côte-Nord, QC	0	35	30	0	65
James Bay, QC	33	53	5	0	91
Southern QC	0	89	0	0	89
Churchill, MB	0	0	42	0	42
Cambridge Bay, NU	0	0	0	40	40
Ulukhaktok, NT	0	0	39	33	72

2.3.5.4. **Infections intensity and risk factors**

To evaluate the effect of species, sex, age, latitude, and BCI on the infection intensity of *T. gondii*, we used a generalized linear model (linear regression) in IBM SPSS (ver. 26; Armonk, New York, USA). We found no outliers (Cook's distance was less than 0.5) and no heteroskedasticity (Zuur et al., 2010). Infection intensity of *T. gondii* (tachyzoite-equivalents per gram) was not normally distributed, and data was thus log 10 transformed (negative foxes were not included in the analysis). Foxes with missing data were also excluded from the model. An omnibus test was performed to evaluate the model coefficients (see Additional file 2.2: Text S1, Table S1a-b).

2.4. **Results**

2.4.1. **Samples**

Overall, seroprevalence was 36% ($CI_{95\%}$: 32-39) and tissue prevalence was 27% ($CI_{95\%}$: 24-31). Prevalence and number of samples per species, sex, age, geographic location, and BCI are shown in Table 2.2.

Table 2.2 Prevalence and risk factors in foxes (*Vulpes* spp; n=749) harvested from Canada.

		Seroprevalence[a] ($CI_{95\%}$) / N	Tissue prevalence ($CI_{95\%}$) / N
Species	Red fox	41% (36-45) / 530	30% (26-34) / 548
	Arctic fox	21% (16-28) / 169	20% (15-27) / 171
	Unknown species	31% (17-49) / 29	23% (12-41) / 30
Sex	Male	36% (32-41) / 387	27% (23-32) / 397
	Female	31% (26-37) / 293	25% (20-30) / 302
	Unknown sex	60% (46-73) / 48	44% (31-58) / 50
Age	Young (≤1 yo)	22% (18-27) / 370	19% (16-24) / 383
	Mature (2-4 yo)	37% (30-44) / 180	24% (18-30) / 187
	Older (≥5 yo)	64% (52-74) / 66	45% (34-57) / 66
	Unknown age	62% (52-70) / 112	50% (41-59) / 113
Region	Northern Labrador, NL	34% (21-50) / 38	24% (13-39) / 38
	Central Labrador, NL	38% (18-61) / 16	38% (21-57) / 24
	Western Labrador, NL	38% (25-52) / 48	21% (12-34) / 48
	Ungava Bay, QC	29% (22-36) / 154	19% (14-25) / 165
	Eastern Hudson Bay, QC	65% (54-75) / 74	51% (40-62) / 75
	Côte-Nord, QC	31% (21-43) / 64	23% (15-35) / 65
	James Bay, QC	31% (22-41) / 91	26% (18-36) / 91
	Southern QC	65% (55-74) / 89	52% (41-62) / 89
	Churchill, MB	24% (13-39) / 42	19% (10-33) / 42
	Cambridge Bay, NU	20% (11-35) / 40	18% (9-32) / 40
	Ulukhaktok, NT	10% (5-19) / 72	11% (6-20) / 72
BCI	1 (no to very little fat)	32% (26-38) / 250	25% (20-30) / 256
	2 (moderate fat)	28% (23-34) / 293	21% (16-25) / 297
	3 (abundant fat)	42% (32-52) / 91	30% (22-40) / 92
	Unknown BCI	64% (54-73) / 94	51% (41-60) / 104
Total		36% (32-39) / 728	27% (24-31) / 749

[a] Individuals not tested (no heart fluid) = 21.
N: Number of individual tested.
CI: Confidence intervals.
Yo: Year old.
BCI: Body condition index.

2.4.2. **Specificity of serological assays**

No cross-reactivity was observed on positive serum samples for *H. hammondii* or *N. caninum* antibodies using ELISA or IFAT.

2.4.3. **Cut-off values and choice of serological assay**

2.4.3.1. **Evaluation of sample dilutions for ELISA and IFAT**

Discordance was observed between dilutions of both ELISA and IFAT and the three groups of foxes on MC-qPCR for only 2 animals: dilution 1:2 on IFAT for a fox in Group N (ID BJ2016-223), and one in Group LP (ID W2015-218) (Table 2.3). Since the IFAT results were discordant for dilution 1:2, dilution 1:50 was used for further analyses, as per Bouchard et al. (Bouchard et al., 2019). Since the four dilutions on ELISA gave similar results for all individuals, we used the cut-off value of 1:2 as recommended by the manufacturer. One MC-qPCR positive fox (ID W2015-218) showed negative results on both serological tests (except dilution 1:2 for IFAT), suggesting that antibody levels had not yet been reached, or had declined below detection limits post-exposure/infection. The results are presented in Table 2.3.

Table 2.3 Comparison of *Toxoplasma gondii* results for heart fluid samples using serological methods (ELISA and IFAT) relative to magnetic capture real-time PCR to determine the best dilution for cut-off values.

	ID	ELISA S/P%				IFAT			
Dilutions		ND	1:2	1:4	1:8	1:2	1:10	1:50	1:100
N	BJ2016-223	27,31	13,84	13,3	11,56	1+	N	N	N
	W2015-221	13,3	2,33	1,68	1,19	N	N	N	N
	Mi2015-214	21,34	2,99	1,47	1,36	N	N	N	N
LP	We2015-248	243,81	237,62	227,31	195,87	3+	3+	2+	1+
	We2015-253	344,84	325,35	296,58	281,05	3+	3+	2+	2+
	W2015-218	17,92	8,31	6,13	2,5	1+	N	N	N
HP	We2015-250	351,79	332,19	323,13	295,77	3+	3+	3+	3+
	W2015-227	297,94	272,58	239,47	203,53	3+	3+	2+	1+
	FN21	331,65	309,55	291,8	244,46	3+	3+	2+	2+

Positive results: ELISA ≥ 70% and IFAT ≥ 1+.
N: Negative.
LP: Low positive on magnetic capture (Cp value >30).
HP: High positive on magnetic capture (Cp value ≤30).
ND: No dilution.
ELISA: Enzyme linked immunosorbent assay.
IFAT: Indirect fluorescent antibody test.

2.4.3.2. **Comparison of serological methods (IFAT and ELISA) relative to MC-qPCR**

Antibodies to *T. gondii* were detected in 53% (83/158) of foxes using ELISA, and in 46% (72/158) using IFAT. There was excellent agreement for the between-reader repeatability of the IFAT (k=0.88). There was no statistical difference between serological and molecular results using the McNemar Chi-square test (ELISA: X^2=0.643, df=1, p=0.423, n=158; IFAT: X^2=1.161, df=1, p=0.281, n=158). There was substantial agreement for ELISA (k=0.82) and only moderate agreement for IFAT (k=0.61) when compared to MC-qPCR. The relative sensitivity and specificity of ELISA was 94% and 89% respectively, compared to 76% and 85% for IFAT. ELISA was therefore used for subsequent analyses.

2.4.4. **Final agreement between ELISA and MC-qPCR**

Seventy foxes had positive serology and negative molecular tests, nine were serologically negative but tissue positive, 190 were positive on both, and 459 were negative on both. There was no statistical difference between serological and molecular results using the McNemar chi square test (X^2=45.6, df=1, p<0.001, n=728). There was substantial agreement between the two tests (k=0.75).

2.4.5. **Agreement between quantitative MC-qPCR and ELISA results**

A moderate, negative, statistically significant correlation was found between ELISA antibody concentration (S/P%) and Cq values on MC-qPCR (Spearman's correlation coefficient = -0.419, p <0.001). As Cq values are inversely related to DNA quantity, this means higher antibody levels were associated with higher amounts of DNA.

2.4.6. **Detection of *T. gondii* antibodies and associated risk factors**

Antibodies to *T. gondii* were detected in 36% ($CI_{95\%}$: 32-39) of foxes using ELISA (heart fluid was missing for 21 individuals). Seropositivity increased from young (22%, $CI_{95\%}$: 18-27), to mature (37%, $CI_{95\%}$: 30-44), to older (64%, $CI_{95\%}$: 52-74) foxes. In general, seroprevalence was highest in foxes in eastern Hudson Bay (65%, $CI_{95\%}$: 54-75) and southern QC (65%, $CI_{95\%}$ 55-74), and lowest in the western Canadian Arctic (10%, $CI_{95\%}$: 5-19). The results are presented in Table 2.2 and Figure 2.1.

Our logistic regression included 583 complete observations. Our final model for seroprevalence included sex, age, latitude, and BCI as significant risk factors. The odds of presence of *T. gondii* antibodies were 3 time (odds ratio: 2.65, $CI_{95\%}$: 1.11-6.92, p=0.036) higher in male than female foxes. Mature and old foxes had significantly higher exposure to *T. gondii* than young foxes; two times (odds ratio: 1.99, $CI_{95\%}$: 1.31-3.02, p=0.001) and 7 times (odds ratio: 6.86, $CI_{95\%}$: 3.74-12.96, p<0.001), respectively. The probability of being exposed to *T. gondii* decreased at higher latitude, as well as for foxes having moderate to little fat (Table 2.4).

According to previous studies (Ducrocq et al., 2021; Goyette et al., 2014b; Sampasa-Kanyinga et al., 2012), exposure of *T. gondii* in Inuit was 8% (n=362, $CI_{95\%}$: 4-12) in the Inuvialuit region, 32% (n=1923, $CI_{95\%}$: 29-36) in Nunavut, 37% (n=2735, $CI_{95\%}$: 33-40) in eastern Nunavik, 56% (n=3532, $CI_{95\%}$: 52-61) in western Nunavik, 11% (n=310, $CI_{95\%}$: 6-16) in Nunatsiavut, and 9% (n=266, $CI_{95\%}$: 6-13) in James Bay. Regional human seroprevalences were compared to regional fox seroprevalences in the present study in Figure 2.2.

Table 2.4 Coefficients of the final logistic regression model and risk factors associated to *Toxoplasma gondii* serological and tissue prevalence in foxes (*Vulpes* spp.) in Canada.

Variables	β (SE)	p-value	df	OR ($CI_{95\%}$)
Variables for seroprevalence				
Species, Red fox	0.42 (0.50)	0.404	1	1.52 (0.59-4.28)
Sex, Male	0.97 (0.46)	0.036*	1	2.65 (1.11-6.92)
Age, Mature	0.69 (0.21)	0.001*	2	1.99 (1.31-3.02)
Age, Old	1.93 (0.32)	<0.001*	2	6.86 (3.74-12.96)
Latitude	-0.07 (0.02)	<0.001*	1	0.93 (0.88-0.98)
BCI, Low	-0.60 (0.30)	0.042*	2	0.55 (0.31-0.98)
BCI, Medium	-0.66 (0.29)	0.024*	2	0.52 (0.29-0.92)
Species^Sex, Male Red fox	-0.73 (0.51)	0.156	1	0.48 (0.17-1.29)
Variables for tissue prevalence				
Species, Red fox	0.14 (0.59)	0.807	1	1.15 (0.39-4.02)
Sex, Male	1.48 (0.53)	0.005*	1	4.39 (1.66-13.88)
Age, Mature	0.36 (0.24)	0.137	2	1.43 (0.89-2.29)
Age, Old	1.13 (0.33)	<0.001*	2	3.10 (1.62-5.86)
Latitude	-0.08 (0.03)	0.003*	1	0.92 (0.87-0.97)
BCI, Low	-0.48 (0.32)	0.139	2	0.62 (0.33-1.18)
BCI, Medium	-0.66 (0.33)	0.044*	2	0.52 (0.28-0.99)
Species^Sex, Male Red fox	-1.19 (0.59)	0.042*	1	0.30 (0.09-0.91)

BCI: Body condition index.
Species^Sex: Interaction term.
β: Estimate coefficient.
SE: Standard error.
df: Degree of freedom.
OR: Odd Ratio.
$CI_{95\%}$: 95% confidence interval.
*Statistically significant at $p<0.05$.

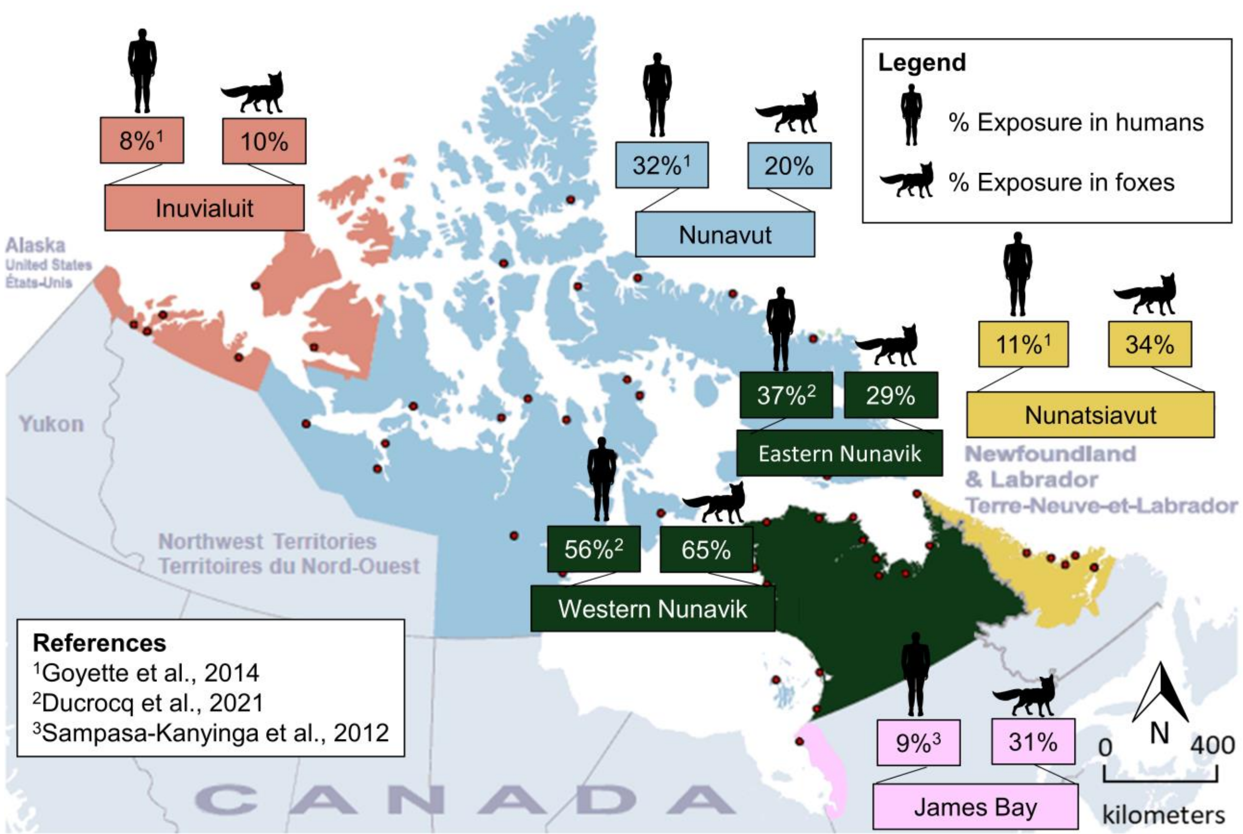

Figure 2.2 Exposure of *Toxoplasma gondii* in people and foxes harvested in Inuvialuit (NT), Nunavut (NU), Nunavik (QC), Nunatsiavut (NL), and James Bay (QC), Canada. Red dots indicate northern communities. Results from both fox species were combined. Credit map: Statistics Canada

2.4.7. **Detection of *T. gondii* DNA and associated risk factors**

DNA of *T. gondii* was detected in 27% ($CI_{95\%}$: 24-31) of foxes using MC-qPCR. Older foxes (45%, $CI_{95\%}$: 34-57) were more frequently infected than mature (24%, $CI_{95\%}$: 18-30) and young (19%, $CI_{95\%}$: 16-24) foxes. A trend in location similar to seroprevalence was also observed for detection of *T. gondii* DNA in foxes. The results are presented in Table 2.2 and Figure 2.1.

Our logistic regression included 593 complete observations, and revealed that sex, age, latitude, BCI, and interaction of sex and species were significantly associated with the presence of *T. gondii* DNA. The odds of presence of *T. gondii* DNA were 4 times (odds ratio: 4.39, $CI_{95\%}$: 1.66-13.88, p=0.005) higher in male than female foxes. Old foxes had 3 times higher odds of being positive for DNA of *T. gondii* in tissues (odds ratio: 3.10, $CI_{95\%}$: 1.62-5.86, p<0.001) than young foxes. The probability of being infected with *T. gondii* decreased at higher latitude, as well as for male red foxes, and foxes having moderate fat (Table 2.4).

2.4.8. **Infection intensity of *T. gondii* and associated risk factors**

Mean infection intensity was 8879 TEG ($CI_{95\%}$: 4278-13480). Mean (SE and $CI_{95\%}$) TEG among different classes of risk factors is shown in Table 2.5. Our generalized linear model included 123 positive foxes with complete data, and revealed that interaction between BCI and latitude were significantly associated with infection intensity. Coefficients of the generalized linear models and potential risk factors for infection intensity (log transformed) of *T. gondii* in foxes are provided in Table 2.6.

2.4.9. **Genotyping results**

Out of 113 fox samples with Cq value < 30, no samples amplified following multiplex multilocus nested PCR-RFLP.

Table 2.5 Infection intensity (tachyzoites equivalent per gram) of *Toxoplasma gondii* in foxes (*Vulpes* spp.) in Canada.

Variables	Category (N)	Mean TEG ($CI_{95\%}$); SE
Species	Arctic foxes (30)	27117.46 (5939.70-48295.21); 10354.71
	Red foxes (137)	5315.85 (1824.39-8807.31); 1765.54
Age	Young (67)	13137.21 (2938.79-23335.84); 5108.04
	Mature (40)	6755.22 (-817.35-14327.79); 3743.81
	Old age (24)	4154.19 (-2916.23-11224.62); 3417.88
Sex	Male (98)	11210.20 (3794.33-18626.07); 3736.48
	Female (58)	6319.44 (576.25-12062.62); 2868.06
BCI	High (24)	5609.90 (-3793.16-15012.96); 4545.49
	Medium (51)	15687.50 (3030.87-28344.14); 6301.34
	Low (56)	5632.37 (-596.50-11861.25); 3108.15

BCI: Body condition index.
N: Number of foxes in the respective category for which TEG was estimated (TEG was not estimated for all positive tissues on MC-qPCR due to lack of data, e.g. missing heart or brain).
TEG: Tachyzoite-equivalents per gram.
$CI_{95\%}$: 95% confidence interval.
SE: Standard error.

Table 2.6 Coefficients of the generalized linear model and potential risk factors for infection intensity (tachyzoites equivalent per gram of tissues; log transformed) of *Toxoplasma gondii* in foxes (*Vulpes* spp.) in Canada.

Variables	β (SE)	95 % Wald confidence interval		Hypothesis test		
		Lower	Upper	Wald-Chi-Square	df	p-value
First Model						
Species, Arctic fox	0.228 (0.3744)	-0.506	0.962	0.371	1	0.542
Sex, Male	0.209 (0.2568)	-0.294	0.713	0.664	1	0.415
BCI, Low	0.113 (0.3259)	-0.526	0.751	0.119	1	0.730
BCI, Medium	0.581 (0.3327)	-0.071	1.233	3.046	1	0.081
Latitude	0.020 (0.0302)	-0.039	0.079	0.448	1	0.503
Age, Young	0.384 (0.3200)	-0.244	1.011	1.438	1	0.231
Age, Mature	0.320 (0.3377)	-0.342	0.982	0.898	1	0.343
Final Model						
BCI^lat, low BCI	0.033 (0.0212)	-0.008	0.075	2.478	1	0.115
BCI^lat, medium BCI	0.042 (0.0207)	0.002	0.083	4.152	1	0.042*
BCI^lat, high BCI	0.030 (0.0216)	-0.013	0.072	1.895	1	0.169

BCI: Body condition index.
β: Estimate coefficient.
SE: Standard error.
df: Degree of freedom.
BCI^lat = Interaction term.
*Statistically significant at p<0.05.

2.5. **Discussion**

This is the first large-scale study on *T. gondii* in a naturally infected wild animal species, using validated serological and molecular testing. The scope of this work allowed geographic comparisons that are not possible when individual or regional studies are conducted using different methods and over different time frames. It also represents a spatio-temporal baseline against which future changes in prevalence driven by climate change can be compared. Finally, we compared prevalence and distribution of *T. gondii* in foxes in four Inuit regions with human exposure data collected through the International Polar Year Inuit Health Survey in 2007-08 and the Nunavik Health Survey in 2017 (Ducrocq et al., 2021; Goyette et al., 2014b), as well as James Bay Cree (Sampasa-Kanyinga et al., 2012), and found that foxes sampled in our study were likely good sentinels for human seroprevalence in most regions (Fig. 2.2).

After determining optimal dilutions and conducting our pilot study on heart fluid, we detected better agreement with MC-qPCR using ELISA compared to IFAT. In contrast, Sharma et al. (Sharma et al., 2019a) reported excellent agreement between both ELISA and IFAT for antibodies to *T. gondii* in wolverines (*Gulo gulo*), using MC-qPCR as a reference test. IFAT has subjective endpoint criteria based on visual inspection which can lead to bias when reporting seroprevalence. ELISA results are quantitative and therefore more objective, and had higher relative sensitivity and specificity than IFAT in the current study. For these reasons, we used ELISA as our primary serological test.

We detected antibodies to *T. gondii* in 36% of the foxes using ELISA, whereas we detected DNA of *T. gondii* in 27% of foxes. Sixty-seven foxes were positive on ELISA but negative on MC-qPCR. This was not unexpected, as seroprevalence is often higher than tissue prevalence (De Craeye et al., 2011; Dubey et al., 2021; Herrmann et al., 2012). This discrepancy can be explained by the presence of cysts in tissues other than brain and heart, or by a lower tissue-infection intensity than the detection limit of the technique (Gilbert et al., 2013). On the other hand, nine foxes were positive on MC-qPCR and negative on serology, possibly due to acute infection (where tachyzoites or early tissue cysts are present but antibodies have not yet been produced at detectable levels) or senescent infections (where antibody levels have declined, with tissue cysts persisting in a non-immunogenic state) (Opsteegh et al., 2010; Robert-Gangneux & Darde, 2012). Even though the

agreement between ELISA and MC-qPCR was substantial (k=0.75), using both serological and molecular tests concurrently maximised detection of *T. gondii* in foxes in the current study.

The overall detection of antibodies was higher in red foxes compared to Arctic foxes, but this was not significant when both species were present at the same latitude. The species effect in our model is likely hidden by the latitude effect (see additional file 2.1: Figure S1). As most red foxes were trapped below the tree line, higher prevalence was expected due to the presence of sympatric wild felids, such as lynx, or outdoor domestic cats that could contribute to environmental contamination and higher infection prevalence in prey species of foxes. In contrast to the southern bias in prevalence, a higher mean infection intensity (tachyzoites equivalent per gram) was observed in Arctic foxes compared to red foxes. Higher parasitic intensity in Arctic foxes could be due to a higher infection intensity of *T. gondii* in food sources of Arctic foxes, as they may be less exposed to oocysts, and possibly due to higher level of carnivorous specialization in the Arctic versus red fox (Szuma & Germonpré, 2020). As well, Arctic foxes could be more susceptible to infection than red foxes, or there could be a more transmissible genotype in northern Canada (Gisbert et al., 2018). Further studies are warranted to explore these hypotheses. Also, we noted that older foxes had significantly higher seroprevalence and tissue prevalence than young foxes. Older age is often associated with *T. gondii* exposure (Ferreira et al., 2019; Sharma et al., 2019b), likely because the likelihood of exposure increases with age, and both tissue cysts and antibodies are long-lived (Rougier et al., 2017). However, mean infection intensity was more than double in young compared to mature and old foxes, which could be due to lower immunity in young animals, allowing more parasite replication in tissues, either as tachyzoites or bradyzoites.

Higher seroprevalence and tissue prevalence were observed in foxes from Hudson Bay (65% and 51%) versus Ungava Bay (29% and 19%) in Nunavik, northern QC. This difference was unexpected, as lynx (potential sources of *T. gondii*) are present in Ungava Bay, but not Hudson Bay. A similar pattern is seen in people, with a seroprevalence of 36% in Ungava Bay, and 56% in Hudson Bay (Ducrocq, 2021). The seroprevalence in Inuit seems to increase with consumption of marine mammals (especially seal), fish, and birds (Ducrocq, 2021). According to the health survey 'Nutrition and Food Consumption Among the Inuit of Nunavik' (Blanchet & Rochette, 2008), the consumption frequency of marine mammals was higher in Hudson Bay, whereas land

animals were most frequently consumed in Ungava Bay. Carnivores in coastal regions, including the majority of the trapped foxes in our study, would also have access to fish and carcasses of marine mammals. Environmental contamination by oocysts may therefore not be a major source of exposure for foxes. Sources of infection with *T. gondii* for marine animals are thought to be oocysts washed into the sea through water run-off and transported by marine currents (Mikaelian et al., 2000; Shapiro et al., 2019a; Shapiro et al., 2019b; Simon et al., 2013c). Marine mammals (such as belugas, seals, polar bears, and walrus) could get infected by consuming filter feeding fish and invertebrates (such as clams and polar cods) acting as mechanical reservoirs of oocysts (Bahia-Oliveira et al., 2017; Fung et al., 2021). We also detected a higher seroprevalence and tissue prevalence in foxes in southern QC (65%) versus subarctic QC (31%). This difference could be explained by higher densities of both wild and outdoor domestic cats in southern QC, thus increasing contamination of food and water with oocysts for both foxes and their prey.

Our overall geographical findings indicate widespread exposure to and infection with *T. gondii* in foxes across northern Canada; however, both fox and human seroprevalence were lower in the western Canadian Arctic, which is drier and may experience less run-off of oocysts from subarctic regions (Ford et al., 2016). For instance, western Hudson Bay had substantially lower sero- and tissue prevalence than eastern Hudson Bay, and the lowest fox and human seroprevalence was in the ISR in the western-most Canadian Arctic. This is also consistent with observation of low (3%) seroprevalence of antibodies to *T. gondii* in people in Alaska (Miernyk et al., 2019). Prevalence in foxes also decreased as latitude increased, suggesting that colder and drier climate may affect the survival or the infectivity of *T. gondii* parasite in more northern regions. Our results in foxes were surprisingly well-correlated with previous studies in Inuit populations, demonstrating a lower seroprevalence in ISR (8%) and Nunavut (32%) compared to the more southern region of Nunavik (Goyette et al., 2014b). However, Inuit from Nunatsiavut and Cree from James Bay had a seroprevalence of 11% (Goyette et al., 2014b) and 9% (Sampasa-Kanyinga et al., 2012), respectively, compared to 34% and 31% in foxes, possibly reflecting dietary preferences in Inuit in Nunatsiavut and James Bay Cree for cooked meat and/or consumption of terrestrial (versus marine) wildlife (Messier et al., 2009). Indeed, Inuit from Nunavut and Nunavik consume high quantities of seal meat (Ducrocq, 2021; Egeland, 2010a), but not in ISR and Nunatsiavut, where *T. gondii* seroprevalence is much lower. Caribou meat is the most consumed

country food in ISR and Nunatsiavut (Egeland, 2010b; Egeland, 2010c), which would be expected to have low prevalence of *T. gondii* in tissues (Kutz et al., 2001).

Determining the genotypes/strains of *T. gondii* present in foxes across the Canadian Arctic would provide valuable information about the population structure and transmission of this parasite; for example, if strains in foxes were identical to those in marine mammal species, or felids in subarctic regions. However, as we found, recovering sufficient DNA of *T. gondii* from naturally-infected wildlife can be challenging, as they have low tissue burden and therefore yield low levels of DNA (Galal et al., 2019). In future, minimizing freeze-thaw cycles and using larger amounts of tissue for extraction could maximize DNA yield. Future studies, including DNA characterization assays, are needed to determine genotypes of *T. gondii* circulating in foxes in northern Canada.

The effect of climate change on *T. gondii* ecology still remains uncertain in northern regions, but may be more severe in the western North American Arctic, where the parasite is not currently as successful as in the Eastern Arctic, and the magnitude of rapid and directional climate change is more pronounced than almost anywhere in the globe. The western Canadian Arctic (Yukon, Northwest Territories and Nunavut) is currently experiencing the largest increases in warming and precipitation due to climate change, which could bring increases in survival and infectivity of oocysts in the future (Bush & Lemmen, 2019). As a result, the range and abundance of *T. gondii* may expand via aquatic environments and migratory wildlife in these ecosystems. The presence of fish and marine invertebrate species in new areas that were previously too cool for their survival represents a risk for increased spread, since they can accumulate oocysts in their gills and filtration organs (Gajadhar & Allen, 2004). As well, with warmer climate, the distribution of lynx and their prey species is predicted to move northward with the tree line, facilitating local oocyst transmission (Jenkins et al., 2013). Increased precipitation could also enhance survival and transport of oocysts from water sources flowing from south to north.

2.6. **Conclusion**

This study supports the hypothesis that foxes are appropriate sentinels for potential human exposure and transmission of *T. gondii* in a future of climate change. Foxes are widespread across

northern Canada, year-round residents, and exposed through multiple routes of transmission likely overlapping with those of humans in most of the Canadian Arctic. While the western Arctic may be more affected by the consequences of climate change, the higher prevalence in the eastern Canadian Arctic warrants continued study to monitor prevalence of *T. gondii* in wildlife species that are consumed in northern communities. Future research could investigate additional predictors of *T. gondii* presence and prevalence in foxes, such as diet (terrestrial vs marine food sources). The geographic scale of the present study speaks to significant community engagement from trappers and territorial, provincial, and Indigenous governments. Synergizing sentinel animal surveillance with human disease surveillance is critical to identifying and addressing the potential human and animal health risks associated with altered transmission of *T. gondii* in a rapidly changing Arctic.

2.7. **Acknowledgements**

We would like to thank all fur trappers and coordinators that contributed to this study by submitting samples, without whom this project could not have taken place. In particular, we would like to acknowledge Elena Berthe, Eddie Kumarluk, Lasarusie Tukai, Ian Winters, Frank Phillips, Luke Parsons, Andrew Andersons, Greg Flowers, and Thomas Lane. We also thank Liz Pijogge, Paul McCarney, Mark Basterfield, Frankie Jean-Gagnon, Agathe Allibert, Ellen Avard, Claude Grenier, Ariane Massé, Michaël Bonin, Richard Neville, Hugh Whitney, and Bruce Rodrigues for valuable help in sample logistics. We are also thankful to Brent Wagner, Champika Fernando, Michelle Sniatynski, and Pratap Kafle for help in laboratory analyses, and to Brad Scandrett as an internal reviewer for the Canadian Food Inspection Agency. Additionally, we would like to thank Marsha Branigan, Christine Menno, Verna Pokiak and Matilde Tomaselli for their assistance with fox collection and necropsies in the ISR and Nunavut. We are grateful to the personnel of the Churchill Northern Studies Centre, the Faculté de Médecine Vétérinaire and the Centre québécois sur la santé des animaux sauvages (CQSAS), the Nunavik Research Centre (Makivik Corporation), regional and local Nunavimmi Umajulivijiit Katujaqatigininga (LNUK and RNUK), and wildlife division office at the Department of Fisheries, Forestry and Agriculture in Goose Bay for storage of carcasses, logistic help, and transport. We would like to respectfully acknowledge Inuit Lands and thank the Nunatsiavut Government for its cooperation and support in providing fox samples.

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2.8. **Ethics approval and consent to participate**

In accordance with the Canadian Council on Animal Care guidelines, this research was exempt from Animal Research Ethic Board review in Canada because all samples were collected from animals previously harvested for non-research purposes. We obtained wildlife research permits and appropriate export permits from the Governments of Northwest Territories (WL0666), of Nunavut (WL2019-003), Manitoba (WB18911, WB21856), and Newfoundland and Labrador (WLR2018-40, WLR2019-45). No permits were required for Québec, where we worked closely with the Minister of Forests, Wildlife and Parks.

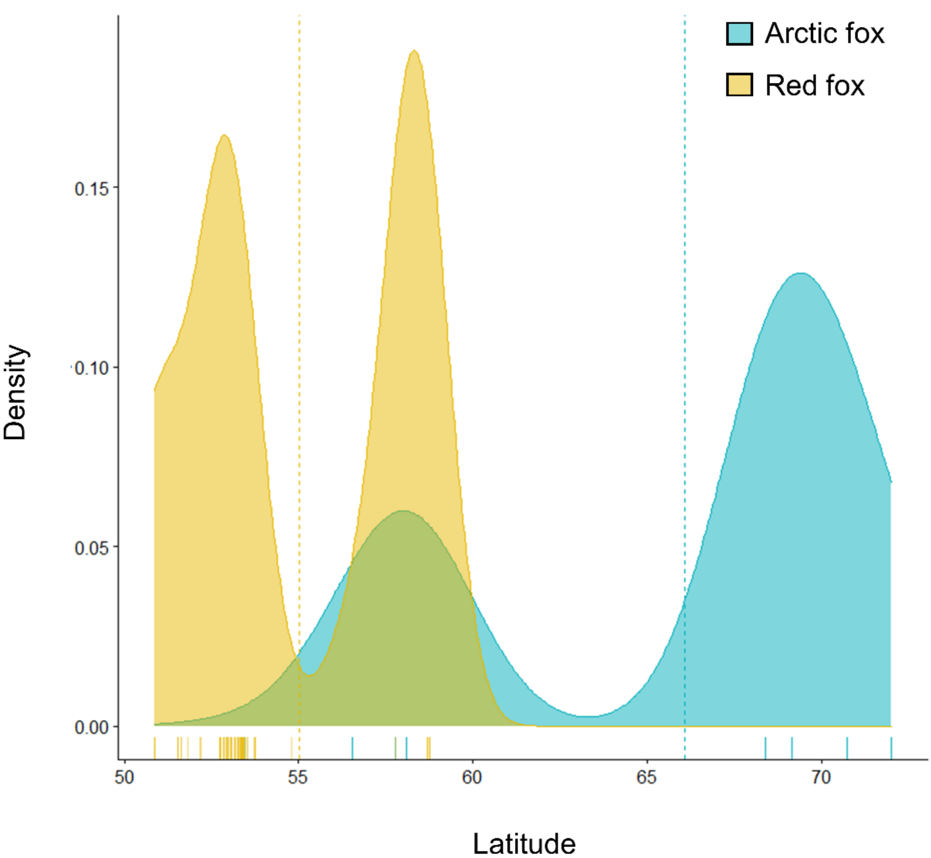
2.9. Supplementary information

Additional file 2.1: Text S1. For serological and tissue prevalence, we tested the relevance of possible interaction terms by comparing models with Akaike information criterion (AIC), where models with AIC < 2 were considered equally plausible. Models were tested against the null model to see if there was a significative amelioration (Table S1). Values of the variance inflation factor (VIF) exceeded 10 for species^latitude interaction, indicating collinearity (Fig. S1). This term was therefore not included in the model. However, we elected to retain species since the species^sex interaction term was significant.

Additional file 2.1: Table S1. Akaike's information criterion model selection results for hypotheses of risk factors influencing *Toxoplasma gondii* serological and tissue prevalence in foxes in Canada.

Model	Models for serological prevalence	∆AIC	AIC weight	Log likelihood	k
1	ELISA ~ species^sex + lat + age + BCI	0.00	0.36	-324.98	9
2	ELISA ~ species^BCI + sex+ lat + age	0.30	0.31	-324.13	10
3	ELISA ~ species^BCI + lat + age + species^sex	0.50	0.28	-323.23	11
4	ELISA ~ species^age + sex +lat + BCI	3.72	0.06	-325.84	10
5	ELISA ~ species^sex^age + lat + BCI	9.57	0.00	-323.77	15
6 Null	ELISA ~ 1	56.34	0.00	-361.15	1
	Models for tissue prevalence				
1	MC ~ species^sex + lat + age + BCI	0.00	0.64	-275.27	9
2	MC ~ species^BCI + lat + age + species^sex	1.92	0.25	-274.23	11
3	MC ~ species^BCI + sex+ lat + age	4.20	0.08	-276.37	10
4	MC ~ species^age + sex +lat + BCI	6.18	0.03	-277.36	10
5	MC ~ species^sex^age + lat + BCI	9.40	0.01	-273.97	15
6 Null	MC ~ 1	20.10	0.00	-293.32	1

As per lme4 notation, the ^ indicates that the model ran the fixed effect of each factor independently and the 2 and 3 ways interactions / AIC: Akaike's information criterion / ∆AIC: change in AIC relative to top model / k: the number of model parameters / MC: Magnetic capture / ELISA: Enzyme-linked immunosorbent assay / lat: study site latitude / BCI: Body condition index



Additional file 2.1: Figure S1: Density of data for each species (Arctic fox in blue and red fox in yellow) compared to latitude in decimal coordinates indicating strong relationships (VIF=146).

Additional file 2.2: Text S1. For infection intensity, AIC was used for the selection of the final model; model (s) with lower AIC_C value (finite sample corrected AIC) was (were) considered better than the other models. Models with AIC < 2 were considered equally plausible. BCI and latitude were associated with infection intensity and interaction was observed between them; we therefore included BCI and latitude as interaction in the final model. Further models were tested for including BCI^lat interaction term along with other possible risk factors influencing *T. gondii* infection intensity in foxes in Canada. However, BCI^lat interaction was kept in the final model as aforementioned (Table S1 a-b).

Additional file 2.2: Table S1 a). Model selection results for hypotheses of risk factors influencing *Toxoplasma gondii* infection intensity in foxes in Canada.

Model	**Models for infection intensity**	Δ AIC_C	**AIC_C**	**Log Likelihood**	**Omnibus Test**		
					LR Chi-sq	**df**	**p**
1	ii ~ sex + species + age + BCI+ lat	0	421.272	-200.839	12.214	7	0.094
2	ii ~ sex + age + BCI+ lat	1.959	419.313	-200.025	11.843	6	0.066
3	ii ~ age + BCI+ lat	1.706	417.607	-201.317	11.260	5	0.046
4	ii ~ BCI + lat	2.434	415.173	-202.330	9.233	3	0.026
5	ii ~ BCI^lat	0.943	414.230	-201.859	10.176	3	0.017*

Additional file 2.2: Table S1 b). Model selection results for hypotheses including "BCI^lat" interaction term and other possible risk factors influencing *Toxoplasma gondii* infection intensity in foxes in Canada.

Model	**Models for infection intensity**	Δ AIC$_C$	**AIC*$_C$***	**Log Likelihood**	**Omnibus Test**		
					LR Chi-sq	**df**	**p**
1	ii ~ BCI^lat+ species + age + sex	0	420.257	-200.332	13.229	7	0.067
2	ii ~ BCI^lat + age + sex	1.968	418.289	-200.292	12.865	6	0.045
3	ii ~ BCI^lat + age	1.676	416.613	-200.819	12.254	5	0.031
4	ii ~ BCI^lat	2.383	414.230	-201.859	10.176	3	0.017*

ii: Infection intensity
lat: Study site latitude
BCI: Body condition index
BCI^lat: Interaction term
AIC$_C$: Finite sample Corrected Akaike's Information Criterion
ΔAIC: change in AIC$_C$ relative to top model
LR: Likelihood ratio
df: Degree of freedom
*Statistically significant at $p<0.05$

TRANSITION STATEMENT CHAPTER 3

After investigating the exposure and infection status in foxes from different regions of northern Canada in Chapter 2, in Chapter 3 I followed up on potential dietary reasons for the significant differences in prevalence observed between two regions of Nunavik, northern Québec. As first author on this manuscript, I wrote the paper, conducted field, laboratory, and data analyses, and coordinated information and comments from coauthors. In collaboration with a coauthor at Université Laval, I tested fox hair and muscle with isotope analysis to obtain information on summer and winter diet composition. This information sheds light on routes of transmission and the role different prey species play in exposing foxes to *T. gondii*. By combining the contribution of each prey type in fox diet with the status of infection using a Bayesian isotopic mixing model technique, I generated valuable information on how the parasite is introduced and maintained in this specific ecosystem. The approach I took in this Chapter, by combining trophic analysis using stable isotope signatures with infection status, allows identification of transmission pathways for zoonotic parasites that can also be applied to other foodborne pathogens in free-ranging wildlife populations and environments where humans co-exist. In addition, our findings are consistent with recent reports that marine prey and migratory birds are linked to human exposure to *T. gondii* in Nunavik.

CHAPTER 3: USE OF STABLE ISOTOPES TO REVEAL TROPHIC RELATIONSHIPS AND TRANSMISSION OF A FOOD-BORNE PATHOGEN

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Citation

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3.1. **Abstract**

Predators in food webs are valuable sentinel species for zoonotic and multi-host pathogens such as *Toxoplasma gondii*. This protozoan parasite is ubiquitous in warm-blooded vertebrates globally, and can have serious adverse effects in immunocompromised hosts and foetuses. In northern ecosystems, *T. gondii* is disproportionately prevalent in Inuit people and wildlife compared to temperate regions, in part due to multiple routes of transmission. We combined data on *T. gondii* infection in foxes from Nunavik (northern Québec, Canada) with stable isotope data tracking trophic relationships between foxes and their prey species, to determine sources of food-borne transmission for *T. gondii*. Red (*Vulpes vulpes)* and Arctic fox (*Vulpes lagopus*) carcasses were collected by local trappers from 2015-2019. We used magnetic capture PCR to detect DNA of *T. gondii* in heart and brain, and enzyme-linked immunosorbent assay to detect antibodies in blood. By linking infection status with diet composition derived from stable isotope analyses, we showed that infected red and Arctic foxes had a higher probability of consuming marine and migratory food sources, respectively, suggesting that these may be important sources of *T. gondii* transmission in the Arctic. The use of microbial methods and stable isotope analysis to reveal parasite transmission pathways can be applied more broadly to other foodborne pathogens, and provides evidence to assess and mitigate potential human and animal health risks associated with *T. gondii* in northern ecosystems. These data also represent a baseline against which to detect changes as a result of climate and landscape alterations, which are more rapidly advancing in the Canadian Arctic than almost anywhere else in the globe.

Key Words

Toxoplasma gondii, stable isotopes, foxes, trophic relationships, diet composition, northern ecosystems, foodborne pathogens

3.2. **Introduction**

The protozoan *Toxoplasma gondii* is a ubiquitous, zoonotic parasite of public health significance. In humans, *T. gondii* can cause life-threatening infections, especially in immunocompromised hosts and foetuses (Dubey, 2010; Montoya & Liesenfeld, 2004). Exposure to the parasite in some Inuit communities in Nunavik, northern Québec, Canada, is much higher than in other parts of North America (43% seroprevalence compared to 10-15%) (Ducrocq et al., 2021). Inuit are thought to be exposed through handling and consumption of Arctic wildlife (Messier et al., 2009). Still, transmission of *T. gondii* in terrestrial arctic ecosystems is complex, potentially involving food, water and vertical routes, and food-borne source attribution is often unclear. Northern transmission of *T. gondii* could involve food or water contaminated with oocysts (Ducrocq et al., 2021; Prestrud et al., 2007); oocysts of *T. gondii* have been observed in aquatic filter feeders (i.e., fish, oyster, clam, snail) (Arkush et al., 2003; Marino et al., 2019). However, true food-borne transmission (versus exposure to oocysts) is thought to be significant in the Arctic, as wild and domestic felids, the only known source of oocysts in the environment, are mostly absent in tundra and high Arctic ecosystems. Therefore, northern hosts may be exposed through the consumption of cysts of *T. gondii* in the tissues of chronically infected migratory prey, such as caribou (*Rangifer tarandus*), arctic-nesting geese (e.g. *Branta canadensis, Anser caerulescens)* or marine mammals (Bachand et al., 2018; Elmore et al., 2015; Jenkins et al., 2013; Prestrud et al., 2007) (Fig. 3.1).

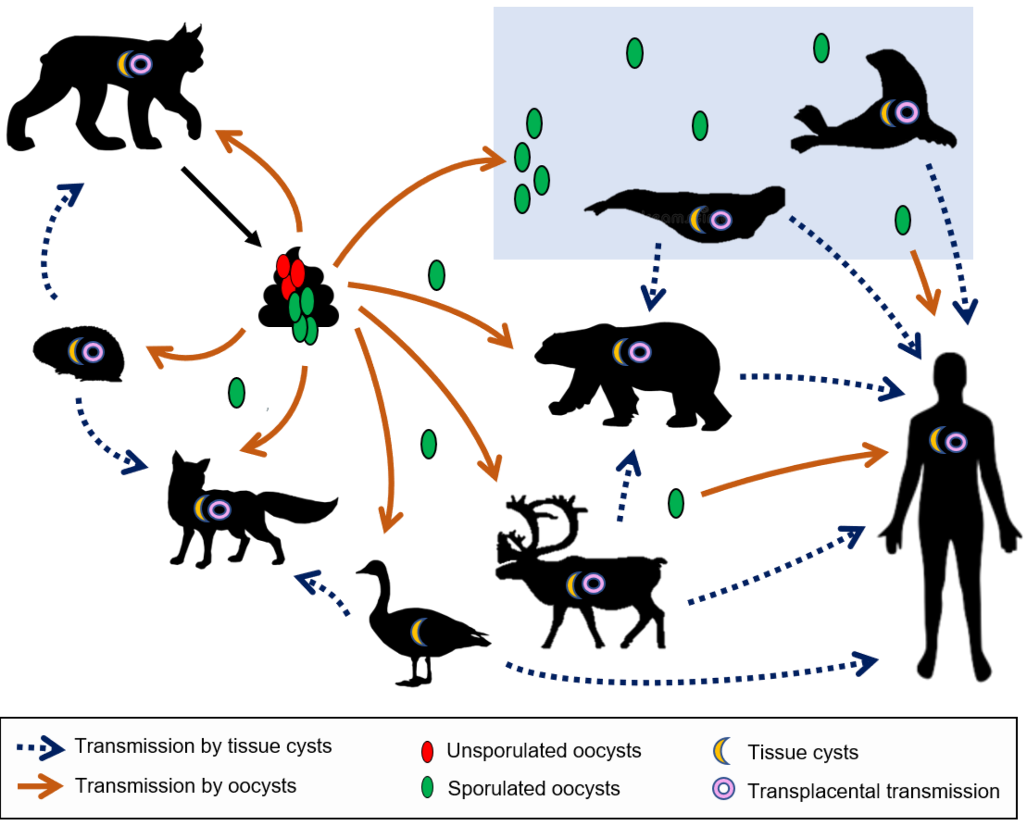

Figure 3.1 Potential routes of transmission of *Toxoplasma gondii* in the North, with focus on free-ranging hosts and the shared environment (Reprinted by permission from Springer Nature: Springer, Toxoplasmosis in Northern Regions, Bouchard *et al*. (Bouchard et al., 2022a))

Given their generalist predator and/or scavenger foraging behaviors across multiple trophic levels, and their close association with human activities, foxes (*Vulpes* spp.) are potentially good sentinel species to investigate transmission through food webs, and exposure (i.e., seroprevalence) in foxes tracks closely with that of people in many regions of the Canadian North (Bouchard et al., 2022b).

Documenting trophic routes through which foxes in remote regions are infected with *T. gondii* remains challenging. Stable isotope analysis, an indirect diet reconstruction approach, has been used to study the diet of terrestrial species, including foxes (Hoekstra et al., 2003; Lecomte et al., 2011; Roth, 2002; Roth, 2003; Savory et al., 2014). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values of consumer tissues reflect the composition of its diet, after accounting for trophic enrichment against heavier isotopes and assimilation process (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981). Carbon isotopic ratios distinguish between terrestrial and marine food sources, while nitrogen isotopic ratios are a relative index of trophic position of individuals or species (Bonin et al., 2020; Kelly, 2000). Different body parts provide dietary information that is integrated over different time scales (e.g., days to months), due to the turnover rate of isotopes (Boecklen et al., 2011; DeNiro & Epstein, 1978; DeNiro & Epstein, 1981). For example, isotopic ratios of blood cells and muscle tissue provide insights on the food habits of a consumer over the past few weeks, while hair reflects the diet of the animal during the entire growth period of the hair since the last molt (Boecklen et al., 2011).

In this study, we combined data on stable isotope analysis and pathogen status to determine possible routes of transmission of *T. gondii* in a highly affected region of the Canadian Arctic. If oocysts in water are the primary route of exposure, then there should be no links between diet and infection with *T. gondii*. If predation of rodents infected by consumption of oocysts in the environment are the primary route of exposure, then foxes consuming rodents should have high prevalence. It has been suggested that *T. gondii* may enter the terrestrial Arctic ecosystem of northern Norway via migratory birds (Sandström et al., 2013); an estimated 7% of barnacle geese (*Branta leucopsis*) on Svalbard are exposed to the parasite where no wild or domestic felids are present (Prestrud et al., 2007). Also considering previous work on migratory geese and marine mammals in Nunavik, and recent findings in the Nunavik Inuit Health Survey (Bachand et al.,

2019; Ducrocq et al., 2021), we hypothesized that migratory geese and marine intermediate hosts consumed by foxes may be primarily responsible for transporting *T. gondii* to the Arctic and disseminating the parasite within northern ecosystems. Given the zoonotic risk posed by *T. gondii* in Inuit communities, documenting its prevalence and transmission routes in northern wildlife is crucial to understanding and addressing the risk of infection in vulnerable animal and human populations. More broadly, we demonstrate that linking microbial and ecological approaches provides a powerful method to unravel transmission of *T. gondii* and other food-borne pathogens with complex transmission routes.

3.3. **Materials and Methods**

3.3.1. **Study area**

Our study area was located in Nunavik, northern Québec (Canada) (Fig. 3.2). Potential terrestrial food sources (through predation and scavenging) for Arctic and red foxes include lemmings (*Lemmus* spp.), arctic hares (*Lepus arcticus*), arctic ground squirrels (*Urocitellus parryii*), migratory caribou (*Rangifer tarandus*), muskoxen (*Ovibos moschatus*), and Arctic nesting geese (e.g. *Branta canadensis*, *Anser caerulescens*) (Bercuson et al., 2021; MFFP, 2019). Both fox species rely heavily on lemmings and voles (Chester, 2016). Foxes may also scavenge carcasses of marine wildlife, including beluga (*Delphinapterus leucas*), walrus (*Odobenus rosmarus*), seals, and fish, as natural mortalities or harvested by human residents.

Figure 3.2 Regions where foxes (*Vulpes* spp.) were collected during winter 2015-2019 by trappers from Nunavik, Canada. Black circles indicate northern communities involved. ©Makivik Corporation

3.3.2. **Fox sampling**

This study was part of a larger research project on *T. gondii* in foxes across northern Canada (Bouchard et al., 2022c). Fox carcasses were harvested by local trappers during regular, licensed fur-trapping activities during winters of 2015-2019 in two regions of Nunavik: eastern Hudson Bay (Arctic foxes=23, red foxes=51) and southwestern Ungava Bay (Arctic foxes=19, red foxes=146) (Fig. 2). Following consultation and presentations at community meetings, local coordinators were hired to help with the collection on site, and ensure that samples were stored at −20 °C until shipped for necropsy. The communities were chosen according to their interest in participating in the project.

Frozen carcasses were sent for necropsies at the Western College of Veterinary Medicine in Saskatoon (SK), the Faculté de Médecine Vétérinaire in Saint-Hyacinthe (QC), and the Nunavik Research Centre in Kuujjuaq (QC). We collected whole hearts, brains, hair (root to tip from the hind right paw) and thigh muscles for each fox. Brain and heart are predilection sites for *T. gondii* in animals (Gisbert et al., 2018; Juránková et al., 2014; Koethe et al., 2015). We recorded harvest location, species, and sex for all individuals. In accordance with the Canadian Council on Animal Care guidelines, this research was exempt from Animal Research Ethic Board review in Canada because all samples were collected from animals legally harvested for non-research purposes.

3.3.3. **Serological analysis**

As validated in Sharma *et al*. (Sharma et al., 2019b) and described in Bouchard *et al*. (Bouchard et al., 2022c), we detected antibodies to *T. gondii* in fluid from thawed hearts (diluted 1:2) using the commercially available ID Screen® Toxoplasmosis Indirect Multi-species Enzyme-linked immunosorbent assay (ELISA) (IDvet, Grabels, France).

3.3.4. **Molecular analyses**

As validated by Opsteegh *et al*. (Opsteegh et al., 2010) and described in Bouchard *et al*. (Bouchard et al., 2022c), sequence specific DNA of *T. gondii* was extracted by magnetic capture from whole heart and brain combined for each fox followed by real-time PCR using the Tox 9F (5´-aggagagata tcaggactgtag-3´) and Tox 11R (5´-gcgtcgtctc gtctagatcg-3´) primers, for the detection of the 188 bp *T. gondii* sequence within the 529 repeat-element.

3.3.5. **Collection of food sources**

Potential food sources of foxes were collected from a concurrent study (Bonin et al., 2020). Briefly, hair and muscle samples were collected in 2016-2017 and 2017-2018 and over the same spatial scale as samples of foxes. We selected three dietary endpoints based on literature review of red and Arctic fox diets: lemmings (hair, *Dicrostonyx hudsonius*), Canada geese (muscle tissue of *Branta canadensis*, also representing isotopic values of eggs), and fish (muscle tissue of Arctic char (*Salvelinus alpinus*) and lake trout (*Salvelinus namaycush*)).

3.3.6. **Stable isotope analysis**

We collected hair and muscles from foxes trapped in winter. Since foxes molt twice per year (Chesemore, 1970; Roth, 2002), with the autumn molt starting in September, stable isotope signatures from winter fur in our study represented diet composition of foxes from the previous summer/fall seasons. Isotopic ratios of muscle tissues from foxes in our study represented their diet composition during winter (Roth, 2003). Variations in lipid concentration can significantly influence $\delta^{13}C$ measurements (Rau et al., 1992); therefore, we cleaned fox hair with distilled water, soaked 3 times for 10 min in a 2:1 chlorophorm:methanol solution to remove lipids, then rinsed hair again in distilled water before drying at room temperature for 48 hours. We cut multiple, complete hairs in 1 mm segments into tin cups to achieve a total weight of 1 mg from each fox. We lyophilised muscle tissues (foxes and food sources) for 36 h and ground them in a ball mill. To remove lipids, we added a 2:1 chlorophorm:methanol solution to each muscle sample, mixed with a tube stirrer, and centrifuged for 8 min at 10,000 RPM. The supernatant was removed, and we repeated the procedure until the supernatant was clear. The samples were left to dry for 24h, then 1 mg was weighed into tin cups.

We analysed samples from foxes and prey tissues for nitrogen and carbon at the Laboratoire d'Océanographie of Laval University, Québec (Canada). Isotopic analyses were performed by continuous-flow isotope ratio mass spectrometer (Thermo Electron Delta Advantage) using an ECS 4010 Elemental Analyzer/Zero Blank Autosampler (Costech Analytical Technologies). Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from V-Pee

Dee Belemnite (carbon) and AIR (nitrogen) international standards. Measurement precision was ± 0.2‰ for $\delta^{13}C$ and ± 0.1‰ for $\delta^{15}N$.

3.3.7. **Statistical analysis**

3.3.7.1. **Serological and molecular test agreement**

Proportion of positive results was compared between ELISA and MC-qPCR, using McNemar's Chi-square tests for paired data. The kappa coefficient (k) was used to determine the level of agreement between the two tests. Analyses were performed using IBM SPSS (ver. 26; Armonk, New York, USA).

3.3.7.2. **Prevalence and risk factors**

Seroprevalence, tissue prevalence and their 95% confidence intervals (CI) were calculated from the proportion of positive results using EpiTools epidemiological calculators (Sergeant, 2018). We used linear regression to test for the effects of sex, species, region, and status of infection on stable isotope values of fox hair and muscles. We used a logistic regression with package lme4 v.1.1-26 (Bates et al., 2015) in R v.3.6.3 (R-Core-Team, 2019) to evaluate the effect of species, sex, region, terrestrial, marine, and migratory diet on *T. gondii* prevalence (exposure and infection results combined). Foxes with missing data were not included in the regression. We tested the relevance of possible interaction terms by comparing models with Akaike information criterion (AIC), where models with $\Delta AIC < 2$ were considered equally plausible. Models were tested against the null model to see if there was a significant amelioration (Table 3.1). We did not include year as a fixed effect in our model as it was dependent on region. We did include year as random effect to take into consideration annual variation in ecological conditions that may influence the prevalence of *T. gondii* in the fox population (e.g. climate-related factors, fox density, lemming density).

Table 3.1 Akaike's information criterion model selection results for hypotheses of risk factors influencing *Toxoplasma gondii* prevalence in foxes (*Vulpes* spp.) in Nunavik, QC.

Model	Models late summer/fall diet	ΔAIC	AIC weight	Log likelihood	k
1	Toxo ~ Region*Species + Species*Dietmarine + Species*Dietmigration + (1|Year)	0.00	0.99	-75.04	9
2	Toxo ~ Region*Dietmarine + Region*Dietmigration + (1|Year)	9.70	0.01	-82.13	7
3	Toxo ~ Dietmarine + Dietmigration + (1|Year)	13.64	0.00	-87.35	4
4	Toxo ~ Region + Sex + Species + (1|Year)	15.99	0.00	-87.46	5
5 Null	Toxo ~ 1+ (1|Year)	18.75	0.00	-92.00	2
	Models for winter diet				
1	Toxo ~ Region*Species + Species*Dietmarine + Species*Dietmigration + (1|Year)	0.00	0.77	-109.11	9
2	Toxo ~ Dietmarine + Dietmigration + (1|Year)	2.52	0.22	-115.71	4
3	Toxo ~ Region*Dietmarine + Region*Dietmigration + (1|Year)	8.03	0.01	-115.29	7
4	Toxo ~ Region + Sex + Species + (1|Year)	16.03	0.00	-121.42	5
5 Null	Toxo ~ 1 + (1|Year)	13.68	0.00	-123.35	2

AIC: Akaike's information criterion

ΔAIC: change in AIC relative to top model

k: the number of model parameters

3.3.7.3. **Stable isotope analysis**

To calculate the percentage of fox diet that was derived from terrestrial, migratory, and marine food sources, we used the $\delta^{13}C$ values of lemming, goose, and fish, respectively. As $\delta^{13}C$ and $\delta^{15}N$ values were very similar for goose muscles and goose eggs (t(2)=0.01, p=0.49), only goose muscles were used in the analysis. Prey items of foxes were all isotopically distinct (Supplementary material, Table S1-S2).

We corrected red fox samples for isotopic discrimination by using values previously calculated in captive red foxes, i.e., hair: 2·6‰ for $\delta^{13}C$ and 3·2‰ for $\delta^{15}N$ ratios, muscle tissue: 1·1‰ for $\delta^{13}C$ and 3·3‰ for $\delta^{15}N$ ratios (Roth & Hobson, 2000). For Arctic foxes, we used values from Lecomte *et al*. (Lecomte et al., 2011), i.e., hair: 2·2‰ for $\delta^{13}C$ and 3·3‰ for $\delta^{15}N$ ratios, muscle tissue: 0·4‰ for $\delta^{13}C$ and 1·8‰ for $\delta^{15}N$ ratios. We accounted for uncertainty in fractionation estimates in our mixed model analysis for both fox species using estimates from Lecomte *et al*. (Lecomte et al., 2011) for muscle (SD of ^{13}C = 0.1‰ and SD of ^{15}N = 0.5‰) and for hair (SD of ^{13}C = 0.4‰ and SD of ^{15}N = 0.6‰). We used SIMMR (R-package SIMMR), a Bayesian stable isotope mixing model, to estimate the proportional contributions of each dietary endpoint (terrestrial, marine, and migratory) to fox diets. Each model consisted of four Markov Chain Monte Carlo of 1,000,000 iterations, tinned by 100 and with an initial discard of the first 1000 iterations. We performed all statistical analyses for prevalence, risk factors and stable isotope analyses using R (R-Core-Team, 2019).

3.4. **Results**

3.4.1. **Agreement between ELISA and MC-qPCR**

Twenty-six foxes were positive on serology and negative on molecular test, one was serologically negative but tissue positive, 66 were positive on both, and 135 were negative on both. There was statistical difference between serological and molecular results (X^2=21.3, *df*=1, p<0.001, n=228), but substantial agreement between the two tests (k=0.74). As a result, for subsequent data analyses, a fox was considered positive if it was positive on either serology or molecular testing.

3.4.2. **Detection of *T. gondii* antibodies and DNA**

Antibodies to *T. gondii* were detected in 40% ($CI_{95\%}$: 34-47) of foxes using ELISA. *Toxoplasma gondii* DNA was detected in 29% ($CI_{95\%}$: 23-35) of foxes using MC-qPCR. As previously described (Bouchard et al., 2022c), seropositivity and tissue prevalence was higher in Hudson Bay versus Ungava Bay foxes (Table 3.2).

Table 3.2 Prevalence (% animals positive) of antibodies to *Toxoplasma gondii* in blood and DNA of *T. gondii* in brain and heart, and demographic variables in foxes (*Vulpes* spp; n=239) harvested in Nunavik, QC.

		Seroprevalence[a] ($CI_{95\%}$) / N	**Tissue prevalence ($CI_{95\%}$) / N**
Species	Red fox	38% (32-45)/188	25% (20-32)/197
	Arctic fox	50% (35-65) /40	45% (31-60)/42
Sex	Male	41% (33-49) /144	28% (21-36) /150
	Female	39% (30-50) /84	31% (22-41) /88
Regions	Ungava Bay, QC	29% (22-36) /154	19% (14-25) /165
	Hudson Bay, QC	65% (54-75) /74	51% (40-62) /74
Total		40% (34-47) /228	29% (23-35) /239

[a] Individuals not tested (no heart fluid) = 11

N: Number of individuals tested

CI: Confidence intervals

3.4.3. Risk factors for *T. gondii* exposure

3.4.3.1. Linear regression for $\delta^{13}C$ and $\delta^{15}N$

Male foxes had a significantly lower input of $\delta^{13}C$ compared to female, and positive foxes had a significantly higher input of $\delta^{13}C$ as well as $\delta^{15}N$ (Fig. 3.3, Table 3.3), in both late summer/fall and winter diet.

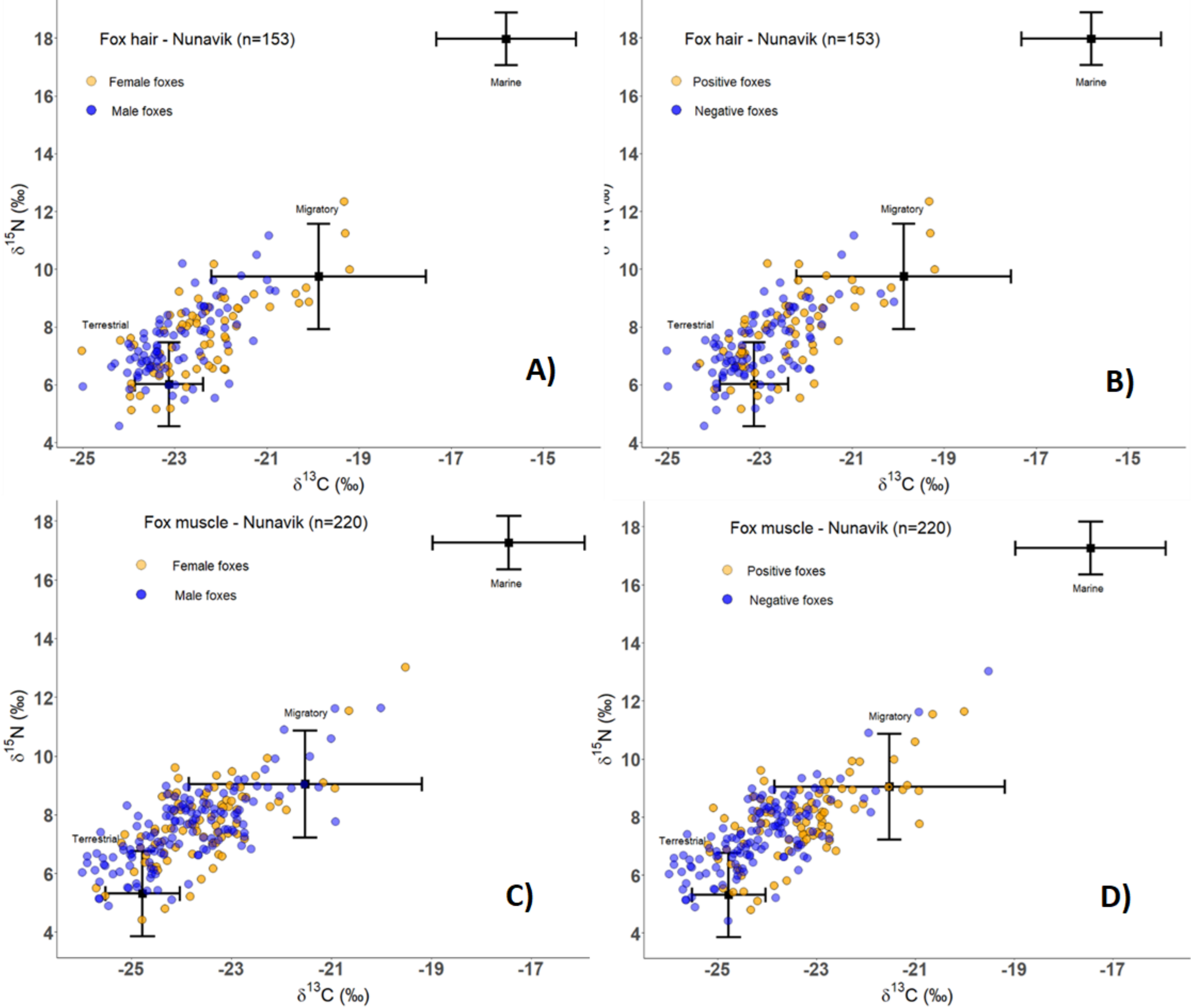

Figure 3.3 Comparisons of $\delta^{13}C$ and $\delta^{15}N$ ratios of dietary endpoints for Nunavik foxes in late summer/fall (based on hair) and winter (based on muscle) for sex (A and C), and *Toxoplasma gondii* infection status (B and D). For both periods, female foxes consumed more marine food sources, and positive foxes were more likely to consume migratory and marine prey ($\delta^{13}C$) on different trophic levels ($\delta^{15}N$).

Table 3.3 Summary of species, sex, region and status of infection effects on dietary endpoints contributions to fox diet (*Vulpes* spp.) for late summer/fall and winter seasons in Nunavik, QC.

	$\delta^{13}C$			**$\delta^{15}N$**		
	β (SE)	*df*	p	β (SE)	*df*	p
Late summer/fall diet						
Species, Red fox	-0.21 (0.22)	1	0.339	-0.01 (0.30)	1	0.981
Sex, Male	-0.55 (0.16)	1	<0.001*	-0.26 (0.21)	1	0.228
Region, Ungava	0.22 (0.23)	1	0.346	0.24 (0.28)	1	0.389
Status of infection	0.63 (0.18)	1	<0.001*	0.68 (0.24)	1	0.004*
Winter diet						
Species, Red fox	-0.22 (0.18)	1	0.243	-0.03 (0.23)	1	0.896
Sex, Male	-0.28 (0.14)	1	0.044*	-0.22 (0.18)	1	0.219
Region, Ungava	-0.36 (0.20)	1	0.081	-0.21 (0.22)	1	0.333
Status of infection	0.55 (0.16)	1	<0.001*	0.43 (0.19)	1	0.025*

β: Estimate coefficient

SE: Standard error

df: Degree of freedom

* Statistically significant at $p<0.05$

3.4.3.2. **Logistic regression: late summer/fall diet**

When fox species were combined, foxes consuming migratory geese in summer and fall were 2 times more likely to be positive for *T. gondii* (odds ratio:1.86, $CI_{95\%}$:1.17-2.98, p=0.009, Table 3.4). For red foxes, the odds of being positive for *T. gondii* were 3 times higher in foxes consuming marine food sources (odds ratio:2.95, $CI_{95\%}$:1.43-6.05, p=0.003, Table 3.4). Red foxes consuming migratory geese were also less likely to be positive for *T. gondii* compared to Arctic foxes (Table 3.4).

3.4.3.3. **Logistic regression: winter diet**

When fox species were combined, foxes consuming migratory geese/eggs in winter were 1.3 times more likely to be positive for *T. gondii* (odds ratio:1.27, $CI_{95\%}$:1.02-1.58, p=0.030, Table 3.4). In red fox, the risk of being positive for *T. gondii* was higher in foxes from Hudson versus Ungava Bay. As well, red foxes consuming marine food sources were 1.3 times more likely to be exposed to *T. gondii* (odds ratio:1.29, $CI_{95\%}$:1.03-1.63, p=0.029, Table 3.4). Red foxes consuming migratory geese were also less likely to be positive for *T. gondii* compared to Arctic foxes (Table 3.4).

Table 3.4 Coefficients of the final logistic regression model and risk factors associated with *Toxoplasma gondii* prevalence in foxes (*Vulpes* spp.) from Nunavik, QC.

Variables late summer/fall diet	**β (SE)**	**p-value**	***df***	**OR ($CI_{95\%}$)**
Species, Red fox	3.81 (2.80)	0.174	1	44.96(0.19-1.08e+04)
Region, Ungava	-1.59 (1.30)	0.223	1	0.20(0.02-2.62)
Diet marine	-0.82 (0.33)	0.014*	1	0.44(0.23-0.85)
Diet migration	0.62 (0.24)	0.009*	1	1.86(1.17-2.98)
Species^Region, Red fox Ungava	-0.09 (1.40)	0.950	1	0.92(0.06-14.33)
Species^Dietmarine, Red fox	1.08 (0.37)	0.003*	1	2.95(1.43-6.05)
Species^Dietmigration, Red fox	-0.64 (0.25)	0.009*	1	0.53(0.32-0.85)
Variables winter diet				
Species, Red fox	5.71 (3.47)	0.100	1	303.29 (0.34-2.74e+05)
Region, Ungava	0.64 (0.91)	0.484	1	1.89 (0.32-11.26)
Dietmarine	-0.05 (0.05)	0.308	1	0.95 (0.86-1.05)
Dietmigration	0.24 (0.11)	0.030*	1	1.27 (1.02-1.58)
Species^Region, Red fox Ungava	-1.45 (1.00)	0.148	1	0.23 (0.03-1.67)
Species^Dietmarine, Red fox	0.26 (0.12)	0.029*	1	1.29 (1.03-1.63)
Species^Dietmigration, Red fox	-0.24 (0.12)	0.050*	1	0.78 (0.61-1.00)

^ : interaction term

β: Estimate coefficient

SE: Standard error

df: Degree of freedom

OR: Odd Ratio

$CI_{95\%}$: 95% confidence interval

*Statistically significant at $p<0.05$

3.5. **Discussion**

This study combine ecological trophic analysis (through stable isotope signatures) with infection status of a food borne pathogen in terrestrial mammals, demonstrating that red and Arctic foxes positive for *T. gondii* were more likely to consume marine and migratory prey, respectively (versus rodents). Arctic and red foxes were previously hypothesized to be good sentinels for *T. gondii* circulation in northern ecosystems as they are widespread across northern Canada, exposed at similar rates and routes as humans in many northern regions, and can be exposed to both oocysts shed into the environment by felids and tissue cysts in consumed prey (Bachand et al., 2018; Bouchard et al., 2022c). Stable isotope analysis is an ecological approach to assess the structure of the food web. The likelihood of a fox being positive for *T. gondii* increased significantly with stable isotope values of $\delta^{15}N$ (Table 3.3). This suggests that positive foxes feed at higher trophic levels, and/or on prey with high values of $\delta^{15}N$ that are more likely to be infected with the parasite (i.e., other carnivores). This is consistent with transmission through carnivory or scavenging, supporting our hypothesis that this is a major route of exposure for foxes versus direct oocyst transmission from the environment. Since lemmings are year round resident herbivores, exposure through this particular prey is less likely; *T. gondii* infection in rodents has not been reported in arctic tundra ecosystems (Elmore et al., 2015; Prestrud et al., 2007).

Although ultimately of terrestrial origin, *T. gondii* can infect and concentrate in marine organisms, playing a significant role in waterborne transmission (Shapiro et al., 2019a) - a “pathogen pollutant” in marine ecosystems. Sporulated oocysts persist in marine environments and are resistant to temperature variations (Lindsay & Dubey, 2009). Red foxes consuming marine food sources in late summer/fall and winter were more likely to be exposed to the parasite. Spillover to terrestrial wildlife and humans through consumption of marine wildlife in the Arctic is often regarded as a possible hypothesis for transmission (Ducrocq et al., 2021; Messier et al., 2009; Simon et al., 2013b). Coastal areas, in particular, often receive substantial inputs of energy and nutrients from the ocean, and these resources can support large numbers of consumers (Roth, 2003). Roth *et al*. (Roth, 2002) found that the stable-carbon isotope ratios of Arctic fox hair indicated the diet was much more marine in winter, probably due to the presence of sea ice giving access to marine food sources such as carcasses of seals killed by polar bears and/or hunters. Killengreen *et al*. (Killengreen et al., 2011) also found that red foxes close to the coast in winter

had strong isotopic signatures of marine components, and were relying on these resources when terrestrial prey became too scarce. We found that female foxes had a higher input of $\delta^{13}C$, indicating that female foxes consumed more marine food sources. This may be due to higher metabolic demands for female foxes raising pups, resulting in females staying closer to coastal areas than males, who spend more time dispersing and moving between territories (Walton et al., 2021).

Foxes consuming migratory food sources for late summer and fall diet were twice as likely to be positive for *T. gondii*, especially Arctic foxes. This is not unexpected given previous epidemiological studies showing that migratory geese in Nunavik harbor *T. gondii*, that Inuit consuming waterfowl are at higher risk of being exposed to the parasite, and that migratory birds are a likely source of *T. gondii* exposure for foxes (Bachand et al., 2019; Ducrocq et al., 2021). Foxes in our study area would have access to geese as food sources from May until late August, as the highest densities of breeding Canada geese are found in the two main regions of Nunavik where foxes were trapped: coastal lowlands of eastern Hudson Bay and coastal lowlands of southwestern Ungava Bay (Cotter et al., 2013). Although Arctic foxes are thought to rely heavily on fluctuating rodent populations, having a regular large influx of birds (and cached eggs) provides foxes with predictable food resources (Careau et al., 2007; Samelius & Alisauskas, 2000; Samelius et al., 2007). This behavior could explain the higher prevalence of *T. gondii* in Arctic vs red foxes in our study (Table 3.2), with greater reliance on these migratory food sources.

We observed higher sero- and tissue prevalence in foxes from Hudson Bay (65% and 51%, respectively) compared to Ungava Bay (29% and 20%, respectively). A similar pattern is also seen in people, with a seroprevalence of 56% in Hudson Bay and 36% in Ungava Bay (Ducrocq et al., 2021). In people, this was explained by a higher consumption of marine mammals, fish, and birds in Hudson Bay compared to Ungava Bay, where land animals seem to be the preference (Blanchet & Rochette, 2008; Ducrocq et al., 2021). We hypothesize that the same scenario could be happening in foxes, with those in Hudson Bay having greater access to migratory birds, fish, and carcasses of marine mammals, which could be an important source of *T. gondii*. As well, contamination of fishes and marine mammals could be related to the geography of the watersheds that irrigate Nunavik. The Hudson watershed originates mostly from subarctic and boreal regions,

where lynx could be shedding oocysts in the environment, while the Ungava watershed is restricted to the tundra for the most part (Ducrocq et al., 2021; Québec, 2020; Simon et al., 2013b).

Study limitations include disagreement between detection methods for *T. gondii:* 26 foxes were positive on serology, but DNA was not detected in tissues. This discrepancy is not unexpected and could be explained by antibodies out-living active infection, low tissue infection intensity (below the detection limit of the molecular technique), and/or non-uniform distribution of tissue cysts of *T. gondii*. As well, false-positive results could have happened due to the high blood content in heart juice which may have interfered with antibody binding (Mecca et al., 2011), or from cross-contamination between samples (less likely since negative controls remained negative). Only one fox was positive for DNA in tissues and negative on serology. This individual could be acutely infected and had not yet developed antibodies, or harbor a senescent infection i.e., antibodies against *T. gondii* have declined, with tissue cysts persisting in a non-immunogenic state (Opsteegh et al., 2010; Robert-Gangneux & Darde, 2012). As well, the use of stable isotope analysis is an indirect method for determining animal diets. As there is no *a priori* information available for the diet of foxes in the present study, we should be cautious when interpreting the results since there can be considerable inter- and intra-population variability in diet (Phillips et al., 2014). Finally, we may have missed food sources in our choice of dietary endpoints that could have been relevant for foxes at the local scale (e.g., scavenging on caribou carcasses). Still, our findings support that stable isotope analysis is a powerful tool for tracking food-borne parasite transmission through food webs.

3.6. **Broader applications and future work**

Using stable isotope analysis to reveal connections between terrestrial, migratory, and marine components in food webs can have far-reaching implications for understanding circulation of food-borne pathogens such as *T. gondii*. While parasites and trophic relationships have been studied before (Sabadel et al., 2018; Wilson et al., 2020), very few have looked at linking status of infection by a protozoan with trophic relationships using stable isotopes (Cabezón et al., 2016). Although our work relied on tissue collection post-mortem, our results suggest that non-invasive hair sampling coupled with serology holds promise for *in vivo* approaches to source attribution of food-borne pathogens, especially in wildlife of conservation significance, and in people. We found

that migratory and marine food sources are a significant contributor to the transmission of *T. gondii* in northern regions. As a consequence of temperature and precipitation change, with increased climate variability and extreme weather events, the ecology of *T. gondii* is expected to shift, including routes of transmission (Patz et al., 2000). The melting of ice and permafrost, in addition to increased precipitation, will likely boost the transport of oocysts in northern ecosystems. As well, warmer temperatures will favor oocyst survival and development (Lindsay et al., 2002). As the tree line moves northward, the habitat range of lynx and their prey species will likely follow (Lenoir et al., 2008).

Combining both microbial and ecological methods provided important tools to unravel the complex transmission of a ubiquitous food-borne pathogen in a remote environment. The coupling of such approaches is key to understanding how intimately linked parasites such as *T. gondii* are within food webs. Understanding current trophic relationships and parasite transmission in foxes as a sentinel system will allow us to detect and predict changes in a rapidly warming Arctic, including altered zoonotic risk for northern human populations.

3.7. **Acknowledgements**

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3.8. Supplementary information

Table S1. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios (‰, mean ± SD) of food sources used in stable isotope analyses of foxes with corresponding tissue types and sample sizes.

Food sources	Dietary endpoints	$\delta^{13}C$, ‰	$\delta^{15}N$, ‰	Tissue type	Sample size
Lemming	Terrestrial	-25.52 ± 0.7	2.76 ± 1.5	Hair	3
Goose	Migratory	-22.53 ± 1.3	6.45 ± 1.0	Muscle	26
Fish	Marine	-18.2 ± 1.5	14.71 ± 0.9	Muscle	13

Table S2. A) Summary of seasonal stable isotope analyses between regions in both species of foxes from Nunavik, QC. Results are given as mean proportion of dietary endpoints in the diet of foxes at the population scale.

Dietary endpoints	Late summer/fall diet				Winter diet			
	Red fox		Arctic fox		Red fox		Arctic fox	
	x̄	$CI_{95\%}$	x̄	$CI_{95\%}$	x̄	$CI_{95\%}$	x̄	$CI_{95\%}$
Ungava Bay	n=99		n=12		n=143		n=19	
Terrestrial	88	(82-91)	82	(66-93)	86	(81-90)	67	(43-82)
Migratory	4	(1-11)	11	(2-30)	6	(1-14)	20	(3-52)
Marine	8	(5-10)	6	(1-13)	8	(5-10)	13	(3-22)
Hudson Bay	n=30		n=12		n=38		n=21	
Terrestrial	84	(73-92)	75	(58-87)	77	(63-86)	56	(36-70)
Migratory	9	(1-23)	16	(2-37)	14	(2-15)	22	(3-52)
Marine	7	(2-12)	9	(2-16)	9	(3-15)	22	(11-30)

Table S2. B) Summary of seasonal stable isotope analyses between *Toxoplasma gondii* exposure status in both species of foxes from Nunavik, QC. Results are given as mean proportion of dietary endpoints in the diet of foxes at the population scale.

Dietary endpoints	Late summer/fall diet				Winter diet			
	Red fox		Arctic fox		Red fox		Arctic fox	
	x̄	$CI_{95\%}$	x̄	$CI_{95\%}$	x̄	$CI_{95\%}$	x̄	$CI_{95\%}$
Positive foxes	n=50		n=11		n=69		n=19	
Terrestrial	82	(72-88)	72	(55-85)	76	(64-84)	56	(35-70)
Migratory	8	(1-23)	18	(3-40)	13	(2-33)	24	(3-54)
Marine	10	(5-15)	9	(2-17)	10	(4-15)	20	(10-29)
Negative foxes	n=79		n=13		n=112		n=21	
Terrestrial	91	(85-95)	82	(67-93)	89	(84-92)	64	(41-80)
Migratory	4	(1-11)	12	(2-29)	5	(1-13)	22	(3-54)
Marine	5	(2-8)	6	(1-13)	6	(3-8)	14	(4-24)

TRANSITION STATEMENT CHAPTER 4

My previous work in Chapters 2 and 3 demonstrated that foxes are likely getting infected with *T. gondii* through carnivorism, act as sentinel intermediate hosts in northern environments, and that migratory and marine food sources likely play an important role in transmission. The coupling of microbial and ecological methods was critical in unravelling complex trophic relationships between foxes and their prey species, and understanding better how this parasite can thrive in northern ecosystems. The remaining missing piece of the puzzle of *T. gondii* transmission in northern ecosystems was to determine if lynx are serving as definitive hosts that can produce and shed oocysts in the tundra regions. In Chapter 4, I seek evidence of intestinal infection with *T. gondii*, as lynx are the proposed definitive host in subarctic and boreal regions. Efforts to detect oocysts in feces of lynx have not yet been successful. Therefore, I used fecal based PCR methods which have increased sensitivity compared to fecal flotation and allow characterization of genotypes. Finally, although previous work has shown that lynx were exposed to *T. gondii* via serology, this is the first effort to use molecular methods to detect DNA of *T. gondii* in tissues of lynx, demonstrating their dual role in the life cycle as both definitive and intermediate hosts. This chapter is published in a special issue of Zoonotic Diseases. As first author, I wrote the paper, conducted field, laboratory, and data analyses, and coordinated information and comments from coauthors instrumental to collection of samples from Yukon and Québec.

CHAPTER 4: CANADA LYNX (*LYNX CANADENSIS*) AS POTENTIAL RESERVOIRS AND SENTINELS OF *TOXOPLASMA GONDII* IN NORTHERN CANADA

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4.1. **Simple Summary**

Toxoplasma gondii is considered one of the most successful parasites in the world, potentially infecting any vertebrates, including humans. Its complex life cycle encompasses many transmission pathways, which remain enigmatic in northern ecosystems. The aim of our study was to assess Canada lynx as potential reservoir of and sentinel for *T. gondii* by 1) using serological and molecular assays to detect *T. gondii*, 2) determining if lynx are definitive hosts for the parasite, and 3) identifying potential risk factors that may contribute to lynx exposure. For the first time, *T. gondii* DNA was found in tissue and DNA consistent with that of *T. gondii* was detected in feces of Canada lynx, indicating that they can be both intermediate and definitive hosts in northern regions. Our findings would help identify high-risk regions for infections in northern Canada, with lynx serving as indicator for potential human exposure of *T. gondii*.

4.2. **Abstract**

Toxoplasma gondii is a zoonotic parasite globally infecting a wide range of species, including humans. Felids are the only known hosts that can excrete environmentally resistant oocysts into ecosystems. In boreal regions, Canada lynx (*Lynx canadensis*) are sought by hunters primarily for their fur, and they are occasionally eaten. We examined carcasses salvaged from trappers from boreal regions of eastern (n=97) and western (n=357) Canada. We detected *T. gondii* antibodies in fluid from thawed heart tissue using a commercial enzyme-linked immunosorbent assay, DNA in heart and brain via magnetic capture and real-time PCR assay, and presence of DNA in feces using a real-time PCR with melt curve analysis. We detected antibodies against *T. gondii* and DNA in tissues in 24% and 19% of lynx, respectively. We detected DNA consistent with that of *T. gondii* in feces of Canada lynx, which could indicate intestinal infection and potential for shedding oocysts. Our results indicate that lynx may be a useful sentinel species for monitoring environmental circulation of *T. gondii* in northern boreal regions, and may pose a risk for transmission to other wildlife and to people handling or consuming lynx.

Keywords: Canada lynx, ELISA, MC-qPCR, melting-curve analysis, sentinel species, *Toxoplasma gondii*, zoonosis

4.3. **Introduction**

Toxoplasma gondii is a global, zoonotic protozoan parasite, capable of infecting and adversely impacting a wide range of vertebrates, especially if the immune system is compromised or if a mammal becomes infected while pregnant (Tenter et al., 2000). Three infective stages characterized *T. gondii*: sporulated oocysts, bradyzoites and tachyzoites. The life cycle of this parasite is complex; felids are the only natural definitive hosts excreting environmentally resistant oocysts in their feces, and virtually all warm-blooded animals may act as intermediate hosts, following consumption of sporulated oocysts (from contaminated water/soil) or tissue cysts containing bradyzoites in other infected hosts (Dubey, 2010). Therefore, once introduced into a food web, *T. gondii* can be maintained through trophic interactions (such as predation) and vertical transmission (tachyzoites from mother to foetus).

In addition to the domestic cats as definitive hosts, wild Felidae have the potential to shed millions of oocysts into the environment (Miller et al., 1972). For instance, following a waterborne outbreak of toxoplasmosis in humans (Bowie et al., 1997), *T. gondii* was isolated from feces of two naturally infected cougars (*Puma concolor*) from British Columbia, Canada (Aramini et al., 1999). Ingestion of infected tissues is likely the most efficient means of transmission of *T. gondii* among felids (García-Bocanegra et al., 2010; Hatam-Nahavandi et al., 2021). Canada lynx (*Lynx canadensis*) likely acquire infection from consuming infected prey, which themselves may become infected by consumption of oocysts in the environment or possibly by vertical transmission. Lynx primarily prey on snowshoe hares (*Lepus americanus*) and red squirrel (*Tamiasciurus hudsonicus*). Although infrequent, lynx can also demonstrate cannibalism or scavenging behavior, especially in years of food shortage (Jung, 2021; Mowat et al., 2000; Peers et al., 2020). Lynx are hunted primarily for their fur, but are also occasionally consumed by local people (Zarnke et al., 2001). Thus, infected lynx could represent a risk of transmission of *T. gondii* to people that consume their meat (containing tissue cysts) if not thoroughly frozen or cooked.

In the Nearctic boreal region, Canada lynx are often the only wild felid present. To date, efforts to detect oocysts in feces of Canada lynx have not been successful (Simon et al., 2013a), possibly due to the short period of time that oocysts are shed, and the difficulty in detecting the small

oocysts on routine fecal examination. Previous studies have reported Canada lynx seropositive for *T. gondii*, indicating exposure to the parasite (Al-Adhami et al., 2016; Labelle et al., 2001; Simon et al., 2013a), but active tissue infection based on detection of DNA in tissues has not been demonstrated. Therefore, this study was conducted with the primary objective to understand whether lynx could act as a definitive and intermediate host for, as well as sentinels of, *T. gondii* in northern Canada. We performed an enzyme-linked immunosorbent assay (ELISA) on fluid from thawed heart; magnetic capture and real-time (MC-PCR) technique on tissues; a real-time quantitative PCR (qPCR) assay with fluorescent melting curve analysis (MCA) on fecal samples that can detect, differentiate, and identify DNA from multiple coccidian species, including *T. gondii* (Lalonde & Gajadhar, 2011); and fecal flotation on suspicious samples. Association between *T. gondii* exposure and potential risk factors was also assessed.

4.4. **Materials and Methods**

4.4.1. **Sample locations and lynx sampling**

During the fur trapping seasons of 2015-2020, we collected carcasses of skinned lynx or gastrointestinal tracts (GIT) from licensed fur trappers in boreal regions of eastern (Labrador and Québec, n=97) and western (Yukon, n=357) Canada (Figure 1). Carcasses and GIT were kept frozen at -20°C until shipped to the Department of Environment in Whitehorse, Yukon; the Western College of Veterinary Medicine in Saskatoon, Saskatchewan; the Faculté de Médecine Vétérinaire in Saint-Hyacinthe, Québec; the Nunavik Research Centre in Kuujjuaq, Québec; or the wildlife division office at the Department of Fisheries, Forestry and Agriculture in Goose Bay, Newfoundland and Labrador. We collected whole hearts, brains, and feces, and recorded sex, age, body condition, and harvest location when possible. We determined sex during necropsy by inspecting internal reproductive organs (Jung et al., 2020). We counted cementum annuli (Matson's laboratory, Manhattan, Montana, USA) to determine age, or used the total body length when not possible. We classified lynx as juveniles (≤1 year old) and adults (≥2 years old) (Slough, 1996). A body condition index (BCI) was obtained by visually estimating the relative amount of fat deposits, using a scale of 1–3 as follows: 1, no to very little visceral fat deposits in abdominal and peritoneal cavity; 2, moderate visceral fat deposits; 3, abundant visceral fat deposits, as described in Bouchard et al. (2022).

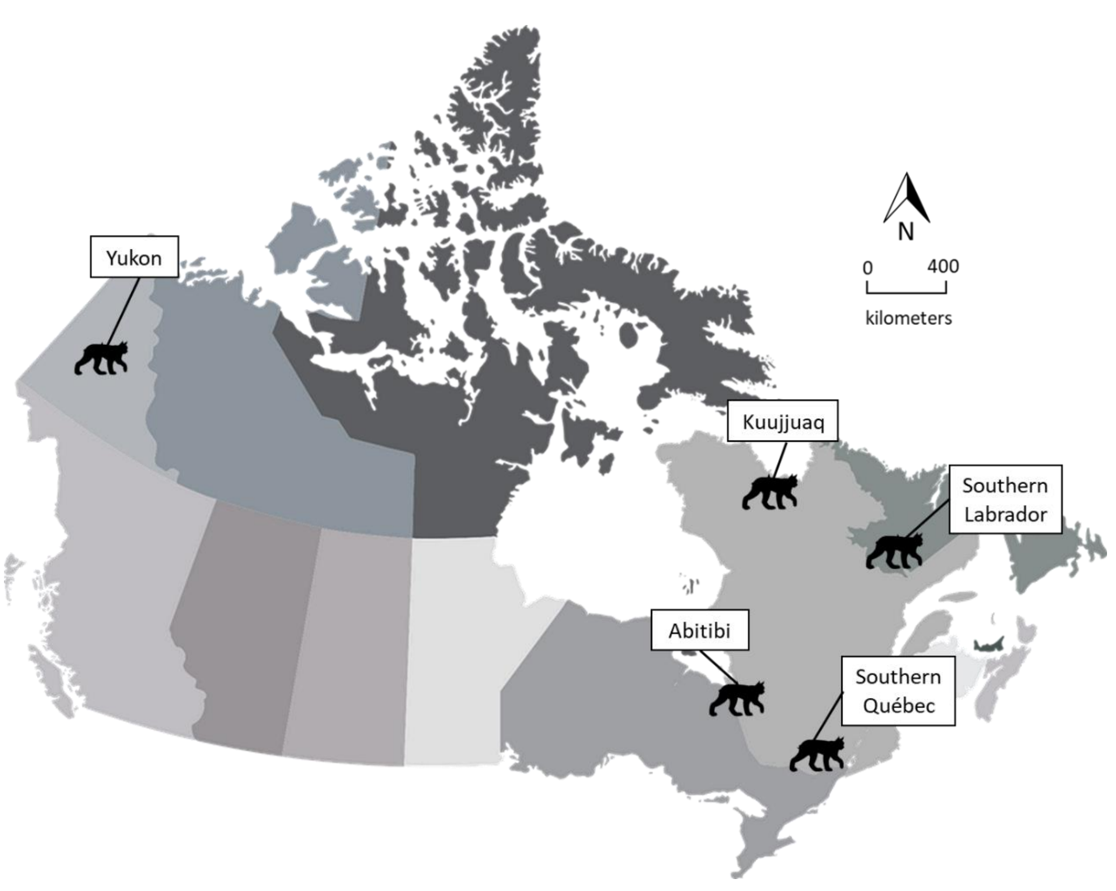

Figure 4.1 Regions where lynx (*Lynx canadensis*) were collected during 2015–2020 by trappers from eastern and western Canada.

4.4.2. **Serological analysis**

Since commercially available serological assays are not validated in lynx, we evaluated (using a subset of samples) heart fluid dilutions for two different techniques, ELISA and the indirect fluorescent antibody test (IFAT), using magnetic capture (MC)-qPCR as the gold standard. As validated in Sharma et al. (2019) and described by Bouchard et al. (2022), we detected antibodies to *T. gondii* in fluid from thawed hearts (diluted 1:2) using the commercially available ID Screen® Toxoplasmosis Indirect Multi-species Enzyme-linked immunosorbent assay (ELISA) (IDvet, Grabels, France). Heart fluid was collected from the heart of individual lynx and processed as per the work of Bouchard et al (2022) before transferring to a labeled 1.5-ml Eppendorf tube. Heart fluids were stored at 4 °C for use within the next 3 days or at − 20 °C until used for testing.

4.4.3. **Molecular analysis**

4.4.3.1. **Detection of DNA of *T. gondii* in tissue samples**

As validated by Opsteegh et al. (2010) and described by Bouchard et al. (2022), we extracted sequence-specific DNA of *T. gondii* from whole heart and brain combined (or heart only if brain was not available) for each lynx using the MC-qPCR technique to determine infection status using the Tox 9F (5'-aggagagata tcaggactgtag-3´) and Tox 11R (5´-gcgtcgtctc gtctagatcg-3´) primers, for the detection of the 188 bp *T. gondii* sequence within the 529 repeat-element. We quantified parasites using the following formula (1):

$$\log10\ (\text{tachyzoites}) = ((43.3 – Cq)\ /3.07) \qquad (1)$$

We expressed this value as the number of tachyzoite-equivalents (TE) and calculated the intensity of infection by dividing TE by the weight of the tissue processed, expressed as tachyzoite equivalents per gram (TEG) (Bachand et al., 2019).

As described in the work of Bouchard et al. (2022), we sent frozen aliquots of template DNA (50μL) to a specialized laboratory (Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA) for further genetic characterization, using a multiplex multilocus nested PCR-RFLP with ten genetic markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico) (Su et al., 2010).

4.4.3.2. **Detection of DNA of *T. gondii* in fecal samples**

Genomic DNA from 300 mg of fecal sample was extracted using the Fast-DNA kit for feces (MP Biomedicals, Sohon, Ohio, USA), followed by a PCR Purification kit (Qiagen, Valencia, California, USA) to remove any additional inhibitors. We detected coccidian species in each fecal sample using a universal coccidia primer cocktail designed to amplify a ~315-bp region of 18S rDNA in a real-time quantitative PCR assay (Lalonde & Gajadhar, 2011). Genetically distinct coccidian species can be differentiated by the melt curve shape and the PCR product melting temperature (on the basis of the nucleotide sequence, length, and G-C content) and identified by comparison to in-run controls and/or sequencing (Lalonde & Gajadhar, 2011). We performed all PCR analyses using the Bio-Rad CFX 96 DNA thermal cycler (Bio-Rad, Hercules, California, USA) as described previously (Lalonde & Gajadhar, 2011). The final PCR assay reaction included 1X EvaGreen Supermix (Bio-Rad), 20mg/ml of bovine serum albumin (Sigma), 400 nM of Crypto-F, Crypto-R, Cyclo-F, Cyclo-R, Toxo-R, and Sarco-R, 800 nM of Toxo-F, 2 ml of DNA, and PCR-grade water adjusted to a final volume of 25 ml. Each PCR reaction plate included a negative and two no-template controls, a standard curve consisting of DNA from *Hammondia* (10^5 to 10^1 oocysts) provided by the Centre for Food-Borne and Animal Parasitology (CFAP), and wells containing DNA from *T. gondii* (genomic, American Type Culture Collection (ATCC), Virginia, USA), *Cryptosporidium parvum* (genomic, ATCC, Virginia, USA), and *Cystoisospora* (genomic, ATCC, Virginia, USA) as positive controls. All samples were run in duplicates. Melt curves from positive samples were visually compared to the controls for preliminary identification, and amplified DNA from positive reactions were sequenced (Macrogen, Seoul, South Korea) for confirmation using original primers. Forward and reverse sequences were assembled with PreGap4 and Gap4 (Staden Package) and consensus sequences were compared with reference sequences in GenBankTM using the nucleotide Basic Local Alignment Search Tool (BLASTN, https://blast.ncbi.nlm.nih.gov/).

For one positive sample, the sequence was assembled in CLC Main workbench and exported for BLAST and alignment. For alignment and analysis, the reference sequences of Ogedengbe et al. (2016) were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). These were aligned to the sample sequence using the

online version of MAFFT (https://mafft.cbrc.jp/alignment/server/) with default parameters. The resulted matrix was viewed, edited and truncated in MEGA7 (Kumar et al., 2016). A maximum likelihood analysis was conducted in RAxML8 (Stamatakis, 2014) using the GTRCAT approximation and invoking the autoMRE bootstopping criterion for calculating bootstrap support. The resulting tree was viewed in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and finalised in Corel PaintshopPro X8.

4.4.4. **Fecal flotation**

To detect oocysts of *T. gondii* in feces, fecal flotation was performed. We thawed, weighed, and mixed 4 g of feces thoroughly in a paper cup with 40 ml of Sheather's sucrose solution to create a homogenized mixture. After sieving the fluid through a cheesecloth into a second cup, we poured a 10 ml aliquot (~25%, representing 1 gram of feces) into a test tube then topped it up with ~5 ml of Sheather's sucrose solution to form a slight convex meniscus. We placed a coverslip on top and centrifuged the tube at 491 rcf for 10 min, after which we lifted the coverslip and placed it on a glass slide. We viewed slides under the microscope at 10–40X magnification, and identified coccidian oocysts based on size and morphology (Dryden et al., 2005).

4.4.5. **Statistical analysis**

4.4.5.1. **Serological and molecular test agreement**

We compared the proportion of positive results between the ELISA and MC-qPCR, using McNemar's Chi-square tests for paired data. If not significantly different, we used the kappa coefficient (k) to determine the level of agreement between the two detection methods. We performed analyses using IBM SPSS (ver. 26; Armonk, New York, USA).

4.4.5.2. **Association between quantitative MC-qPCR and ELISA results**

We used a Spearman's rank-order correlation coefficient to determine the relationship between antibody concentration (S/P%) and cycle quantification (Cq) values obtained from ELISA and MC-qPCR, respectively.

4.4.5.3. **Prevalence and risk factors**

An animal was considered sero-positive and tissue positive if found positive using ELISA and MC-qPCR, respectively. Seroprevalence and tissue prevalence, along with 95% confidence intervals (CI), were calculated online from the proportion of positive results using EpiTools (Sergeant, 2018). The term "prevalence" indicates proportion of positive results (positive on at least one test).

We built binary logistic regression models to test for (1) the presence of antibodies to *T. gondii* (sero-positive or sero-negative), (2) the presence of *T. gondii* DNA (tissue-positive or tissue-negative), and (3) the presence of antibodies to and/DNA of *T. gondii* (at least positive on one test or negative on both tests). We evaluated age-class (juvenile and adult), sex (female and male), body condition score (low, medium, and high), and location (eastern or western Canada) for inclusion in our final multivariable model. Stepwise forward multivariable regression analysis was performed to include risk factors and confounders in the final model. Variables associated at a significance level of $p < 0.05$ were retained in the final model. Goodness-of-fit of the final model was evaluated by the Hosmer Lemeshow test. To estimate the degree of the association between each predictor and *T. gondii* status, we calculated odds ratios and respective 95% confidence intervals. We performed all statistical analysis using IBM SPSS (ver. 24; Armonk, New York, USA).

4.4.5.4. **Infection intensity and risk factors**

To evaluate the effects of predictors on the infection intensity of *T. gondii*, we used a generalized linear model (linear regression) in IBM SPSS (ver. 26; Armonk, New York, USA). We excluded age and BCI as there were insufficient data collected for these variables. Infection intensity of *T. gondii* (tachyzoite-equivalents per gram) was not normally distributed, so we log 10 transformed the data to approximate normality (negative lynx were not included in the analysis). An omnibus test was performed to evaluate the model coefficients.

4.5. Results

4.5.1. Descriptive analysis

In total, 409 whole lynx carcasses and 45 GIT (28 from southern QC, 17 from Yukon) were collected from the year 2015 to 2021. Male to female ratio was 3:2 (238 of 397 [60%] vs 159 of 397 [40%]); information on sex was not available for 57 lynx). BCI score was determined for 303 lynx; the highest proportion (61%, 186 of 303) of lynx had a medium BCI score, followed by low (30%, 92 of 303) and high (8%, 25 of 303). More juvenile than adult were collected (64% vs 36%; age was determined only for 100 lynx). Most lynx (79%: 357 of 454) were from western Canada. Moreover, the number of lynx varied annually (range = 28-137), with no carcass submitted during the winters of 2015-2016 and 2020-2021 from eastern Canada (Table 4.1).

Table 4.1 Number of lynx (*Lynx canadensis*) per region for each sampling year in Canada.

Region	Year						Total
	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020	2020-2021	
Kuujjuaq, QC	0	1	0	1	5	0	7
Abitibi QC	0	0	50	0	0	0	50
Southern QC	0	28[1]	0	0	0	0	28
Southern Labrador	0	0	0	10	2	0	12
Yukon	28	99	87	46	51	46	357

[1] Only GIT collected (due to logistic limitations)

4.5.2. **Cut-off values and best-performing serological assay**

Samples from nine lynx known to be positive and negative on magnetic capture were used to determine dilution cut-off values, as described in Bouchard et al. (2022). Since the IFAT results were discordant for dilution 1:2, 1:8, and 1:128, dilution 1:32 was used for further analyses. Since all dilutions on ELISA gave similar results for all individuals, we used the cut-off value of 1:2 as recommended by the manufacturer. The results are presented in Table S1.

To determine the best-performing serological test, heart fluid from 60 randomly chosen lynx were tested (in duplicate) by ELISA and IFAT, and results were compared using magnetic capture PCR as a reference test, as described in Bouchard et al. (2022). Antibodies to *T. gondii* were detected in 37% (22 of 60) of lynx using ELISA, and in 45% (27 of 60) using IFAT. There was almost perfect agreement for the between-reader repeatability of the IFAT (k=0.90). The McNemar Chi-square test comparing the ELISA and IFAT serological assays to the molecular assay was significant (ELISA: X^2=4.17, df=1, p=0.041, n=60; IFAT: X^2=, df=1, p=0.003, n=60), meaning that there was a difference between results from both serological assays and MC-qPCR. The relative sensitivity and specificity of ELISA was 100% and 86%, respectively, compared to 100% and 75% for IFAT. ELISA was therefore used for subsequent analyses. The results are presented in Table S2.

4.5.3. **Association between serological and molecular tests**

We found that 308 lynx (75%) were negative on both serology and PCR, 75 (18%) were positive on both, 22 (5%) were seropositive and tissue negative, and four (1%) were seronegative but tissue positive. Using the McNemar chi-squared test ($X^2_1 = 11.1$, $p < 0.001$, $n = 409$), we found a significant difference between serological and molecular results; a Kappa test was thus not performed (Dohoo et al., 2010). No correlation (Spearman's correlation coefficient = −0.064, p = 0.522) was found between antibody concentration (S/P%) and quantification cycle (Cq) values on MC-qPCR.

4.5.4. **Prevalence and potential risk factors associated with *T. gondii***

Overall, seroprevalence (exposure to *T. gondii* indicated by presence of antibodies) was 24% (95% CI=20-28) and tissue prevalence (infection of *T. gondii* indicated by presence of DNA)

was 19% (95% CI=16-23) (Table 4.2). Prevalence of *T. gondii* (on the basis of positivity on at least one test) was 25% (95% CI=21-29). Results of univariate logistic regressions (for the three models) are given in Table 4.2. Because a significant difference was observed between the results of ELISA and MC-qPCR, and due to higher sensitivity when using both test together, multivariate analysis was performed only for the third model (positive on ELISA and/or MC-qPCR).

Table 4.2 Univariate analysis on variables associated with *Toxoplasma gondii* sero-positivity, tissue-positivity and overall positivity among Canada lynx (*Lynx canadensis*).

Variable		Seroprevalence[1]			Tissue-prevalence[2]			Prevalence[3]		
		% (95% CI); P/T	OR (95%CI)	p value	% (95% CI); P/T	OR (95%CI)	p value	% (95% CI); P/T	OR (95%CI)	p value
Sex	Female[4]	19 (13-26); 27/145			16 (11-23); 23/145			20 (14-27); 29/145		
	Male	29 (23-35); 62/215	1.8 (1.1-3)	0.029	23 (18-29); 49/215	1.6 (0.9-2.7)	0.11	30 (24-36); 64/215	1.7 (1.0-2.8)	0.039
Age-class	Juvenile[4]	8 (4-18); 5/60			7 (3-16); 4/60			8.3 (4-18); 5/60		
	Adult	59 (42-74); 20/34	15.7 (5.0-49.2)	<0.001	44 (29-61); 15/34	11.1 (3.3-37.4)	<0.001	59 (42-74); 20/34	15.7 (5.0-49.2)	<0.001
BCI	Low[4]	13 (8-21); 12/92			11 (6-19); 10/92			13 (8-21); 12/92		
	Med	19 (14-26); 36/186	1.6 (0.8-3.2)		14 (10-20); 27/186	1.4 (0.6-3.0)		20 (15-26); 37/186	1.7 (0.8-3.4)	
	High	32 (17-52); 8/25	3.1 (1.1-8.8)	0.094	20 (9-39); 5/25	2.1 (0.6-6.7)	0.468	32 (17-52); 8/25	3.1 (1.1-8.8)	0.091
Region	Eastern	41 (30-52); 28/69	2.7 (1.6-4.6)	<0.001	32 (22-44); 22/69	2.3 (1.3-4.2)	0.004	41 (30-52); 28/69	2.4 (1.4-4.3)	0.001
	Labrador, NL	33 (14-61); 4/12			33 (14-61); 4/12			33 (14-61); 4/12		
	Abitibi, QC	36 (24-50); 18/50			24 (14-37) 12/50			36 (24-50); 18/50		
	Kuujjuaq, QC	86 (49-97); 6/7			86 (49-97); 6/7			86 (49-97); 6/7		
Region	Western[4]	20 (16-25); 69/340			17 (13-21); 57/340			22 (17-26); 73/340		
	Yukon									
Total		24 (20-28); 97/409			19 (16-23); 79/409			25 (21-29); 101/409		

[1] heart fluid was tested for exposure to *T. gondii* using ELISA
[2] tissues (heart and/brain) were tested to detect DNA of *T. gondii* using MC-qPCR
[3] animal was considered positive if found positive on at least one test
[4] reference category
P=positive on particular test, T=number of samples tested, 95% CI= 95% Confidence Interval, OR=odds ratio

In general, prevalence was higher in lynx in eastern (41%, 95% CI=30-52) as opposed to western (22%, 95% CI=17-26) Canada. Prevalence was higher in adult (59%, 95% CI = 42–74) as opposed to juvenile (8%, 95% CI = 4–18) lynx. Due to a large number of missing values for age (354 of 454) and BCI (151 of 454), only sex and location were included in the multivariable analysis, which included 360 complete observations. The odds of being positive to *T. gondii* were two times (OR = 1.83, 95% CI = 1.10–3.06, p = 0.021) higher in male than female lynx, and two and half times higher in lynx from eastern (OR = 2.49, 95% CI = 1.41–4.40, p = 0.002) than those from western Canada (Table 4.3).

Table 4.3 Multivariable analysis on variables associated with *Toxoplasma gondii* positivity among Canada lynx (*Lynx canadensis*).

Variables (N=360)		Odds Ratio	95% CI	p value
Sex	Female [1]			0.021
	Male	1.83	1.096-3.055	
Region	Western [1]			0.002
	Eastern	2.49	1.409-4.404	

N: complete observations

[1] reference category

4.5.5. **Infection intensity of *T. gondii* and associated risk factors**

A total of 72 observations with complete data were included in our generalized linear model, with a mean infection intensity of 4361 TEG (95% CI: 2193–6584). Since more than 10% missing values were observed for age and BCI, only sex and region were included. The mean infection intensity for the western (n = 51) and eastern (n = 21) regions were 5479.14 (2233.25–8725.03) and 1645.05 (173.10–3117.00), respectively. The mean infection intensity for males (n = 49) and females (n = 23) were 4389.69 (1274.28–7505.11) and 4299.43 (800.48–7798.39), respectively. Our generalized linear model revealed a significant association between region and TEG (Table 4.4).

Table 4.4 Coefficients of the generalized linear model and potential risk factors for infection intensity (tachyzoites equivalent per gram of tissues; log transformed) of *Toxoplasma gondii* in Canada lynx (*Lynx canadensis*).

Variables	β (SE)	95 % Wald CI		Wald-Chi-Square	df	p value
Sex, female	0.345 (0.241)	-0.128	0.818	2.039	1	0.153
Region, eastern	-0.710 (0.251)	-1.202	-0.218	7.991	1	0.005*

*Statistically significant at p<0.05.

4.5.6. **CoproPCR and DNA sequencing**

Of 345 fecal samples tested, only one lynx from eastern Canada tested positive for *T. gondii* on real-time PCR with melt-curve analysis. Sequence from this sample was aligned and analysed, and the resulting matrix consisted of 102 sequences with a length of 2362 base pairs. The sample sequence formed a well-supported monophyly with the *T. gondii*, *Hammondia* spp. and *Neospora* spp. sequences (bs = 91) but lacked species level resolution (Figure 4.2). Inferring species identity using phylogeny was therefore not possible by analysing this molecular marker. A BLAST search did, however, return *T. gondii* (LC416238) as the best match with a query cover of 98% and a shared identity of 97.39%. As well, the sequence was sent to CFAP for verification, trimmed to ensure that any low-quality portions were not included, and the BLAST result gave 98.8% similarity with *T. gondii*. The PCR product melting temperature was similar to the in-run controls of *T. gondii* genomic DNA (83°C). Of 17 lynx tissue samples (Cq value < 30), none amplified following sequencing.

0.2

M97703_Toxoplasma_gondii_strain_RH
EF472967_Toxoplasma_gondii_strain_RH
L24381_Toxoplasma_gondii
L37415_Toxoplasma_gondii
Coccidian_Lynx
AF096498_Hammondia_hammondi
KF854253_Hammondia_hammondi_strain_H.H_20
KT184369_Hammondia_hammondi_strain_H_H20
KT184370_Hammondia_heydorni_strain_MEO2015
JX220986_Hammondia_heydorni_clone_3
GQ984222_Hammondia_triffittae_isolate_HtRtVulpes_1981_N
GQ984224_Hammondia_heydorni_isolate_HhCanis_2000_1_N
JX220987_Hammondia_heydorni_clone_5
AJ271354_Neospora_caninum_isolate_NC_PV1
L24380_Neospora_caninum
EU334134_Nephroisospora_eptesici_isolate_MN_2007_
AY833646_Besnoitia_besnoiti
KT184359_Cystoisospora_cf._ohioensis_strain_MEO2015a
KF854252_Cystoisospora_suis_strain_MEO2010_CS2
KF854251_Cystoisospora_suis_strain_MEO2010_CS1
KT184365_Cystoisospora_cf._ohioensis_strain_MEO2015b
KT184366_Cystoisospora_cf._ohioensis_strain_MEO2015c
KT184367_Isospora_rivolta_strain_MEO2015
FJ357797_Isospora_sp._Harbin/01/08
AY618555_Cystoisospora_cf._ohioensis
KT184361_Cystoisospora_canis_strain_MEO2015c
KT184363_Cystoisospora_canis_strain_MEO2015b
KT184368_Cystoisospora_canis_strain_MEO2015a
AB519675_Cystoisospora_sp._2_MM
KT184364_Cystoisospora_felis_strain_MEO2015
L76471_Isospora_felis
KT184362_Cystoisospora_canis_strain_MEO2015e
KT184360_Cystoisospora_canis_strain_MEO2015d
AF298623_Hyaloklossia_lieberkuehni
KC209737_Sarcocystis_tenella_isolate_S9_
KF831274_Sarcocystis_alces
EF467656_Sarcocystis_tarandivulpes_isolate_Stv1RtI
EF056010_Sarcocystis_grueneri_isolate_Sg1RtN
KF831277_Sarcocystis_taeniata_isolate_AaC1.7_clone_1
EF056011_Sarcocystis_rangi_isolate_Sr1RtN
KC209740_Sarcocystis_cruzi_isolate_B4.21
KF831276_Sarcocystis_alceslatrans_isolate_AaC1.21
KF831294_Sarcocystis_hjorti_isolate_Aa36.6
EF056016_Sarcocystis_rangiferi_isolate_Srf2RtN
JN226122_Sarcocystis_silva_isolate_NoCc11.4_clone_1
GQ250969_Sarcocystis_tarandi_isolate_St3RtN_clone_3
KC209744_Sarcocystis_bovifelis_isolate_B4.9
KC209733_Sarcocystis_gigantea
GU120092_Sarcocystis_rileyi
HM185742_Sarcocystis_rileyi_strain_Europe
KT184371_Sarcocystis_neurona_strain_MEO2015
KF854255_Sarcocystis_neurona_strain_TWC_2013
KT184345_Eimeria_maxima_strain_Guelph_strain_74
U67117_Eimeria_maxima
KT184346_Eimeria_maxima_strain_M6
U67116_Eimeria_brunetti
KT184337_Eimeria_brunetti_strain_Guelph_80
FJ236370_Eimeria_praecox_clone_70_35
KT184351_Eimeria_praecox_strain_Guelph_2012
KT184352_Eimeria_praecox_strain_Guelph_2012
KT184333_Eimeria_acervulina_strain_NC3
U67115_Eimeria_acervulina
DQ136187_Eimeria_acervulina_strain_Shanghai
FR775302_Eimeria_mitis
U67118_Eimeria_mitis
FR775307_Eimeria_mitis_mivati_type_3
KC305176_Eimeria_adenoeides_isolate_line_1_clone_50
FR745914_Eimeria_adenoeides
KC305180_Eimeria_adenoeides_isolate_line_2_clone_14
KC305191_Eimeria_meleagrimitis_isolate_line_3_clone_31
KC305196_Eimeria_meleagrimitis_isolate_line_4_clone_16
KT184349_Eimeria_necatrix_strain_Guelph_84
EF210325_Eimeria_tenella_strain_Shanghai_
KT184354_Eimeria_tenella_strain_USDA_80
U67121_Eimeria_tenella
AF111183_Cyclospora_cayetanensis
KT184356_Eimeria_zuernii_strain_OVC_2007
AB769656_Eimeria_zuernii
KT184339_Eimeria_falciformis_strain_Chob2
KT184340_Eimeria_falciformis_strain_Chob2
AF080614_Eimeria_falciformis
KT184357_Isospora_sp._strain_MEO_xd24
KF854254_Isospora_gryphoni_strain_UWO_2010
AF080613_Isospora_gryphoni
KF648871_Isospora_sp._MAH_2013b_strain_MTZ2
KF648870_Isospora_sp._MAH_2013a_strain_MTZ1
FJ829323_Eimeria_trichosuri_clone_I
KT184331_Caryospora_cf._bigenetica_strain_MEO2015a
KT184332_Caryospora_cf._bigenetica_strain_MEO2015b
AF060976_Caryospora_bigenetica_clone_2
AF060975_Caryospora_bigenetica_clone_1
AY603402_Babesia_bigemina_isolate_Kunming
HQ264112_Babesia_bovis_isolate_USDA_IA_clone_6
AY309955_Babesia_caballi
HQ895972_Theileria_parva_clone_B15e
KF559356_Theileria_annulata
AB049999_Babesia_rodhaini_gene_for_18S_rRNA_partial_sequence
EU400394_Hepatocystis_sp._MFRC11_clone_A_
AF463507_Plasmodium_juxtanucleare
AB489196_Plasmodium_malariae
JQ627158_Plasmodium_vivax_isolate_SV6
M19172_P_falciparum

Figure 4.2 A Maximum likelihood topology generated using the RAxML GTRCAT approximation. All nodes with bootstrap support of higher than 75 are marked with blue dots. The sample sequence is indicated in red bold text.

4.5.7. **Fecal flotation**

We tested 62 samples following suspicious results on melting-curve analysis. In the one positive sample following sequencing, we observed a dozen oocyst-like structures resembling *T. gondii* (Figure 4.3) with similar shape and size (spherical, 10-12 µm).

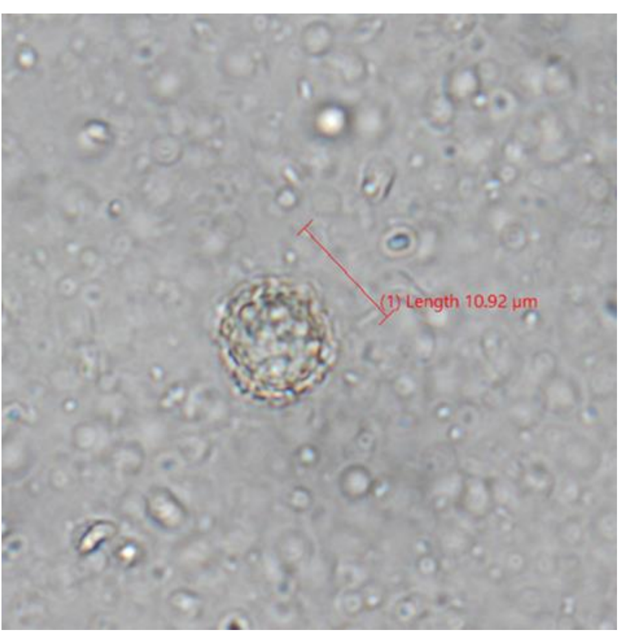

Figure 4.3 Photograph of oocysts-like structure in lynx (*Lynx canadensis*) feces following fecal flotation visualized on compound microscopy.

4.6. **Discussion**

Our study supports that Canadian lynx are both intermediate and likely definitive hosts for *T. gondii*; they may thus play an important role in contaminating the freshwater environments and infecting wildlife (Chester, 2016; Simon et al., 2013b) in boreal regions. They may also pose a risk for transmission to people consuming lynx. These findings provide critical data in understanding better the epidemiology of *T. gondii* in northern ecosystems and highlight the impact lynx may have, which was previously unknown, on contaminating the environment with oocysts in northern regions and maintaining transmission by infecting their prey.

We detected DNA of *T. gondii* in lynx tissues for the first time, which indicates that lynx are suitable intermediate hosts for *T. gondii*. Lynx are occasionally consumed by hunters and trappers which, in addition to skinning the animal for fur, may result in the pathway for transmission to humans (McDonald et al., 1990; Zarnke et al., 2001). Most reports of *T. gondii* in wild felids are from serosurveys (Hatam-Nahavandi et al., 2021), which give little information on tissue infection. Detection of *T. gondii* DNA in tissues is thus of greater significance from a food safety perspective. As we only tested brain and heart, future research should target consumed tissue (i.e., skeletal muscle) and consumption trends in order to provide more insight on the food safety risks in this species. Canada lynx can also be preyed on by coyote (*Canis latrans*), wolverine (*Gulo gulo*), wolf (*Canis lupus*), cougar, and fisher (*Pekania pennanti*) (Lavoie et al., 2019), thus representing a risk of transmission to other carnivore species and maintaining *T. gondii* in wildlife populations regardless of shedding. However, further studies are needed to determine the degree of infectivity of the parasite in tissue cysts from these lynx populations.

Our finding of DNA consistent with that of *T. gondii* (but also closely related coccidians) in one sample of feces emphasizes lynx as a potential source of oocyst contamination in freshwater and snowmelt run-off in boreal regions of Canada, as well as transporting oocysts north through marine currents (Simon et al., 2013c). Multiple oocyst-like structures of around 10-12 μm fit the description of *T. gondii*, but lacked definition. However, we should be cautious when interpreting these results, and the negative results on fecal flotation on 61 other individuals since oocysts are likely shed only on first exposure and for only a few weeks in the lifetime of the animal. Moreover, the intestines and feces underwent ≥1 freeze thaw cycles that could have affected the structural

integrity of the oocysts. Of note, the fragment of the sequence analyzed was non-discriminatory for *T. gondii*, *Hammondia hammondi* and *Neospora caninum*. DNA of *N. caninum* in felid feces is not likely reflective of true infection (canids are the usual hosts), and could have been found in feces following ingestion of *N. caninum* oocysts through canid prey or feces, but *T. gondii* DNA would be more likely. *Hammondia hammondi* has never been described in Canada lynx but cannot be ruled out since felids are potential hosts. Nevertheless, we are confident in our finding since the melting temperature was a match to the control, our sequences were of high quality, and the BLAST match was above 98%. Lynx may therefore be a significant reservoir and useful sentinel species for environmental circulation in boreal regions.

We found some disagreement following serological and molecular testing. As is often the case, seroprevalence tends to be higher than tissue prevalence (Bouchard et al., 2022c; Dubey et al., 2021). Tissue burdens in lynx may simply be below the detection level of the MC-PCR technique, or present elsewhere than heart or brain (Gilbert et al., 2013). On the other hand, four lynx were serologically negative but tissue positive. Acute infection (tissue cysts are present but antibodies not yet produced) or chronic infection (tissue cysts persist while antibody response has faded), could explain these results.

Adult lynx had higher seroprevalence and tissue prevalence compared to juveniles. This likely reflects an increase of exposure with age due to the persistence of both tissue cysts and antibodies, often described in other wildlife studies (Bouchard et al., 2022c; Ferreira et al., 2019; Sharma et al., 2019b). Low exposure in juveniles suggests that vertical transmission (mother to fetus) might not represent an important route of transmission in lynx (García-Bocanegra et al., 2010). Moreover, male lynx were found to be at higher risk of being positive to *T. gondii* compared to females. This may be due to males requiring greater food intake, thus increasing the chance of ingesting contaminated prey, or differences in diet between the sexes.

Higher seroprevalence and tissue prevalence were observed in eastern compared to western Canada. Seroprevalence of 41% in eastern regions was quite similar to 36% reported in a previous study on *T. gondii* in lynx from the boreal region of Québec, eastern Canada (Labelle et al., 2001). However, Simon et al. (2013) found a seroprevalence of 14% in the same region, which suggests

spatio-temporal dynamics that may be a result of variation in prey-predator cycles (Simon et al., 2013a). Methodological differences between studies (modified agglutination tests vs. ELISA) could also explain these divergent results, although they both proved to be good serological techniques to detect *T. gondii* antibodies (Bachand et al., 2019; Bouchard et al., 2019). Our finding of 20% seroprevalence in lynx from the Yukon is comparable to 15% in previous work from Alaska (Zarnke et al., 2001). These results are also similar to the low seroprevalence (3%) found in people in Alaska (Miernyk et al., 2019) and in recent Inuit health surveys (~8% in the Inuvialuit Settlement Region) (Goyette et al., 2014b), supporting genuinely lower levels of circulation of *T. gondii* in northwestern North America.

It is unlikely that the difference in prevalence between eastern and western regions are caused by variations in diet since lynx consume mostly snowshoe hare throughout their range; however, the proportion of hare in the diet may vary annually with their abundance, which cycles on a 9-11 year basis (Elton & Nicholson, 1942; Krebs et al., 2001). During period of increasing density of lynx and hares, we would thus expect higher transmission of *T. gondii* in both species (Simon et al., 2013a). Due to the non-uniform distribution of samples in different years from both regions in the present study, we were not able to explore this further. Abiotic factors such as a colder and drier climate could also explain a lower prevalence in western northern boreal regions, thus affecting the survival or the infectivity of *T. gondii*. Sporulated oocysts can remain infectious for at least 18 months in moist conditions (Frenkel et al., 1975). A higher prevalence in the eastern lynx population, especially in Kuujjuaq (86%), could be due to them being adjacent to large watershed that are potentially disseminating infectious sporulated oocysts coming from temperate regions into northern coastal environment and surrounding wildlife. Snowshoe hares could thus be more exposed from contaminated herbage (from snowmelt and water run-off for example), and potentially through meat. Hares have been seen scavenging a variety of different species that could act as intermediate hosts for *T. gondii*, including lynx (Peers et al., 2018). Future studies investigating prevalence in snowshoe hare from different regions are required to understand better *T. gondii* transmission pathways in northern boreal regions. Contrary to prevalence, the infection intensity in lynx from the Yukon was significantly higher than from eastern Canada. The parasite stage when infected, strain virulence as well as age could play a role in intensity of infection (Bouchard et al., 2022b; Gisbert et al., 2018; Koethe et al., 2015). Younger individuals with lower

immunity could have been trapped in the Yukon, as observed for wolverine (*Gulo gulo*), a species similarly trapped in the Yukon (Kukka et al., 2017). Alternatively, a more transmissible genotype could be present in the western boreal forest.

Knowledge of genotypes and strains circulating in a particular geographical region is thus important to better understand the epidemiology of *T. gondii*. We were not successful in genotyping even our strongly positive samples from tissues, which is not uncommon when working with previously frozen tissue samples from naturally exposed wildlife (in which amplification in vivo or in vitro is not possible). Unrecognized genetic diversity has been increasingly reported in North American wildlife, but findings still remain scarce (Bouchard et al., 2022a; Dubey et al., 2011). Some strains may have more localized circulation in wild felids and their prey species, which would allow us to better understand the molecular epidemiology and biological significance of genetic diversity of this parasite in boreal regions and connected ecosystems. However, recovering sufficient DNA in naturally infected wildlife to determine which genotypes/strains are present remains a challenge (Bachand et al., 2019; Bouchard et al., 2022c). To maximize chances of recovering sufficient target DNA, future studies should seek fresh tissues from which the parasite can be cultured and amplified to recover sufficient DNA to determine genotypes of *T. gondii* circulating in lynx and other wildlife species in northern regions.

Annual temperature and precipitation is projected to increase throughout northern Canada (Bush & Lemmen, 2019); a changing climate will likely accelerate the development of *T. gondii* in endemic areas. As a consequence of temperature and precipitation change, the distributional range of lynx and their primary prey, the snowshoe hare, will likely move northward, thus facilitating oocyst transmission in more northern ecosystems. Moreover, lynx can serve as a source of infection for humans consuming its meat or handling carcasses. Wearing gloves during the skinning process, disinfecting tools, and thoroughly freezing and cooking meat before consumption is recommended. Further studies aiming at detecting viable *T. gondii* tissue cysts are needed to truly assess food safety risks to trappers and consumers of lynx. As well, further investigation into the genetics of *T. gondii* is required to better understand the ecological drivers for its introduction, persistence, and emergence in northern latitudes, especially the western North American where prevalence in wildlife and people is currently relatively low.

4.7. Acknowledgments

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4.8. **Supplementary material**

Table S1 Comparison of *Toxoplasma gondii* results for heart fluid samples using serological methods (ELISA and IFAT) relative to magnetic capture real-time PCR to determine the best dilution for cut-off values.

	ID	ELISA S/P%				IFAT			
Dilutions		ND	1:2	1:4	1:8	1:2	1:8	1:32	1:128
N	19	33.65	7.76	7.59	2.62	N	N	N	N
	31	112.34	97.30	69.49	46.34	P	P	N	N
	45	24.89	12.16	11.51	2.62	N	N	N	N
LP	2	124.15	109.98	85.92	67.26	P	P	P	N
	16	114.56	121.93	106.50	87.53	P	P	P	P
	17	46.86	59.07	56.97	50.78	P	P	P	P
HP	7	133.22	131.56	109.29	81.69	P	P	P	N
	18	150.35	174.59	163.30	142.89	P	P	P	P
	24	211.51	216.04	198.30	182.87	P	P	P	P

Positive results: ELISA ≥ 40% and IFAT ≥ 1+.

N: Negative.

LP: Low positive on magnetic capture (Cp value >30).

HP: High positive on magnetic capture (Cp value ≤30).

ND: No dilution.

ELISA: Enzyme linked immunosorbent assay.

IFAT: Indirect fluorescent antibody test.

Table S2 Comparison of optimized serological methods (ELISA and IFAT) for *Toxoplasma gondii* relative to magnetic capture qPCR.

	MC-qPCR		
	Positive	Negative	Total
ELISA			
Positive	16	6	22
Negative	0	38	38
Total	16	44	60
McNemar test (p value)	0.041*		
Relative sensitivity	100		
Relative specificity	86.36		
IFAT			
Positive	16	11	27
Negative	0	33	33
Total	16	44	60
McNemar test (p value)	0.003*		
Relative sensitivity	100		
Relative specificity	75		

MC-qPCR: Magnetic capture qPCR

ELISA: Enzyme linked immunosorbent assay

IFAT: Indirect fluorescent antibody test

*: Significant p value <0.05

Note: A subset of randomly chosen lynx (n=60) were tested.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

Toxoplasma gondii is considered one of the most successful parasites in the world, with any vertebrates susceptible to infection, including humans. As described in this thesis, its complex life cycle encompasses many transmission modes and routes, which remain enigmatic in northern ecosystems. The main purpose of this research was to assess the use of carnivores such as foxes and lynx as reservoir and sentinel species for *T. gondii* in northern regions of Canada, to help elucidate ways of transmission in this particular environment.

In Chapters 2 and 4, I documented exposure and prevalence in foxes and lynx across northern Canada, comparing different detection techniques and generating baseline data. My results suggested that these species are likely suitable sentinels for *T. gondii*, with relatively similar seroprevalence to humans when compared with recently published surveys from the same regions. While human health surveys can be considered more of a direct assessment of risks for exposure to *T. gondii*, they are ethically challenging, costly, and time consuming. The use of sentinels can therefore be useful alternatives, especially in a northern context. By combining brain and heart in laboratory analysis, two sites of predilection for *T. gondii*, and by using multiple detection methods, I increased the chances of detection and took into account the lack of gold standards in wildlife studies. These findings may help identify high-risk regions for infections in northern Canada, with foxes and lynx serving as indicators for potential human exposure of *T. gondii*.

The significant differences in prevalence that were found in foxes between eastern and western Nunavik highlighted the need to gather more information on transmission pathways in this particular region. In Chapter 3, I used stable isotopes of fox hair and muscle to investigate trophic relationships by reconstituting their diet over a certain period of time. This approach allowed me to link prey items with status of infection and brought to light the significant role of marine and migratory geese as an important route of transmission for *T. gondii*, especially in the Hudson Bay region. Even though definitive hosts are largely absent in northern ecosystems, they can still be a source of *T. gondii* for local wildlife by shedding oocysts in boreal and sub-Arctic regions, also through watershed and ocean currents bringing oocysts up North. In Chapter 4, I aimed at finding evidence of intestinal infection in lynx, which has never been achieved before. For the first time,

DNA consistent with that of *T. gondii* was found in feces of a Canada lynx in boreal region of Québec, suggesting that they can be both intermediate and definitive hosts, and could thus locally contaminate northern regions. Combining traditional fecal flotation with molecular techniques such as real-time PCR with melting-curve analysis improved our chance of detection.

It is important to recognize uncertainty and implications of biased samples in wildlife disease studies (Lachish & Murray, 2018). As for many of these studies, my study extended over several years and only tested a small fraction of the studied population. The foxes and lynx in my project were not randomly chosen and derived mostly from convenience sampling (i.e., hunter and trapper based samples). Therefore, geographical and trapping/hunting biases were likely present and could have contributed to a higher prevalence in my study population that the general fox population. The animals trapped were often adjacent to northern human settlements, thus likely clustered where carcasses of hunted animals, such as marine mammals, are often left on the land and could represent an increased risk of *T. gondii* transmission. Moreover, behavioral/physiological traits such as size, sex, or health status possibly affected which animals where collected (Biro, 2013; Kay et al., 2000). Infection with *T. gondii* can make hosts more adventurous and likely to engage in risky behaviors. If so, the proportion of positive animals in my study may overestimate the incidence of toxoplasmosis in the population because infected animals would be more likely to be caught. In contrast with hunted animals, where experienced hunters often avoid harvesting sickly or abnormally behaving animals, trapped animals are not screened out. A more systematic approach would have been ideal, but not possible due to logistic and financial constraints. These implications can definitely affect my inferences about the general fox and lynx populations since my results reflect only the populations sampled. But despite these potential biases, my findings are still of interest to public health, veterinary and wildlife management.

In Canada, the North has historically faced health disparities as well as food and water insecurity and is now experiencing climate change at rates four times that of the global average (Rantanen et al., 2022). Vector, food, and water-borne diseases have been identified as the infectious diseases most likely to emerge in a future of climate change in Canada, and zoonoses continue to pose threats to wildlife and public health in the Canadian North, including

toxoplasmosis that can cause abortion and congenital disease in multiple species. With climate change modifying the North at an incredibly fast pace, it is crucial to keep monitoring wildlife and investigating *T. gondii* effects in order to determine the potential human and animal health risks associated to *T. gondii* infection.

5.1. Carnivores are good sentinel species for *T. gondii* in northern regions of Canada

Wildlife are intrinsically connected to the health of an ecosystem. They can provide insightful information about environmental changes on spatial, temporal, and trophic scales (Tabor & Aguirre, 2004). More specifically, wild carnivores are a sensitive indicator of *T. gondii* contamination in the environment. In this thesis, we chose foxes and lynx as particularly relevant in regard to the complexity of *T. gondii* epidemiology. By monitoring both intermediate and definitive hosts, I was able to factor in various ways of transmission, such as the trophic (through consumption of tissue cysts by carnivorism) and environmental routes (through consumption of oocysts shed by lynx). Foxes are ideal sentinels to monitor for this parasite; they are highly adaptable to ecosystems and human-impacted environments, they can develop a subclinical response in an otherwise healthy individual, and they occupy a high trophic position as carnivorous scavengers (Halliday et al., 2007). In the context of my research, foxes were easily accessible through hunter/trapper organisations, in close proximity to the target population, were exposed through consumption of similar food items consumed by the local residents, and widespread across northern Canada. For the first time, we monitored foxes across the entire Canadian Arctic (Chapter 2) to demonstrate regional differences in prevalence in foxes that mirror observed differences in human prevalence, supporting higher levels of the parasite circulating in the eastern vs western Arctic, especially in Nunavut and Nunavik, where marine food sources are highly consumed. By having a closer look at the fox diet in Nunavik through stable isotope analysis (Chapter 3), we were able to support the hypothesis that *T. gondii* transmits from marine to terrestrial ecosystems, not just from land to sea as has previously been well described. Foxes have thus proven to be great indicators of possible source of *T. gondii* infection. As for lynx, a similar trend was observed, with a lower prevalence in western boreal regions. The colder and drier climate in the western Canadian Arctic likely disadvantage *T. gondii* survival and infectivity. As well, finding *T. gondii* DNA in feces of one lynx suggests that lynx are local definitive hosts, thus creating local environmental contamination where lynx are present. Determining exposure in hares as the main prey of lynx

could help better understand the true prevalence of *T. gondii* in boreal regions. Ultimately, to test an assemblage of different species from different trophic levels and ecological roles would be an ideal scenario to better grasp the multiple variables that make the *T. gondii* life cycle so hard to decipher in a northern context. Animal sentinels certainly represent an important but underused surveillance tool in regard to infectious disease (Rabinowitz et al., 2005). A better integration between disciplines is needed to value animal sentinels for enhanced pathogen detection such as *T. gondii*.

5.2. The use of multiple detection techniques and the importance (and challenges) of genotyping parasite isolates from wildlife

Despite a human burden of food borne disease similar to salmonellosis or campylobacteriosis, toxoplasmosis is a neglected and underreported disease (Kijlstra & Jongert, 2009). One of the main reasons why monitoring programs are not implemented is the ubiquity of the parasite (no countries are considered free of the parasite) and the absence of standardised validated diagnostic tests in meat and food product, hampered by the lack of available reference sera and/or tissues. *Toxoplasma gondii* is microscopic and does not affect the appearance of food. Oocysts survive long periods in the environment and are resistant to many natural and artificial conditions, making control even harder (Gajadhar & Allen, 2004). Moreover, there is no rapid, inexpensive, and accurate test available, making transmission control a challenge (FAO/WHO, 2014). To be effective, these tests should also have comparable specificity and sensitivity across multiple species. Developing and implementing such standardized methods would help answer to the demand for food safety measures, especially in a northern context where country food is culturally important to the well-being of people and often consumed in ways that do not inactivate tissue cysts.

I used various techniques of detection in this thesis, including serological, molecular, and microscopy. The comparison of two serological techniques, IFAT and ELISA, using magnetic capture as gold standard, allowed me to take in account the uncertainty when dealing with a lack of gold standard in wildlife, as seen in Chapters 2 and 4. I also conducted pilot studies to determine which cut-off values should be used since these assays are usually developed for domestic animals or people, not wildlife species. In my research, ELISA was determined to be the best-performing

serological test, showing higher sensitivity and specificity. This is contrary to a previous study in arctic foxes, where IFAT was considered the test of choice when compared to ELISA and the direct agglutination test (Elmore et al., 2016). In our case, using the magnetic capture technique as the gold standard for determining which test is best to use has likely improved our chance to find true positive. Magnetic capture has been increasingly used in wildlife studies due to its high sensitivity by testing large amount of tissue (up to 100g) compared to traditional molecular methods that use only a few mg (Bachand et al., 2018; Bachand et al., 2019; Bouchard et al., 2022c; Sharma et al., 2019a). For the first time, we detected *T. gondii* DNA in tissues of Canada lynx using the magnetic capture technique, definitively demonstrating that this species is a suitable intermediate host. Despite testing only brain and heart, it is likely that tissue cysts are present in other organs and muscles, thus representing a risk for people consuming lynx meat (a delicacy in some regions), although more studies are needed to confirm tissue predilection in lynx. Also, DNA consistent with that of *T. gondii* was detected in the feces of one individual lynx, supporting the hypothesis that Canada lynx are definitive hosts for the parasite. A previous study by Simon et al. (2013) looked in 84 Canada lynx using fecal flotation but did not detect any *T. gondii*-like oocysts, which is not surprising as felines generally shed once a lifetime for only a few weeks. In this thesis, we used a molecular approach, with a melting-curve analysis real-time PCR that uses a universal primer cocktail that is known to amplify many coccidia species, including *T. gondii*. This technique has increased sensitivity compared to microscopic and morphological methods that rely only on visual identification of oocysts. We sequenced our positive sample to achieve a definitive diagnosis since false positives could arise from other organisms having the same melting temperature as *T. gondii*. Finally, in order to confirm our finding, microscopy was used to search for *T. gondii* oocysts in feces. In that particular individual, many oocyst-like structures were seen but lacked definition, most likely due to multiple freeze-thaw cycles that affected the integrity of the oocyst wall. Combining various detection methods increased our confidence in our findings, something we couldn't have achieve solely with microscopy.

Genotyping of *T. gondii* can help understand the routes of transmission and track the source of contamination, potentially linking spatial patterns of *T. gondii* to the genetic structure of both parasites and hosts (Galal et al., 2020; Shapiro et al., 2019b; Su et al., 2010). The pathogenicity of *T. gondii* will also vary depending on the genotype (Maubon et al., 2008). Determining the genetic

diversity of this parasite in lynx and foxes in subarctic regions may contribute to find any unrecognized genotype(s) with increased (or moderate/minimal) virulence that might be present in the North, and give us clues on how it is introduced. Unrecognized genetic diversity have in fact been increasingly reported in North American wildlife (Dubey et al., 2011). It may also clarify the extent of genetic diversity and the relative roles of sexual recombination (oocysts) vs clonal propagation (asexual reproduction). The fact that *T. gondii* has a highly clonal nature provides evidence that other ways of transmission often bypass sexual reproduction in the definitive host, whether it is by carnivorism or vertical transmission (Hide et al., 2009; Sibley & Boothroyd, 1992). Moreover, the number of oocysts and prevalence of shedding by felids might be different depending on which type of strain is in play (Shapiro et al., 2019a). However, genotyping techniques (such as RFLP) require larger amounts of DNA, which is especially difficult in wild animals with low tissue burden and samples that are previously frozen and cannot be cultured in vitro or passaged in vivo (Galal et al., 2019).

In Chapter 2 and 4, I sought to determine which genotypes were present in infected foxes and lynx, but did not succeed despite testing strong positives. In a previous study, type II was detected in a Nunavik goose (Bachand et al., 2019), which is the most commonly involved strain in human cases (Dubey, 2010). However, only one genetic marker was used, which could have limited the detection of non-clonal strains as most genetic markers distinguish two of the three clonal lineages (Su et al., 2006). We planned to use 10 different markers in our work, through multiplex multilocus nested PCR-RFLP; however, we did not recover sufficient DNA. Future effort should be made in testing bigger amount of tissues, minimizing freeze-thaw cycles to reduce the degradation of DNA, and seeking to recover fresh samples from which live parasite can be amplified in vitro. More information is needed concerning genotypes in northern ecosystems to assess the degree of clonality of *T. gondii* strains in infected animals and elucidating the population structure and transmission routes of this parasite. Despite being costly in terms of animal use and under ethical constraint, bioassays are often considered to be the gold standard for detection of viable *T. gondii* in tissue samples. This technique could allow recovery of sufficient DNA in order to successfully genotype *T. gondii* in naturally infected wildlife.

5.3. ***Toxoplasma gondii* and its multiple routes of transmission – You are what you eat**

Toxoplasma gondii is one of the most successful parasites in the world, and can infect nearly all warm-blooded animals, including over 350 mammalian and avian species (Dubey, 2010; Tenter et al., 2000). Only felids act as definitive hosts and can shed the parasite in the environment (Dubey et al., 2004). The three major routes of transmission are faecal-oral transmission (environmental route through oocysts), transmission by carnivorism (with infected animals developing tissue cysts), and transplacental transmission (Robert-Gangneux and Darde, 2012). This parasite is thus highly successful in finding ways to get to its host and spread, especially by bypassing sexual reproduction in felids.

In Chapter 3, to help elucidate pathways of transmission in foxes, we combined trophic analysis using stable isotope signatures with infection status of *T. gondii*, predicting a higher exposure to the parasite in foxes consuming marine and migratory bird food sources. We used stable isotopes of red and Arctic fox muscle and hair to test these predictions. Stable isotope ratios of naturally occurring and anthropogenic foods are highly distinct, and these dietary "signatures" are preserved in fox tissues, as those tissues grow or turn over. Our findings regarding status of infection on the stable isotope values of $\delta^{13}C$ supports the hypothesis that marine and migratory food sources could be a significant source of *T. gondii* exposure in Nunavik. Knowing that foxes are exposed through multiple routes of transmission likely overlapping with those of humans in most of the Canadian Arctic (as described in Chapter 2), these findings were consistent with human risk factors for exposure to *T. gondii* in Nunavik, the most highly affected region of the Canadian Arctic, where nearly 43% of people are exposed to the parasite (Ducrocq et al., 2021). In a rapidly changing Arctic, these data remain essential, especially to improve the protection of vulnerable groups from the consequences of toxoplasmosis.

This application of stable isotope analysis is particularly relevant for elucidating complex transmission pathways for multi-host parasites such as *T. gondii*, for which food-borne source attribution is often elusive for both animal and human hosts. The combined ecological and microbiological approach for *T. gondii* is relatively unexplored and can be more broadly applied to other foodborne pathogens in free-ranging wildlife populations and even humans, using non-invasive methods (hair and blood samples, for example). In this research, the coupling of microbial

and ecological methods was critical in unravelling complex trophic relationships between foxes and their prey species, and understanding better how this ubiquitous parasite can thrive, even in remote northern ecosystems. As well, our findings put emphasis on which species have higher risks of transmission to people if consumed.

Canada's North is experiencing food insecurity at far higher rates than the rest of the country, as high as 70% in households in Nunavut (Egeland, 2010a). The remoteness of northern communities, combined with a high cost of living and the dire impacts of climate change, contribute to making food hard to access. Country foods play thus a significant role in food security in the North. Eating raw or traditionally prepared food is culturally, nutritionally, and economically important, especially in Indigenous and remote regions. But consuming country food is not without risk since it can harbor infectious pathogens, such as *T. gondii*. A food safety testing program such as the currently well-established *Trichinella* monitoring program at the Nunavik Research Centre would provide valuable information on the risks of consuming country foods in regard to *T. gondii*. With respect to my results, the testing of marine and migratory animals (such as ringed seal, walrus, and caribou) and species/animal parts often consumed raw would be particularly relevant to minimize risk of infection. While the combined use of molecular and serological technique, such as conventional PCR and ELISA, would give a good insight on the food safety risk of *T. gondii* in a particular species/tissue (and logically feasible in a northern context), the fact that *T. gondii* cysts are not homogeneously distributed within the animal and tissue makes it hard to determine the true safety of consuming it. Recommendations to freeze tissues for at least 72 hrs at less than -12 ºC (which may be more culturally acceptable than cooking) should thus be strongly emphasis, especially for pregnant woman and immunocompromised individuals. Nevertheless, implementing such program for the North and by the North would allow to provide much needed baseline data on which species and tissues are more at risk to harbor *T. gondii*, as well as raise awareness on toxoplasmosis and the health risks associated to it, as it has been successfully demonstrated by the *Trichinella* monitoring program (Larrat et al., 2012).

Prevention and implementation of policies to control its transmission is thus critical, but hard to achieve due to many unknowns. It is therefore crucial to understand better the complexity of its transmission pathways. By doing so, it will be easier to develop effective and targeted

prevention and control measures, especially where higher risks are present due to cultural preferences (Gilot-Fromont, 2012; Pinto-Ferreira et al., 2019).

5.4. *Toxoplasma gondii* and the One Health paradigm in a northern context

According to the Centers for Disease Control and Prevention, One Health is a "collaborative, multisectoral, and transdisciplinary approach with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment". One Health approaches are particularly relevant to study infectious diseases such as zoonotic parasites. As mentioned in Chapter 1, *Toxoplasma gondii* is a poster parasite for the One Health approach. It can be found in a vast array of ecosystems, from Arctic to hot desert areas, in terrestrial or aquatic environments, and in virtually all vertebrates. Approximately one third of the world human population has been exposed to it, and can cause significant disease in animals and humans (Tenter et al., 2000), being the 3rd most important foodborne parasite worldwide and 2nd in the EU. It can also infect its hosts through multiple infective stages and transmission routes, being deemed one of the most successful parasites in the world (Dubey, 2010). To facilitate a better understanding of its transmission and impacts, it thus requires synergy among multiple disciplines, requiring transdisciplinary study, integrative research, and capacity building (Aguirre et al., 2019; Polley, 2005; Schurer et al., 2016; Thompson, 2013), a perfect scenario for a One Health approach.

To prevent toxoplasmosis, it is important to understand in depth the ecology of *T. gondii* in its hosts but also its interactions with humans (Wood et al., 2012). For example, in the context of my research, remote northern communities have limited transportation and communication infrastructures, extreme weather conditions, as well as historical colonization and trust issues. These realities make surveillance and outreach a challenge regarding risks associated with harvested wildlife and zoonotic parasites (Hueffer et al., 2019). To understand and mitigate the risk of transmission, we must understand the context of livelihoods of the region studied, so that control efforts do not threaten other aspects of well-being of the community. A solution that is intended to reduce harm in one sector can lead to more harm in another sector (MacGregor & Waldman, 2017; Sleeman et al., 2019). For example, by telling northern Indigenous peoples to stop eating raw meat to reduce *T. gondii* transmission, this will overall lead to detrimental effects on health since a subsistence lifestyle is often part of the living culture of indigenous peoples and

is central to their well-being. Taking away traditional food practices can have detrimental effects on physical and mental health, thus doing more harm than good (FAO, 2013). Large screening programs that target wild animals, but also domestic animals (by limiting free-roaming domestic cats in communities for example), humans, and the surrounding environment would be necessary to assess the true "One Health" impact of *T. gondii* (Jones et al., 2018). This would require the input of local and scientific knowledge through consultations and engagement of different groups.

While trying to tend towards a more collaborative and transdisciplinary approach, it would be inaccurate to qualify my work as One Health as it was not developed under its framework. Addressing aspects of environmental and health sciences, behavior, and governance would have been necessary. I quickly came to realize that many challenges remain in order to improve and achieve a One Health approach in the North, such as data sharing (low internet connections, language barriers, trust barriers), synching research interests of communities and science, timelines (summers dedicated to hunting on the land may not be compatible with sampling and knowledge translation schedules), or budget constraints (Arctic science is expensive!). I believe addressing the previous points described above and prioritizing a two-way exchange will help strengthen many other aspects and expand the limits to what is possible for an approach in a northern context. An increased awareness of the interconnectivity of the health of people, domestic animals, wildlife and to the integrity of ecosystems is crucial in order to tackle the complexity of *T. gondii*. This is especially relevant to research in the North where close connections among human, animals and the environment is part of everyday life and vital to well-being. Upscaling and sustaining One Health approaches in the circumpolar North will enable collaborations throughout all stages of research as well as bringing long-term solutions for the benefit of all in the One Health community.

5.5. ***Toxoplasma gondii* in a changing Canadian North**

With climate change affecting the Arctic at unprecedented rates, *T. gondii* distribution and transmission, as well as ecology of its hosts, may be adversely influenced (or positively influenced from a parasite perspective!) (Jenkins et al., 2015; Mills et al., 2010; Polley & Thompson, 2009). The potential impacts of climate change on *T. gondii* can be conceptualized using the epidemiological triad as a basic framework for understanding. Infection with *T. gondii* is not synonymous with the disease toxoplasmosis, a highly complex process that can be influenced by

both infectious and non-infectious factors. Therefore, impacts on any of the three components of the triad – agent, host and environmental factors - can influence disease development, progression and outcomes significantly (Fig. 5.1). Because of the complexity of agent-host-environment relationships, the impact on *T. gondii* in northern Canada can be hard to predict. However, many mechanisms are understood and can be predicted based on existing knowledge.

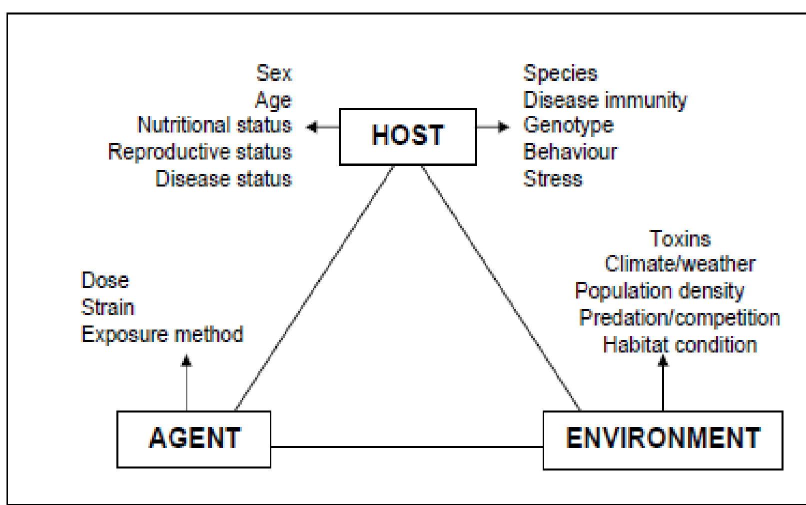

Figure 5.1 Classical triad representing the relationship among agent, host and environment (Fraser & Parmley, 2009).

First, climate change has already had observable effects on the **environment**. Changes in rainfall including more frequent and intense drought, floods and storms, heat waves, rising sea levels, melting glaciers and warming oceans, altering entire ecosystems, in which *T. gondii* may thrive or fail (Rocklöv & Dubrow, 2020). For example, the melting of the ice layer in Arctic ecosystems as well as increased precipitation might bring a range expansion of *T. gondii* via aquatic environments. Being highly resistant to external environmental conditions, *T. gondii* oocysts are easily disseminated in fresh or sea water. Filter feeding invertebrates and fish may filter and concentrate the oocysts from the marine environment, and serve as carriers (Massie et al., 2010). The presence of invertebrate species in new areas that were previously too cool for their survival represents a risk for increased spread of *T. gondii* (Gajadhar & Allen, 2004). With an increase of oocysts in marine systems due to terrestrial run off caused by melting and precipitation, there is a higher risk of contamination of drinking water, for which treatment infrastructures in northern communities are already suboptimal. Moreover, the relationship between predators and

prey can be disrupted by warmer weather, and can contribute to increase in transmission of *T. gondii*. For example, more animal mortalities due to extreme weather events are likely to happen, which could be a source of tissue cysts for carnivores and scavengers, such as foxes (Burek et al., 2008).

Many factors related to climate could affect **pathogens** directly. For example, higher temperatures can increase the rate of development of some temperature sensitive pathogens. However, higher temperatures can also have the opposite effect on survival rates of pathogens that thrive in colder climates, underscoring the complexity of these relationships (Ogden, 2018). For *T. gondii*, unsporulated oocysts sporulate faster at warmer temperatures, but may experience higher mortality under warmer conditions (Dubey, 2010). As well, dryness and prolonged exposure to extremely low (<-21°C) and high temperatures (>45°C in water, >67°C in meat) will inactivate sporulated oocysts (Lelu et al., 2012). Since oocysts rely on warm and humid conditions to thrive, rising temperature and precipitation in Canada's North will likely increase the survivability of *T. gondii* oocysts on land and water (Jenkins et al., 2013). Higher seroprevalence was seen in polar bears following wetter summers (Pilfold et al., 2021), and an increase in toxoplasmosis infection rates was seen in felines following rainy periods (Afonso et al., 2010). This could apply to lynx in northern regions, therefore increasing viable oocysts loads on land and in water. Climate change may also facilitate evolution of pathogen virulence and infectivity. With new host species moving northwards, they may bring new strains of *T. gondii*, creating novel epidemiological risks and uncertainties regarding *T. gondii* evolution (Proesmans et al., 2021).

The effect of climate change on the movements of animal **host** populations can also increase the likelihood of exposure to *T. gondii*. Many terrestrial species are expected to undergo range shifts to higher elevations or latitudes as a result of climate warming (Seebacher & Post, 2015), including lynx. Moreover, extreme weather events may cause an increase in the stress of wildlife hosts which could alter breeding and feeding patterns (Dash et al., 2021), which may render some species more susceptible to develop serious effects from toxoplasmosis by reducing their immunity. The decrease in habitat connectivity such as loss of sea ice can also contribute to stress by leading to less access to food resources (Bush & Lemmen, 2019). Another result of warming climate and stress is the loss of species, which can alter species composition in an area

and increase encounter rates between *T. gondii* and new hosts, therefore increasing disease risk for humans and wildlife which can be exposed to different infection intensities or strains (Ostfeld, 2017). On the other hand, climate change can also lead to increased density of hosts resulting in greater animal crowding and interactions between species which can impact *T. gondii* transmission (Dobson, 2004; Polley et al., 2010).

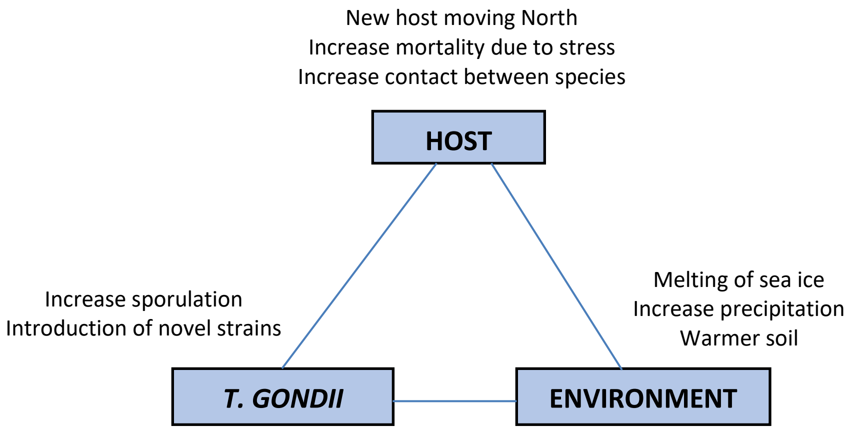

Figure 5.2 Epidemiological triad representing the relationship among *Toxoplasma gondii*, host and the environment in a changing North.

All these factors are intertwined and may be happening simultaneously, making it very difficult to assess the risks of transmission in a future of climate change. As mentioned above, changes in multiple factors such as food webs, stress, and weather patterns will influence disease transmission and frequency (Stephen & Soos, 2021). Cumulative impacts of climate change on the agent-host-environment triad, and on already struggling populations and ecosystems should be recognized as increasingly important for wildlife health and conservation. While the extent of impacts remain uncertain, systematic surveillance of *T. gondii* exposure in wildlife due to climate changes in Canada are needed in order to understand the ways in which climate change and other anthropogenic stressors synergize to impact human and wildlife health. These many uncertainties around the potential impacts of climate change on *T. gondii* epidemiology highlight the need for timely action to help inform mitigation, adaptation and decision-making.

5.6. **Community engagement and northern research**

In northern communities, the close relationship with the land and wildlife is crucial for food security, cultural practices and livelihoods, but also represent a risk for exposure to *T. gondii*. Efforts to raise awareness as well as monitoring *T. gondii* in wildlife, people and the environment is thus key in preventing transmission. However, conventional scientific monitoring alone is often not enough to achieve reliable monitoring for pathogens in the Arctic. Bringing together multiple stakeholders and sources of knowledge is especially important in northern context, where logistical, financial, and weather constraints often pose challenges (Brook et al., 2009; Mallory et al., 2018). In such contexts, the co-management and direct engagement of Indigenous people in research, as well as the integration of local and traditional knowledge, is necessary and should be the case whenever possible. It can considerably improve the detection of emerging trends and generate deeper insight into changes in the environment. It is however not always the case due to a lack of systematic, transparent, and practical aspects of bridging these knowledges together (Tomasini, 2018). To avoid miscommunication and frustration on the process and/or outcomes, an adaptable framework that includes local and traditional knowledge in addition to scientific knowledge should be prioritised to strengthened the approach, for example by using hunter-based samples, interviews, and conventional scientific monitoring altogether (Peacock et al., 2020). There is a need to shift methods in northern research in order to better support community priorities and leadership, and make research results accessible to community and territorial decision-makers.

Through this work, I had the chance to visit multiple communities in order to consult the people on their knowledge, share my findings and raise awareness on the health risks that represent *T. gondii* in harvested country food. The amount of collaboration and northern partners involved in this thesis is hard to quantify and has been key in the success of my research. By recruiting Inuit coordinators, guides to navigate the land, performing necropsies with high school students, doing presentations at community meetings, attending cultural events, or translating key findings and reports in indigenous languages, I was able to develop trust, partnerships, and facilitate knowledge transfer. While this was not developed as a participatory community project, through hunter based samples and local coordinators, communities were a key part of this work and strongly engaged in the research activities. Still, I have also seen much frustration and disappointment towards southern-based researchers, especially in Nunavik often overwhelmed by research projects from

the South not always providing tangible and lasting benefits for Nunavimmiut and their communities. Many voiced their concerns regarding the come and go of researchers without any words on the final results which are often inaccessible to communities; the primary benefactors of northern research often remain researcher themselves. While I tried to stay away from colonialism practices, it can be hard to achieve due to the current reality of academia and government budgets. Funding support must be adjusted to allow for unconventional methods such as prioritizing on-the-land engagement with communities, and including Indigenous methodologies (i.e., based on relationships, storytelling, personal reflection). A better approach would be to directly involve Inuit partners as lead investigators, shifting the focus from capacity building to capacity sharing to enable decision for what is best for the community; research results should profit those outside academia.

I was fortunate enough to already have connections in the North through my supervisors who were familiar with these contexts and already had northern partners, as well as colleagues already involved in communities. However, this is often not the case. Navigating the complex relationship-building required in northern research, with limited fundings and timelines, is very hard to achieve (definitely the hardest part of my PhD). Establishing mandatory courses and support for science communications, in regard to northern communities and their realities, would help break down silos between researchers and communities, and lead to culturally sensitive and better collaborative research. Nevertheless, during the course of my PhD, I saw the research paradigm starting to shift in northern Canada, supporting a leading role for Inuit in northern research. For example, SIKU, the Indigenous Knowledge Social Network, was recently created to facilitate self-determination in research, education and stewardship by providing tools and services for ice safety, language preservation and weather services. As well, a centralized research and approval process is currently being developed in Nunavik, the only region in Inuit Nunangat without one. This will support researchers and community research needs by improving communications and integrate research management and governance, by Inuit, for Inuit.

5.7. **Conclusion**

Environmental degradation and climate change due to anthropogenic impacts are one of the most pressing ecological health concerns of today: an increase in global average temperature

and ocean heat content, melting of land and sea ice and rising of sea level are just a few examples. The data obtained in this thesis represent a baseline against which to detect changes resulting from climate and landscape alterations, which are more rapidly advancing in the Canadian North than almost anywhere else in the globe. It also highlights the value of comparing *T. gondii* prevalence among different ecological groups and ecosystem types for a more comprehensive understanding of the epidemiology of *T. gondii*. My work shed new light on the current status of *T. gondii* in wildlife in northern Canada and helped identify high-risk regions for infection in northern Canada. *Toxoplasma gondii* was more prevalent in foxes and lynx in subarctic Nunavik than anywhere else in the Canadian Arctic, similar to serosurveys in people. It is important for northern communities to be aware of regional differences in risk of exposure to *T. gondii* through foodborne routes, while balancing these risks against the many nutritional, cultural, and economic benefits of consuming locally harvested wildlife. Targeted public health messaging is needed to raise awareness about specific foodborne parasitic zoonoses, as well as safe country food handling practices. Finally, working hand in hand with communities towards wildlife health surveillance and country food safety testing should be a priority to help stakeholders to identify One Health issues relevant to the North.

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APPENDIX A

HOST AND GEOGRAPHIC DIFFERENCES IN PREVALENCE AND DIVERSITY OF GASTROINTESTINAL HELMINTHS OF FOXES (*VULPES VULPES*), COYOTES (*CANIS LATRANS*) AND WOLVES (*CANIS LUPUS*) IN QUÉBEC, CANADA

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Abstract

Wild canids are hosts to a wide range of parasites and can play a role in transmission of zoonoses. As many parasites are transmitted through food webs, and wild canids are at high trophic levels, parasite prevalence and diversity in wild canids can serve as excellent indicators of ecosystem health. Our main objectives were to update knowledge on the composition of gastrointestinal helminths in wild canids from Québec, Canada, and to describe differences in parasite prevalence and diversity among canid species and regions. Hunters and trappers provided whole carcasses of red foxes (*Vulpes vulpes*) (N = 176), and intestinal tracts of coyotes (*Canis latrans*) (N = 77) and gray wolves (*Canis lupus*) (N = 23) harvested for non-research purposes over the winter of 2016–2017. A modified Stoll's centrifugation sucrose flotation on feces of 250 wild canids was used, and eggs of one family and eight genera of parasitic helminths were recovered: diphyllobothriids, *Taenia/Echinococcus* spp., *Capillaria* spp., *Toxascaris* sp., *Toxocara* sp., *Trichuris* sp., *Uncinaria* sp., and *Metorchis* sp. Adult *Taenia* spp. cestodes were recovered from 61 of 276 (22%) canids. Six different species (*T. hydatigena*, *T. twitchelli*, *T. crassiceps*, *T. polyacantha*, *T. krabbei,* and *T. pisiformis*-"like") were differentiated based on DNA sequenced from 65 individual adult cestodes using primers for the nicotinamide adenosine dinucleotide dehydrogenase subunit 1 (ND1) and cytochrome *c* oxidase subunit 1 (CO1) mitochondrial DNA loci. *Alaria* sp. trematodes infected 89 of 276 canids (32%). A subset were identified as *A. americana* at the CO1 locus. The marine trematode *Cryptocotyle lingua* was reported for the first time in foxes in the province of Québec. These results help us understand more fully the predator-prey relationships within this group of canids. This baseline data in regional parasite prevalence and intensity is critical in order to detect future changes following ecological disturbances due to climate and landscape alterations.

Keywords: canids, wildlife, parasites, cestodes, nematodes, trematodes

1. Introduction

Wild canids, such as red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*) and gray wolves (*Canis lupus*), are reservoirs of several zoonoses, including parasites, that can spillover to humans and domestic animals (Aguirre, 2009). Despite the widespread distribution of wild canids in Québec, little information is available on their parasite communities or the prevalence and intensity of particular species in this region. This type of baseline data is necessary to monitor ecological changes. Parasite surveillance is thus an important tool to assess the population health of wildlife, particularly in canid populations in close contact with human settlements (McCallum and Dobson, 1995).

Among the factors that influence the gastrointestinal parasite communities of wild canids are their diets and trophic relationships. For example, red foxes feed mainly on voles and other small rodents, but also forage on a variety of other prey species, with dietary patterns influenced by season and prey availability (Larivière and Pasitschniak-Arts, 1996). The broad distribution and diverse diet of this opportunistic predator expose it to diverse parasites. In Canada, nine cestode, 14 nematode, and 11 trematode parasites have been reported in red foxes. It includes nematodes *Ancyclostoma caninum* (hookworm) and *Toxascaris leonina* (roundworm), as well as parasites that pose potential threats to domestic animals or humans, such as *Toxocara canis* (roundworm), *Dirofilaria immitis* (canine heartworm), and *Echinococcus multilocularis* (a zoonotic tapeworm that causes liver disease in dogs and people) (Curtis et al., 1988; Robbins, 2018; Schurer et al., 2018).

Coyotes are considered opportunistic and generalist predators and scavengers, capable of exploiting many habitats, including urban areas (Watts et al., 2015; Breck et al., 2019). Their diet consist mainly of rodents and lagomorphs, and the scavenged remains of larger wildlife (Wells and Bekoff, 1982). Previous surveys of parasites in coyotes in Canada reported several zoonotic species, including *Echinococcus* spp. cestodes (Schurer et al., 2018), *Toxocara canis* (Bridger et al., 2009), and the intestinal trematodes *Alaria arisaemoides* and *Alaria americana* (Luong et al., 2018).

Gray wolves are broadly distributed across Canada, with the exception of the Maritime provinces and Newfoundland (Mech and Boitani, 2004). The main prey of gray wolves are large ungulates, but their diet also includes secondary prey species, such as hares, foxes, beavers, small rodents, and birds (Hénault and Jolicoeur, 2003; Chester, 2016). At least 25 helminth species have been reported in gray wolves in Canada. These commonly include *Taenia* spp. and *Echinococcus* spp. cestodes, *Uncinaria stenocephala* (northern hookworm) and *Toxascaris leonina* nematodes, and several species of *Alaria* (Craig and Craig, 2005).

In addition to host trophic ecology, the transmission and distribution of canid parasites is affected by ecological disturbances, such as rapid urbanization, global warming, or habitat destruction, with climate change being recognized as one of the top individual drivers (Patz et al., 2000; Semenza et al., 2016). Many factors driven by climate change could potentially affect parasite distribution in wildlife. These include biodiversity loss and latitudinal and altitudinal host range shifts, such as wild canids moving northward (Jenkins et al., 2013), as well as decreased habitat connectivity (Cable et al., 2017). In Canada, climate has been, and continues to be, impacted by human activity. Canada has a rate of surface warming more than twice the global rate (Bush and Lemmen, 2019). As a consequence, this may alter seasonal and geographic patterns of parasite transmission, as well as the development and survival of environmental stages of parasites (Jenkins et al., 2011). Climate-driven mismatch between prey and host may also add variability to the epidemiological outcomes of parasite transmission (Altizer et al., 2013). Landscape changes, such as deforestation, may also favor paratenic and intermediate hosts of parasites, such as rodents. These changes may increase risk of transmission of helminth parasites through carnivory (Romig et al., 2006).

Despite the ecological and distributional differences among wild canid species, as high-trophic-level predators and scavengers throughout eastern Canada, these hosts share many parasites through similar habitats and prey species. Parasite communities in these hosts can serve as indicators of intact trophic relationships for indirectly transmitted parasites, one example being the important zoonotic cestodes of the genus *Echinococcus*. Urbanization impacts ecosystems, affecting the gut microbiota of generalist canid species following consumption of anthropogenic food, resulting in higher parasite susceptibility (Sugden et al., 2020). Still, studies describing

parasite diversity and prevalence in wild canids from the province of Québec are lacking (Table 1). There are very few reports on helminths in red foxes (Swales, 1933; Curtis et al., 1988; Schurer et al., 2018), no published studies on parasite diversity in coyotes, and only a few studies of gray wolves, the latter including reports of *Echinococcus granulosus*/*E. canadensis*, *Taenia hydatigena*, *Taenia krabbei*, *Taenia pisiformis*, *Alaria* spp., and *Dioctophyme renale* (giant kidney worm) (McNeill and Rau, 1984; Curtis et al., 1988; Hénault and Jolicoeur, 2003; Schurer et al., 2018). With unprecedented climate and landscape change in Canada, and with little baseline data in Québec, we address a pressing need to update knowledge on parasites hosted by wild canids and their distribution to better understand risk to human and animal populations. Initially driven by the need for *Echinococcus* surveillance in wild canids (published previously in Schurer et al., 2018), here we describe the composition of other gastrointestinal helminths among foxes, coyotes, and wolves, as well as differences in parasite prevalence and diversity based on gross examination and fecal flotation in canids in two different climate regions of Québec (QC), Canada.

2. Materials and methods

2.1 Study area

Hunters and trappers in QC provided whole carcasses of red foxes (N=176), and intestinal tracts of gray wolves (N=77) and coyotes (N=23) that were harvested during regular fur-trapping activities of winter 2016-2017. Fox samples were collected in two main climate regions: Subarctic (N=87) and Humid Continental (N=89), whereas coyotes and wolves were harvested throughout the Humid Continental climate region (Fig. 1) (Beck et al., 2018). These two regions differ in climate, vegetation, and prey abundance and diversity. The Subarctic climate has long winters, among the coldest in eastern Canada, with warm but short summers. Boreal forest covers most of the southern Subarctic region, with spruce, lichen and moss in the northern part (MFFP, 2019). This climate region supports large numbers of woodland caribou (*Rangifer tarandus caribou*), as well as moose (*Alces alces*), beaver (*Castor canadensis*), Canada lynx (*Lynx canadensis*), black bears (*Ursus americanus*), wolves, snowshoe hare (*Lepus americanus*) and a variety of birds (Bercuson et al., 2021). The Humid Continental climate region includes most major human population centres, and has warm, humid summers and long, cold winters. Deciduous and mixed forest characterise the region, with precipitation abundant throughout the year, in contrast to the Subarctic climate (MFFP, 2019). White-tailed deer (*Odocoileus virginianus*) thrive in this region,

as well as smaller mammals such as squirrels, mink (*Neovison vison*), raccoons (*Procyon lotor*), muskrats (*Ondatra zibethicus*), skunks (*Mephitis mephitis*), rabbits, groundhogs, mice and moles (Bercuson et al., 2021).

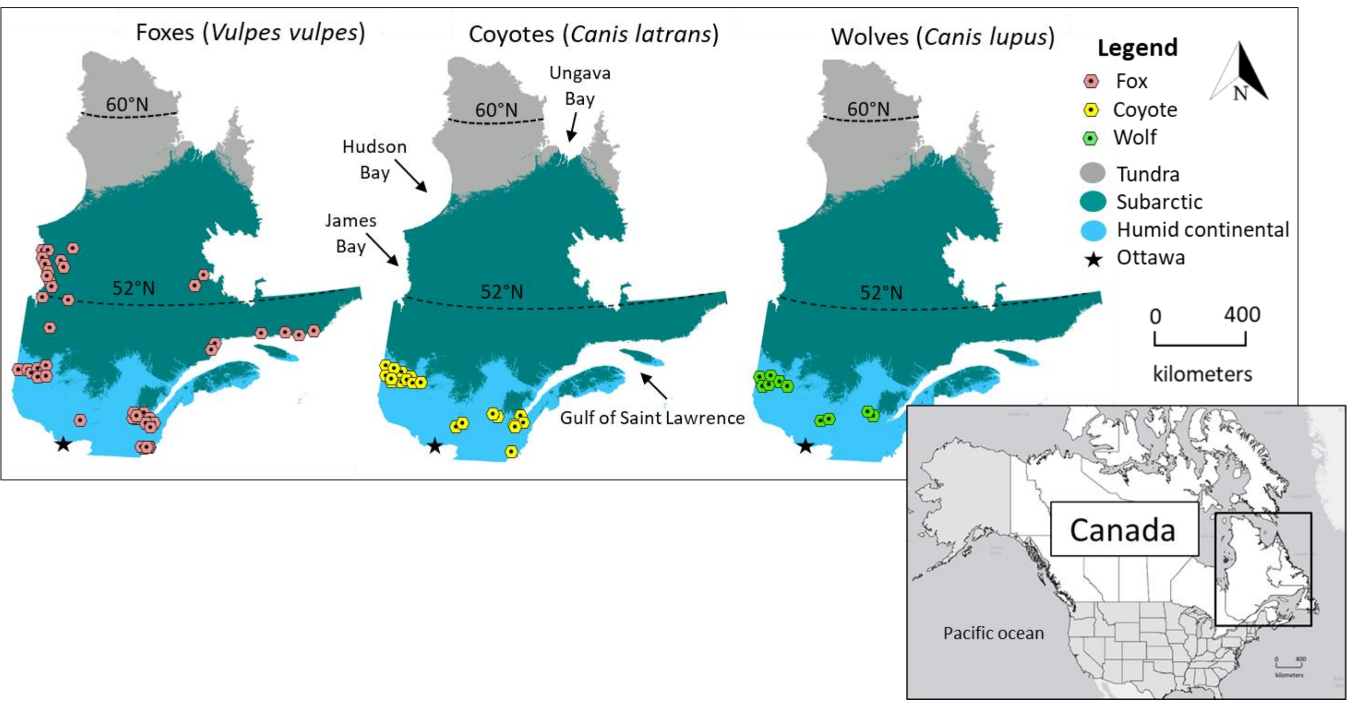

Fig. 1. Köppen climate regions and sampling distribution of foxes (*Vulpes vulpes*, N=176), coyotes (*Canis latrans*, N=77), and wolves (*Canis lupus*, N=23) collected during winter 2016-2017 by hunters and trappers from Québec, Canada. Arrows indicate major waterways.

2.2 Gross examination

Our research team performed fox necropsies at the Faculté de Médecine Vétérinaire in Saint-Hyacinthe (QC) where intestinal tracts (including feces) of all canids were stored at -20°C until shipped to the University of Saskatchewan (SK). They were then frozen at -80°C for at least five days prior to examination to inactivate infectious Echinococcus eggs as per World Health Organization recommendations for safe handling (Eckert et al., 2001). We collected helminths from small intestines by the scraping, filtration, and counting technique (SFCT) (Gesy et al., 2013). Briefly, we divided each small intestine into four equal parts, opened them longitudinally, and placed them in a sealable glass jar with 250 ml of tap water. After shaking vigorously for 1-2 min, we scraped the intestines and washed the contents through a large mesh sieve (one mm pore size, 20.3 cm diameter, USA standard test sieve no. 18, Fisher Scientific Company, Ottawa, Ontario,

Canada) prior to counting and identifying adult helminths. Our study team recorded the presence of adult nematodes, primarily ascarids, but did not identify them morphologically, relying on detection of characteristic eggs on flotation for species level identification. We stored cestodes in 90% ethanol prior to morphological examination and molecular analysis. For the trematodes, we morphologically examined the adults, and recorded their presence. We fixed several Alaria specimens (n=17) in 90% ethanol for molecular identification to species level. After obtaining feces from the rectum, colon, and/or distal ileum, we stored them at -20˚C prior to conducting fecal egg counts (FEC) (Schurer et al., 2014). We identified non-Echinococcus cestodes (Taenia and diphyllobothriids) by morphological examination.

2.3 Modified Stoll's centrifugation sucrose flotation

We thawed 4 grams of feces, weighed and mixed them thoroughly in a paper cup with 40 ml of Sheather's sucrose solution (specific gravity of 1.2) to create a homogenized mixture. After sieving the fluid through a cheesecloth into a second cup, we poured 10 ml aliquot (~25%, representing 1 gram of feces) into a test tube then topped it up with ~5 ml of Sheather's sucrose solution to form a slight convex meniscus. We then placed a coverslip on top, centrifuged the tube at 491 rcf for 10 minutes after which we lifted the coverslip and placed it on a glass slide. One technician examined one slide per sample from each canid, viewed helminth parasite ova under the microscope at 10 - 40X objective lens, and identified them based on size and morphology (Dryden et al., 2005). The whole slide was counted. The detection limit per egg count was five eggs per gram (epg) of feces (Nielsen et al., 2010). Fecal infection intensity, defined as the concentration of helminth eggs infecting a host, was categorised using the following semi-quantitative scale: 1+ 1-50 epg; 2+ 51-250 epg; 3+ 251-1000 epg; 4+ >1000 epg. While not ideal, we used a semi-quantitative method based on ordinal measures (1+ to 4+) instead of egg counts to reduce technician time.

2.4 Molecular methods

Our study team identified *Taenia* cestodes to species level by molecular analysis. Using fine tipped scissors, we macerated approximately 25 mg of tissue from two representative *Taenia* specimens per host in separate microcentrifuge tubes, from which we extracted DNA using the DNeasy Blood and Tissue Kit as per manufacturer's instructions, except eluting 150 µl of DNA in

the final step to increase DNA concentration (Qiagen Inc., Valencia, California, USA). We conducted Polymerase Chain Reaction (PCR) (25ul, 5ul of DNA) with two primer sets, previously designed and validated, targeting a 471 base pair (bp) region of the nicotinamide adenosine dinucleotide dehydrogenase subunit 1 (ND1) and a 366 bp region of the cytochrome *c* oxidase subunit 1 (CO1) mitochondrial DNA (Bowles et al., 1992; Bowles and McManus, 1993). After resolving PCR products by electrophoresis on a 1.5% agarose gel, we purified them using the QIAquick PCR Purification Kit following manufacturer's instruction (Qiagen Inc., Valencia, California, USA), and sent for sequencing (Macrogen Inc., Seoul, Korea). We trimmed forward and reverse sequences, aligned them, and identified them using the BLASTn tool to compare sample to reference sequences in the nucleotide database of GenBank. We used Clustal (https://www.ebi.ac.uk/Tools/msa/clustalo/), a multiple sequence alignment program, for sequences that did not have strong matches to GenBank to look for any similarity, as many had low query coverage but were most similar to the same accession numbers. We recorded mixed infections (i.e. infection with multiple species of taeniid cestodes). Methodology for *Echinococcus* spp. identification was described by Schurer et al. (2018). For trematodes in the genus *Alaria*, we extracted DNA using the DNeasy Blood and Tissue Kit as per manufacturer's instruction (Qiagen Inc., Valencia, California, USA). We conducted PCR (25ul, 5ul of DNA) with validated primers targeting the ~366 bp COI region (Moszczynska et al., 2009; Van Steenkiste et al., 2015) on a subset of samples (n=17) followed by sequencing and comparison with published data through BLASTn searches. Sequences were aligned with those published from other representatives of *Alaria* using MAFFT (Katoh et al., 2002) and sequence similarity was calculated and visualized in a neighbour-joining tree constructed in Geneious Prime (Biomatters, Auckland, NZ). All PCRs used both negative and positive controls (generated in house).

2.5 Statistical analysis and mapping

Canids were considered infected if we observed (1) parasite adults on SFCT, or (2) ova of helminths, for which wild canids are known definitive hosts, by FEC. We conducted a two-sided Fisher's Exact test to determine if parasite prevalence (percentage of canid host species infected with one or more helminth species) differed between canid host species based on gross examination, and when combined with fecal flotation. The same test was used to determine if parasite prevalence differed between geographic regions (comparing only foxes), when combining

gross examination and fecal flotation. Freeman–Halton's extension was used when contingency tables were greater than 2×2. We used a one-way ANOVA post hoc test to detect differences in the number of parasite genera between canid host species (defined as parasite genus richness), determined by gross examination and fecal flotation combined. We corrected for multiple pairwise comparisons using Bonferroni correction, with a threshold for significance of 0.05/n (n=3). We also used one-way ANOVA to determine if there were significant differences in the number of parasite genera between foxes from Subarctic and Humid Continental climate regions. We used the Mann-Whitney U-Test to evaluate median infection intensity differences among canid host species, when based on gross examination. Median infection intensity is defined here as the concentration of adult helminths infecting a host. All test were performed at a significant level of 5%. We generated 95% confidence intervals with the Wilson's method using Epitools (Sergeant, 2018). We mapped the distribution of infected and uninfected canids by entering the geographic coordinates (latitude, longitude) of trap sites into QGIS version 3.12.1 (QGIS Development Team, 2020). We did not include host sex or age in our analyses because these were known for only a few individuals. Data on *Echinococcus* spp. reported previously in Schurer et al. (2018), were included in the taeniid analysis. For statistical purposes, we assumed that all Alaria were the same species as the 17 specimens identified by DNA sequence comparison. All statistical tests were performed using SPSS version 26 (IMB Corporation, Armonk, New York, USA).

In accordance with the Canadian Council on Animal Care guidelines, this research was exempt from Animal Research Ethic Board review in Canada because all samples were collected from animals previously harvested for non-research purposes.

3. Results

Examination of small intestinal contents revealed seven helminth parasites: cestodes, including diphyllobothriids (likely *Dibothriocephalus* spp., the broad fish tapeworm, known previously as *Diphyllobothrium* spp. (Scholz et al., 2019; Waeschenbach et al., 2017)), *Echinococcus canadensis*, and *Taenia* spp.; ascarid nematodes (*Toxocara canis* and/or *Toxascaris leonina*); and trematodes, including *Alaria* sp. and *Cryptocotyle lingua* (Table 2a). We observed eggs from nine gastrointestinal helminths on fecal flotation, including cestodes diphyllobothriids, *Taenia* spp. and/or *Echinococcus* spp.; nematodes *T. canis*, *T. leonina*, *Trichuris vulpis* (canine whipworm),

U. stenocephala (northern hookworm), and *Capillaria* spp. (various species, adults of which can live in the intestine, airways, and bladder); and trematodes *Metorchis conjunctus* (North American liver fluke) and *Alaria* sp. Eggs of *T. leonina* were the most prevalent in foxes and taeniid eggs were most prevalent in coyotes and wolves (Table 3).

We found that by combining gross examination and fecal flotation, 89% of foxes (138/155, 95% CI:83-93), 73% of coyotes (53/73, 95% CI:61-82), and 73% of wolves (16/22, 95% CI:52-87) were infected with at least one gastrointestinal parasite (Table 3). Parasite genus richness ranged from zero to five for foxes, and zero to four for coyotes and wolves (Fig. 2). Fewer foxes were uninfected than coyotes (p = 0.006). More foxes were infected by two parasite genera than coyotes (p = 0.004) (Table 4a). No significant difference was seen in foxes between Subarctic and Humid Continental climate regions and parasite genera (Table 4b).

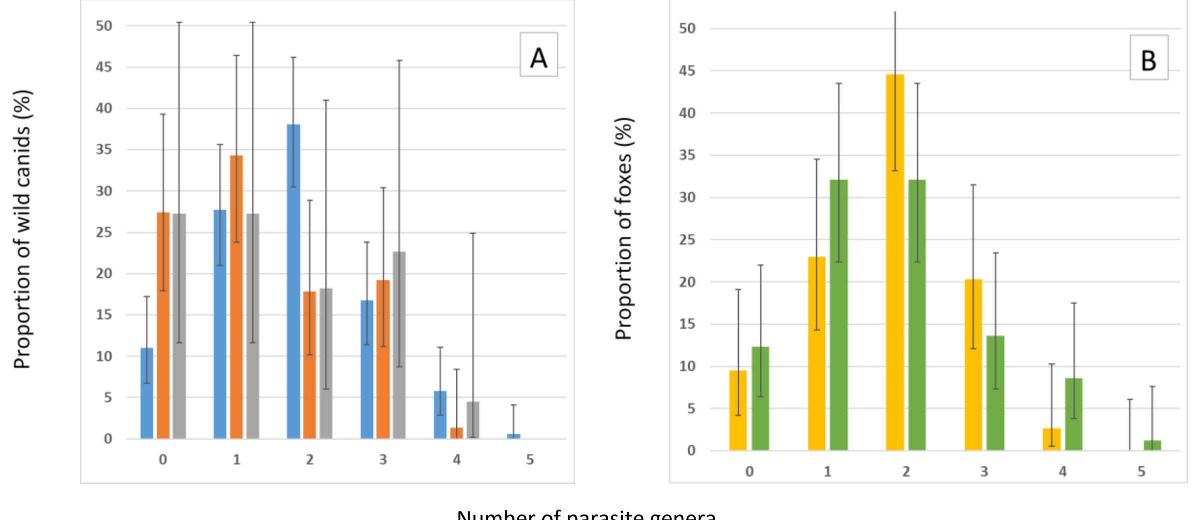

Fig. 2. A) Parasite genus richness in foxes (*Vulpes vulpes*, blue), coyotes (*Canis latrans*, orange), and wolves (*Canis lupus*, grey) from Québec, Canada, determined by gross examination and fecal flotation combined (N=250). Fewer foxes were uninfected than coyotes (p = 0.006). More foxes were infected by two parasite genera than coyotes (p = 0.004). B) Parasite genus richness between Subarctic (yellow) and Humid Continental climate (green) in foxes from Québec, Canada, determined by gross examination and fecal flotation combined (N=155). No significant difference in parasite genera was seen in foxes between Subarctic and Humid Continental climate regions. Parasites counted in both histograms were: diphyllobothriids (likely *Dibothriocephalus* spp.),

Echinococcus spp., *Taenia* spp., *Capillaria* spp., *Toxascaris* sp., *Toxocara* sp., *Trichuris* sp., *Uncinaria* sp., *Alaria* sp., *Cryptocotyle* sp., and *Metorchis* sp. Parasites observed in both fecal and gross examination were only counted once. Bars represent 95% confidence intervals.

Based on gross examination, the median infection intensity of *E. canadensis* was 120 ± 813 cestodes/coyote (min-max: 6-2378), and 460 ± 1110 cestodes/wolf (min-max: 5-2900), with no significant difference between canid species (p = 0.32). For *Alaria americana*, the median infection intensity was 12 ± 77 trematodes/fox (min-max: 1-390), 3 ± 9 trematodes/coyote (min-max: 1-30), and 10 ± 35 trematodes/wolf (min-max: 1-80), with more abundant infections in foxes than in coyotes (p = 0.02). *Cryptocotyle lingua* was found only in foxes, with a median infection intensity of 48 ± 1724 trematodes/fox (min-max: 1-6000). Finally, combined nematode intensity was 5 ± 15 nematodes/fox (min-max:1-120), and 8 ± 13 nematodes/coyote (min-max: 1-33), with no significant difference between both species (p = 0.83). No nematodes were detected in wolves on gross examination. Eggs of *T. leonina* had the highest fecal infection intensity in foxes (>1000 epg), followed by *Trichuris vulpis* and *A. americana* (251-1000 epg). In coyotes, taeniid eggs, *Toxocara canis* and *Uncinaria* eggs had moderate fecal infection intensity (51-250 epg) while in wolves, all parasite species recorded had low infection intensity (1-50 epg).

Out of 84 non-Echinococcus adult cestodes, we morphologically identified 65 as *Taenia* spp. We detected six different species through DNA sequencing: *T. hydatigena*, *T. twitchelli*, *T. crassiceps*, *T. polyacantha*, *T. krabbei*, and *T. pisiformis*-"like" (Table 2b). Of 53 specimens, eight were *T. hydatigena* (99-100% similarity), ten were *T. twitchelli* (97-100% similarity), five were *T. crassiceps* (97-100% similarity), three were *T. polyacantha* (100% similarity), and three were *T. krabbei* (99% similarity). The remaining 23 high-quality sequences were 91-92% similar to *T. pisiformis* and identical to each other. Four other high-quality *Taenia* sequences had less than 90% similarity to any sequences in Genbank. The nine remaining sequences did not align well in GenBank (low query coverage, ≤ 91%), but six had high similarity to each other after generating multiple alignments of these sequences with the program Clustal. Findings of *Echinococcus* canadensis genotypes in wolves and coyotes are reported in Schurer et al. (2018).

Sequences of CO1 from 17 specimens of *Alaria* (16 from fox, 1 from wolf; geographic origins: Subarctic 13, Humid Continental 4) were identified as *A. americana* based on a match with a specimen from a fox in Nova Scotia (MH536507, Locke et al., 2018). Mean CO1 variation in this cluster of 18 sequences of *A. americana* was 0.81% (range 0-2.51%). By comparison, CO1 mean variation among 49 sequences of *Alaria alata* sampled across Europe (Fig. 3) was 0.88%, range 0-2.87%, and among five sequences of *Alaria* sp. in Argentina was 0.3% (range 0-0.8%). Mean interspecific distances among 73 CO1 sequences, including the 17 obtained in the present study, and four species of *Alaria* (the two named and two unnamed species in Fig. 3) were 8.05% (range 5.96-10.53%).

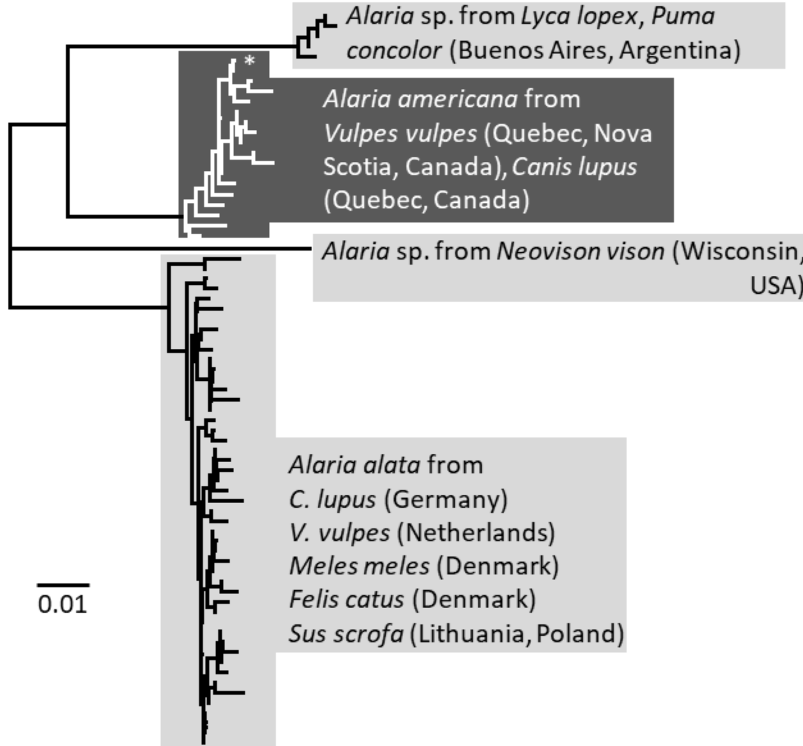

Fig. 3. Neighbour-joining tree of Jukes-Cantor distances among sequences of CO1 (alignment 450 bp using all sites) from *Alaria* available on GenBank as of 7 July 2021. Data from *Alaria americana*, including data from the present study, indicated by darker shaded cluster and white font. Sequences from *A. alata* are HM022221-3, KF751233-4, KP123416-20, KP123422-5, KX962374, KX962392, KX962395, KX962397-8, KX962402, KX962406, KX962415, KX962421, KX962433, KX962437, KX962454-5, KX962471-2, KX962481, KX962491, KY012317, MT103215-31; from *Alaria* sp. in Argentina KF572949, MH892076, MT328804-6;

from *Alaria* sp. in Wisconsin, USA KT223036; from *A. americana* MZ605217-33 (present study) and MH536507 (indicated with an asterisk).

Mixed taeniid infections were present in coyotes and wolves of the Humid Continental region (Fig 4). In the 13 foxes infected by taeniids, only single taeniid infections were observed (of *T. twitchelli*, *T. crassiceps*, or *T. polyacantha*, Table 2b).

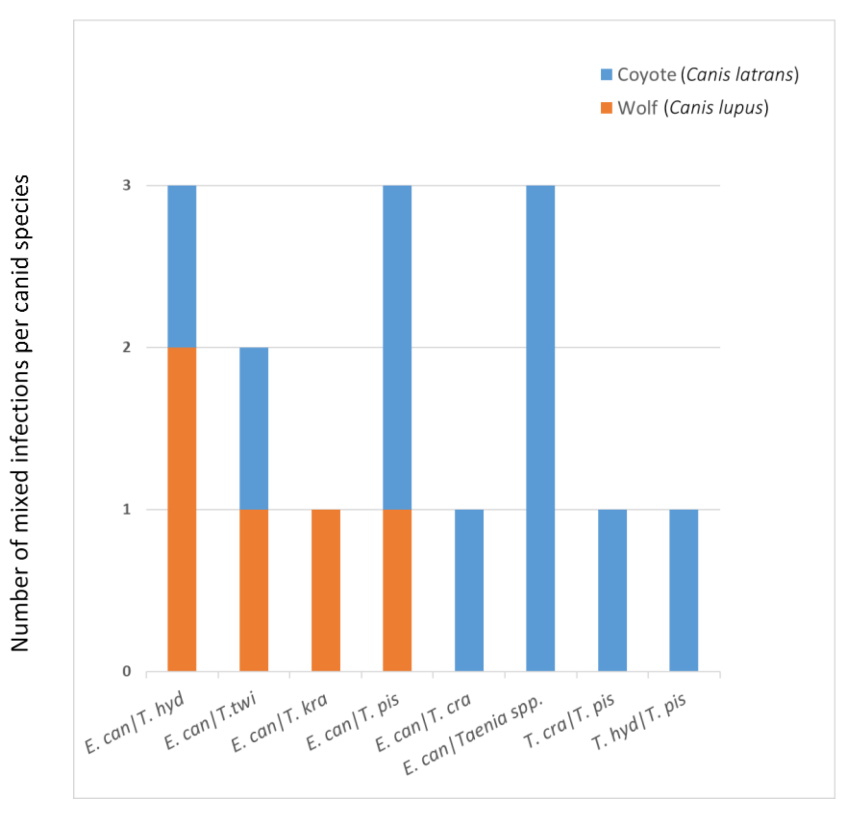

Fig. 4. Mixed taeniid infections in the Humid Continental climate in coyotes (*Canis latrans*) and wolves (*Canis lupus*) from Québec, Canada, following molecular analyses. Abbreviations on x-axis: *E. can*, *Echinococcus canadensis*; *T. hyd*, *Taenia hydatigena*; *T. twi*, *T. twitchelli*; *T. kra*, *T. krabbei*; *T. pis*, *T. pisiformis*-“like”; *T. cra*, *T. crassiceps*.

In the Humid Continental climate region, prevalence of *T. canis*, *T. vulpis*, and *A. americana* was higher in foxes compared to other canid hosts ($p < 0.05$). In coyotes and wolves, taeniid cestodes and diphyllobothriids were more prevalent than in foxes ($p < 0.05$). In foxes

collected in the Subarctic region, *T. leonina*, *T. vulpis*, *A. americana* and *Cryptocotyle lingua* were the dominant gastrointestinal helminths. We observed multiple foxes (n=26) infected with *Cryptocotyle lingua* trematodes, with the majority concentrated along the St. Lawrence estuary (Fig. 5). Finally, *Capillaria* spp. and *M. conjunctus* were only observed once in foxes (Table 3).

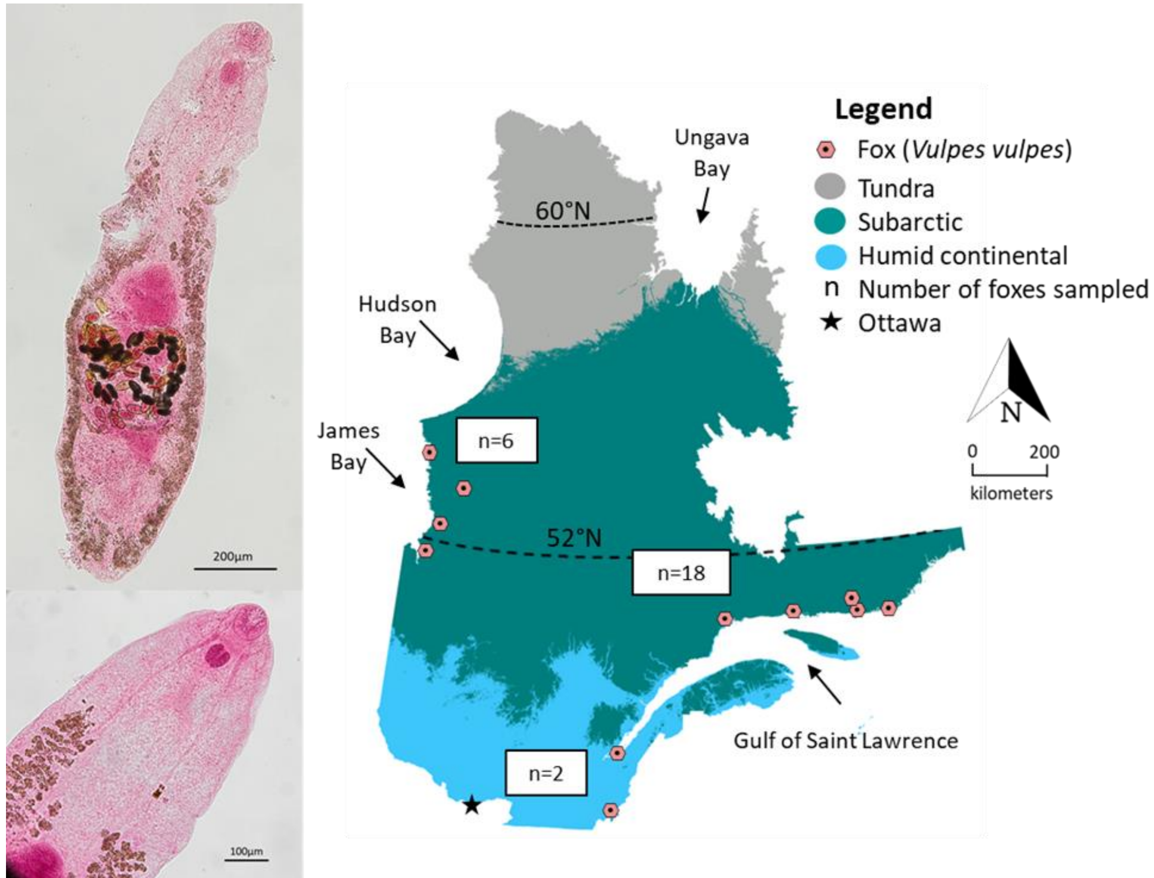

Fig. 5. On the left, whole mounted *Cryptocotyle lingua* adult trematode stained with borax carmine (credit: Brent Wagner). On the right, distribution of foxes (*Vulpes vulpes*) infected with *C. lingua* in the Subarctic (samples (n) collected along James Bay and the St Lawrence estuary) and Humid Continental climate collected during winter 2016-2017 by trappers from Québec, Canada. Arrows indicate major waterways.

We found significant geographic differences in parasite prevalence in foxes for taeniids ($p = 0.02$), *Toxascaris leonina* ($p < 0.001$), *Toxocara canis* ($p = 0.002$), *Trichuris* ($p = 0.02$), and *Cryptocotyle lingua* ($p < 0.001$) (Table 3). Foxes from more southerly areas had significantly higher prevalence of *Toxocara canis* than those from the north ($p = 0.002$), whilst the contrary

applied for *Toxascaris leonina*, which was less prevalent in southern than northern latitudes ($p < 0.001$) (Fig. 6) (Table 3). Overall parasite prevalence in foxes was similar between both regions (no significant difference on fecal flotation ($p = 0.16$) and combined with gross examination ($p = 0.62$)) (Table 3).

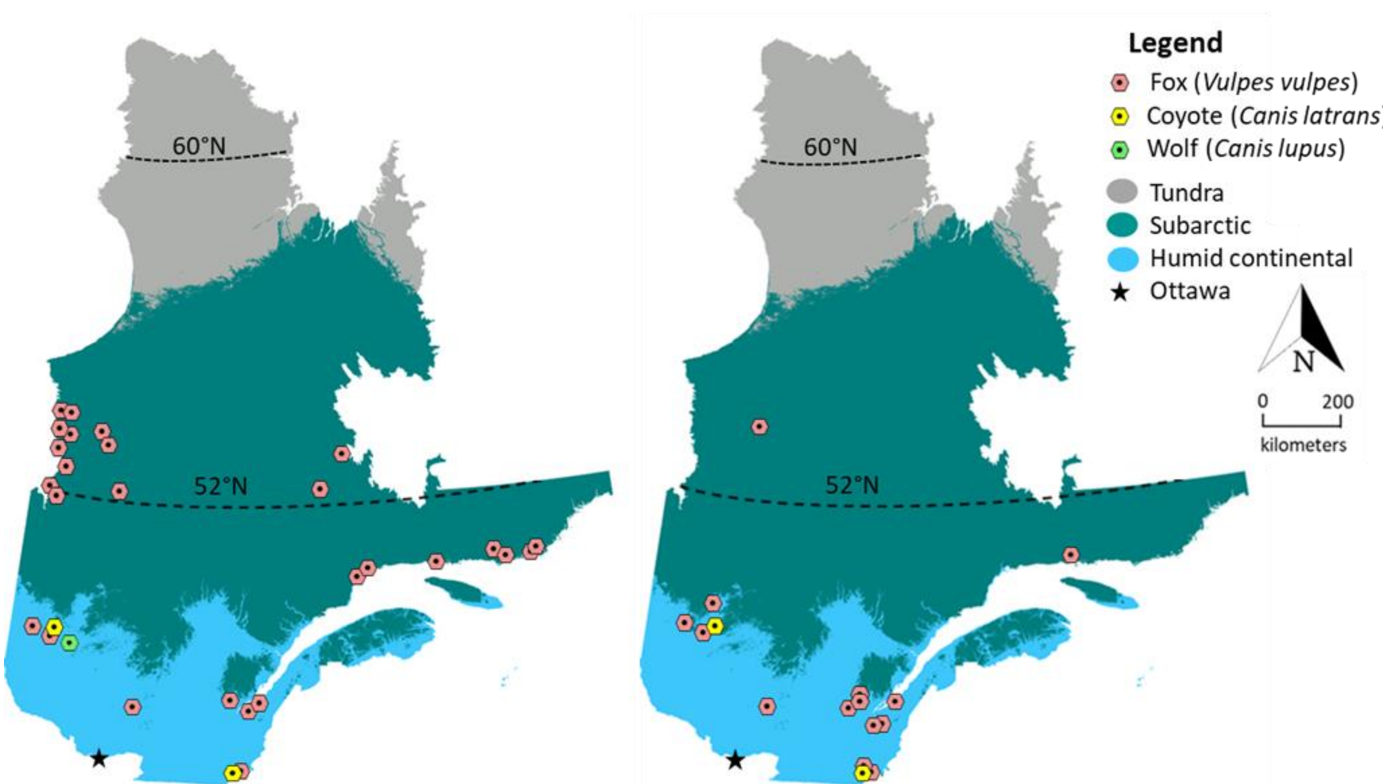

Fig. 6. Distribution of foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and wolves (*Canis lupus*) infected with *Toxascaris leonina* (left, N=55) and *Toxocara canis* (right, N=19) in the Subarctic and Humid Continental climate collected during winter 2016-2017 by hunters and trappers from Québec, Canada.

4. Discussion

We encountered regional and host-specific differences in gastrointestinal parasitism in wild canids from two climate regions of Québec, Canada, combining adult parasite recovery and fecal flotation with morphological and molecular methods to improve detection and maximize taxonomic resolution. Using combined methods was especially important for cestodes in coyotes

and wolves, for which prevalence is underestimated when diagnosis is based solely on eggs in feces (Schurer et al., 2016).

4.1 Parasitism, host diet and ecology

4.1.1 Cestodes

Host diet can play a key role in shaping gastrointestinal helminth diversity. Our findings of *T. twitchelli*, *T. crassiceps*, and *T. polyacantha* in foxes highlight predation on rodents (lemmings, voles, and mice) and lagomorphs (Stien et al., 2009). Coyotes can hunt and scavenge larger wildlife such as cervids, accounting for findings of *T. hydatigena* and *E. canadensis* (Schurer et al., 2018). Recovery of *Taenia* species using rodents and lagomorphs as intermediate hosts (e.g., *T. twitchelli*, *T. pisiformis*-like, *T. crassiceps*, and *T. polyacantha*) from coyotes are consistent with the higher proportion of small mammals in coyote diets compared to wolves (Keith et al., 1986; Rausch and Fay, 1988). Detection of *T. hydatigena* and *T. krabbei* in wolves highlight the importance of big game such as moose, deer and caribou in the diet of wolves, which serve as intermediate hosts for these parasites. Beavers, an important secondary prey of wolves, can also harbor *T. hydatigena* (Gable et al., 2018; Spickler, 2020). Our findings of *T. twitchelli* and a *T. pisiformis*-like species also reinforce observations that wolves prey on lagomorphs and porcupines (*Erethizon dorsatum*), which serve as intermediate hosts for these cestodes (Holmes and Podesta, 1968; Craig and Craig, 2005). Also, our results on mixed infection in coyotes and wolves support laboratory-based studies demonstrating that complete protective immunity against recurrent taeniid infection with different species is unlikely in canids (Jenkins and Rickard, 1985). As well, mixed infections indicate broad ranges of dietary sources for individual wild canids, with each host harbouring a potentially unique community of trophically transmitted parasites.

The higher prevalence of adults and eggs of diphyllobothriids in coyotes (Table 2a-3) suggests a diet that includes more piscine intermediate and paratenic hosts containing plerocercoids (Ching, 1984; Jenkins et al., 2013; Reid et al., 2018). Given the known geographic distribution of diphyllobothriids, and the distance of collected specimens from marine environments, the most likely identification for these cestodes in our data set are the freshwater species *Dibothriocephalus latus* (previously *Diphyllobothrium latum*) or *D. dendriticus* (previously *Diphyllobothrium dendriticum*) (Jenkins et al., 2013).

4.1.2 Nematodes

Trichuris vulpis, which was more common in foxes (Table 3), has a direct life cycle with no paratenic hosts. Canids acquire *T. vulpis* by ingesting larvated eggs that persist for years in the environment (Kirkova and Dinev, 2005). The higher prevalence in foxes versus coyotes and wolves may reflect higher density of foxes in urban areas due to smaller home range (Goszczyński, 2002), and more potential for contact with environments contaminated with feces from stray and domestic dogs in peri-urban settings in southern regions (Traversa, 2011). We did not examine large intestines (the infection site of adult *T. vulpis*), and consequently our data may underestimate overall prevalence. In addition, some *T. vulpis* eggs observed may have originated from ingested prey species. Eggs of *Uncinaria*, another potentially directly transmitted nematode, were found in all canid species. This parasite can be acquired through consumption of hatched third stage larvae (L3) in the environment, indirectly through consumption of L3 in paratenic hosts, or, rarely compared to *Ancylostoma*, through cutaneous invasion of L3.

4.1.3 Trematodes

All three trematodes detected in Québec canids, *Alaria*, *Metorchis*, and *Cryptocotyle*, are acquired from aquatic environments. *Alaria* sp. requires aquatic snails and amphibians as intermediate hosts, and can also be transmitted by paratenic hosts such as snakes and rodents (house and deer mouse), which are all prey items of wild canids (Möhl et al., 2009). Eggs of *Metorchis conjunctus* were only detected in one fox from the Subarctic climate. Multiple *Cryptocotyle lingua* were detected on gross examination of small intestines in 26 foxes, mostly from the northeastern shore of the St. Lawrence estuary in the Subarctic climate region. Foxes may become infected with this fluke by feeding on fish remains, particularly euryhaline and diadromous species, along the shorelines of marine waters, rivers and ponds (Gibson, 1996; Saeed et al., 2006).

These findings illustrate relationships between trophically transmitted parasites and diet in wild canids (cestodes and trematodes) and between directly transmitted parasites such as *T. vulpis* and host ecology and habitat use. This is expected as many helminth species are acquired through the ingestion of infective larval stages, from the environment or from infected prey serving as intermediate or paratenic hosts (Lafferty, 1999). In the present study, foxes had significantly more

parasite species richness compared to coyotes. Although smaller in body size, this could be explained by a greater dietary breadth than coyotes (Dodd and Whidden, 2018). Other features such as environmental factors, population density, and geographical range, can also play a role as determinants of parasite species richness (Kamiya et al., 2014; Villalobos-Segura et al., 2020).

4.2 Cryptic species and molecular challenges

In the present study, molecular methods were critical for many parasite identifications. For example, even though species of *Taenia* can be distinguished through rostellar hook morphometrics, freeze-thaw cycles impede recovery of intact worms, making scolices difficult to recover, and strobila alone have limited utility for morphological identification. Molecular methods are therefore a useful alternative. Previously reported in wolves from northern and western Canada (Schurer et al., 2016), we demonstrated coinfection of coyotes and wolves with multiple taeniid genera and species. Following molecular analyses, we found mixed infections of *E. canadensis* – *Taenia* (10%) and *Taenia* species (3%) in coyotes, as well as *E. canadensis* – *Taenia* (22%) in wolves. As we did not conduct PCR on all taeniid cestodes, and no coyotes and wolves were harvested in the Subarctic region, our molecular approach likely underestimated the true prevalence of mixed infections. Our results also highlight the need for morphologically confirmed identifications in molecular databases, and the utility and limitations of widely used loci such as the CO1 mtDNA gene. We found 23 isolates with CO1 91-92% similar to *T. pisiformis*, suggesting that these specimens belong to a closely related and as-yet-unsequenced species of *Taenia* circulating in wild canids in North America. Future work could include genetic comparisons of putative *T. pisiformis* from across its global distribution. We also recovered six identical sequences from *Taenia* spp. specimens that did not align well (query coverage ≤ 91%) with any sequences in GenBank; this could also be an existing *Taenia* sp. for which sequence has not yet been published, or a new species. Many *Taenia* were often observed in gross examination, and molecular identification was not conducted in all specimens because of the costs and resources necessary for sequencing. A multiplex PCR or restriction fragment length polymorphism PCR could be an alternative for future large-scale studies (Al-Sabi and Kapel, 2011; Huttner et al., 2009).

4.3 Distribution ranges and limits of distribution

In our study, *T. canis* was detected in 11% (17/155) of foxes, mostly in the Humid Continental region, and 3% (2/73) of coyotes following fecal flotation. The highest latitude at which *T. canis* was detected was 53°43'60.0"N, in a fox in the Subarctic climate region (Fig. 6). In addition to decreasing prevalence when moving north, this finding supports the hypothesis that *T. canis* does not thrive at northern latitudes (Jenkins, 2020). The eggs of *T. canis* have low freeze-tolerance but can persist for many years at temperatures of 10-30°C, which are required for the eggs to fully embryonate (Azam et al., 2012). Antibodies to *T. canis* have been reported at low prevalence (3.9%) of people in Subarctic QC, and the parasite has been reported in dogs, but is not well documented in QC wildlife (Cameron et al., 1940; Messier et al., 2012). Several factors may increase risks of *T. canis* infection in northern latitudes with current and future climate change, including growth in human and domestic dog populations, increasingly close contact with wildlife such as foxes, northward shifts in red fox distribution, lack of veterinary services, and enhanced egg survival in warmer and wetter weather (Jenkins et al., 2013). Co-infection with both *T. canis* and *Toxascaris leonina* is well documented in wild canids (Okulewicz et al., 2012). Within our study, *T. leonina* was found in both climate regions and in all canid species, with a higher prevalence in foxes from the Subarctic climate (Fig. 6). This is consistent with earlier coprological studies from arctic fox and in wild canids in general in Canada (Aguirre et al., 2000; Meijer et al., 2011; Elmore et al., 2013; Jenkins, 2020). The higher prevalence of *T. leonina* in foxes in the Subarctic (55%) compared to the Humid Continental climate region (8%) could be explained by increased competition with *T. canis* at more southern latitudes (Okulewicz et al., 2012), a higher reliance on rodent paratenic hosts as part of fox diet in subarctic regions, and negative effects of urbanization on rodent-transmitted parasites in southern regions (Reperant et al., 2007).

Prevalence of *T. vulpis* was higher in foxes from the south ($p = 0.02$), with no infections north of 53°45'16.4"N in the Subarctic climate. This may reflect the higher temperature and moisture requirements of eggs of *T. vulpis* (Spindler, 1929; Dubin et al., 1975; Miterpáková et al., 2009). Hookworm eggs, most likely *Uncinaria stenocephala* given the geographic context, were seen at relatively similar prevalence in foxes (8%) and other canids (4%). *Ancylostoma caninum* (southern hookworm) is usually found in temperate, tropical, and subtropical regions, and is rarely seen in Canada (Craig and Craig, 2005). *Uncinaria* is an ancyclostomid occupying a similar niche in

northern latitudes, with reports in arctic foxes from Europe, Iceland and Greenland (Meijer et al., 2011).

Finally, *Alaria americana*, identified in a subset of samples, has previously been considered a synonym of *A. marcianae* (Locke et al., 2018), which may have led to underreporting of *A. americana* in eastern Canada. Another trematode, *Cryptocotyle lingua*, has been reported in foxes from the eastern Canadian provinces of Prince Edward Island, New Brunswick, and Nova Scotia (Smith, 1978; Wapenaar et al., 2013; Robbins, 2018). This is the first report of *C. lingua* in foxes from QC, where this parasite has been recorded once in a great black-backed gull (*Larus marinus*) near Ungava Bay (Ferguson et al., 2012).

4.4 Zoonotic potential

Although rarely associated with clinical disease, diphyllobothriid cestodes are zoonotic. People become infected the same way as wild canids, by eating contaminated raw fish. The nematode *Toxocara canis* is another helminth with zoonotic potential, and is the agent of human toxocariasis, responsible for visceral larva migrans, ocular larva migrans and neurotoxocariasis (Macpherson, 2013). Commonly found in wild canids from temperate regions, *T. canis* has been reported as the most common parasite in dogs from urban animal shelters across Canada (Villeneuve et al., 2015). *Toxascaris leonina*, another ascarid nematode but with very limited zoonotic potential, has also been detected. *Uncinaria* is thought to have much lower zoonotic potential and animal health significance than *Ancylostoma*, the more pathogenic species in canids (Bowman et al., 2010). Cases of human intraocular infection with *Alaria* have been demonstrated in a few cases, likely caused by ingestion of undercooked contaminated frog legs (Otranto and Eberhard, 2011). As for *Metorchis conjunctus*, canids and people acquire this parasite from ingestion of metacercaria in catostomid fishes (suckers). Human infections caused by parasitic helminths remain of particular importance given their possible gravity and the close proximity between people, domestic animals, and wild canids.

4.5 Methodological limitations

Among limitations of our study were sampling that was uneven regionally and in terms of host species, especially in the Subarctic climate region. Given the constraints of sample acquisition and

the scale of our study, our methods of parasite detection and identification were generally robust and multidisciplinary, and our data likely reflect real biological patterns, but deficiencies were nonetheless unavoidable. As we did not target or record presence of adult hookworm nematodes from small intestines, as well as for large intestines, we likely underestimated infection levels. Fecal floats were not always conducted, as fecal matter was sometimes absent. Moreover, fecal floats do not reveal non-patent infections, and freezing at -80°C can affect egg integrity and detection. Indeed, morphological changes were observed in eggs of *T. canis* and to a lesser extent *T. leonina* following freezing at -80°C, although eggs of both these nematodes were still readily identified. In contrast, *Uncinaria stenocephala* infection levels are likely underestimated due to small sample sizes and the fragile nature of hookworm eggshells which rupture easily following freezing at -80°C (Schurer et al., 2014). Finally, eggs of the trematode *Metorchis conjunctus* were only detected in one fox. As we did not examine bile ducts on necropsy, and eggs of this parasite are dense and may not float well, our record likely underestimates true prevalence.

Necropsies are not always feasible or ethically possible in wildlife; therefore, fecal flotation remains a useful technique (especially for fecund nematodes such as ascarids). Results from flotations are quick, inexpensive, and safer than gross examination when samples are properly handled, including freezing at -80°C for a few days prior to processing to inactivate eggs of *Echinococcus* spp. On the other hand, gross examination is more sensitive for cestodes and trematodes which may not reliably shed eggs in feces or may not have eggs that float well in standard flotation solutions, and enables recovery of adult specimens suitable for morphological and/or molecular identification of species, i.e. to distinguish among species of *Echinococcus* and *Taenia* (Schurer et al., 2016).

5. Conclusion

In this study, we used a combined morphological and molecular approach to obtain new information on parasite communities of wild canids in Québec, Canada, and provided much needed baseline data on canid parasites that may undergo changes in distribution and prevalence in the rapidly changing climate in Subarctic and Arctic regions of Canada. We report two previously unsequenced *Taenia* species, describe northern distributional limits for two important canid nematodes, *Toxocara canis* and *Trichuris vulpis*, clarified the prevalence of a canid intestinal

trematode in northeastern North America (*Alaria americana*), and reported a new geographic record for the trematode *Cryptocotyle lingua* in foxes. We observed that foxes had more nematodes and fewer cestodes than wolves and coyotes (Table 2a). Many of the parasites observed are transmitted through prey, which informs our understanding of diets among wild canids. Finally, thinning boundaries between wild and domestic canids, as well as human disturbance, likely impact foraging and use of food resources by wild canids. As a result, this could alter the risk of transmission of these wildlife reservoired parasites, all of which can transmit to dogs, and many to people (*Echinococcus*, diphyllobothriid cestodes, *Toxocara*, *Metorchis*, and *Alaria* spp.) (Manlick and Pauli, 2020; Sugden et al., 2020). Continued studies of host-parasite interaction and parasite distributions are needed to detect changes in parasite communities as a result of climate and landscape alterations.

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Table 1. List of gastrointestinal helminth species recorded from foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and wolves (*Canis lupus*) observed by gross examination and/or fecal flotation in Québec, Canada, including the present study and references.

	Foxes[a,b]	**Coyotes[a,b]**	**Wolves[a,b]**
Cestodes			
Diphyllobothriids (likely *Dibothriocephalus* spp.)	●	●	●
Dipylidium caninum	● (1)		
Echinococcus granulosus/canadensis		●● (4)	●● (2,3,4)
Taenia crassiceps	●	●	
Taenia hydatigena		●	●● (2)
Taenia krabbei			●
Taenia pisiformis		●	●
Taenia polyacantha	●		
Taenia twitchelli	●	●	●
Nematodes			
Ancylostoma caninum	● (1)		
Capillaria spp.	●● (1)		
Toxascaris leonina	● (1,3)	●	●● (3)
Toxocara canis	●	●	
Trichuris vulpis	●● (1)	●	●
Uncinaria stenocephala	●● (1)	●	●
Trematodes			
Alaria americana	●● (1)	●	●
Alaria marcianae			● (2)
Cryptocotyle lingua	●		
Metorchis conjunctus	●		

[a] Grey dot indicates gastrointestinal helminth species found in the present study.
[b] (1) Swales, 1933; (2) McNeill and Rau, 1984; (3) Curtis et al., 1988; (4) Schurer et al., 2018

Table 2a. Gastrointestinal helminth prevalence in foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and wolves (*Canis lupus*) observed by gross examination (N=276) in Québec, Canada.

	Foxes (N=176)		Coyotes (N=77)		Wolves (N=23)		Difference in parasite prevalence among canid host species (N=276)		
	N	**%[d]**	**N**	**%[d]**	**N**	**%[d]**	**N**	**%[d]**	**P[e]**
Cestodes[a]	22	13 (8-18)	49	64 (52-73)	15	65 (45-81)	86	31 (26-37)	**<0.001**
Diphyllobothriids (likely *Dibothriocephalus* spp.)	1	1 (0-3)	8	10 (5-19)	1	4 (1-21)	10	4 (2-6)	**<0.001**
Echinococcus canadensis[b]	0	0 (0-2)	9	12 (6-21)	8	35 (19-55)	17	6 (4-9)	**<0.001**
Taenia spp.	13	7 (4-12)	36	47 (36-58)	12	52 (33-71)	61	22 (18-27)	**<0.001**
Nematodes[a]	79	45 (38-52)	5	7 (3-14)	0	0 (0-14)	84	30 (25-36)	**<0.001**
Trematodes[a]	87	47 (40-54)	15	20 (12-30)	6	26 (13-46)	108	38 (33-44)	**<0.001**
Alaria americana	68	37 (30-44)	15	20 (12-30)	6	26 (13-46)	89	31 (26-37)	**0.008**
Cryptocotyle lingua	26	14 (10-20)	0	0 (0-5)	0	0 (0-14)	26	9 (6-13)	**<0.001**
Overall parasite prevalence after gross examination[c]	134	76 (69-82)	53	69 (58-78)	17	74 (54-87)	204	74 (68-79)	0.46

[a] Canids infected with at least one gastrointestinal helminth species from the helminth classes (cestodes, nematodes, trematodes) based on gross examination
[b] *E. canadensis* results already published and discussed in Schurer et al., 2018
[c] Canids infected with at least one gastrointestinal helminth species based on gross examination
[d] 95% confidence intervals are displayed in parentheses
[e] P values are from Fisher's Exact test

Table 2b. Comparison of *Taenia* spp. in foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and wolves (*Canis lupus*) based on molecular analyses (N=65) following gross examination (N=276) in Québec, Canada.

	Foxes (N=176)		**Coyotes (N=77)**		**Wolves (N=23)**		**Difference in *Taenia* prevalence among canid host species (N=276)**		
	N	**%[b]**	**N**	**%[b]**	**N**	**%[b]**	**N**	**%[b]**	**P[c]**
***Taenia* spp.**									
T. hydatigena,	0	0 (0-2)	5	6 (3-14)	3	13 (5-32)	8	3 (1-6)	**<0.001**
T. twitchelli	5	3 (1-6)	2	3 (1-9)	3	13 (3-35)	10	4 (2-7)	0.08
T. crassiceps	4	2 (1-6)	1	1 (0-7)	0	0 (0-14)	5	2 (1-4)	1.0
T. polyacantha	3	2 (1-5)	0	0 (0-5)	0	0 (0-14)	3	1 (0-3)	0.66
T. krabbei	0	0 (0-2)	0	0 (0-5)	3	13 (5-32)	3	1 (0-3)	**<0.001**
T. pisiformis- "like"	0	0 (0-2)	21	27 (19-38)	2	9 (2-27)	23	8 (6-12)	**<0.001**
Unidentified	1	1 (0-3)	11	14 (8-24)	1	4 (1-21)	13	5 (3-8)	**<0.001**
Overall *Taenia* prevalence after gross examination[a]	13	7 (4-12)	36	47 (36-58)	12	52 (33-71)	61	22 (18-27)	**<0.001**

[a] Canids infected with at least one *Taenia* species based on gross examination
[b] 95% confidence intervals are displayed in parentheses
[c] P values are from Fisher's Exact test

Table 3. Gastrointestinal helminth prevalence in foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and wolves (*Canis lupus*) observed by gross examination and fecal flotation combined (N=250), and compared by climate regions of Québec, Canada. Fecal infection intensity is categorized using a semi-quantitative scale.

	Subarctic			**Humid Continental**												**Difference in parasite prevalence between regions (only foxes, N=155)**		
	Foxes=74			Foxes= 81			Coyotes=73			Wolves=22			Difference in parasite prevalence among canid host species in the Humid Continental climate (N=176)					
	N	**%[b]**	**I[a]**	**N**	**%[b]**	**I[a]**	**N**	**%[b]**	**I[a]**	**N**	**%[b]**	**I[a]**	**N**	**%[b]**	**P**	**N**	**%[b]**	**P**
Cestodes																		
Diphyllobothriids (likely *Dibothriocephalus* spp.)[c]	4	5 (2-13)	1+ to 2+	1	1 (0-7)	1+	8	11 (6-20)	1+	1	5 (1-22)	0	10	6 (3-10)	**0.02**	5	3 (1-7)	0.19
Taeniid (*Echinococcus* spp. and/or *Taenia* spp.)	2	3 (1-9)	1+	11	14 (8-23)	0	37	51 (39-62)	1+ to 2+	14	64 (43-80)	1+	62	35 (29-43)	**<0.001**	13	8 (5-14)	**0.02**
Nematodes																		
Capillaria spp.	0	0 (0-5)	1+	1	1 (0-7)	1+	0	0 (0-5)	0	0	0 (0-15)	0	1	1 (0-3)	1.0	1	1 (0-4)	1.0
Toxascaris leonina	41	55 (44-66)	1+ to 4+	10	12 (7-21)	1+ to 4+	3	4 (1-11)	1+	1	5 (1-22)	1+	14	8 (5-13)	0.14	51	33 (26-41)	**<0.001**
Toxocara canis	2	3 (1-9)	1+	15	19 (12-28)	1+ to 2+	2	3 (1-10)	1+ to 2+	0	0 (0-15)	0	17	10 (6-15)	**<0.001**	17	11 (7-17)	**0.002**
Trichuris sp.	16	22 (14-32)	1+ to 3+	33	41 (31-52)	1+ to 2+	6	8 (4-17)	1+	2	9 (3-28)	1+	41	23 (18-30)	**<0.001**	49	32 (25-39)	**0.02**
Uncinaria sp.	7	10 (5-18)	0	5	6 (3-14)	1+	3	4 (1-11)	1+ to 2+	1	5 (1-22)	1+	9	5 (3-9)	0.89	12	8 (5-13)	0.55
Trematodes																		
Alaria americana	29	39 (29-51)	1+ to 2+	42	52 (41-62)	1+ to 3+	18	25 (16-36)	1+	7	32 (16-53)	1+	67	38 (31-45)	**0.002**	71	46 (38-54)	0.15
Cryptocotyle lingua	24	32 (23-44)	0	2	3 (1-9)	0	0	0 (0-5)	0	0	0 (0-15)	0	2	1 (0-4)	0.62	26	17 (12-23)	**<0.001**
Metorchis sp.	1	1 (0-7)	1+	0	0 (0-5)	0	0	0 (0-5)	0	0	0 (0-15)	0	0	0 (0-2)	1.0	1	1 (0-4)	0.48
Overall parasite prevalence after fecal flotation[d]	**57**	**77 (66-86)**	**-**	**53**	**65 (54-75)**	**-**	**22**	**30 (20-42)**	**-**	**6**	**27 (12-50)**	**-**	**81**	**46 (39-54)**	**<0.001**	**110**	**71 (63-78)**	0.16
Overall parasite prevalence combining gross examination/fecal flotation[d]	**67**	**91 (82-95)**	**-**	**71**	**88 (79-93)**	**-**	**53**	**73 (61-82)**	**-**	**16**	**73 (52-87)**	**-**	**140**	**80 (73-85)**	**0.05**	**138**	**89 (83-93)**	0.62

[a] Fecal infection intensity : 1+ 1-50 eggs per gram of feces (epg) / 2+ 51-250 / 3+ 251-1000 / 4+ >1000

[b] 95% confidence intervals are displayed in parentheses

[c] Most likely *D. latus* (previously *Diphyllobothrium latum*) or *D. dendriticus* (previously *Diphyllobothrium dendriticum*)

[d] Canids infected with at least one gastrointestinal helminth species observed by fecal flotation, or combining gross examination and fecal float

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APPENDIX B

ACQUISITION DE CONNAISSANCES
SUR LA FAUNE

UN MONDE INSOUPÇONNÉ DE PARASITES : portrait chez les canidés sauvages au Québec

Par Émilie Bouchard
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Souvent méconnus, les parasites font partie de nos écosystèmes et nous renseignent sur l'écologie des animaux qui sont leurs hôtes. Que savons-nous sur l'éventail des parasites chez les canidés sauvages au Québec? Quels sont les risques pour l'humain? Voici un premier portrait des parasites détectés chez les renards, les coyotes et les loups du Québec.

Pourquoi s'intéresser aux parasites?

L'étude des parasites présents chez les animaux sauvages nous renseigne sur plusieurs choses. Par exemple, la prévalence et la diversité des parasites gastro-intestinaux chez les canidés sauvages peuvent être d'excellents indicateurs pour améliorer notre compréhension des écosystèmes. Les parasites transmis par des proies nous renseignent également sur les régimes alimentaires de leurs prédateurs. Dans le cas de canidés sauvages présents à proximité des habitations, l'identification des parasites qui peuvent être transmis à l'humain et aux animaux de compagnie est particulièrement utile pour cibler les précautions à prendre afin de réduire les risques de contamination.

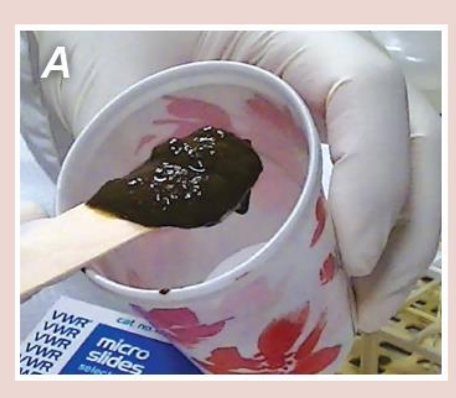
Le projet de recherche

Un projet de recherche a été réalisé afin de dresser un portrait des principaux parasites gastro-intestinaux présents chez les canidés sauvages à travers différentes régions du Québec. L'étude visait à évaluer la présence et la diversité des parasites présents chez le loup, ainsi que chez le renard roux et le coyote, que l'on retrouve parfois à proximité des humains.

Comment avons-nous fait?

Grâce à la collaboration de trappeurs et chasseurs, nous avons pu analyser le tractus gastro-intestinal de 176 renards roux, 77 coyotes ainsi que 23 loups gris récoltés au cours des hivers 2015-2016 et 2016-2017. Les renards ont été récoltés dans 2 régions climatiques du Québec, soit le climat subarctique et le climat continental humide, tandis que les coyotes et loups ont été récoltés dans le climat continental humide seulement. Nous nous attendions à observer des différences au niveau de la composition parasitaire entre les espèces dont le régime alimentaire diffère. Nous avons tenté de vérifier si la composition parasitaire reflète les différences en termes de climat, de végétation et d'abondance de proies entre les climats subarctique et continental humide.

Plusieurs méthodes ont été utilisées pour détecter les parasites dans les tractus gastro-intestinaux des canidés. L'examen des fèces (techniques de centrifugation et flottation d'une suspension fécale) de 250 canidés a été effectué afin de repérer la présence d'œufs de parasites (Figure 1).



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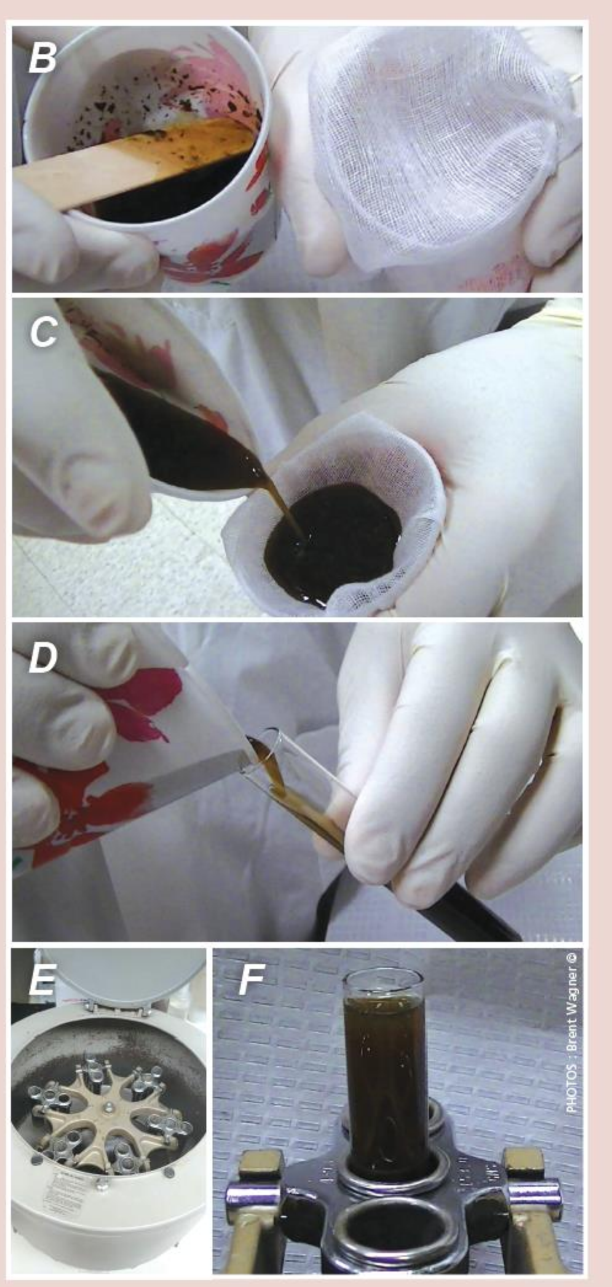


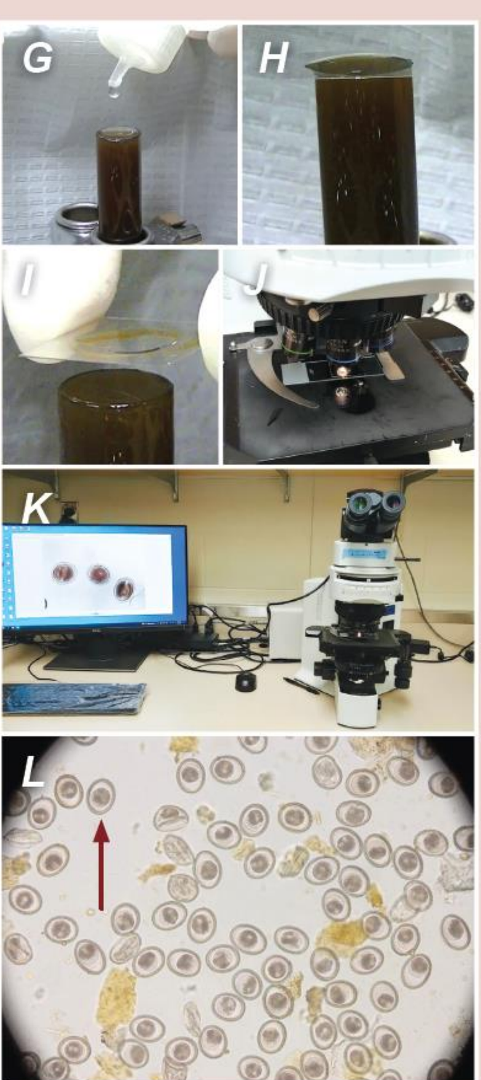


FIGURE 1. LES DIFFÉRENTES ÉTAPES DE L'EXAMEN DES FÈCES AFIN DE DÉTECTER LA PRÉSENCE D'ŒUFS DE PARASITES. LA MÉTHODE CONSISTE À FILTRER UNE SOLUTION CONTENANT LES FÈCES (A À D) ET À CENTRIFUGER L'ÉCHANTILLON (E À H), CE QUI PERMET AUX ŒUFS DE PARASITES DE COLLER SUR LA LAMELLE DÉPOSÉE SUR LE TUBE (I), POUR ENFIN OBSERVER AU MICROSCOPE LES ŒUFS DE PARASITES (J À L ET FLÈCHE)

...suite ▸

Saviez-vous que…

La grande majorité des œufs de parasites ont une forme, une taille et une couleur qui leur sont propres?

Ce sont ces caractéristiques qui permettent leur identification au microscope. Lorsque ce n'est pas possible, nous utilisons des outils moléculaires (test PCR) pour faire l'identification.

Par la suite, nous avons fait un examen macroscopique, c'est-à-dire visible à l'œil nu, de 276 échantillons provenant du petit intestin des canidés (Figure 2). Cette technique permet de détecter différents groupes de parasites adultes, c'est-à-dire les vers ronds, appelés nématodes, ainsi que les vers plats, comme les cestodes et les trématodes.

Figure 2. Technique de filtration permettant de détecter les parasites adultes présents dans le petit intestin des canidés. La méthode consiste à couper et ouvrir le petit intestin en 4 sections égales, de mélanger le contenu avec de l'eau et de brasser vigoureusement le bocal afin de récolter les parasites attachés à la paroi intestinale (A et B), de filtrer le contenu afin de recueillir les vers adultes visibles sur le filtre (C et D) pour enfin conserver et observer au binoculaire le contenu du liquide qui pourrait contenir de plus petits parasites (E et F)

Finalement, nous avons réalisé des analyses moléculaires (test PCR) pour détecter la présence de gènes spécifiques aux vers plats du genre *Taenia*, puisqu'il est difficile de différencier les diverses espèces de *Taenia* par les méthodes décrites précédemment (l'identification au binoculaire ou au microscope n'est souvent pas possible)!

Qu'avons-nous observé?

L'examen des fèces a permis d'identifier les œufs de 9 espèces parasitaires, tandis que l'examen macroscopique des échantillons du petit intestin a permis de détecter 7 espèces adultes. En combinant les résultats de ces 2 méthodes, nous avons observé qu'au moins 89 % des renards roux ainsi que 73 % des coyotes et des loups étaient infectés par au moins une espèce parasitaire (Figure 3). La présence accrue de parasites chez les renards peut s'expliquer par une plus grande diversité de proies dans leur régime alimentaire comparativement aux coyotes et aux loups. D'autres variables telles que l'environnement, la densité ainsi que la distribution géographique des canidés peuvent également jouer un rôle.

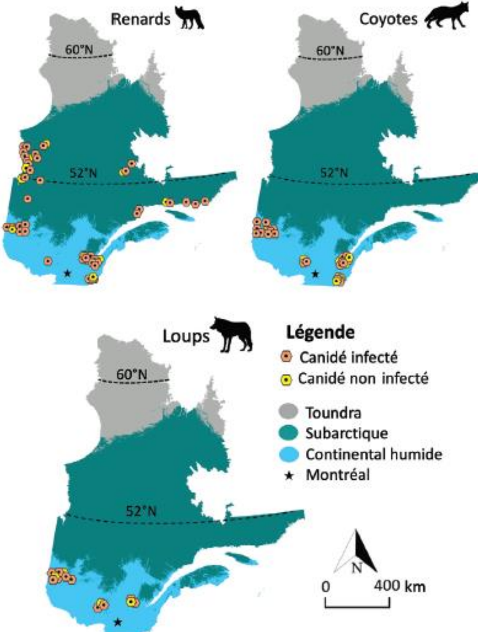


Figure 3. Distribution des renards (N=176), coyotes (N=77) et loups (N=23) récoltés par des trappeurs et chasseurs dans le cadre de l'étude. Les canidés infectés par au moins un parasite sont en rose et les non infectés en jaune

Nos résultats démontrent également que la composition parasitaire peut varier grandement selon l'espèce de canidés. Par exemple, les renards sont davantage infectés par des nématodes (vers ronds) comparativement aux coyotes et aux loups (Figure 4). Ces nématodes se transmettent généralement chez les canidés par contact avec un environnement contaminé par des fèces d'autres canidés contenant des œufs de parasites. La contamination des renards pourrait aussi être favorisée en zones périurbaines et urbaines, où les chiens infectés par des vers ronds peuvent également contaminer l'environnement.

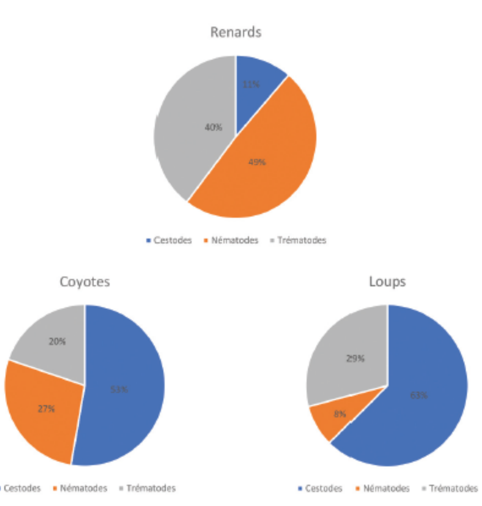


Figure 4. Composition des parasites intestinaux détectés chez les renards, coyotes et loups échantillonnés au Québec

Quant aux coyotes et aux loups échantillonnés dans notre étude, ceux-ci sont majoritairement contaminés par les vers plats de la classe des cestodes et des trématodes (Figure 4). Contrairement aux vers ronds, ces parasites doivent plutôt passer par un hôte intermédiaire, comme une proie, afin de se retrouver chez les canidés.

Pour ce qui est des cestodes, 6 espèces de *Taenia* ont été détectées à la suite des analyses moléculaires. La diversité des *Taenia* observée s'explique en grande partie par le fait que ces parasites ont chacun leurs hôtes intermédiaires particuliers. Par exemple, certains *Taenia* présents chez les renards (*T. twitchelli*, *T. crassiceps* et *T. polyacantha*) sont caractéristiques des rongeurs, ce qui met en évidence la prédation sur les rongeurs (lemmings, campagnols et souris) et les lagomorphes. D'autres parasites (*T. hydatigena* et *T. krabbei*), habituellement présents chez le gros gibier comme l'orignal, le cerf et le caribou, nous renseignent sur l'importance de ces proies dans le régime alimentaire des loups. Quant aux vers plats de la classe des trématodes, aussi transmis par l'ingestion de proies, ils sont généralement acquis dans des environnements aquatiques en consommant par exemple des escargots, des amphibiens ou des poissons le long des côtes.

Saviez-vous que…

Votre chien peut s'infecter en consommant accidentellement des œufs de parasites expulsés dans les selles de canidés sauvages? Il peut donc contribuer à contaminer son environnement et exposer des personnes.

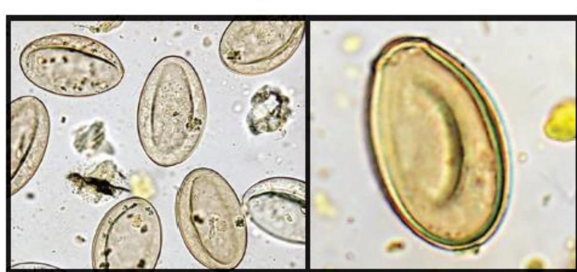
Afin de réduire le risque d'infection chez les chiens, les propriétaires d'animaux de compagnie devraient consulter leur vétérinaire afin de vermifuger leurs chiens qui sont à risque d'être en contact avec ces parasites (ex. : chiens qui chassent de petits mammifères ou qui ont accès à des carcasses/viscères). En étant vigilant pour limiter les contacts avec des animaux morts et en adoptant de bonnes pratiques d'hygiène, les risques de contamination pour les chiens ainsi que pour les humains peuvent être réduits significativement.

Quels parasites sont transmissibles à l'humain?

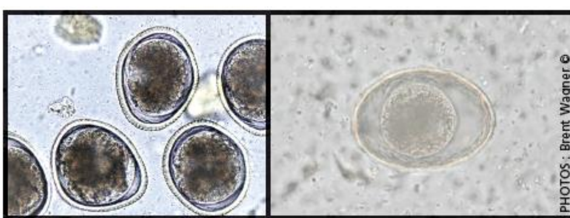
Certains des parasites détectés dans notre étude sont zoonotiques, c'est-à-dire qu'ils peuvent se transmettre de l'animal à l'humain. Généralement, une personne s'infecte par la consommation accidentelle des œufs de parasites qui se retrouvent dans l'environnement (sol et eau contaminés) ou sur une surface de travail contaminée par des fèces de canidés, ou encore par la fourrure de canidés lors de la manipulation de carcasses.

Parmi les parasites zoonotiques, nous avons détecté 2 vers plats (*Diphyllobothrium* et *Metorchis conjunctus*), qui peuvent se transmettre chez les canidés ou les humains en consommant du poisson cru contaminé. Le ver rond *Toxocara canis*, quant à lui, est l'agent de la toxocarose humaine et peut causer plusieurs problèmes de santé, surtout chez les jeunes enfants qui ont ingéré des œufs après un contact avec un sol contaminé. Un autre ver rond, le *Toxascaris leonina*, peut également se transmettre aux humains, mais est beaucoup plus rare. Bien que les infections causées par ces parasites ne soient pas fréquentes chez l'humain, la prudence est de mise puisqu'elles peuvent causer de graves symptômes.

…suite



Diphyllobothrium *Metorchis conjunctus*



Toxocara canis *Toxascaris leonina*

Conclusion

Les parasites sont infiniment petits, mais ils sont bien présents dans la faune du Québec. Ce premier portrait des parasites intestinaux présents chez les canidés sauvages du Québec n'est probablement pas exhaustif, mais il permet de comprendre que les types de parasites chez les renards, les coyotes et les loups sont intimement liés à leur écologie et aux relations prédateur-proie chez ces espèces. Notre étude rappelle l'importance d'avoir de bonnes pratiques lors de la manipulation des carcasses de canidés sauvages puisque certains parasites détectés peuvent être transmis à l'humain. Ceci est d'autant plus important sachant que certains canidés sauvages comme les renards et les coyotes vivent parfois à proximité des humains et de leurs animaux de compagnie.

Recommandations pour les trappeurs

Lors des manipulations de carcasses, il est important de prendre certaines précautions pour éviter de s'infecter :

- Porter des gants imperméables et un tablier lors de la manipulation de carcasses animales afin de minimiser le risque d'infection;
- Laver ses mains et ses avant-bras avec de l'eau et du savon après toute manipulation de carcasses animales;
- Nettoyer et désinfecter la surface de travail et tout le matériel utilisé lors de la manipulation des carcasses avec une solution composée d'une partie d'eau de Javel (hypochlorite de sodium 5-6 %) pour 9 parties d'eau. Les œufs de parasites sont microscopiques, c'est pourquoi il vaut mieux prendre des précautions pour éviter la contamination;
- Consulter un vétérinaire pour administrer le traitement antiparasitaire approprié à son chien;
- Éviter de nourrir son chien avec de la viande de gibier crue, des abats ou des viscères. Dans la mesure du possible, éviter que son chien soit en contact avec des carcasses animales ou qu'il consomme des rongeurs.

Remerciements

Nous tenons à remercier tous les trappeurs, chasseurs et coordonnateurs qui ont contribué à cette étude en soumettant des échantillons, sans lesquels ce projet n'aurait pu avoir lieu. Un merci particulier à Pierre Canac-Marquis, Pierre-Yves Collin, Jean-Pascal Trudeau, Alexandre Fournelle, Yves Jetté, Claude Grenier et Audrey Simon. Nous remercions également les chercheurs du projet (Janna Schurer, Temitope Kolapo, Brent Wagner, Sean Locke, Patrick Leighton et Emily Jenkins). Nous sommes reconnaissantes envers le personnel de la Faculté de médecine vétérinaire et du Centre québécois sur la santé des animaux sauvages (CQSAS) pour l'entreposage des carcasses, l'aide logistique et le transport. Cette étude a été soutenue par le Conseil de recherches en sciences naturelles et en génie du Canada, le US National Center for Veterinary Parasitology, les réseaux de centres d'excellence ArcticNet, Polar Knowledge et la Weston Family Foundation.

Pour en savoir plus…

Lire les articles portant sur ce projet concernant les parasites des canidés sauvages :

Bouchard, É., Schurer, J.M., Kolapo, T., Wagner, B., Massé, A., Locke, S.A., Leighton, P., Jenkins, E.J., 2021. Host and geographic differences in prevalence and diversity of gastrointestinal helminths of foxes (Vulpes vulpes), coyotes (Canis latrans) and wolves (Canis lupus) in Québec, Canada. International Journal for Parasitology: Parasites and Wildlife. 16:126-137.

Schurer, J.M., Bouchard, É., Bryant, A., Revell, S., Chavis G., Lichtenwalner, A., Jenkins, E.J., 2018. Echinococcus in wild canids in Québec (Canada) and Maine (USA). PLOS Neglected Tropical Diseases. 12(8):e0006712.

Bouchard É. L'échinococcose chez les canidés sauvages au Québec : un premier portrait épidémiologique. Le Veterinarius, OMVQ. Hiver 2020. Vol 36 (1). Veterinarius_hiver 2020.indd (omvq.qc.ca)