TOXOPLASMA GONDII IN WILDLIFE TRADITIONALLY HARVESTED BY INUIT OF NUNAVIK, CANADA

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By

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ABSTRACT

For centuries, Inuit have consumed wildlife. For decades, high levels of Inuit exposure to *Toxoplasma gondii* have been reported in Nunavik, Canada. This is puzzling given the rare occurrence of felids, the only definitive host for *T. gondii*, throughout this region. The handling and/or consumption of wildlife, more particularly the consumption of raw tissues, have been identified as risk factors for *T. gondii* exposure in Inuit. Yet, little is known about wildlife as reservoirs for *T. gondii* throughout Nunavik, largely due to lack of a sensitive direct detection method needed for a large-scale study. The rationale for this thesis was to determine whether wildlife poses a risk for Inuit exposure to *T. gondii* in Nunavik. This thesis first set out to confirm whether the magnetic capture and real-time PCR technique can be used to detect DNA of *T. gondii* in wildlife by assessing the PCR prevalence in tissues of foxes trapped throughout Nunavik. Then, seroprevalence (MAT) and PCR prevalence (MC-PCR) of *T. gondii* were compared in ringed seals, geese, ptarmigan and caribou to determine whether serological results can predict an animal's infection status. The probability that Inuit are exposed to *T. gondii* through consumption of goose tissues was then estimated in a quantitative exposure assessment. Lastly, we determined whether Inuit awareness, knowledge and risk perceptions of parasites in wildlife influence the adoption of health-protective behaviors that mitigate exposure to foodborne parasites based on multivariable logistic regression analysis. DNA of *T. gondii* was detected in 44% (95% CI: 28-60%) of foxes from four locations in Nunavik. DNA of *T. gondii* was also detected in 9% (CI: 3-15%) of geese, but not in other wildlife species including 20% (95% CI: 12-31%) of ringed seals and 26% (95% CI: 14-43%) of caribou seropositive on MAT. In geese, parasite load was quantified as highest in heart, followed by brain, breast muscle, liver, and gizzard. Overall, given the

consumption of 4 goose tissues, there was a 32% probability that Inuit were exposed to at least 1 bradyzoite during a one month period. Finally, approximately 61% (95% CI: 53-69%) of Inuit were aware of parasites and 47% (95% CI: 39-56%) were knowledgeable about their transmission. Both perceived severity and response efficacy positively influenced the adoption of health-protective behaviors. This is the first account of *T. gondii* detection in wildlife using the MC-PCR technique. In Nunavik, foxes are a good sentinel for *T. gondii*. Wild geese harbor the parasite and hunter-harvested geese are a plausible source of *T. gondii,* although the probability of exposure is low based on consumer data collected during this thesis. Risk communication messages should provide information on specific zoonotic parasites, as well as remind people that cooking their meat above 67ºC is effective at reducing their probability of exposure. An alternative, perhaps more culturally-appropriate means of inactivating the parasite could be by freezing at -12º C or colder for 3 days. Future research is needed to validate the use of serological assays in wildlife as a screening tool for food safety decision-making. For now, it is not recommended to extrapolate serological results on the infection status of individual animals for wildlife species included in this thesis. Future research is also needed to identify whether other wildlife species consumed by Inuit are infected with *T. gondii* including other migratory birds potentially highly exposed in the south.

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I would first like to thank Dr. Emily Jenkins for accepting to become my director when I applied from the middle of the African bush in Gabon. Thank you for your support and for always having that quick solution when the *going got tough*.

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Lastly, I would like to thank my family for their emotional support and understanding during certain (many) times throughout the last 5 years.

DEDICATION

I am dedicating this thesis to Dr. Bettina Salle (aka *Belle Doc*), a lifelong friend who left this world too precipitously and whom I shall always remember as the underlying reason for finally pursuing this path. I am choosing to believe that, wherever you may be, you are sharing the fruition of this work with me.

I am also dedicating this to my parents (Janine Divens and Robert Bachand). Behind great achievements are often great parents, which is definitely the case here.

I would also like to dedicate this to Willhem Bachand (and his potential sibling(s)) which I have yet to meet, but who is (are) already inspiring me to be for them what my parents have been for me.

And finally,

I am dedicating this also to all western lowland gorillas who are victims of the bushmeat trade in Africa; especially *Gimenu*, an orphaned gorilla who inspired me in 2006 to begin the path of graduate studies with the hope of eventually being able to help more. Your life story will be known to only few, but the impact you have had on me will hopefully carry through to many.

Gimenu (2018)

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LIST OF ABBREVIATIONS

AIC: Akaike's Information Criterion
AIDS: acquired immune deficiency syndrome
BLAST: basic local alignment search tool
CAC: codex alimentarius commission
CDC: Center for Disease Control and Prevention
CFAP: Centre for Foodborne and Animal Parasitology
CI: confidence interval
CIAC: competitive internal amplification control
CLB: cell lysis buffer
Ct: cycle threshold
DAT: direct Agglutination Test
Dim: dimension
Df: degrees of freedom
DNA: deoxyribonucleic acid
ELISA: enzyme-linked immunosorbent assay
EPA: United States Environmental Protection Agency
FAO: Food and Agriculture Organization
fg: femtogram
FITC: fluorescein isothiocyanate
g: gram
gDNA: genomic DNA
GR herd: George River herd
IFA: immunofluorescent assay
IFAT: indirect fluorescent antibody test
IHAT: indirect hemagglutination test
HBM: health belief model
H-L: Hosmer-Lemeshow
IgG: immunoglobulin G
IgM: immunoglobulin M
ISR: Inuvialuit Settlement Region
ITS-1 gene: internal transcribed spacer-1 gene
Kg: kilogramme
LAT: latex agglutination test
LCI: lower confidence interval
LNUK: Local Nunavimmi Umajulivijiit Katujaqatigininga
$\log_{10}$: logarithm with base 10
LR: Leaf River herd
Max.: maximum

MAT: modified agglutination test
MCA: multiple correspondence analysis
MC-PCR: magnetic capture and real-time PCR
Min.: ninimum
MLik: most likely
Mngt: management
n: sample size
neg: negative
NP: not performed
NTC: no template control
OD: optical density
OR: odds ratio
PBS: phosphate-buffered saline
PCR: polymerase chain reaction
pmol: picomole
PMT: protection motivation theory
pos: positive
qPCR: quantitative PCR
rDNA : ribosomal DNA
rf : reduction factor
RFLP : restriction fragment length polymorphism
RNUK: Regional Nunavimmi Umajulivijiit Katujaqatigininga
s: number of positive samples
SD: standard deviation
SE: standard error
SF dye test: Sabin-Feldman dye test
SEU: subjective expected utility
TE: Tris and EDTA buffer
UCI: upper confidence interval
µ: mean
µl: microliter
USA: United States of America
USDA: United States Department of Agriculture
WHO: World Health Organization

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. *Toxoplasma gondii* and its epidemiology in Nunavik

1.1.1. The agent: life cycle and stages

Toxoplasmosis is one of the most common infections in humans and animals worldwide (Halonen and Weiss, 2013). The zoonotic pathogen, *Toxoplasma gondii (T. gondii)*, is an obligate intracellular apicomplexan parasite capable of infecting a broad range of warm-blooded hosts including humans, mammals and birds as intermediate hosts (Tenter et al., 2000). There are three infectious life stages in its life cycle (Figure 1.1): 1. sporozoites within oocysts shed in the environment by felid hosts (the only known definitive host); 2. tachyzoites which can travel through blood and across blood barriers (e.g. placental, ocular and brain), and; 3. bradyzoites contained within cysts in several tissues and organs of both definitive and intermediate hosts (Hill and Dubey, 2002). Asexual replication can occur in both definitive and intermediate hosts (Dubey and Jones, 2008), allowing for clonal propagation between intermediate hosts via the ingestion of tissue cysts (Su et al, 2003). This, unlike other Apicomplexa*,* allows perpetuation of the parasite without the need for the active presence of the definitive host (Saeij et al., 2005).

Felid hosts, the only known definitive hosts for *T. gondii*, shed unsporulated oocysts into the environment through their feces. Then, when intermediate hosts ingest sporulated oocysts, sporozoites released from these sporulated oocysts convert into tachyzoites within the intestinal tract which then cross into the bloodstream to invade most cells (Tenter et al., 2000). Similarly, bradyzoites contained within tissue cysts consumed by either definitive or intermediate hosts also revert back to the tachyzoite stage within the intestinal tract.

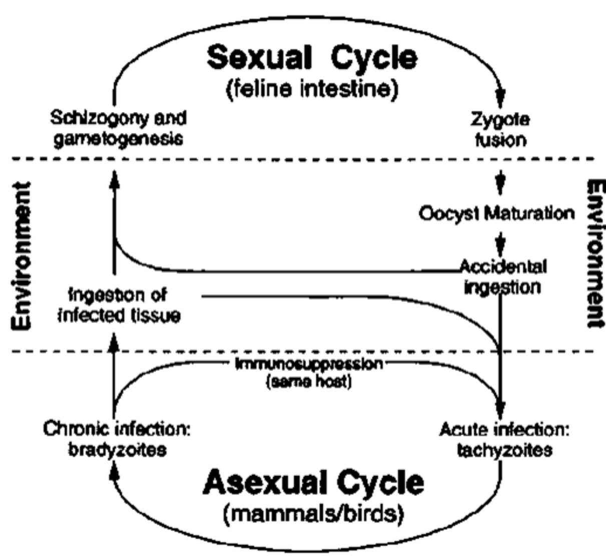

FIGURE 1.1: THE THREE INFECTIVE LIFE STAGES WITHIN THE *T. GONDII* LIFE CYCLE (© Black and Boothroyd, 2002)

(Dubey,1998). Once across the intestinal barrier and into the bloodstream, tachyzoites disseminate throughout the host to invade several tissues and can eventually also cross the cerebral, ocular and placental blood barriers (Lachenmaier et al., 2011). In seronegative pregnant women exposed for the first time, tachyzoites can therefore reach the fetus (Dubey, 2004). However, in most healthy individuals with previous exposure to the parasite, they are usually cleared by the immune system (Tenter et al., 2000). The fast-replicating tachyzoites within blood can, however, evade the immune system by penetrating several host tissues where they become slowly-replicating bradyzoites encapsulated within tissue cysts (Lyons et al., 2002). Bradyzoites within tissue cysts continue to replicate asexually throughout the life of the host (Dubey, 2004). Eventually, if tissue cysts rupture, bradyzoites can revert back to tachyzoites which can once again invade the blood stream.

1.1.2. Routes of *T. gondii* transmission in people

Transmission of *T. gondii* in humans can occur through the ingestion of food or water contaminated with sporulated oocysts, ingestion of raw or undercooked animal tissues infected with cysts, trans-placental migration of tachyzoites during pregnancy and, more rarely, transfusion of blood contaminated with tachyzoites (Robert-Gangneux et al., 2012). Although people can be congenitally infected with *T. gondii* tachyzoites during pregnancy, infection by consumption of bradyzoites (within tissue cysts) or sporozoites (within oocysts) are considered to be more prevalent (Aroussi et al., 2015). Based on a European multicentre study in pregnant women, foodborne transmission of *T. gondii* through consumption of meat accounted for

between 30% and 60% of all *T. gondii* human infections (Cook et al., 2000). Major sources of *T. gondii* transmission in people are highlighted in Figure 1.2.

1.1.3. *T gondii* molecular epidemiology

Because not all *T. gondii* strains are equally virulent in different hosts (Howe and Sibley, 1995), it is important to understand the strain-specific health significance of *T. gondii* for both people and wildlife. Despite the occurrence of a sexual life cycle in felid hosts, *T. gondii* populations are considered to be mainly clonal with three distinct lineages: Type I, Type II and Type III (Howe and Sibley, 1995). In experimentally infected mice, type I strains are more virulent compared to type II or III strains (Sibley and Boothroyd, 1992). Epidemiological studies have indicated that type II strains are common in humans in North America (Su et al., 2010), which are responsible for the majority of congenital infections and in people with AIDS (Howe et al., 1997). However, in Europe, Type I strains have also been disproportionately associated with severe congenital toxoplasmosis (Fuentes et al., 2001.) Atypical strains were also reported in a waterborne human outbreak in Canada (Aramini et al., 1999). Although it is generally believed that seropositive pregnant women are immune to reinfection with *T. gondii*, one experimental study showed that reinfection of chronically infected mice is possible by different *T. gondii* genotypes (Dao et al., 2001). It is unknown whether this is also applicable to people.

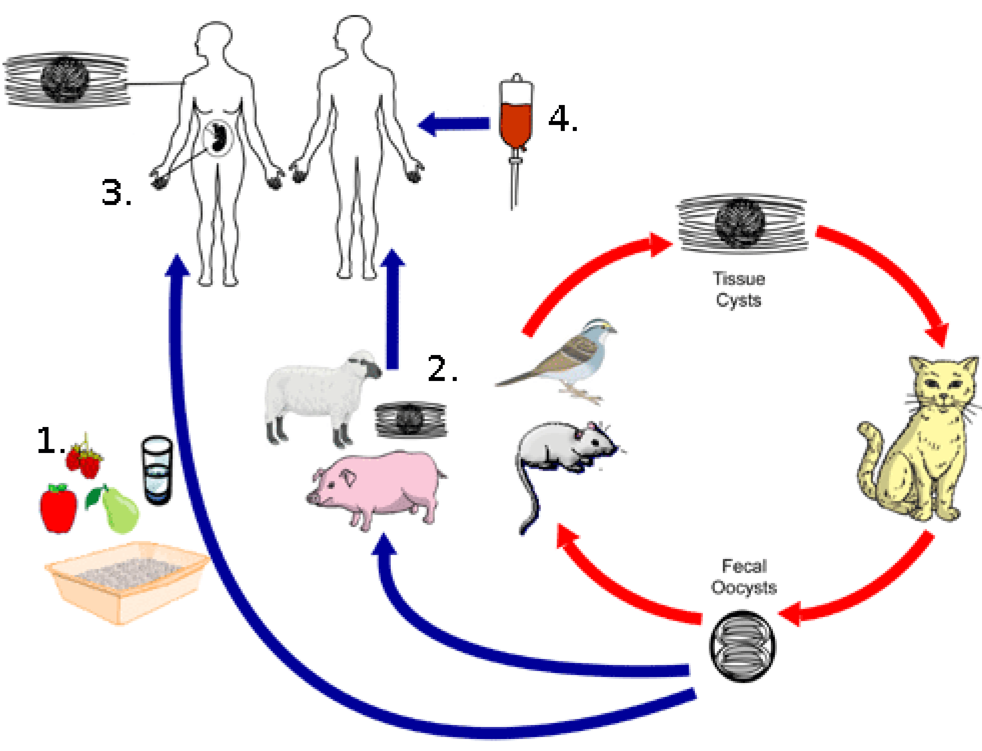

FIGURE 1.2: MAJOR SOURCES OF *T. GONDII* IN PEOPLE (CDC): 1. Food or water contaminated with oocysts, 2. Animal tissues infected with tissue cysts, 3. Through the placenta in seronegative women exposed to the parasite for the first time, and 4. Transfusion of blood containing tachyzoites.

1.1.4. *T. gondii* pathogenesis

The consequences of infection with *T. gondii* depend on its genotype and the host species it has infected (Su et al., 2010). In most healthy people, lymphadenopathy and flu-like signs may develop (Schluter et al., 2014). However, *T. gondii* poses a higher health risk for individuals with a poorly developed or weakened immune system which include the very young, the elderly and immunocompromised people (Tenter et al., 2000). Toxoplasmosis can lead to several possible adverse health outcomes, but the more commonly reported ones include congenital toxoplasmosis, ocular toxoplasmosis and toxoplasmic encephalitis (Flegr et al., 2014). Congenital toxoplasmosis involves a range of possible clinical manifestations from mild symptoms, the development of retinochoroiditis later in life, to more severe cases of hydrocephalus, microcephaly seizures, mental retardation and even foetal death (McLeod et al. 2000). Generally, the risk of infection increases throughout the pregnancy, but the severity of clinical signs declines.

1.1.5. *T. gondii* diagnostics

Indirect detection

Detection of IgM antibodies is usually possible within a week of exposure and these can remain for months to years (Liu et al., 2015). Therefore, their detection does not always imply the occurrence of an acute infection. The gold standard serological test for *T. gondii* is the Sabin-Feldman Dye test, though this test is highly technical and potentially hazardous to the person doing the test (Dubey, 2010). Most serological tests fall into two main categories, namely

primary tests (those that detect antibodies) and secondary tests (those that detect antibody-antigen complexes). The Enzyme-Linked Immunosorbent Assay (ELISA) detects IgG antibodies and its main advantage is that interpretation is not subject to human subjectivity (Liu et al., 2015). The Indirect Fluorescent Antibody Test (IFAT) is a simple test that detects both IgG and IgM antibodies (Montoya, 2002). However, it requires species-specific conjugates, a challenge when dealing with wildlife, and cross-reaction with rheumatoid factor and anti-nuclear antibodies can occur (Afssa, 2005). The most commonly used secondary serological tests include Direct or Modified Agglutination Tests (DAT, MAT), the Latex Agglutination Test (LAT) and the Indirect Hemagglutination Test (IHAT). These tests detect IgG antibodies and are generally easy to perform (Dubey and Desmont, 1987). The IgG avidity test is a test used to differentiate between acute and chronic infections (Su et al., 2010)

Direct detection

To confirm whether an individual is actually infected, direct detection methods are needed. The gold standard method for *T. gondii* is bioassay in cats or mice; cat bioassays are considered more specific than mice bioassays since oocysts can usually be recovered from cats (Dubey, 2010; Liu et al., 2015). Bioassays are also more sensitive than conventional DNA extraction kits in terms of tissue analysed since much larger amounts of tissue can be analysed (Prestrud et al., 2008). The lower sensitivity of conventional DNA extraction kits, with only milligrams of tissues used for extracting target DNA, is compounded by the fact that *T. gondii* cysts are not homogeneously distributed within tissues and that parasite burdens are usually low in naturally-infected animals (Hill et al., 2006). The major disadvantages of bioassays are that they are expensive to perform, time-consuming and laden with ethical concerns (Opsteegh et al., 2010). Other disadvantages are that they may preferentially recover certain strains (experimentally-infected animals may not

always be susceptible to a particular strain and thus may not display clinical signs) and testing is usually limited to a single tissue due to cost and ethical constraints linked to the use of experimentally-infected animals (Lindstrom et al., 2008). An attractive alternative is the magnetic capture and real-time (MC-PCR) technique, which has been used to detect DNA of *T. gondii* in up to 100 grams of tissue/sample from several domestic livestock species in Europe (Opsteegh et al., 2010; Jurankova et al., 2014; Aroussi et al., 2015; Koethe et al., 2015; Gomez-Samblas et al., 2015). The technique allows for the isolation of small amounts of parasite DNA from large amounts of host tissue (up to 100 grams) using magnetic beads labelled with sequence-specific parasite DNA probes that capture low concentrations of parasite DNA, even against high backgrounds of host DNA and inhibitory PCR products (Opsteegh et al., 2010).

Once DNA has been recovered, molecular methods are used to confirm the presence of target DNA. They are broadly categorised as conventional, nested and quantitative real-time PCR assays (Afssa, 2005). Real-time quantitative PCR assays are less labor-intensive, they allow for quantification of the microbial agent load in tissues and they use single tube reactions which reduces the potential for contamination among samples (Dubey, 2010). Molecular assays often target a DNA sequence that is "repetitive" within the target organism's genome. For *T. gondii,* the three most commonly used repetitive sequences include the B1 gene (35 copies), the 529-repeat element (200-300 copies) and the internal transcribed spacer (ITS-1) or 18S rDNA sequences (110 copies) (Su et al., 2010). The 529 bp repeat-element is considered to be the most sensitive of the three repetitive sequences; in fact, it is more than 10-100 times more sensitive compared to the B1 gene (Homan et al., 2000; Reischl. et al., 2003; Kasper et al., 2009). The disadvantage of molecular assays, besides not providing information on parasite viability, is that information is limited to a positive versus negative diagnosis (Su et al., 2010). Therefore, DNA

characterization assays are needed for a better epidemiological perspective of *T. gondii* transmission since different clonal and atypical *T. gondii* strains, reported to each have varying effects in different intermediate hosts, circulate among animals and people (Sibley et al., 2009).

1.1.6. *T. gondii* in Inuit of Nunavik

In the Arctic, north of the treeline, foodborne transmission is believed to be an important route of exposure for Inuit since felid hosts responsible for shedding *T. gondii* oocysts are rare to absent in this region (Messier et al., 2009) and Inuit consume several wildlife organs and tissues prepared in ways that may not inactivate the parasite e.g. consumption of raw animal tissue (Food Safety Network, 2009; McDonald et al., 1990). Nunavik, Canada, is home to over 12,000 Inuit living among 14 remote communities along Ungava Bay, Hudson Strait and Hudson Bay. Based on an Inuit health survey conducted in 2004, the *T. gondii* seroprevalence was 62.8% in people from Hudson Bay and 58.4% in people from Ungava Bay (Messier et al., 2009). These values are high compared to a seroprevalence of 11 % reported in the remainder of North America, but corroborate similar results from a previous study where *T. gondii* seroprevalence was 61% and 69% among Inuit from Kuujjuaq and Salluit, respectively (Tanner et al., 1987; Jones et al., 2018). It is also high compared to other Canadian Inuit settlements such as Nunatsiavut in Labrador, the Inuvialuit Settlement Region (ISR) and Nunavut where human T. *gondii* seroprevalence has recently been reported to be 11.3% (95% CI: 6.2-16.3%), 7.5% (95% CI: 3.5-11.5%) and 32.4% (95% CI: 29.4-35.6%), respectively (Goyette et al., 2014). Moreover, the reported incidence of congenital toxoplasmosis of 1.7% in Nunavik exceeds the 0.2-0.8% reported incidence in the remainder of Canada (Lavoie et al., 2008). Risk factors for Inuit

exposure to *T. gondii* in Nunavik include skinning wildlife, frequent consumption of caribou (*Rangifer tarandus*), eating raw caribou, eating dried ringed seal (*Pusa hispida*) and eating seal liver (Tanner et al., 1987; Curtis et al., 1988; McDonald et al., 1990). In a separate study, other risk factors included drinking reservoir water, as well as consuming seal meat and waterfowl (Messier et al., 2009). Risk factors for Inuit exposure to *T. gondii* in Nunavik are summarized in Table 1.1. Since meat consumption and prevalence in wildlife are considered to be major risk factors for human *T. gondii* infection (Schluter et al., 2014), the next section discusses the importance of wildlife as a source of country food in Nunavik and what is known about *T. gondii* in wildlife of Nunavik.

TABLE 1.1: SUMMARY OF PUBLISHED STUDIES ON *T. GONDII* SEROPREVALENCE AND RISK FACTORS IN INUIT OF NUNAVIK

Location	n	Test	% (95% CI)	Risk Factors	Odds Ratio	Reference
Nunavik	759	IH	48%	Not evaluated	-	Tanner et al., 1987
Salluit **Kuujjuaq**	264	IH IH	61% 69%	Not evaluated	-	Curtis et al., 1988
Kuujjuaq (pregnant women)	22	IFA	50%	Skinning of wildlife Frequent consumption (caribou) Seal liver Seal meat (dried)	16.5 8.7 6.9 4	McDonald et al., 1990
Ungava Bay	917	ELISA	52.3% (47.9-56.8%)	Water exposure Water reservoir (cleaning) Eating seal	1.97 0.55 1.69	Messier et al., 2009
Hudson Bay		ELISA	62% (61.5-69.7%)	Eating waterfowl	2.05	

IH: Indirect Haemagglutination; ELISA: Enzyme-linked immunosorbent assay

1.2. The role of wildlife as country food in Nunavik

1.2.1. Wildlife as country food in Nunavik

Wildlife represents an important source of food security for Inuit communities living in the Canadian Arctic (Chan et al., 2006; Lambden et al., 2006). Still today, consumption of country food remains an important component of Inuit livelihoods (van Oostdam, 2005; Furgal and Rochette, 2007). The expression "country food" refers to terrestrial/marine mammals, land/sea birds, fish, plants and berries harvested from the local environment for consumption as food (McGrath-Hanna, 2003). Indeed, a recent 2004 human health survey conducted in 14 Inuit communities of Nunavik, Canada showed that game (meat from wildlife) contributes on average 40% to people's daily protein intake, as well as many vitamins, minerals and fatty acids (Blanchet and Rochette, 2008). On average, Inuit consumed game more than five times per week throughout the year and wildlife species most commonly consumed (≥ 11 times on an annual basis) included caribou (*Rangifer tarandus),* goose (*Branta canadensis*)*,* ptarmigan (*Lagopus leucura)* and beluga (*Delphinapterus leucas*) (Blanchet and Rochette, 2008). The consumption of raw and undercooked meat from wildlife is a long-standing tradition in the Inuit culture and is still a common practice within Inuit communities (Food Safety Network, 2009). Several studies have reported on the high nutritional value of country food as a source of proteins, lipids, vitamins and minerals (Van Oostdam, 1999; Blanchet et al., 2000). Country food is also believed by some to contain medicinal properties evidenced by several reports from Nunavik residents on the importance of consuming country food when illness strikes (O'Neil, 1997; Furgal and

Rochette, 2007). Finally, in addition to its nutritional and medicinal benefits, country food is also a motivation for Inuit hunters to remain physically active as an added benefit to overall health (Pufall et al., 2011)

Country food also plays an important role in ensuring food security. In Nunavik, one out of 4 households do not have access to sufficient food according to a regional health survey (Blanchet and Rochette, 2008). Food insecurity implies that individuals or households do not have reliable access to food, there is insufficient availability of nutritious food, and/or there is limited access to high-quality food (FAO, 1999). In Nunavik, common causes of food insecurity include the prohibitive cost of store-bought foods, low household income, and unemployment. Less nutritious store-bought foods are often more readily available (and less expensive) than more nutritious foods (Blanchet and Rochette, 2008). A study in the community of Kangiksujjuak reported cost, inconsistent food quality, food unavailability and a lack of food variety as sources of food insecurity (Lawn and Harvey, 2004). For these reasons, Inuit rely on traditional harvesting activities that provide access to country food as a readily available and nutritious source of food.

1.2.2. Potential foodborne hazards in wildlife as country foods

Food can contain several chemical, biological and physical human health hazards (Pufall et al., 2011) and meat from wildlife carries a risk for cross-species transmission for several pathogens of public health importance (Wolfe et al., 2005). Because Inuit are frequently exposed to a variety of country foods, and because many types of country foods are consumed raw or partially cooked, there has been interest in quantifying risks of adverse health events through their

consumption (McDonald et al., 1990; Proulx et al., 2002). Since the early 1960s, risk assessments have been conducted in many fields including that of public health and food safety. However, in Nunavik, the focus of human health risk has been mostly on chemical contaminants rather than zoonotic parasites. Table 1.2 highlights human outbreaks of infectious foodborne diseases in Nunavik.

1.2.3. Challenges inherent to wildlife studies

Despite an important body of knowledge surrounding *T. gondii* in domestic animals (Tenter et al. 2000; Dubey, 2010), not as much information is known in wildlife. This is because studying wildlife presents several challenges compared to livestock species. The first challenge is estimating prevalence in wildlife, since we seldom have a "denominator" (the known population size) or suitable sample size from a representative source population. Logistical challenges also make it hard to ensure high-quality samples (e.g. tissue degradation, contamination with debris, storage conditions during transport, transport delays) (Ryser-Degiorgis, 2013). Unlike people or livestock which live in controlled environments, wildlife is not easily observable, their clinical signs are often masked, and capturing individual animals is rarely feasible (Rhyan et al., 2010). Obtaining biological or ecological data is also not always feasible for wildlife (e.g. identifying risk factors), making the full epidemiological picture of *T. gondii* difficult to fully understand (Stallknecht, 2007). Lastly, validated diagnostic tests are usually not available for wildlife (Ryser -Degiorgis, 2013). Without known test performance characteristics (e.g. diagnostic sensitivity and specificity) and population characteristics from which wildlife is sampled (e.g. population size, expected disease prevalence), interpreting tests results to understand the health

TABLE 1.2: EXAMPLES OF INFECTIOUS FOODBORNE OUTBREAKS LINKED TO THE CONSUMPTION OF COUNTRY FOODS IN INUIT OF NUNAVIK

Year	Foodborne pathogen	Risk factors	Tissue	# outbreaks (cases)	Reference
1982-1984	*Trichinella*	Walrus	Raw meat	4 (34)	Maclean et al., 1989
1987	*Toxoplasma*	Caribou	Raw meat*	1 (5)	McDonald et al., 1990
1985-2005	*Clostridium botulinum*	Seal Walrus Fish	Meat and fat Meat Meat & head	82 (134)	Leclair et al., 2013
1995-2009	*Trichinella*	Walrus	Raw meat *	Several	Larrat et al., 2012

*Unconfirmed

significance of *T. gondii* within and across populations is difficult (Kuiken, 2005; Boadella et al., 2011).

1.2.4. Knowledge about *T. gondii* in wildlife of Nunavik

Despite these challenges, exposure to *T. gondii* has been reported in several wildlife species commonly harvested in northern communities (Table 1.3). Seroprevalence of antibodies to *T. gondii* in caribou ranges between less than 1% up to 29% (Leclair and Doidge, 2001; Kutz et al., 2001; Johnson et al., 2010). Waterfowl are also frequently consumed by Inuit, and *T. gondii* seroprevalence has been reported in geese (4.2%) and ptarmigan (2.5%) in Nunavik (Leclair and Doidge, 2001). Another category of highly praised country foods that contribute to the daily protein intake of Inuit communities are marine mammals. Seroprevalence studies have shown that *T. gondii* occurs in walrus (5.6 %, southeastern Alaska) and many seal species (5.9-23.1 %) in several sites throughout the Canadian Arctic (Dubey et al., 2003; Simon et al., 2011). Species-specific risk factors for wildlife exposure to *T. gondii* are not well understood for reasons previously explained, and prevalence of *T. gondii* in meat from different wildlife species destined for human consumption is not available. Table 1.3 summarizes what is known about the seroprevalence of *T. gondii* in wildlife endemic to Nunavik.

As previously mentioned above, not all *T. gondii* strains affect people (and animals) equally. From an epidemiological perspective, especially in Nunavik where human contact with wildlife is frequent, it is therefore important to understand which strains circulate in wildlife. Most North American and European *T. gondii* strains belong to one of three distinct clonal lineages, though the *T. gondii* clonal lineage II seems to predominate in both domestic animals and wildlife in Europe (Schluter et al., 2014).

TABLE 1.3: EXAMPLES OF *T. GONDII* SEROPREVALENCE STUDIES IN WILD FELIDS AND WILDLIFE HARVESTED IN NORTHERN QUEBEC, CANADA

Species	Location	Test	Sample type	Prevalence % (n)	Reference
Caribou	Kuujjuaq	SF Dye test	Serum	63.5% (25/40)	McDonald et al., 1990
Caribou	LR herd	MAT	Serum Pos:≥ 1:25	0.7% (4/535)	Leclair and Doidge, 2001
Caribou	GR herd	MAT	Serum Pos:≥ 1:25	1.2% (1/82)	Leclair and Doidge, 2001
Ringed seal	Nunavik	MAT	Serum	14% (4/28)	Leclair and Doidge, 2001
Ringed seal	Sanikiluaq	DAT	Serum	8% (18)	Simon et al., 2011
Harp seal	St-Lawrence river	MAT	Serum	0% (out of 112)	Measures et al., 2004
Hooded seal	St-Lawrence river	MAT	Serum	1.7 % (1/60)	Measures et al., 2004
Harbor seal	St-Lawrence river	MAT	Serum	9% (3/34)	Measures et al., 2004
Grey seal	St-Lawrence river	MAT	Serum	9% (11/122)	Measures et al., 2004
Beluga whale	St-Lawrence river	MAT	Serum	27% (6/22*)	Mikaelien et al., 2000
Canada goose	Nunavik	MAT	Serum	4.2% (1/24)	Leclair and Doidge, 2001
Ptarmigan	Nunavik	MAT	Serum	2.5% (2/79)	Leclair and Doidge, 2001
Bobcat	Southern Quebec	MAT	Heart clots	40% (4/10)	Labelle et al., 2001
Lynx	Southern Quebec	MAT	Heart clots	44.3% (47/104)	Labelle et al., 2001

LR: Leaf River; GR: George River
*All 22 animals were found stranded along the St-Lawrence River

However, genotyping methods are biased since analyses are usually limited to individual animals or organs with inherently high concentrations of DNA of *T. gondii* (Schluter et al., 2014) and restricted to only those animal species and host tissues from which detection has been targeted. There is also evidence that co-infection with two different genotypes is possible in cats (Saeij et al., 2005), which explains why genetically distinct strains could be generated from a single infected animal. Another study has reported on the genetic diversity of *T. gondii* in North American wildlife in which the most common genotypes isolated among 169 wildlife isolates were genotype strain Type II (28%), genotype strain type III (10%), as well two non-clonal genotypes (47%) (Dubey et al., 2011). These non-clonal genotypes, genotypes 1 and 2, have recently been assigned as the 4th clonal lineage which is considered to be a common genotype in North American wildlife (Khan et al., 2011). This is important from an epidemiological perspective since severe cases of toxoplasmosis have occurred in immunocompetent people believed to be infected with atypical *T. gondii* genotypes (Vaudaux et al., 2010). Table 1.4 summarises the different *T. gondii* strains identified from North American wildlife.

TABLE 1.4: A BRIEF SUMMARY OF STUDIES ON THE STRAINS OF *T. GONDII* DETECTED IN NORTH AMERICAN WILDLIFE

Species	Location	Test	Tissue	Genotype	Reference
Black bear	Kuujjuaq, Canada	PCR-RFLP (10 markers)	Tongue	r I/III	Dubey et al., 2008
Canada goose	Mississippi, USA	PCR-RFLP (SAG2)	Brain	III	Dubey et al., 2004
Canada Goose	Maryland, USA	PCR-RFLP (10 markers)	Heart	II, III, #4, #266, #267	Verma et al., 2016
Black bear **Bob cat** **Red fox**	Pennsylvania, USA Georgia, USA Georgia, USA	PCR-RFLP (SAG 2)	Heart Heart Heart	II, III, r II/III II II	Dubey et al., 2004
Arctic fox **Red fox** **Wolf**	Alaska, USA Alaska, USA Alaska, USA	PCR-RFLP (11 markers)	Heart Heart Tongue	II, Atypical II Atypical	Dubey et al., 2011

1.2.5. Evaluating the safety of country food in Nunavik

Risk assessment, one of three components in the risk analysis framework (Figure 1.3), is a structured analytical approach devised to better understand and address risks posed by different foodborne pathogens in humans (Fazil, 2005). It is a decisional tool intended to help risk managers address food safety hazards of public health importance. Several risk assessment frameworks exist, but Codex Alimentarius Commission (CAC) guidelines are internationally recognised and provide a list of eleven principles applicable to foodborne microbial risk assessments (CAC, 1999). These guidelines are subdivided into four steps, namely: 1. hazard identification, 2. exposure assessment, 3. hazard characterization and 4. risk characterization. Generally, the purpose of any microbial risk assessment is to estimate the likelihood and severity of illness(s) resulting from specific food-pathogen combinations in a given population (or population segment) based on a. the amount of viable pathogen ingested in a food commodity and b. effects of that pathogen on human health (Health Canada, 2000; Forsythe, 2002). The former is evaluated as part of an exposure assessment that factors hazard characteristics (e.g. prevalence, concentration) and consumer patterns (e.g. frequency, amount). The latter corresponds to the hazard characterization step which addresses the probability and magnitude of different health effects given varying levels of a hazard in food (FAO/WHO, 2009). Exposure assessment and hazard characterization outcomes are then combined to provide a final estimate of the risk of infection or disease. Besides providing risk estimates for different food-pathogen combinations, risk assessments can also be used to identify the most effective risk mitigation strategies applicable to different segments along the food-to-table chain. Finally, risk assessments can help to highlight data and knowledge gaps needed to reduce levels of uncertainty in future risk assessment efforts (Bassett et al., 2012).

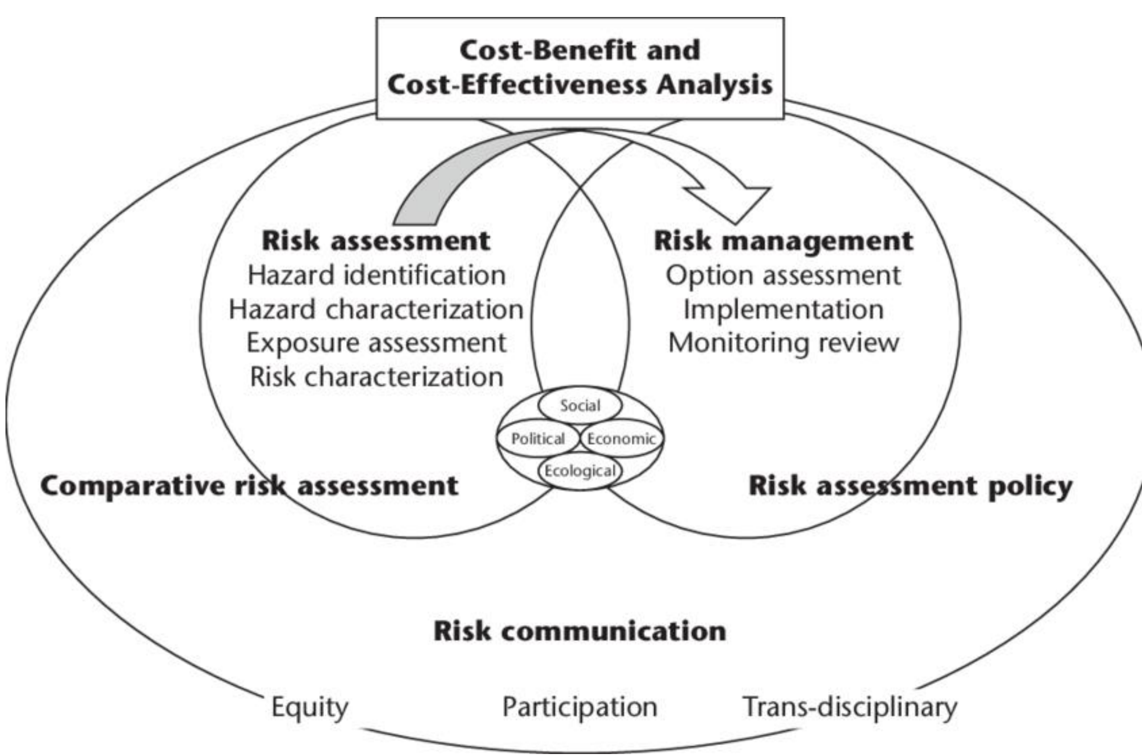

FIGURE 1.3: THE RISK ANALYSIS FRAMEWORK (© WHO)

Risk assessments can be qualitative, quantitative or semi-quantitative (USDA/EPA, 2012). Qualitative risk assessments provide a descriptive or categorical (e.g. non numerical) estimate of risk (e.g. negligible, low, medium and high); whereas a quantitative risk assessment output is numerical (e.g. number of illnesses per 100,000 people or 100,000 servings). Both types of assessments are subject to quality requirements inherent to guidelines of the CAC risk analysis framework which include that they 1. be soundly based on science, 2. be transparent and well documented, 3. are developed using high-quality data, and 4. describe uncertainty throughout each step of assessment (CAC, 1999). Whereas qualitative assessment methods characterise exposure in terms of descriptive likelihood categories (e.g. low, medium, high), quantitative assessment methods estimate exposure based on deterministic or stochastic models (USDA/EPA, 2012). Stochastic models are usually preferred over deterministic ones since they factor randomness to a larger extent and therefore are likely more representative of natural systems (Bassett et al., 2012). Quantitative exposure assessments also factor the effect of different food processes on pathogen growth or inactivation dynamics along the food production chain (e.g. partitioning, freezing, cooking) using predictive microbiology when this is applicable and feasible.

Only a few *T. gondii* food safety risk assessments, all done for domestic animals, have been published from several countries worldwide (Table 1.5). These assessments have identified important uncertainties and knowledge gaps, such as the lack of data on the concentration of *T. gondii* tissue cysts or bradyzoites in naturally-infected species or tissue type (Guo et al., 2015). Based on experimental infection studies, the tissue concentration of bradyzoites varies between tissues and species (Opsteegh et al., 2010; Jurankova et al., 2013). Moreover, tissue concentration data from experimental infections based on large infectious doses or

administration routes are not representative of the natural setting, which can represent an important bias when estimating the risk of exposure (Guo et al., 2017). It is also challenging to assess tissue concentration of bradyzoites, since the number of bradyzoites per tissue cyst varies between different-sized cysts and because tissue cysts are not homogeneously distributed throughout tissues (Dubey et al., 1986). Another major drawback in most risk assessments is that *T. gondii* prevalence is usually estimated based on serology, which does not correlate consistently with infection status and/or tissue concentration in infected animals (Dubey et al., 2005; Opsteegh et al., 2011). Several studies have shown that seronegative animals can be infected with *T. gondii*, and vice versa, so a better understanding of the relationship between serological and infection status is needed at the species level (Mie et al., 2008). A risk assessment outcome using *T. gondii* prevalence based on direct detection of the parasite or its DNA would logically provide a more useful estimate in terms of food safety, especially if some measure of infection intensity (quantification of DNA copies) is possible. Determining tissue concentration at the species and tissue level would also provide more accurate estimates of risk (Dubey et al., 2005). Lastly, there is often little knowledge of consumer behaviors with respect to consumption volume, frequency and preparation methods for different types of meat commodities at the local scale, and extrapolations are often made from national food production data as a proxy for consumption (Opsteegh et al., 2010; Guo et al., 2017).

TABLE 1.5: EXAMPLES OF QUALITATIVE AND QUANTITATIVE *T. GONDII* FOOD SAFETY RISK ASSESSMENTS IN DOMESTIC ANIMALS

Species/Food commodity	Nature of Assessment	Outcome Measurement	Outcome	Reference (Country)
Pork **Beef** **Chicken**	Quantitative	Probability of purchasing meat contaminated with *T. gondii* in a 10-year period	48% 0% 0%	Dubey et al., 2005 (USA)
Ready-to-eat processed meats	Qualitative	Likelihood that a product contains *T. gondii* cysts	Variable (low to medium)	Mie et al, 2008 (Australia)
Sheep **Beef** **Pork** **Mixed (beef & pork)**	Quantitative	Predicted number of human infections per year in the general population	395,000 1.9 million 316, 469 199,994	Opsteegh et al., 2010 (The Netherlands)
Fresh pork **Processed pork** **Beef** **Conventional chicken** **Free range chicken** **Goat meat** **Organic pork** **Organic lamb meat**	Qualitative	Exposure risk to *T. gondii* from the consumption of a food commodity	Low Medium Low Low High High High High	Guo et al., 2015 (USA)
Pork	Quantitative	Probability of infection per year per portion Annual number of new infections due to fresh pork	3.2×10^{-7} - 9.5×10^{-6} 94,600	Guo et al., 2017 (USA)

1.3. Risk perception and its role in country food safety

Technical food safety risk assessment estimates reflect the extent and magnitude of health consequences given exposure to specific foodborne hazards, but lay people have more complex judgements of risk (Sjoberg, 2000). Differences between expert and lay people's assessment of risk explain why little weight is usually attributed to expert risk assessments by the general population (Covello et al., 1986; Slovic, 1996). Therefore, a better understanding of risk perception is needed for developing more effective risk mitigation interventions. The concept of risk can be described based on at least two perspectives, namely *what is known* about the impact and magnitude of exposure to a hazard and *what is felt* about it. Knowing which factors influence how the target audience assesses risk (e.g. risk perception) is therefore important for the effective communication of risk (Frewer, 2000). Several theoretical frameworks have been proposed to assess how risk perception is appraised (Leppin and Aro, 2009). These can be broadly categorised into those that evaluate risk at aggregate (broad) versus individual (granular) levels.

1.3.1. Aggregate-level appraisal of risk perception

The psychometric paradigm contends that cognitive maps of laypeople's judgement of different hazards can be reconstructed into risk dimensions using multivariate regression analyses based on people's quantitative assessments (e.g. scores) of several psychological variables. The main psychological and social variables used to define these risk dimensions are described in Table 1.6. Because some of these variables can be correlated, they are combined into factor "spaces" that grossly classify hazards in terms of dread (composed of perceived lack of control,

catastrophic potential, inequitable distribution of risks, fatal consequences and dreadful) and of the unknown (composed of observability, knowledge level, delay effect and novelty) (Slovic, 1987). For example, nuclear power is recognised as a hazard characterised by a high level of dread and a low level of familiarity, which consequently leads to a higher level of risk perception expressed by the general population (Sjoberg, 2000). However, the assumption that aggregate-level data generated through analyses of the psychometric paradigm are applicable at the individual level remains to be verified.

Developed in 1982, the cultural risk theory is another aggregate-level appraisal of risk which was founded on the premise that risk perception is affected by an individual's culture and is assessed in terms of four worldviews: fatalism, hierarchy, individualism and egalitarianism (Rippl, 2002). These worldviews constitute the social and cultural norms that influence or affect how individuals perceive risk. For example, one study showed that cultural minorities in the US approach risk mitigation differently than the majority, and emphasized the need to capture the cultural component of risk perception when devising risk management plans (Gierlach et al., 2010). The economic situation of a cultural group can also influence risk perception: for example, when sustaining one's livelihood prevails over the consequence of being exposed to a presumed risk (Vaughan, 1993). Much like the psychometric paradigm though, the cultural setting is known to influence how individuals perceive or respond to risk, but does not measure risk perception at the individual level.

TABLE 1.6 PSYCHOLOGICAL AND SOCIAL FACTORS THAT INFLUENCE RISK PERCEPTION AT THE AGGREGATE LEVEL (SCHMIDT, 2004)

Variable	Description and effect on risk perception
Voluntariness	Perception of risk is amplified if it is imposed, but attenuated if it is not
Control	Risks perceived to be under one's controls are considered to be more acceptable
Natural versus Manmade	Natural processes are generally better accepted
Familiarity	A risk that is present for a long time is attenuated due to habituation even if the technical risk remains the same
Benefit linked to risk	Risks associated with clear benefits are usually more accepted
Fairness	Greater acceptance if people don't constantly have to endure risk
Risk management process	Wider acceptance when the process is transparent, open and responsive
Memorability	Diffused over time or delay (greater acceptance) versus catastrophic (lesser acceptance)
Dread	Some risks are more dreaded than others
Diffusion	A rare event that kills many people will cause less acceptance than a regular event that kills a smaller number of people
Risk group (morality)	Affects adults (greater acceptance) versus children (lesser acceptance)
Risk communicator	By trustworthy person (greater acceptance) versus dishonest or unconcerned manager (lesser acceptance)

The Social Representation Theory, or Social Amplification of Risk Framework, refers to how social dynamics influence the way individuals perceive and react to risk events (Kasperson et al., 1996). It is based on a systematic approach that evaluates how a technical assessment of risk is overestimated or underestimated under different psychological, social, institutional and cultural processes (Pidgeon et al., 2003). Perception of a risk event can change over time depending on the various external factors that influence individual perceptions of risk (Kasperson et al., 2003). Similarly, risk perception can vary from one locality to another based on the occurrence of one or more of these external factors; for example, a community that experiences the loss of community members in a local outbreak of foodborne poisoning linked to beef burgers are likely to have a higher level of risk perception associated with beef burgers in the future (Strachan et al., 2011). The media can also play an important role in the social amplification of risk by sometimes overemphasizing the importance of "low probability" risk events using dramatic and emotional language (Leppin and Aro, 2009). Issues of trust and blame can also be key factors in the amplification and attenuation of risk perceptions (Johnson and Slovic, 1995); for example, Inuit may be skeptical of food safety recommendations provided by public health authorities when these recommendations contrast with their cultural beliefs about health benefits of country foods. Aggregate-level models investigate whether hazards are perceived as being associated with low or high risks and extrapolate these broad categories to every person in the general population. They answer the question "*how are risk perceptions formed?*" rather than "*how does risk perception influence behavior?*" which is addressed by expectancy-value models (Leppin and Aro, 2009), the focus of the following section.

1.3.2. Individual-level appraisal of risk perception

Expectancy-utility models are founded on the subjective expected utility (SEU) framework, which posits that individuals make decisions based on a threat probability assessment combined with the utility or desirability of different protective options. In the health field, similar to the technical definition of risk, the Health Belief Model (HBM) and the Protection Motivation Theory (PMT) describe risk perception as an individual's perception of the likelihood that he/she will be exposed to a health hazard (termed personal vulnerability or personal susceptibility) multiplied by his/her perception of the severity of that disease (termed personal severity). The risk perception outcome is then used to evaluate how individuals make decisions about applying various measures to avoid exposure to the threat. Although both the HBM and the PMT models share similar features, a distinctive feature of the PMT model is that it factors self-efficacy, an individual's perception of his/her ability to apply protective measures that reduce or eliminate a threat. It is generally believed that even if a threat is perceived as being high, people may not be motivated to act toward its reduction if they have the perception of low self-efficacy – i.e. there is nothing that they can do, personally, to protect against it (Maddux and Rogers, 1983). Moreover, even if people believe they are capable of applying protective measures, they may not be motivated to act if they don't believe that the proposed measure is indeed protective, which is referred to as response-efficacy (Prentice-Dunn and Rogers, 1986). Consequently, though it is important to understand how risk perception is appraised by individuals, it is also relevant to understand how risk perception influences individual behaviors that are needed to mitigate risks. Because the PMT theory factors self-efficacy, and because this variable can influence people's decisions to follow food safety recommendations with respect to appropriate health-protective behaviors, it is a good choice for assessing risk perceptions of parasites from wildlife in Nunavik

where people are in frequent contact with and regularly consume wildlife.

1.3.3. Risk perception and safety of country food in Nunavik

Practices surrounding harvesting and consumption of country food are integral to maintaining Inuit culture (Berkes and Farkas, 1978). Even if alternative foods are available (e.g. store-bought foods), the cultural value attributed to hunting and consumption of wildlife cannot be underestimated (Van Oostdam, 2005). For Inuit, meat from the land is associated with good health and "the true meaning of being Inuk" (O'Neil, 1997). Moreover, harvesting activities represent opportunities for sharing traditional knowledge which is essential to cultural sustainability (Houde, 2007). Having a sense of control and independence over food acquisition of country foods is also highlighted as being influential in choosing to consume country food (Bone, 1985; Bernier, 2003).

Few studies of risk perception on the safety of country food have been done in Nunavik, with the bulk of the work being focused on chemical contaminants. Food contaminants (e.g. mercury, PCB) in country food have been measured for several wildlife species since the early 1990's (Furgal and Rochette, 2007). Surprisingly though, a recent regional survey done in Nunavik showed that levels of awareness concerning contaminants in country foods were low in the general population (Furgal and Rochette, 2007). A few respondents who reported being aware of contaminants did report a minor reduction in their levels of country food consumption, especially with respect to marine wildlife and fish (presumably because the high-fat content of marine wildlife and fish promotes the absorption of contaminants). However, the authors suggest that the main reason why the "contaminant discourse" does not promote more behavioral changes

than expected is because country food is valued for its nutritional, cultural, economic, medicinal and spiritual benefits. In other words, benefits clearly outweigh the risks. Therefore, health risks attributed to the consumption of country food compromises, from an Inuit perspective, a lot more than just the consumption of that "food" (van Oostdam, 2005). As such, Inuit from Nunavik have reported that they would continue to consume country food even if they are told that it may be contaminated (O'Neil, 1997; Furgal, 1999). There is a lack of knowledge on Inuit awareness of parasites from wildlife and their perceptions as a threat to their health (Pufall et al., 2011). This information is needed to complement any future country food safety risk assessment by improving the effectiveness of risk communication messaging while factoring the benefits and cultural importance of country foods.

THESIS STATEMENT

The original intent of this thesis was to perform a risk assessment of *Toxoplasma gondii* (*T. gondii*) in country foods of Nunavik, Canada. Based on a regional Inuit health survey done in 2004, *T. gondii* antibody levels were shown to be unusually high in Inuit of Nunavik compared to the rest of North America. This latter survey, combined with an outbreak investigation and a few other epidemiological studies (Tanner et al., 1987; McDonald et al., 1990; Messier et al., 2009), highlighted wildlife as the likely culprits for these unusually high levels of exposure in Inuit. In order to complete a risk assessment of *T. gondii* (the hazard) from wildlife (the food), several gaps first needed to be filled and these became the foundation of this PhD thesis.

Chapter 1 addressed the first step of a risk assessment (hazard identification) and highlights several features that make *T. gondii* a successful parasite in people and animals worldwide. The chapter also provides a synthesis on the value and consumption patterns of country food in Nunavik. Because communicating risk assessment results can be challenging, and because effective risk communication is intimately influenced by the public's risk perceptions, the last part of this introductory chapter focuses on describing what risk perception is and ways to evaluate it.

There are no recent *T. gondii* prevalence data for wildlife of Nunavik. From a food safety perspective, a drawback in several wildlife studies is that *T. gondii* seroprevalence (exposure status) is reported rather than infection status. Extrapolating infection status from seroprevalence data brings inherent uncertainty in any food safety risk assessment. **Chapter 2** therefore describes the prevalence of *T. gondii* in foxes to evaluate their possible role as sentinels of *T. gondii* in Nunavik; whereas **Chapter 3** describes the prevalence of *T. gondii* in several wildlife species consumed by Inuit. Both these chapters relied on the use of a novel direct detection technique (the

magnetic capture and real-time PCR technique) which was set-up in house at the Zoonotic Parasite Research Unit (ZPRU), Western College of Veterinary Medicine, in Saskatoon, Saskatchewan, as part of this thesis. Because tissue parasite load is another important gap in *T. gondii* risk assessments, **Chapter 3** also tackled the quantification of parasite loads in tissues of naturally-infected wildlife. Finally, this chapter attempted to describe the genetic diversity of *T. gondii* in wildlife of Nunavik.

Yet another gap in country food risk assessments is consumer data for different wildlife species consumed in Nunavik. Wildlife is not a generic term and consumer habits (i.e. consumption frequency and tissue preparation methods) vary according to each type of wildlife species. In **Chapter 4**, the objective was to describe consumer data for tissues of *T. gondii*-positive wildlife identified in Chapter 3. Then, species prevalence and tissue infection intensity data were combined with consumer data to develop an exposure assessment.

While visiting Nunavik in April 2015, I noticed a local Inuk woman handing a piece of raw goose liver to her 1 year-old grandson as she was processing a freshly harvested Canada goose with her bare hands (see Appendix VI). This made me realize that some Inuit may not perceive handling or consumption of raw tissues or organs as risky, which reinforced the relevance of **Chapters 2-4**. But it also raised the question of whether a science-based assessment of *T. gondii* exposure from country food would be well received by Inuit who may not themselves recognise country food as risky. Therefore, results of any risk or exposure assessment may not be effective in and of themselves at promoting behaviors that help to minimise risk without factoring socioeconomic and cultural factors that may affect how people make decisions concerning risk. **Chapter 5** therefore placed attention on providing more clarity concerning current levels of Inuit knowledge and

awareness of parasites in wildlife, as well as on people's perceptions of risks related to parasites. Lastly, this chapter aimed at determining which factors influence the adoption of health-protective behaviors that help reduce exposure to foodborne parasites. The main intent was to provide evidence-based data that could possibly make risk communication messages regarding *T. gondii* exposure from wildlife more effective and culturally-appropriate.

The strength of this thesis is rooted, from the very start of this project, in the input and direct involvement of local Inuit impacted by information generated through this work using a participatory approach. Before initiating the research project, representatives of Makivik Corporation (an organization based in Montreal mandated to represent the interest of Inuit in Nunavik) were consulted to gauge whether the thesis content could be of any interest to Inuit, which they indeed agreed that it would. Many gaps of knowledge concerning *T. gondii* in wildlife of Nunavik and its potential public health implications for Inuit needed to be filled. This project was then presented at the 2014 annual regional hunter association (RNUK) meeting in Kangisualujjuaq, Nunavik, in front of all 14 community leaders. Two months later, visits took place in 6 of the 14 communities (2 communities per each of the three regions in Nunavik) to obtain input from local communities on the development of a successful wildlife sampling strategy and useful project outcomes. At this time, three communities were selected and a community liaison was hired in each community to help coordinate wildlife sampling efforts and sample shipment. Finally, a progress report and results were communicated back to community leaders during each of the subsequent (2015 and 2016) annual regional hunter association meetings in Kuujjuaraapik and Salluit, respectively.

THESIS OBJECTIVES

Chapter 2

Assess *T. gondii* exposure and infection status in foxes of Nunavik as potential sentinels for foodborne parasites.

Chapter 3

Assess *T. gondii* exposure and infection status in ringed seals, ptarmigan, geese (Canada and Snow geese), caribou and walruses, as well as quantify tissue parasite load.

Chapter 4

Describe consumer patterns with respect to wildlife species that tested positive for *T. gondii* in Chapter 2, as well as develop a *T. gondii* exposure assessment to predict the probability that Inuit are exposed to *T. gondii* from country food.

Chapter 5

Describe Inuit knowledge and awareness of parasites in wildlife, and assess the influence of efficacy beliefs and risk perceptions of wildlife parasites on the adoption of health-protective behaviors by Inuit in Inukjuak, Nunavik, Canada.

CHAPTER 2: FOXES (*VULPES VULPES*) AS SENTINELS FOR PARASITIC ZOONOSES*, TOXOPLASMA GONDII* AND *TRICHINELLA NATIVA*, IN THE NORTH EASTERN CANADIAN ARCTIC

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Author Contributions

NB coordinated field data collection. NB, EJ, AR, CS and PL contributed to study design. NB and KK did laboratory work. CF, MN contributed to laboratory mentoring and support. NB drafted the manuscript. All authors reviewed and approved the final manuscript.

2.1. CHAPTER TRANSITION

For decades, it has been hypothesized that wildlife in Nunavik act as reservoirs of *Toxoplasma gondii*, a zoonotic parasite that can be transmitted to people through the foodborne route. A regional health survey done among all 14 communities in Nunavik has demonstrated that Inuit from this region display high levels of antibodies against *T. gondii* compared to other North Americans. This finding is unusual since felid hosts, the only known definitive hosts for this parasite, are rare to absent in Nunavik. Because foxes are carnivorous animals that can be exposed to either oocysts in the environment or tissues cysts through carnivory, thus increasing the likelihood of exposure to the parasite, they may represent a good sentinel for investigating the occurrence of *T. gondii* in Nunavik. Similarly, because *Trichinella* (another foodborne parasite) is transmitted through carnivory, foxes could be good sentinels for investigating the circulation of this parasite in the terrestrial ecosystem of Nunavik. Because these parasites are expected at a higher prevalence in carnivorous or omnivorous wildlife species compared to herbivorous or piscivorous wildlife species, the aim of this chapter was to determine whether these two foodborne parasites of public health importance occur in Nunavik based on serological and molecular detection in foxes.

2.2. ABSTRACT

Outbreaks of *Toxoplasma gondii* and *Trichinella* spp. have been recurring for decades among Inuit of Nunavik, northeastern Canada. Contact with wildlife has been identified as a risk factor for Inuit exposure to *T. gondii,* but reservoirs have yet to be confirmed based on direct detection of DNA or organism. Similarly, little is known about the occurrence of *Trichinella* spp. in wildlife species of Nunavik other than walrus (*Odobenus rosmarus*) and bears *(Ursus americanus, Ursus maritimus)*. Foxes (*Vulpes vulpes*) were targeted as possible sentinels for *T. gondii* and *Trichinella* spp. because of their high trophic position within the Arctic food chain as carnivorous scavengers. A total of 39 red foxes were sampled from four communities in southern and western Nunavik between November 2015 and September 2016. For the first time in wildlife, a novel magnetic capture DNA extraction and real-time PCR technique was used to isolate and detect *T. gondii* DNA from the heart and brain of foxes. A double separatory funnel digestion method followed by multiplex PCR was used to recover and genotype larvae of *Trichinella* spp. from tongues of foxes. Seroprevalence based on detection of antibodies to *T. gondii* was 41% (95% CI: 27-57%) using a commercially available modified agglutination test (MAT). Detection of DNA of *T. gondii* and larvae of *Trichinella nativa* (T2) occurred in 44% (95% CI: 28-60%) and 36% (95% CI: 21-51%) of foxes, respectively. Coinfection with both *T. nativa* and *T. gondii* occurred among 23% (95%CI: 13-38%) of foxes which can be attributed to co-transmission from prey and scavenged species in their diet. There was only moderate agreement between *T. gondii* serology and direct detection of *T. gondii* DNA using the MC-PCR technique (Kappa test statistic: 0.321), suggesting that using both methods in tandem can increase the sensitivity of detection for this parasite. These findings show that foxes are good sentinels for circulation of parasitic zoonoses in terrestrial northern ecosystems since they are

highly exposed, show measurable indicators of infection and do not serve as exposure sources for humans.

Keywords: *Toxoplasma gondii*, *Trichinella nativa*, sentinel species, parasitic zoonoses, Arctic

2.3. INTRODUCTION

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

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

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

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

FIGURE 2.1: MAP OF NUNAVIK, CANADA (© Lemire et al, 2015)

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

Because *T. gondii* and *Trichinella* spp. have a broad range of possible hosts, preliminary investigations using a key sentinel species can be a cost-effective way of identifying their presence in the local environment before attempting detection in other target animals or humans (Pei Shan Neo and Huan Tan, 2017). An ideal sentinel species for *T. gondii* is one that can be exposed to both oocysts in the environment and tissue cysts in foods of animal origin. Other important characteristics of a key sentinel species include having a widespread distribution, a restricted home range, the ability to bioaccumulate a hazard, susceptibility to the hazard, and continuous residency in the local environment (Basu et al., 2006; Landres et al., 1988). An ideal sentinel species should also have increased exposure (higher trophic position in the food chain) compared to the target population, yet not pose a risk for pathogen transmission to the target

population (Bowser and Anderson, 2018). Foxes can be exposed to both oocysts and tissue cysts of *T. gondii* and have a high probability of being infected with *T. gondii* if it occurs in the local environment. Lynx (*Lynx canadensis*) are a potential source of oocysts in southern Nunavik, but are absent in western and northern Nunavik (Naughton, 2012). Exposure of Arctic wildlife could occur through ingestion of water contaminated with oocysts shed by felids from remote boreal and temperate regions and transported north in freshwater or marine currents (Simon et al., 2013). It is also possible that foxes are exposed through the consumption of migratory birds exposed to oocysts in the South (Prestrud et al., 2007; Sandstrom et al., 2013). Wildlife exposure to *T. gondii* in Nunavik is also supported by a seroprevalence of 4.2% in Canada geese, 2.5% in ptarmigan (*Lagopus lagopus*) and 14% in ringed seals (Leclair and Doidge, 2001).

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

2.4. MATERIALS AND METHODS

2.4.1. Samples

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

2.4.2. Detection of *T. gondii* antibodies

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

2.4.3. Tachyzoite source and plasmid DNA for standard curves

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

To construct *T. gondii* plasmid DNA, a conventional polymerase chain reaction (PCR) was performed in an Eppendorff thermocycler (Mastercycler pro, Eppendorf AG, Germany) using the Tox 9F (5´-aggagagata tcaggactgtag-3´) and Tox 11R (5´-gcgtcgtctc gtctagatcg-3´) primers to generate a 188 bp PCR product within the 529 bp repeat-element of the *T. gondii* genome (Reischl et al., 2003) with slight modifications. Each PCR reaction contained 2 µL of *T. gondii* genomic DNA in a 50 µl reaction with 2.5 U Taq DNA polymerase (Quanta Bio Science, USA) Q), 2.5mM $MgCl_2$, 100 µM dNTP (Invitrogen, Pharmacia Biotech), 20 mM each of primers Tox 9F and Tox 11R, 50 mM KCl, 10 mM Tris/HCl, pH 8.3. PCR reactions were amplified as per Homan et al. (2000). PCR products were then purified using the QIAquick Gel Extraction Kit (Qiagen, Toronto, Canada) and cloned to pGEM-T Easy vector (Promega) according to the manufacturer's protocol. Finally, the plasmid DNA was extracted using EZ-10 spin columns (Bio Basic Inc –ON) and was diluted in 10 fold in TE buffer (Promega) to construct the standard curve. Quantitative real-time PCR was performed in a Bio-Rad CFX 96 DNA thermal cycler (Biorad, Hercules, California, USA) with each reaction containing 1× SYBR Green Supermix

(Bio-Rad Laboratories Canada Ltd., Mississauga, ON), 400 nM each primer Tox 9F and Tox 11, and 2 µL of plasmid DNA, in a total volume of 25 µL All reactions were performed in duplicate and a no-template control (NTC). Reactions were incubated at 95 °C for 10 min followed by 40 cycles each of 15 sec at 95 °C, 1 min at 60 °C.

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

To determine the minimum number of DNA copies detected by the PCR assay, a minimum of 8 replicates were amplified for each ten-fold dilution of *T. gondii* genomic DNA (gDNA) ranging between 2 to 2 million femtograms (fg) per PCR reaction. To evaluate the lowest detectable number of tachyzoites for the MC-PCR method, 100 g beef muscle samples were spiked with 10-fold serial dilutions of tachyzoite concentrations ranging between 12.5 and 2.5 million tachyzoites per 100 g of tissue. Analysis of the lowest detection limits was determined by factoring the number of positive reactions for each concentration into a probit regression model (IBM SPSS Statistics 24, SPSS Statistics, Chicago, IL) for both the genomic DNA and tachyzoite spiking experiments.

2.4.5. Extraction and detection of DNA

DNA extraction from a maximum weight of 60 g each of half the brain (mean: 43 g; standard deviation: 6 g) and the entire heart (mean: 36 g; standard deviation: 10 g) from each fox was performed as per Opsteegh et al. (2010), except that the 5′ end of the competitive internal amplification control (CIAC) probe (5´ agcgtaccaacaagtaattctgtatcgatg 3´) was labelled with

HEX rather than with JOE. Moreover, real-time PCR amplification was done using the Bio-Rad CFX 96 DNA thermal cycler (Biorad, Hercules, California, USA) based on a published protocol for the detection of the 188 bp *Toxoplasma* sequence (Omar et al., 2015). The final PCR assay reaction included 0.5 M of Itaq Supermix, 20uM of TP1 probe (5´ ccggcttggctgcttttcct 3´), 10 uM of Tox 9F, 10 uM of Tox 11R, 2 fg of CIAC, 5 uM of CIAC probe, 6.75 uL of PCR-grade water and 8 uL of template DNA. A positive PCR reaction was defined as any reaction with: 1) a Ct-value smaller or equal to 35, 2) a no-template control with a Ct-value of zero, 3) a negative MC-PCR control, and 4) a positive MC-PCR control (Opsteegh et al., 2016). All reactions where only one of two duplicates amplified, or where CIAC amplification failed to occur, were repeated.

2.4.6. Detection and genetic characterization of *Trichinella* spp

Tongues were collected from each fox and frozen at -20 ºC for <2 months until *Trichinella* larvae were recovered using a double centrifugation and enzymatic digestion assay described by Forbes and Gajadhar (1999). Briefly, approximately 10 grams from each tongue was homogenized in a blender, digested in 1% HCl/Pepsin solution at 37 ºC for 1 h, and concentrated by sequential sedimentation through two different separatory funnels. Sediment was examined under a stereo-microscope at 10-16 x magnification for the presence of larvae. Recovered larvae were washed in PBS and frozen at minus 20 ºC until further analysis. For each positive fox, DNA was extracted from 15 larvae (5 individually and 10 pooled) for further characterization as per Scandrett et al. (2018). The extracted DNA was amplified by a conventional multiplex PCR

using primer sets that generate unique banding patterns on a 2.5% agarose gel for each known species and genotype of *Trichinella* (Zarlenga et al., 1999).

2.4.7. *Statistical analyses*

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

2.5. RESULTS

2.5.1. Samples

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

2.5.2. Detection limit of genomic *T. gondii* DNA and tachyzoites

The estimated 95% lowest detection limit of the PCR assay was 73 fg of *Toxoplasma* genomic DNA per PCR reaction (95% CI: 23–5847) using a probit regression model that fitted the data adequately (Pearson χ^2=0.212, p=0.99). The estimated 95% lowest detection limit for the MC-PCR method was 445 tachyzoites per 100 grams (95% CI: 86-742,000) of beef sample. Model fit was adequate (Pearson χ^2 = 9.494, p=.22).

2.5.3. Detection of *T. gondii* antibodies

Of the 39 foxes, heart fluid from sixteen (41%) were positive to *T. gondii* antibodies with the Modified Agglutination Test (MAT): 5 out of 7 collected from Kuujuaq (75%), 4 out of 13 from Kuujjuaraapik (31%) and 6 out of 19 from Inukjuak/Puvirnituq (37%). Of the sixteen seropositive foxes, no DNA was detected in 5 foxes (Table 2.1). Of the 23 seronegative foxes, DNA was detected in the brain and/or heart of 7 foxes.

2.5.4. Detection of *T. gondii* DNA and tachyzoites

DNA of *T. gondii* was detected in 10 fox brains (26%; CI: 14-41%) and 17 fox hearts (44%; CI: 29-59%) for an overall PCR prevalence of 46% among all 39 foxes. A total of 8 out of 18 PCR-positive foxes were positive for both brain and heart (Table 2.1). There was no significant difference in the proportion of PCR-positive foxes between western (53%) and southern (40%) Nunavik ($\chi^{2\,=}$ 0.22, p=0.85).

TABLE 2.1: PROPORTIONS OF FOXES POSITIVE FOR *T. GONDII* AND *TRICHINELLA NATIVA* IN NUNAVIK (APRIL 2015 – SEPTEMBER 2016)

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

* MAT: Modified Agglutination Test

** MC-PCR: Magnetic Capture and Polymerase Chain Reaction technique

[1] *T. gondii* prevalence based on foxes positive on serology or tissue detection was 59% (95%CI: 43-73%)

TABLE 2.2: COMPARISON OF THE MODIFIED AGGLUTINATION TEST (MAT) AND MC-PCR OUTCOMES FOR THE DETECTION OF *T. GONDII* IN FOXES OF NUNAVIK

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

2.5.5. *Trichinella spp.*

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

2.5.6. Co-infection with *T. gondii* and *T. nativa*

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

2.5.7 *Agreement between MC-PCR and serology for T. gondii*

Using either method of detection (serology or molecular), 23 of 39 foxes (59%) were exposed to and/or infected with *T. gondii*. Five foxes were positive on serology and negative on tissue DNA, 7 were serologically negative but tissue positive, 11 were positive on both, and 16 were negative on both (Table 2.2). There was no statistical difference between serological and molecular results using the McNemar chi square test (p=0.581), and the Kappa test statistic (k=0.321) showed only moderate agreement between the two tests (Dohoo et al., 2010).

2.6. DISCUSSION

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

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

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

We also report for the first time the occurrence of co-infection with *T. gondii* and *T. nativa* in red foxes of Nunavik. The prevalence of *T. nativa* was estimated at 36% in this study compared to a prevalence of 11% (n=28) reported in Arctic foxes of Nunavut and the Yukon Territory (Gajadhar and Forbes, 2010). Because *Trichinella* spp. are only transmitted through ingestion of infected meat, red foxes in Nunavik are most likely exposed to the parasite by scavenging on the carcasses of other carnivores including black bears, polar bears, wolves and other foxes, or even marine mammals in coastal communities (Gajadhar and Forbes, 2010). Larvae of *T. nativa* were

viable even following freezing of tongues, compatible with freeze-resistance as demonstrated through the recovery of viable larvae in the frozen muscle of naturally-infected walruses (Leclair et al., 2004). Our study shows that *T. nativa* occurs in foxes trapped from several communities located remotely from one another, implying that *Trichinella nativa* is widely distributed throughout southern and northwestern parts of Nunavik. Further work is indicated to determine if other species and genotypes (such as T6) of *Trichinella* are circulating in terrestrial and marine ecosystems in Nunavik.

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

parasite's geographic distribution within Nunavik, additional studies should include foxes and other wildlife from other parts of Nunavik and the circumpolar North.

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CHAPTER 3: SEROLOGICAL AND MOLECULAR DETECTION OF *TOXOPLASMA GONDII* IN TERRESTRIAL AND MARINE WILDLIFE HARVESTED FOR FOOD IN NUNAVIK, CANADA

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Author Contributions

NB coordinated field data collection. EA contributed support in the field. NB, EJ, AR, CS and PL contributed to study design. NB did the laboratory work. MN contributed to laboratory support. NB drafted the manuscript. All authors reviewed and approved the final manuscript.

3.1. CHAPTER TRANSITION

Following the successful use of the MC-PCR technique in tissues of naturally-infected foxes of Nunavik (Chapter 2), this chapter aimed to apply the same technique to: a. determine the importance of country foods as a potential food safety concern for Inuit with respect to *T. gondii,* and b. explore hypotheses about the relative roles of environmental oocysts versus food-borne tissue cysts as sources of *T. gondii* exposure in Nunavik. Since herbivorous species can only become infected with *T. gondii* through exposure to oocysts in the environment or congenitally, the oocyst exposure hypothesis was assessed by including endemic herbivorous species of the terrestrial (e.g. caribou, ptarmigan). Canada and Snow geese were also tested to assess the possibility that migratory birds are exposed to oocysts outside of Nunavik and are possible seasonal sources of exposure to *T. gondii* in Nunavik. Serological assays are the most commonly used tests for determining *T. gondii* prevalence in animals, especially in wildlife. Because serology only reflects previous exposure to the parasite rather than current infection status of an animal, we compared serological results with those of the MC-PCR technique used to detect and quantify *T. gondii* DNA in tissues of wildlife harvested for food in Nunavik. This chapter addressed gaps of information needed for the development of a quantitative exposure assessment in Chapter 4.

3.2. ABSTRACT

Toxoplasma gondii, a zoonotic protozoan parasite, infects mammals and birds worldwide. Infection in humans is often asymptomatic, though illnesses can occur in immunocompromised hosts and the fetuses of susceptible women infected during pregnancy. In Nunavik, Canada, 60% of the Inuit population has measurable antibodies against *T. gondii.* Handling and consumption of wildlife have been identified as risk factors for exposure. Serological evidence of exposure has been reported for wildlife in Nunavik; however, *T. gondii* has not been detected in wildlife tissues commonly consumed by Inuit. We used a magnetic capture DNA extraction and real-time PCR protocol to extract and amplify *T. gondii* DNA from large quantities of tissues (up to 100 grams) of 435 individual animals in Nunavik: 166 ptarmigan (*Lagopus lagopus*), 156 geese (*Branta canadensis* and *Anser caerulescens*), 61 ringed seals (*Pusa hispida)*, 31 caribou (*Rangifer tarandus*), and 27 walruses (*Odobenus rosmarus*). DNA from *T. gondii* was detected in 9% (CI: 3-15%) of geese from four communities in western and southern Nunavik, but DNA was not detected in other wildlife species including 20% (95% CI: 12-31%) of ringed seals and 26% (95% CI: 14-43%) of caribou positive on a commercial modified agglutination test (MAT) using thawed heart muscle juice. In geese, tissue parasite burden was highest in heart, followed by brain, breast muscle, liver, and gizzard. Serological results did not correlate well with tissue infection status for any wildlife species. This is the first report on the detection, quantification, and characterization of DNA of *T. gondii* (clonal lineage II in one goose) from wildlife harvested for food in Nunavik, which supports the hypothesis that migratory geese can carry *T. gondii* into Nunavik where feline definitive hosts are rare. This study suggests that direct detection methods may be useful for detection *T. gondii* in wildlife harvested for human consumption and provides

data needed for a quantitative exposure assessment that will determine the risk of *T. gondii* exposure for Inuit who harvest and consume geese in Nunavik.

Keywords: *Toxoplasma gondii*, zoonosis, foodborne pathogen, wildlife, public health

3.3. INTRODUCTION

Harvested wildlife is an important source of "country food" in the Canadian North (Chan et al., 2006; Lambden et al. 2006). Country foods include terrestrial/marine mammals, land/sea birds, fish, plants, and berries harvested as food from the local natural environment (McGrath-Hanna et al., 2003). Consumption of food products of wildlife origin is frequent in Nunavik, northeastern Canada where it contributes up to 25% of people's daily protein requirements and is consumed at least five times weekly year-round (Blanchet and Rochette, 2008). Although country food is beneficial nutritionally and for ensuring food security, it can harbor chemical, physical and biological hazards sometimes harmful to human health including foodborne zoonotic parasites (Havelaar et al., 2015). In the Arctic, Inuit are potentially exposed to a range of pathogens through frequent subsistence hunting and consumption of raw or undercooked animal tissues from different wildlife species (Jung et al., 2017). Not all foodborne hazards can be observed grossly through visual inspections undertaken by hunters or even during controlled, systematic meat inspection (Hathaway and McKenzie, 1991). Understanding which zoonotic pathogens are found within wildlife reservoirs in the North is, therefore, needed to evaluate health risks for people who rely on the frequent consumption of wildlife.

Toxoplasmosis, a common infection in humans globally, is caused by the zoonotic parasite *Toxoplasma gondii* (Halonen and Weiss, 2013; Tenter et al., 2000). Its life cycle involves three distinct infectious life stages: 1. sporozoites contained within oocysts excreted in the feces of its definitive host (felids); 2. tachyzoites that travel through blood and cross blood barriers (e.g. placental, ocular and brain) in both definitive and intermediate hosts, and; 3. bradyzoites

contained within cysts in tissues of definitive and intermediate hosts (Hill and Dubey, 2002). This zoonotic parasite can persist lifelong in its hosts as bradyzoites that divide and multiply slowly within tissue cysts that remain latent (Schluter et al., 2014) This lifelong persistence within animal tissues is a key feature of the epidemiology of *T. gondii* in humans since the parasite can persist through trophic interactions of intermediate hosts (carnivory) without a need for sexual reproduction in the definitive host (Tenter et al., 2000). In areas where definitive felid hosts are rare to absent, such as the Canadian Arctic, this could explain how people and animals are exposed to *T. gondii*.

One third of the global human population has been exposed to *T. gondii*, compared to 60% of Inuit in Nunavik, Canada [Blanchet and Rochette, 2008; Cook et al., 2000). Foodborne transmission is considered an important route of exposure for Inuit since definitive hosts (felids) that shed oocysts are rare to absent north of the treeline (Messier et al., 2009). Inuit regularly consume organs and tissues from several wildlife species raw or undercooked, a food preparation method considered as high risk for exposure to viable *T. gondii* tissue cysts (Food Safety Network, 2009). Two studies in Nunavik have identified the consumption and/or handling of different wildlife species [caribou (*Rangifer tarandus*), seals (several species) and feathered game] as important risk factors for Inuit exposure to *T. gondii* (McDonald et al., 1990; Messier et al., 2009). A regional serological screening program initiated for pregnant women in the early 1980s showed that congenital toxoplasmosis (seroconversion of the mother during pregnancy) was higher in Nunavik compared to the remainder of Canada (1.8% compared to 0.2%. respectively) (Lavoie et al., 2008). There is therefore a need to determine whether people are

potentially exposed to infected tissues from hunter-harvested wildlife commonly consumed in Nunavik.

Although exposure to *T. gondii* has been serologically demonstrated in over 300 species of mammals and 30 species of birds worldwide (Fledgr et al., 2014), including seals, geese and ptarmigan in Nunavik (Leclair and Doidge, 2001), direct detection of DNA or organism in tissues from wildlife is far less common. This is partly because wildlife pathogen investigations in general present unique challenges due to difficulties with accessing freely-roaming wildlife in remote areas, limited local capacity for testing, and diagnostic tests that are often not validated or optimized for use in wildlife (Ryser-Degiorgis, 2013). Most studies in animals rely on detection of antibodies in blood, but this reflects lifetime exposure to, rather than active infection with, *T. gondii*. Because blood or serum is rarely accessible from carcasses of hunter-harvested wildlife, detection of antibodies to *T. gondii* in meat fluid has also been proposed as a suitable alternative in large-scale monitoring programs (Villena et al., 2012; Berger-Schoch et al., 2011). However, relying on serology as a food safety screening test in wildlife could lead to the rejection of seropositive animals that are not actively infected, which is undesirable in the North where ensuring food security remains an ongoing challenge (Blanchet and Rochette, 2008).

Indirect detection methods for *T. gondii*, such as bioassays, also have limitations (Koethe et al., 2015). Cat bioassays, the gold standard for *T. gondii* detection, require up to 500 grams of tissue in feeding trials. As well, not all strains of *T. gondii* produce clinical disease in every animal model (cat or mouse) since virulence is strain and host specific (Dubey, 2010). Bioassays also have the disadvantage of being time-consuming, costly and requiring high numbers of animals which make the method impractical and unethical for wildlife studies (Gomez-Samblas et al., 2015). For these reasons, direct detection methods for DNA of *T. gondii* are increasingly used in

food safety settings. However, kit-based DNA extraction methods from small tissue quantities (on the order of 25-100 mg) limit detection since *T. gondii* tissue cysts are not uniformly distributed in tissues (da Silva et al., 2001; Hill et al., 2006). As a result, a magnetic-capture DNA extraction and real-time PCR method (MC-PCR) has been developed for testing up to 100 grams of tissue, allowing for improved detection and quantification of parasite DNA (Opsteegh et al., 2010; Jurankova et al., 2014; Koethe et al., 2015; Gomez-Samblas et al., 2015). Briefly, sequence-specific DNA fragments bound to magnetic beads help to capture low concentrations of parasite DNA against high backgrounds of host DNA and inhibitory PCR products (Opsteegh et al., 2008). Once concentrated using a magnet, the captured DNA sequences are amplified using a quantitative real-time PCR assay based on a highly conserved and sensitive 529 bp non-coding DNA fragment present in 200-300 copies per *T. gondii* genome (Homan et al., 2000). The MC-PCR technique is being used in screening of food production animals in Europe for *T. gondii* (Opsteegh et al., 2016), and has recently been used successfully in naturally-infected foxes of Nunavik [Bachand et al., 2018). There is clearly a need, and now a good method, to determine whether *T. gondii* DNA is present in tissues of wildlife commonly consumed by Inuit of Nunavik, and to compare these results with serological findings based on a commonly used agglutination assay.

3.4 MATERIALS AND METHODS

3.4.1. Study design

A cross-sectional study was designed to detect DNA of *T. gondii* in the tissues of migratory geese (*Branta canadensis* and *Chen caerulescens*), willow ptarmigan (*Lagopus lagopus*), and

ringed seals (*Pusa hispida*) harvested by local hunters as part of regular subsistence activities in three communities of southern and western Nunavik, Québec (QC), Canada (Figure 3.1). Hunters were informed of the study by a local community coordinator with consent from the local hunter association. Wildlife samples were submitted on a volunteer basis between April 2015 and September 2016. The target sample size was calculated using prevalence estimates from the literature, a 5% precision level, and a 95% confidence interval as follows: ringed seals (n=104 with expected prevalence of 7.3%); Canada geese (n=140; 35 pools of 5 individuals based on an expected prevalence of 4.2%); and willow ptarmigan (n=95; 19 pools of 5 individuals each based on an expected prevalence of 2.5%) (Leclair and Doidge, 2001; Dohoo, 2010; Simon et al., 2011).

3.4.2. Tissue samples

Local hunters recorded information on species, sex, harvest location, and date. Tissues collected for each animal varied according to wildlife species: seal kits contained the entire heart, at least 100 grams each of diaphragm and liver, and the tongue; goose kits included the head, the heart, the gizzard, the liver and at least 100 grams of breast muscle; and the entire carcass was collected from ptarmigan. Samples were stored at -20 ºC for less than 2 months before analysis in the laboratory. Caribou samples (e.g. brain, heart, muscle, and sera) were collected in 2013 from the Leaf River Herd in Nunavik by biologists and held at -20 ºC until processing in 2016, whereas walrus tongues were collected as part of a regional *Trichinella* spp. monitoring program at the Nunavik Research Centre. Authorizations were obtained from a major body representing Inuit of Nunavik, the Makivik Corporation, and the Regional Nunavimmi Umajulivijiit Katujaqatigininga (RNUK) during a regional hunter association meeting in Kangiqsualujjuaq in

FIGURE 3.1: MAP OF NUNAVIK, CANADA (© Lemire et al., 2015)

November 2014. Since animals were harvested for other purposes, this work was considered Category A by the University of Saskatchewan Animal Research Ethics Board.

3.4.3. *T. gondii* serology

Sera were available only for caribou, while for seals, ptarmigan, and geese, whole hearts kept frozen in individual plastic bags were thawed at room temperature and fluid was collected from the bag using a sterile disposable plastic pipette (Villena et al., 2012). For each species, a modified agglutination test (MAT, New Life Diagnostic LLC, Carlsbad, CA, United States) was used with a threshold dilution of 1:25 (Dubey and Desmonts, 1987). We used both positive and negative controls supplied in the commercial kit, as well as in-house positive controls including heart fluid from naturally-exposed fox and sera from experimentally infected reindeer (Bouchard et al., 2017). Since blood from marine mammals contains lipids that may interfere with the performance of agglutination assays (Blanchet et al., 2011), we removed lipid from seal samples using a chloroform method, re-tested using MAT, and compared serological test results with the ID Screen® Toxoplasmosis Indirect Multiple-Species ELISA kit (IDVet Innovative Diagnostics, Montpellier). ELISA results were measured as optical density percentages (OD %) as per manufacture instructions, where an OD% greater than 50% is positive, between 40-50% is ambiguous, and less than 40% is negative.

3.4.4. Extraction and detection of DNA

DNA was extracted from wildlife tissues as per Opsteegh et al., (2010) with a minor modification for avian (brain, heart) and caribou samples less than 25 grams, which were instead pooled and digested in 50 ml centrifuge tubes rather than stomacher bags. For seals (heart, liver, diaphragm), walrus (tongue) and goose tissues (breast muscle and liver), up to 100g of each

tissue was weighed to determine the required amount of cell lysis buffer (CLB) based on 2.5 ml CLB per gram of tissue. For geese and ptarmigan, aliquots of digest of brain and heart from five birds were pooled. Digests were incubated overnight followed by homogenization by manual vortexing for one minute. For each heart or brain PCR-positive pool, reserved lysate from individual animals (heart and brain) was subsequently analysed separately. Other tissues (liver, gizzard and breast muscle) were analysed for individual geese from PCR-positive brain or heart pools.

Real-time PCR amplification was done in a Bio-Rad CFX 96 DNA thermal cycler (Biorad, Hercules, California, USA) based on published protocols for detection of the 188 bp *Toxoplasma* sequence within the 529 repeat-element with the forward primer TOX 9 (5´ aggagagata tcaggactgt ag3´) and backward primer TOX 11 (5´gcgtcgtctc gtctagatcg3´) as per Opsteegh et al. (2010) and Omar et al. (2015). The final PCR assay reaction included 6.5 ul (0.5M) of Itaq Supermix, 0.25 ul (20uM) of TP1 probe, 1.25 ul (10 uM) of Tox 9F, 1.25 ul (10 uM) of Tox 11R, 0.5 ul (2 femtograms) of CIAC, 1 ul (5 uM) of ciac probe, 6.75 ul of PCR-grade water and 8 ul of template DNA (Bachand et al., 2018). A positive PCR reaction was defined as any reaction with a Ct-value smaller or equal to 35, a control negative PCR with a Ct-value of zero, a negative extraction control with a Ct value of zero and a control positive extraction control with a Ct-value smaller or equal to 40 (Opsteegh et al., 2016). A negative PCR reaction was defined as a reaction with a Ct-value of zero, a positive competitive internal amplification control (CIAC) Ct-value, a negative DNA extraction control with a Ct-value of zero and a positive DNA extraction control with a Ct-value of 40 or less. All reactions for which only one of two replicates amplified, or where CIAC amplification did not occur, were repeated. The DNA from positive PCR products was then purified using the EZ-10 Spin Column PCR Products

Purification Kit (Bio Basic, Markham, Ontario) before being sent for DNA sequencing (Macrogen Inc., Korea). DNA sequences were then analyzed using the online Basic Local Alignment Search Tool (BLAST) tool.

3.4.5. DNA characterization

3.4.5.1. GRA6 DNA extraction

For strongly positive samples (a real-time positive PCR Ct-value less than 32), DNA was extracted from 6-12 ml of frozen lysate using 15 pmol of primers targeting the GRA6 gene (GRA6-CapF and GRA6-CapR) rather than 10 pmol (Opsteegh et al., 2010). The purified DNA product was sent within 24 hours on dry ice to the National Reference Centre for Parasitology, Research Institute of the McGill University Health Centre, Montreal, QC, Canada for further genetic characterization.

3.4.5.2. PCR- RFLP amplification

Amplification of the GRA6 gene was done using a published protocol (Zakini et al., 2006). Briefly, amplification was performed in 50 µl which included 2 µl of DNA template, 5x GoTaq Flexi buffer (Promega), 2 mM $MgCl_2$, 50 pmol of each primer, 0.2 mM of each deoxynucleotide triphosphate and 1.25 U of Taq DNA polymerase. Reactions were incubated at 94ºC for 5 min, followed by 35 cycles of denaturing for 30 sec at 94ºC, annealing for 60 sec at 54ºC, and extension for 90 sec at 72ºC. The final cycle was followed by an extension step of 7 min at 72ºC. Two µl of this final PCR product was then used as template DNA in the secondary PCR which used a forward primer (5'- GTAGCGTGCTTGTTGGCGAC-3') and reverse primer (5'-TACAAGACATAGAGTGCCCC-3') described by (Fazaeli et al., 2000) at an annealing temperature of 60ºC and an extension of 2 minutes with 35 cycles. Five µl of amplicon was run

in a 1.5 % agarose gel containing GelRed at 120 V for 40 minutes with 1× TE buffer prior to being visualised under UV light. The unpurified PCR product was sequenced at McGill University and the Génome Québec Innovation Centre in Montreal, QC, Canada. Nucleotide sequences were applied to a BLAST in order to determine % similarity with the GRA6 sequences deposited in the GenBank. RFLP analyses were then performed on PCR positive samples in order to characterise the strain type. GRA6 positive amplicons were incubated with the MseI enzyme according to the manufacturer's instructions (New England BioLabs) and digested PCR amplicons were visualized by electrophoresis on a 1.6 % agarose gel containing Gel Red.

3.4.6. Data analysis

3.4.6.1. Prevalence

Seroprevalence and PCR prevalence and their 95% confidence intervals were estimated using the Ausvet Epitools epidemiological calculators (Sergeant, 2018).

3.4.6.2. Lowest detection limit and quantification

Determination of the minimum number of DNA copies and tachyzoites detected by the MC-PCR technique has been described elsewhere (Bachand et al., 2018). Ct-values resulting from the amplified DNA recovered from the spiked beef samples for determining the lowest detection limit were then used to estimate the equation that predicts the log10 (concentration) by fitting a generalized linear model in R statistical software version 3.4.4. (Opsteegh et al., 2010).

3.4.6.3. Serological test agreement (seals)

In seals, proportion of positive results was compared between the MAT and the ELISA using McNemar's χ^2 test. If not significantly different, the kappa coefficient was used to determine the level of agreement between the two tests (Dohoo, 2010).

3.5. RESULTS

3.5.1. Samples

A total of 166 willow ptarmigan, 156 geese, and 61 ringed seals were received. Of the 156 geese, 148 were Canada geese (*Branta canadensis*) and 8 were Snow geese (*Chen caerulescens*). A total of 31 caribou sampling kits (16 adult females and 15 calves) from the Nunavik Leaf River herd in 2013, as well as 27 walrus tongues from the Nunavik Research Centre were analyzed. Information on the weight of different tissues analysed for different species is displayed in Table 3.1.

3.5.2. Detection of *T. gondii* antibodies

Antibodies were detected on MAT of heart fluid in 20% of ringed seals (95% CI: 12-31%) and 26% of caribou (95% CI: 14-43%) (Table 3.2). For geese, seroprevalence was estimated at 11% (95% CI: 6-17%) and 2 of the 18 seropositive geese were snow geese (Table 3.2). No detection occurred for ptarmigan (Table 3.2) and serological testing was not possible for walruses. Following lipid removal, no seals were positive on MAT, and positive controls remained positive. Using the ELISA, seroprevalence in seals was estimated at 30% (95% CI: 20-42%) (Table 3.2).

TABLE 3.1: AVERAGE WEIGHT OF TISSUES ANALYSED USING THE MC-PCR METHOD FOR DETECTING *T. GONDII* DNA IN HARVESTED WILDLIFE FROM NUNAVIK, CANADA

Species	n	Tissues	Average weight (g)	Min. weight (g)	Max. weight (g)
Seal	61	Heart	90	32	100
		Liver	74	35	100
		Diaphragm	83	16	100
Geese	156	Brain	16	4	26
		Heart	18	9	26
		Gizzard	72	28	100
		Liver	46	22	100
		Breast muscle	76	36	100
Ptarmigan	166	Brain	7	6	9
		Heart	2	<1	3
Caribou	31	Brain	21	3	91
		Heart / Muscle	52	11	87
Walrus	27	Tongue	51	37	58

TABLE 3.2: SEROPREVALENCE OF *T. GONDII* IN HUNTER-HARVESTED WILDLIFE OF NUNAVIK, CANADA

Species	n	Seroprevalence (%) (95%CI) (# of positive/ total # analyzed)	
		MAT	ELISA
Ptarmigan	166	0%	NP[1]
Caribou	31	26% (14-43%) (8/31)	NP
Adult females	16	31% (14-56%) (5/16)	NP
Calves	15	20% (7-45%) (3/15)	NP
Ringed seals	61	20% (11-31%) (12/61)	30% (20-42%) (18/61)

[1] NP: Not performed

TABLE 3.3: PREVALENCE OF *T. GONDII* BASED ON THE MODIFIED AGGLUTINATION TEST (MAT) AND THE MAGNETIC CAPTURE & REAL-TIME PCR METHOD IN MIGRATORY GEESE HARVESTED IN NUNAVIK, CANADA

Species	n	Seroprevalence (%) (95%CI)	MC-PCR (%) (95%CI) (# of positive/ total # analyzed)					
		Heart fluid	Brain (B)[1]	Heart (H)[1]	H, B[2]	Liver	Muscle	Gizzard
Geese	156	11% (7-18%)	4% (0-8%) (9/41)	4% (0-8%) (9/41)	9% (3-15%) (13/41)	14% - (1/7)	31% - (4/13)	9% - (1/11)

[1] Pools were constituted of 5 individual brains or hearts and "n" is the number of pools

[2] PCR-prevalence based on whether pools were positive on brain, heart or both

3.5.3. Lowest detection limits and quantification

The estimated 95% lowest detection limit for the MC-PCR technique was 445 tachyzoites per 100 grams (95% CI: 86-742,000) (Bachand et al., 2018). For quantification, a generalized linear model was fitted using Ct-values generated from the lowest detection limit experiments with known tachyzoite concentrations used to spiked 100-gram beef muscle samples. The best fitting model was described by Ct = 43.3 – 3.07 $\log_{10}$ [tachyzoite] with the outcome being the number of tachyzoite-equivalents per 100 grams of tissue. The linear regression showed that the Ct-value could statistically significantly be predicted by the log [tachyzoite] with $F(1.92) = 2172$, $p< 0.005$. The log [tachyzoite] accounted for 96% of the explained variability in the Ct-value. The intercept with the y-axis was 43.3 (95% CI: 42.6 to 43.9) and the slope was −3.07 (95% CI: −3.2 to −2.9). This formula was rewritten as ($\log_{10}$ [tachyzoites] = (43.3–Ct) /3.07) to more simply estimate the number of tachyzoite-equivalents from the Ct-value in field samples.

3.5.4. Detection of *T. gondii* DNA from samples

DNA of *T. gondii* was detected in 9% (CI: 3-15%) of geese (Table 3.3) and no detection occurred for any other wildlife species including ringed seals and caribou positive on serology.

3.5.5. Genotyping using the GRA6 gene

Out of 5 goose samples with a qPCR Ct-value between 30 and 33, only one amplified on PCR using primers for the GRA6 gene. On PCR-RFLP, this was identified as the Type II clonal lineage of *T. gondii* which was confirmed by sequencing.

3.5.6. Parasite burden in geese tissues

Based on the $\log_{10}$ [tachyzoites] = (43.3–Ct) /3.07) equation and Ct-values obtained from field samples, the parasite burden defined as the mean number of tachyzoite-equivalents per gram (TE/g) for each tissue and its standard error was: 744 (SE: 476) for heart (n=8), 300 TE/g (SE: 100) for brain (n=9), 104 (SE: 140) for breast muscle (n=4), 33 for liver (n=1) and 8 for gizzard (n=1).

3.5.7. Agreement between serological tests (seals)

In total, heart fluid was available for comparison between the MAT and ELISA for 55 seals: 2 seals were positive on both, 10 samples were positive for MAT but negative for ELISA, 16 were negative for MAT but positive for ELISA, and 27 were negative on both. The McNemar chi square test comparing the MAT and ELISA serological assays was significant (p=0.029), meaning that there was a difference between results from both serological assays in the case of seals. Therefore, a kappa test statistic was not performed (Dohoo, 2010).

3.6. DISCUSSION

We directly detected DNA of *T. gondii* in multiple tissues of naturally-infected geese harvested for food by local hunters of Nunavik. This supports previous epidemiological associations between consuming waterfowl and Inuit exposure to *T. gondii* in Nunavik, where the average regional human seroprevalence is 60% (Messier et al., 2009). This also shows that migratory geese carry *T. gondii* between southern and northern ecosystems (Prestrud et al., 2007; Sandstrom et al., 2013), which is further supported by detection of *T. gondii* on mouse and cat bioassays of heart digests from Canada geese in Maryland, USA (Verma et al., 2016) and brain digests from four Canada geese in Mississippi, USA (Dubey et al., 2004). The MC-PCR

technique used in the current study is specific for *T. gondii* as it targets the highly conserved 529 repeat-element absent in other coccidian parasites such as *Sarcocystis* and *Neospora* spp. (Opsteegh et al., 2010), and is highly sensitive since there are 200-300 copies per *T. gondii* genome (Homan et al., 2000). As well, this technique uses large amounts of tissue (up to 100 g) which increases the probability of including a portion of tissue containing parasite DNA. Nonetheless, prevalence based on direct detection has likely been underestimated because levels of parasites in tissues of naturally-infected wildlife may be below the detection limit of the MC-PCR technique used in this study and because *T. gondii* cysts are not uniformly distributed among and within tissues of infected animals (Dubey, 2010).

DNA of *T. gondii* was detected in several goose tissues destined for human consumption including heart, liver, gizzard, and breast muscle. This suggests that consumption of infected undercooked geese can lead to foodborne transmission of *T. gondii* in Nunavik (Cook et al., 2000). Tissue burdens (number of bradyzoites per gram of tissue) found in this study could be high enough to produce infection given daily goose consumptions trends by Inuit throughout Nunavik (between 0.1-0.3 grams per Kg body weight daily depending on the region) (Lemire et al., 2015) and using infectious doses for experimentally infected cats (10 bradyzoites), in the absence of data for humans (Cornelissen et al., 2014). These findings may have public health implications for Inuit who consume goose tissues raw and undercooked since these may be infected with viable *T. gondii* bradyzoites. Future work includes an exposure assessment for estimating the risk of human exposure to *T. gondii* in Nunavik through goose consumption, a more thorough assessment of the infection status of goose tissues other than heart and brain, as well an assessment of *T. gondii* viability in country foods prepared in traditional ways.

The *T. gondii* strain detected in one goose in this study was characterized as Type II based on the GRA6 gene. This is one of the three main clonal lineages recognised in North America, where Type II strains are responsible for the majority of congenital infections and infections in people with AIDS (Howe and Sibley, 1995). However, because most genetic markers distinguish two of the three clonal lineages, using a single marker can limit the ability to detect non-clonal strains (Su et al., 2006). Characterization results in this study should therefore be interpreted with caution despite the fact that the GRA6 gene is reported to differentiate among the three main clonal lineages (Fazaeli et al., 2000). Recent studies have demonstrated the occurrence of atypical strains in North American wildlife including geese (Verma et al., 2016; Khan et al., 2011). Further studies are therefore needed to better characterize genetic diversity of *T. gondii* in geese harvested in Nunavik.

Evaluating serology against direct detection was an important objective of the paper from a food safety perspective, since detection of antibodies to *T. gondii* in meat juice has been suggested in animals slaughtered for human consumption (Villena et al., 2012). We chose to use MAT since it has been widely used to detect antibodies to *T. gondii* in sera from caribou, geese, ptarmigan, seals and walrus (Leclair and Doidge, 2001; Kutz et al., 2001; Dubey et al., 2003; Measures et al., 2004; Jensen et al., 2012; Simon et al., 2011). Recently it has been used to detect antibodies in meat juice from horses, rabbits, and sheep (Mecca et al., 2011; Villena et al., 2012; Aroussi et al., 2015). In experimentally infected pigs, there was a strong correlation ($r=0.87$; $p<0.001$) between detection of antibodies in serum and meat juice from heart (Wallander et al., 2015). Heart fluid was used in the current study since it was not possible to obtain serum from hunter-harvested wildlife, and to determine if heart fluid could act as a screening test in the field.

Results from this study demonstrated frequent discrepancies between serological and molecular results in both directions (e.g. seronegative animals with positive tissues, and seropositive animals with negative tissues) which could be accounted for by biological reasons, such as waning of antibodies in individuals with chronic infections. In acute infection, it is possible that tissue invasion has not yet occurred despite the occurrence of detectable antibodies. In another study, one cat inoculated with heart digest from four seronegative geese excreted viable *T. gondii* oocysts, which suggests that serological status is not a reliable indicator of infection status (Verma et al., 2016). In addition to biological reasons for these discrepancies, there are a number of sampling, handling, and diagnostic test characteristics that may play a role. In our study, the cut-off value of a 1:25 dilution on the MAT was used to differentiate seronegative from seropositive geese, but it is possible that antibody levels were too low to be detected, leading to the classification of false-negative geese on serology. Dilutions lower than 1:25 (such as 1:5) might be more sensitive, but could also lead to false-positive results (Dubey et al., 2016). It is also possible that the high blood content in the heart juice interfered with antibody binding, which has been observed with ELISA in rabbit meat cuts (Mecca et al., 2011). Freezing and thawing have not been reported to compromise the detection of antibodies even after 120 days of freezing (Mecca et al., 2011). Cross-contamination of *T. gondii* DNA between samples could have occurred (leading to false-positive results on tissue testing), but negative controls remained negative.

Our results suggest that MAT and ELISA assays commonly used as screening tools for exposure to *T. gondii* in marine mammals should be interpreted cautiously. Our seroprevalence estimates in ringed seal (MAT 20%, ELISA 30%) were comparable to previous estimates of 7-14% in seals in Nunavik based on a MAT (Leclair and Doidge, 2001; Simon et al., 2011). However, no

T. gondii DNA was detected in any of the seal tissues analysed in our study, and all seals became seronegative on MAT after lipid removal. While it is possible that tissue burdens in seals may simply be below the detection level of the MC-PCR technique used in this study, this suggests a need to validate the use of serological assays for antibody to *T. gondii* in marine mammals and to carefully interpret previously published findings. As well, serological assays, while useful for obtaining a snapshot of exposure status in wildlife populations, should not be used to make decisions on the possible infection status of tissues from an individual animal (i.e. for food safety decisions or when the decision impacts public health, such as in Nunavik). False-positives on serology, resulting in discarding a healthy animal as a source of food, could compromise food security for individuals who rely on or prefer harvested wildlife, especially in Nunavik where one of four households is considered to be food insecure (Blanchet and Rochette, 2008).

Similar to seals, we did not detect DNA of *T. gondii* in tissues of walruses. We only had access to archived tongue samples for walruses; *T. gondii* has been detected in tongue in some experimentally-infected species (Opsteegh et al., 2016). Future research should include a panel of tissues to determine occurrence and tissue predilection of *T. gondii* in walrus. If tongue proves to be a predilection site, detection of *T. gondii* in tongues of walrus could be added to the currently well-established *Trichinella* monitoring program at the Nunavik Research Centre (Proulx et al., 2002).

We did not detect *T. gondii* DNA in ptarmigan or caribou, two endemic terrestrial wildlife species of Nunavik. Herbivores are generally infected with *T. gondii* via ingestion of oocysts shed in the environment by felid hosts (rare to absent in Nunavik) or tachyzoites that cross the placenta to infect fetuses when a female is infected for the first time in pregnancy. Ptarmigan was the only species in this study which displayed consistent negative serological and molecular

results. One study previously reported a *T. gondii* seroprevalence (using MAT) of 2.5% in 70 ptarmigan from communities in Ungava Bay (Leclair and Doidge, 2001), whereas ptarmigan in the current study originated from Hudson Bay. Imperfect test performance and the use of different media (serum vs tissue fluid) to detect antibodies may also explain these differences. Although MAT has recently been validated for use in chickens, test performance was shown to be poor (Dubey et al., 2016). At the moment, there is little evidence of exposure to or infection with *T. gondii* in ptarmigan of Nunavik, which supports the hypotheses that oocyst transmission is rare in northern ecosystems and that ptarmigan represent a low food safety concern with respect to *T. gondii*.

No DNA of *T. gondii* was detected in caribou tissues (muscle, heart, brain) from Nunavik's Leaf River herd despite detection of *T. gondii* antibodies in the sera of 23% of 30 caribou. This is much higher than the previously reported *T. gondii* seroprevalence of 1.5% (n=268) using another MAT, but lower than the 62.5% (n=40) based on the Sabin-Feldman dye test reported in Kuujjuaq (McDonald et al., 1990; Leclair and Doidge, 2001). Very few studies have attempted to correlate the serological status of an animal with the presence of *T. gondii* in their tissues (Villena et al., 2012). In domestic animals, a correlation between serological and tissue infection status has been reported in pig and sheep, but not in cattle (Opsteegh et al., 2011). In our study, four caribou calves were seropositive, which could be due to transfer of maternal antibodies (one had a seropositive dam), congenital transmission of the parasite, or infection via oocyst consumption. For caribou, only small portions of each tissue (muscle, heart and brain) were available for DNA isolation which limited detection probability. Our seropositive samples, in combination with findings of DNA in all tissues examined in reindeer experimentally exposed to high doses of Type III *T. gondii* oocysts (Bouchard et al., 2018), suggest that further work is

needed to determine the tissue infection status of naturally-exposed caribou. Future research should use large amounts (at least 100 grams) for different caribou tissues in order to provide more insight on the food safety risk of *T. gondii*.

Detection of *T. gondii* DNA in several goose tissues commonly consumed by people may partially explain the high levels of *T. gondii* exposure observed in Nunavik, Canada. However, since both *T. gondii* prevalence and consumption trends (preparation method, consumption frequency) affect the risk of exposure to *T. gondii*, a better understanding of goose consumption trends in Inuit and an exposure assessment are needed to better answer this question. Since serological and molecular results were often discordant, generally biased towards higher seroprevalence than tissue prevalence, our work suggests caution in using serology as a means of screening positive animals as a food safety prevention measure against a backdrop of food insecurity. As well, future research on other wildlife species endemic to Nunavik should aim for higher sample numbers using larger tissue samples (e.g. in caribou and walrus). Finally, because DNA of *T. gondii* was not detected in any terrestrial or marine wildlife species endemic to Nunavik, these results suggest that exposure to *T. gondii* oocysts shed by felids may be less important than foodborne and vertical routes of exposure in the Canadian North.

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CHAPTER 4: A QUANTITATIVE ASSESSMENT OF INUIT FOODBORNE EXPOSURE TO *TOXOPLASMA GONDII* FROM HUNTER-HARVESTED GEESE IN NORTHEASTERN CANADA

Citation

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Author Contributions

NB and AR devised the conceptual model and mathematical models. NB ran the analyses. AR provided technical support. NB drafted the manuscript. All authors reviewed and approved the final manuscript.

4.1. CHAPTER TRANSITION

In Chapters 2 and 3, I demonstrated for the first time that DNA of *T gondii* is present in wildlife of Nunavik, namely in migratory geese and foxes. In this chapter, I tackle the next step of determining whether consuming geese represents a risk for *T. gondii* exposure in Inuit of Nunavik. This quantitative exposure assessment estimates the monthly probability that Inuit are exposed to *T. gondii* through the consumption of geese. This was achieved by combining data from Chapter 3 and goose consumer data collected as part of this chapter. Consumer data specific to goose was missing in the literature, which represented an important gap of information that needed to be filled for a more accurate estimate of the probability of exposure to *T. gondii* in Nunavik. This chapter also aimed to provide information on the relative importance of different goose tissues as possible sources of *T. gondii* exposure, as well as on specific preparation methods that may present higher exposure risks for Inuit. Lastly, the exposure assessment aimed at identifying additional data gaps and future research needs.

4.2. ABSTRACT

The zoonotic parasite *Toxoplasma gondii* is a public health concern worldwide. The incidence of congenital toxoplasmosis and the *T. gondii* seroprevalence are high among Inuit of Nunavik, Canada. Recently, *T. gondii* DNA has been detected in hunter-harvested geese from this region. The objective of this study was to estimate the probability that Inuit from Inukjuak, Nunavik, are exposed to *T. gondii* bradyzoites (the chronic tissue stage of the parasite) through the consumption of 4 goose tissues: heart, liver, gizzard and breast muscle. Consumer data were collected based on a goose consumption frequency (GCF) questionnaire given to 30 Inuit respondents from Inukjuak in May 2018. Every respondent consumed goose and the average number of Canada geese consumed per person was 9.3 (SD: 5.3; Min.: 1; Max.: 30) compared to 3.2 for snow geese (SD: 3.1; Min.: 0; Max.: 10). Half of respondents reported eating goose gizzard raw, and no goose tissues were frozen long enough for *T. gondii* tissue cysts to be inactivated. The number of *T. gondii* bradyzoites contained in a typical tissue serving size was estimated using portion size data from the GCF questionnaire and bradyzoite concentration data from naturally-infected goose tissues. Reduction in the number of viable bradyzoites at different cooking temperatures was estimated based on a log-linear regression model. For each tissue, the probability of exposure to viable *T. gondii* bradyzoites was calculated based on the number of self-reported tissue servings consumed raw (unprocessed) versus cooked (processed) over the 30-day recall period, multiplied by the probability that the tissue is infected with *T. gondii*. For each tissue, the probability of exposure to viable *T. gondii* bradyzoites was obtained by adding the probabilities of exposure to unprocessed and processed servings. The sum of all probabilities for each of the four tissues provided the overall probability of exposure to different viable *T.*

gondii bradyzoite doses during the month of May. Given the consumption of all 4 types of goose tissues, there was a 32% probability that Inuit were exposed to at least 1 bradyzoite during the 30-day recall period. Among people who were exposed, exposure to a mean number of 642 viable bradyzoites occurred throughout the month. Overall, Inuit of Inukjuak in Nunavik are most likely exposed to *T. gondii* bradyzoites through the consumption of processed breast meat, then (in decreasing order) unprocessed gizzard, processed heart, processed gizzard, and unprocessed liver. As well, our findings support the importance attached to harvesting and consumption of geese since all respondents consumed goose. Recommendations to sufficiently cook tissues are supported by this exposure assessment since the sensitivity analysis showed that cooking had the largest influence on the exposure assessment output. However, data are needed in Nunavik on actual finished cooking temperatures for different goose tissues. Until then, mitigation measures could include recommendations to freeze goose tissues for at least 72 hrs at less than -12 ºC, which may be more culturally acceptable than cooking. Next steps would be to consider the consequences of such exposure in terms of clinical toxoplasmosis, to balance these risks against benefits (nutritional, cultural, and economic) of goose consumption, and to manage and communicate these risks in a culturally appropriate manner.

Keywords: *Toxoplasma gondii*, quantitative exposure assessment, public health, risk analysis, heart, liver, gizzard, meat

4.3. INTRODUCTION

The apicomplexan parasite *Toxoplasma gondii* is a zoonotic pathogen with a worldwide distribution and broad range of warm-blooded vertebrate hosts including mammals and birds (Innes, 2010). Major transmission routes for *T. gondii* in humans include the ingestion of food or water contaminated with sporulated oocysts, ingestion of raw or undercooked animal tissue infected with cysts containing bradyzoites, vertical transmission through trans-placental migration of tachyzoites and, more rarely, the transfusion of blood contaminated with tachyzoites (Robert-Gangneux et al., 2012). Bradyzoites result from the conversion of tachyzoites (the fast-replicating life stage of *T. gondii*) which exit the blood stream to invade animal tissues (Dubey, 2010). People are exposed when they consume bradyzoites in tissue cysts from an infected animal, which are responsible for initiating infection in an acutely exposed host (Hill & Dubey, 2002; Guo et al., 2017). . Although toxoplasmosis is usually asymptomatic in humans, clinical manifestations (ocular and neurological) can occur among immunocompromised individuals and congenitally infected children. *T. gondii* can also cause reproductive problems (miscarriage) in pregnant women, especially those infected in the first trimester with *T. gondii* for the first time in their lives (McDonald et al., 1990). Chronic infection has also been associated with the development of mental disorders in animals and humans (Palmer, 2007; Torrey et al, 2012).

Approximately 12,000 Inuit live in the Nunavimmiut settlement (Nunavik) of northeastern Canada (Messier et al., 2009). Based on a 2004 regional human health study done in Nunavik, the *T. gondii* seroprevalence was reported as 62.8% in Inuit of Hudson Bay compared to 58.4% in Inuit of Ungava Bay. These values are much higher than a seroprevalence of 11% reported for the United States (Jones et al., 2018), but are in line with those of a previous study in Nunavik

where *T. gondii* seroprevalence was 61% and 69% among Inuit of the communities of Kuujjuaq and Salluit, respectively (Tanner, 1987). Moreover, in this region, the incidence of congenital toxoplasmosis is 1.7% compared to 0.2-0.8 % reported in the remainder of Canada (Lavoie et al., 2008). For pregnant women, risk factors associated with exposure to *T. gondii* include the skinning of wildlife, frequent consumption of caribou, eating raw caribou, eating dried seal and eating seal liver (Tanner et al., 1987; Curtis et al.,1988; McDonald et al., 1990). In the general Nunavik population, risk factors include drinking reservoir water, the frequent cleaning of water reservoirs, as well as the consumption of seal meat and waterfowl (Messier et al., 2009). Because felid hosts are rare to absent north of the treeline (north of the 56º latitude in Northern Quebec), where most Inuit communities in Nunavik occur, the ingestion of water or food contaminated with oocysts seems a less likely route of exposure for Inuit relative to exposure through the consumption of animals infected with tissue cysts (Elmore et al., 2012).

For centuries, Inuit have relied on land and its natural resources (Kenny et al., 2018). Consumption of traditionally prepared country food continues to be an important contributor to Inuit livelihoods (van Oostdam, 2005; Furgal and Rochette, 2007). Country food includes terrestrial/marine mammals, land/sea birds, fish, plants and berries harvested from the local environment for consumption as food (McGrath-Hanna, 2003). In Nunavik, country food is more than just a food commodity and is viewed as inherently important for maintaining health (van Oostdam, 2005). These foods contribute up to 25% of local people's daily protein requirements as well as many vitamins, minerals and fatty acids (Blanchet & Rochette, 2008). On average, Nunavik Inuit consume country food more than five times weekly throughout the year. Moreover, wildlife species most commonly consumed (≥ 11 times on an annual basis) include

caribou (*Rangifer tarandus),* goose (*Branta canadensis*)*,* ptarmigan (*Lagopus leucura)* and beluga (*Delphinapterus leucas*) (Blanchet & Rochette, 2008).

A previous study on terrestrial wildlife of Nunavik revealed a 4.2% seroprevalence in geese and 2.5% in ptarmigan (Leclair and Doidge, 2001). More recently, we reported a prevalence of 9% (CI: 3-15%) and 44% (CI: 39-51%) of DNA of *T. gondii* in hunter-harvested geese and trapped foxes from four communities of southern and western Nunavik, respectively (Bachand et al., 2018). Migratory geese are commonly consumed in Nunavik, and may harbour *T. gondii* cysts in their tissues following exposure to oocysts on their overwintering grounds.

Although general trends on country food consumption exist for Nunavik, there are information gaps regarding consumption and preparation trends specific to goose. This information is needed to reduce uncertainty within microbial risk assessments. Microbial risk assessment is a structured analytical approach used to better understand the risks to human health for different food-pathogen combinations (Fazil et al., 2005). It is a decisional tool intended to help risk managers address food safety hazards of public health importance. Because Inuit frequently consume a variety of country foods, and because many country foods are consumed raw or partially cooked in Nunavik, it is important to quantify the risk of exposure to *T. gondii* through consumption of wildlife. The objectives of this study were therefore to determine goose consumption patterns in Nunavik, and to determine the probability that Inuit are exposed to goose tissues infected with *T. gondii*. A better understanding of exposure linked to geese could help regional health authorities assess and manage risks, and communicate species-specific health prevention guidelines to reduce exposure to *T. gondii,* especially in high risk sub-populations.

4.4. MATERIALS AND METHODS

4.4.1. Goose consumption patterns

To collect goose consumer data, a goose consumption frequency (GCF) questionnaire was administered by a representative of the Local Nunavimmi Umajulivijiit Katujaqatigininga (LNUK) of Inukjuak (the local hunter association). In May 2018, the GCF questionnaire was given in the form of a structured interview with approval from the University of Saskatchewan Behavioural Research Ethics board, Makivik Coporation and the LNUK. The questionnaire was based on a one-month recall period in order to capture data on consumption patterns for tissues consumed relatively less frequently and which may not be captured using a 24-hour recall period (Blanchet and Rochette, 2004). The month of May was selected since this is the start of the goose harvesting season in Nunavik and one of the months during which exposure to geese is most likely. A total of 30 respondents were solicited via radio announcement by the LNUK representative and this formed a convenience sample in which each gender (male, female) and age group (younger, older) category were equally factored. Information was gathered on a) the number of whole geese consumed, b) the length of time a goose was frozen prior to consumption, c) the types of tissues consumed raw (e.g. heart, liver, breast meat, gizzard, other, none) and d) the number of meals accounted for by a single goose. These tissues were selected because focus-group discussions with six different Inuit communities of Nunavik, Canada, between February and September 2015 indicated that these tissues were consumed frequently (breast muscle), or consumed raw (gizzard, liver and heart). Then, based on a question format similar to a food frequency questionnaire previously used in Nunavik (Blanchet and Rochette, 2008), more detailed information was collected on the consumption frequency (e.g. never, once,

2-3 times monthly, once weekly, 2-3 times weekly, 5-6 times weekly, daily, 2-3 times daily) for four tissues (e.g. heart, liver, gizzard and breast muscle) consumed raw and cooked (Blanchet and Rochette, 20004). Finally, for each tissue, serving size was estimated. Because heart, liver and gizzard are relatively small tissues, respondents were asked to report whether they typically consume the entire organ, half the organ or less than half the organ. For breast meat, respondents were asked to check one of five boxes located below figures that provided a visual estimate of the amount of food consumed during a meal. Finally, general sociodemographic information was collected on the respondent's sex, year of birth and number of people in their household.

4.4.2. Model description / building

Based on guidelines from the Codex Alimentarius Commission (1999) and two *Toxoplasma gondii* microbial risk assessments (Opsteegh et al., 2011; Guo et al, 2017), a conceptual model was adapted to guide the development of a context-specific quantitative exposure assessment model for Nunavik which factored 4 different stages (Figure 4.1): 1. Bradyzoite concentration (unprocessed tissue). 2. Viable bradyzoite concentration (after cooking), 3. Tissue consumption, and 4. *T. gondii* prevalence (goose and tissue levels). The final outcome consisted of the probability that Inuit were exposed to 5 different *T. gondii* bradyzoite doses (0; 1-10; 10-100; 100-1000, ≥1000 bradyzoites) resulting from the consumption of one or all four goose tissues based on the number of tissue servings (exposure events) consumed raw and/or cooked during a one-month period. Analyses were done using a Monte Carlo probabilistic simulation with Latin Hypercube sampling over 500,000 iterations as per Guo et al (2017). An iteration represented the

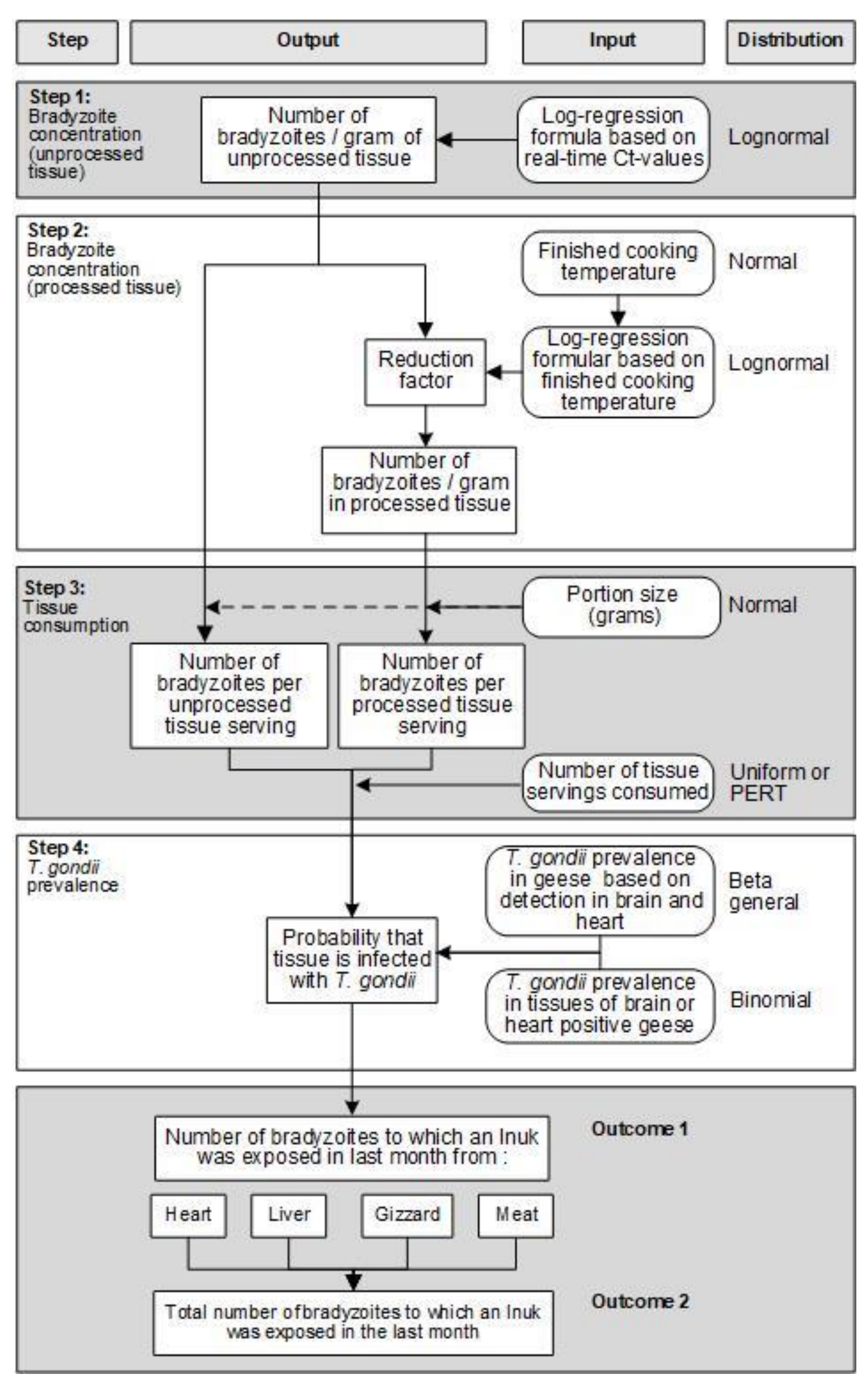

FIGURE 4.1: FLOW CHART OF THE EXPOSURE ASSESSMENT MODEL DEVELOPED TO ESTIMATE THE MONTHLY EXPOSURE DOSE OF BRADYZOITES FOR PEOPLE WHO CONSUME GEESE

path for each of the four tissues for a single goose. Model simulations were run in the Microsoft Excel ™ add-in software @Risk version 7.5.1 (Palisade Inc., Ithaca, New York, USA).

4.4.3. Data source and calculations

Step 1: Bradyzoite concentration in unprocessed tissues

For each type of tissue, bradyzoite concentration was described, rather than tissue cyst concentration, since tissue cysts contain a few to thousands of bradyzoites. Moreover, the number of tissue cysts is usually low in any given tissue, which increases the uncertainty associated with model inputs (Guo et al., 2017). Lastly, most experimental studies measure infectious doses of *T. gondii* in terms of oocysts, tachyzoites, or bradyzoites, rather than tissue cysts (Dubey, 2006).

We used a mean tissue concentration (bradyzoites per gram of tissue) in hunter-harvested geese in Nunavik of 744 bradyzoite-equivalents for heart and 104 for breast muscle, which was also extrapolated to liver and gizzard (Bachand et al., 2018). For heart, a lognormal distribution was truncated at a minimum value of 6 bradyzoites per gram of tissue and a maximum value of 4010 bradyzoites based on the 95% confidence interval (Bachand et al., 2018). For muscle, liver and gizzard, the lognormal distribution was truncated at a minimum value of 17 and a maximum value of 313 (Table 4.1). The final number of bradyzoites per gram of unprocessed tissue serving was determined as follows: bradyzoite concentration (bradyzoites per gram of tissue) multiplied by the estimated weight of a typical tissue serving (in grams) obtained from the goose consumption frequency survey.

Step 2: Viable bradyzoite concentration after cooking

Since *T. gondii* cannot grow within tissues once the host is killed, the number of viable bradyzoites can only be reduced, inactivated or remain unchanged during processing, storage or cooking. For tissues consumed raw, we assumed that the number of tissue bradyzoites was not affected by goose handling procedures (e.g. plucking, dressing) or by freezing for less than 24 hours prior to consumption (the most common practice), based on reports that freezing for at least 72 hours at -12 ºC is required for *T. gondii* cyst inactivation (Djurkovik-Djakovik and Milenkovic, 2000). Therefore, cooking was the only factor considered to reduce or inactivate tissue cysts in this model. The effect of cooking on bradyzoite tissue concentration was modeled based on time-temperature profiles described in experimentally exposed mice (Dubey, 1990) and used in a previous *T. gondii* risk assessment (Guo et al., 2017). This experimental study used the survival proportion of five mice inoculated with a mixture of brain and meat from experimentally infected rodents and pigs, respectively, for determining inactivation of tissue cysts. These temperature values were fitted with a normal distribution as per Guo et al. (2017) and used to fit the log-linear regression equation needed to calculate a reduction factor (rf) for each of the 500,000 iterations done during the simulation based on the finished cooking temperature (Guo et al., 2017). At a temperature less than 49.9 ºC, the reduction factor was set at 0 (no inactivation), whereas at a temperature above 61ºC the reduction factor was set at 1 (complete inactivation). For temperatures above or equal to 49.9 ºC and below or equal to 61 ºC, the reduction factor ranged between 0 and 1 based on the log regression equation $\log(rf)=8.583-0.172*(T^{o}C_{final})$ (Guo et al., 2017). Cooking temperature data were fitted to a normal distribution (mean: 72.7 ºC, standard deviation: 12.8) that was truncated at the minimum reported cooking temperature of 24.4 ºC and maximum cooking temperature of 104ºC based on the Ecosure home cooking

temperature data reported for poultry (Ecolab, 2008). For each iteration, the outcome of this stage represented the product of the tissue concentration for an unprocessed tissue (bradyzoites per gram) multiplied by the reduction factor, which resulted in the number of viable bradyzoites per gram of processed (cooked) tissue.

Then, the number of bradyzoites per unprocessed versus processed tissue serving was calculated by multiplying the respective tissue concentrations by the estimated tissue serving size in grams based on the GCF survey. Respondents reported consuming at least half of the heart, though more often whole, during a single exposure event. The mean heart weight was fitted to a normal distribution (mean: 18.4 grams, standard deviation: 4.5, truncated at 17 and 19.8) based on the heart weight measured from 40 hunter-harvested geese (Bachand et al., 2018). Liver was reportedly consumed whole and the mean weight, fitted to a normal distribution (mean: 46.2 grams, standard deviation: 17.2, truncated at 40.5 and 51.9), was based on measurement from the liver of 35 hunter-harvested geese (Bachand et al., 2018). Gizzard was also reportedly consumed either half or whole. The average weight of an entire goose gizzard was fitted to a normal distribution (mean: 72.2 grams, standard deviation: 22, truncated at 66.4 and 78) based on the weight of gizzards from 55 hunter-harvested geese (Bachand et al., 2019). Finally, respondents reported consuming an average of 228 grams of breast muscle in a single serving, which was also fitted to a normal distribution (standard deviation: 51, truncated at 208.6 and 245.4).

Step 3: Tissue consumption frequency

For cooked heart, raw liver and cooked liver, consumption frequencies were fitted to uniform distributions based on the minimum and maximum number of times each type of tissue was consumed among all respondents during the 30-day recall period. As for raw gizzard, cooked

gizzard and cooked breast meat, monthly consumption frequencies were fitted to PERT distributions based on minimum, most likely, and maximum values. Portion sizes reported for each tissue based on the GCF questionnaire were fitted to a normal distribution.

Step 4: *T. gondii* prevalence in geese and goose tissues

We previously estimated that 9% (95% CI: 3-15%) of hunter-harvested geese were infected with *T. gondii* (Bachand et al. 2018). These values were used to define a beta-general distribution and characterize the variability of *T. gondii* prevalence in naturally-infected geese of Nunavik with the distribution truncated between 0.03 and 0.15 to respect the 95% confidence interval. For tissue prevalence, DNA of *T. gondii* was present in 7 (54%) hearts, 4 (31%) breast muscle samples, 1 liver (14%) and 1 gizzard (9%) (Bachand et al.,2018). The probability that *T. gondii* was present in an infected tissue portion was then fitted into a binomial distribution for each tissue where n (defined as the occurrence of *T. gondii* in tissue) was equal to 1 and p represented the proportion of positive tissues (Bachand et al., 2019). The probability of infection for each tissue was then calculated by multiplying the prevalence of *T. gondii* in geese by the probability of infection for the tissue (Table 4.1).

TABLE 4.1: DESCRIPTION OF PARAMETERS AND DISTRIBUTIONS USED IN THE EXPOSURE ASSESSMENT MODEL

Variable	Description	Distribution / Value	Source
Step 1: Bradyzoite concentration (unprocessed tissues)			
Heart	Number of bradyzoites per gram of tissue	Lognormal (μ, ρ) μ= 744, ρ= 1348 Truncated to (16 , 4010)	Bachand et al., 2018
Liver		Same as muscle	
Gizzard		Same as muscle	
Muscle (meat)		Lognormal (μ, ρ) μ= 104 , ρ= 140 Truncated to (17 , 313)	
Number of bradyzoites per unprocessed tissue serving	Bradyzoite load in a typical serving (unprocessed)	Number of bradyzoites per gram of unprocessed tissue X tissue serving size (grams)	**Calculation**
Step 2: Viable bradyzoite concentration (processed tissue)			
Finished cooking temperature	Consumer-reported internal temperature (ºC) of poultry meat upon removal from heat	Normal (μ, ρ) μ=72.7, ρ= 12.8 Truncated to (24.4, 104)ºC	Ecolab, 2008
Reduction factor (RF)	If finished temperature >61ºC, meat considered free of *T. gondii*	-Reduction factor: 0	Guo et al., 2017
	-If finished temperature is ≤ 49.9ºC, no reduction has occurred (reduction factor: 1)	-Reduction factor: 1	
	-If finished temperature is ≥ 49.9 ºC and ≤ 61 ºC, a log-regression equation was specified to determine the reduction factor	-Log_{10}(rf)=8.583 – 0.172 * ($TºC_{final}$)	

Number of bradyzoites in processed tissue	Bradyzoite load in a typical serving (processed)	Number of bradyzoites per gram of processed tissue X tissue serving size (grams)	**Calculation**
Step 3: Tissue consumption			
Tissue Serving size (grams):			
Heart	Self-report on the quantity of tissue consumed in a typical serving size (grams)	Normal (μ, SD) μ=18.4 , SD=4.5 Truncated to (4.5, 19.8)	Bachand et al., 2018
Liver		Normal (μ, SD) μ=46.2, SD=17.2 Truncated to (40.5, 51.9)	Current study
Gizzard		Normal (μ, SD) μ=72.2, SD=22 Truncated to (66.4, 78)	
Muscle		Normal (μ, SD) μ=228, SD=51 Truncated to (208.6, 245.4)	
Tissue consumption frequency			
Heart: Cooked	Monthly number of tissue servings consumed monthly according to preparation method (none versus cooked)	Uniform (Min,, Max) Min: 0,, Max: 4.5	Current study
Liver : Raw		Uniform (Min,, Max) Min: 0,, Max:1	
Liver: Cooked		Uniform (Min,, Max) Min: 0,, Max: 1	
Gizzard: Raw		PERT (Min, MLik, Max) Min: 0, MLikely: 1, Max: 11.25	
Gizzard: Cooked		PERT (Min, MLik, Max) Min: 0, MLikely: 1, Max: 11.25	
Muscle: cooked		PERT (Min, MLik, Max) Min: 4.5, MLikely:11.25, Max: 24.5	
Step 4: *T. gondii* prevalence			

T. gondii prevalence in geese	Proportion of harvested geese positive on brain or heart (MC-PCR)	Beta general (s+1, n-s+1) s=13 ; n=156 Truncated to (0.03, 0.15)	Bachand et al., 2018
T. gondii prevalence in specific tissues:			
Heart	Proportion of tissues that were positive among geese previously classified as positive based on real-time PCR testing in brain or heart	Binomial (n, p) n=1, p=0.54	Bachand et al., 2018
Liver		Binomial (n, p) n=1 ; p=0.14	
Gizzard		Binomial (n, p) n=; p=0.09	
Muscle (meat)		Binomial (n, p) n=1 ;p=0.31	
Proportion of infected goose tissues	Probability that a tissue is infected with *T. gondii*	Prevalence in geese X tissue prevalence	**Calculation**
Outcome			
Outcome 1a	Probability of exposure to *T. gondii* bradyzoites through the consumption of unprocessed tissues	Number of bradyzoites in un-processed tissue serving X Monthly number of unprocessed tissue servings consumed X Probability that tissue is infected with *T. gondii*	**Calculation**
Outcome 1b	Probability of exposure to *T. gondii* bradyzoites through the consumption of processed tissues	Number of bradyzoites in processed tissue serving X Monthly number of processed tissue servings consumed X Probability that tissue is infected with *T. gondii*	**Calculation**
Outcome 2	Total probability of exposure to *T. gondii* bradyzoites in the last month	Outcome 1a + Outcome 1b	**Calculation**

4.4.4. Sensitivity analysis

A global sensitivity analysis determined which parameters had the highest effect on the overall estimated mean number of bradyzoites to which Inuit were exposed during the 30-day recall period, given the consumption of all 4 tissues. The impact of input parameters on the outcome of the baseline model was evaluated in @Risk Palisade.

4.5. RESULTS

4.5.1. Goose consumption frequency survey

A total of 15 Inuit men and 15 Inuit women participated in the survey. The age distribution was similar for each gender category, namely 7 individuals between the ages of 18-39 years and 8 individuals aged more than 40 years (Maximum age: 56 years old). All had consumed Canada geese in the last month, 83% consumed lesser snow goose (*Chen caerulescens*) at least once, and 47% indicated that they sometimes consumed goose tissues raw. The average number of Canada geese consumed per person in the last month was 9.3 (SD: 5.3; Min.: 1; Max: 30) compared to 3.2 for snow geese (SD: 3.08; Min.: 0; Max: 10). Sixteen people (53%) kept geese cool for less than 12 hours prior to consumption, while the remainder (47%) kept geese cool for less than 24 hours. No single goose was frozen for more than 72 hours. No one reported consumption of raw heart or raw breast muscle tissue, while (raw) liver was only rarely consumed during the 30-day recall period. People consumed raw gizzard a maximum of 11 times (2-3 times per week) during the 30-day recall period. Cooked heart was consumed a maximum of 4.5 times per person during the 30-day recall period, compared to once for cooked liver, 11 times for cooked gizzard (2-3

times per week) and 25 times for cooked breast muscle (5-6 times per week). All respondents reported that a single goose contributes only one meal for a household.

4.5.2. *T. gondii* exposure assessment model: probability of exposure to bradyzoites

Overall, there was a 32% probability that individual Inuit were exposed to at least 1 bradyzoite in the last month based on the average consumption of 21 goose tissue servings (Table 4.2). Probabilities of exposure varied according to different bradyzoite doses; the highest probability of exposure (14.3%) was for a dose between 101 and 1000 bradyzoites.

Based on the mean number of tissues servings consumed among all 30 respondents over the 30-day recall period, there was a 14% probability of exposure to at least 1 viable bradyzoite given the consumption of 2.25 servings of cooked heart compared to 9% for the consumption of 0.5 monthly servings of raw gizzard. The lowest probability of exposure (less than 2%) was associated with the consumption of cooked gizzard (based on the average consumption of 2.5 montly servings per person), whereas there was a 5.8 % probability of exposure with the consumption of breast muscle (average of 12 monthly servings per person).

4.5.3. Sensitivity analyses

The most influential parameter, which contributed to the highest variability in the monthly mean number of viable bradyzoites, was the finished cooking temperature. The next three parameters, which were much less influential, included the *T. gondii* prevalence in breast muscle, the *T. gondii* prevalence in gizzard tissue and the bradyzoite concentration in unprocessed breast muscle (Figure 4.2).

TABLE 4.2: DESCRIPTION OF THE ESTIMATED MEAN NUMBER OF VIABLE BRADYZOITES TO WHICH INUIT WERE EXPOSED DURING MAY 2018, AS WELL AS PROBABILITIES OF EXPOSURE TO DIFFERENT DOSES ACCORDING TO THE TYPE OF GOOSE TISSUE CONSUMED

Tissue-preparation combination	Mean number of monthly servings	Per person monthly exposure probabilities (%) to different doses of T. *gondii* bradyzoites					Mean monthly number of viable bradyzoites to which Inuit were exposed		
		0	1-10	11-100	101-1000	≥1000	Mean (+/- 90%CI)	Min	Max
Heart cooked	***2.3***	86%	0.8%	5.7%	7.4%	0.1%	73 (5)	0	31,500
Liver raw	***0.5***	91%	0%	0.5%	4.5%	4%	25 (1)	0	1,772
Liver cooked	***0.5***	90.2%	1.2%	3.4%	3.5%	1.7%	2 (0.2)	0	929
Gizzard raw	***2.5***	97.7%	1%	0.9%	0.4%	0%	132 (5)	0	18,695
Gizzard cooked	***2.5***	98.4%	0.2%	0.6%	0.6%	0.2%	8 (1)	0	9,237
Breast meat cooked	***12.3***	94.2%	0%	0.2%	2.1%	3.5%	402 (26)	0	127,350
All 4 tissues	***20.5***	68.6%	1%	7%	14.3%	9%	642 (28)	0	127,350

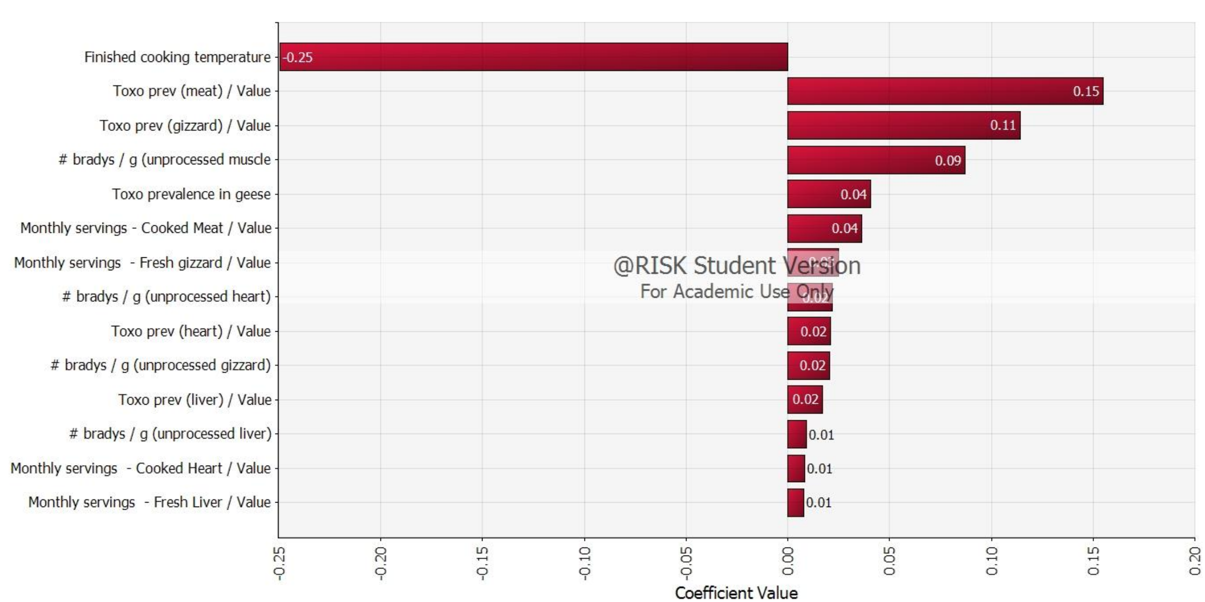

FIGURE 4.2: TORNADO GRAPH DISPLAYING THE SENSITIVITY ANALYSIS OF THE MOST INFLUENTIAL PARAMETERS THAT AFFECTED THE VARIABILITY IN THE NUMBER OF VIABLE BRADYZOITES FROM GEESE TO WHICH 30 INUIT RESPONDENTS WERE EXPOSED DURING THE MONTH OF MAY 2018

4.6. DISCUSSION

This is the first quantitative exposure assessment for a food-borne parasite in country food consumed by Inuit of Nunavik, Canada. Based on the people surveyed in this study, the exposure assessment predicted that there is a 32% probability that Inuit were exposed to at least one viable bradyzoite during the 30-day recall period. On average, people were exposed to 642 viable bradyzoites of *T. gondii,* primarily through consumption of breast muscle. Our consumer survey revealed that all respondents had consumed geese during the 30-day recall period, and half of respondents consumed goose gizzard raw at least once during that same period. Based on an average of 21 goose tissue servings consumed per person during the 30-day recall period, Inuit had a 1% probability of being exposed to a dose between 1-10 bradyzoites, a 7% probability for a dose of 10-100 bradyzoites, a 14% probability for a dose of 100-1000 bradyzoites and 9% for a dose of more than 1000 bradyzoites. This suggests that when exposure occurs, exposure to doses above 100 bradyzoites is more likely. The significance of this is challenging to interpret since nothing is known about infectious dose of viable *T. gondii* bradyzoites in people.

Cooking temperatures above 67 ºC usually inactivate viable *T. gondii* bradyzoites within meat and the time required for inactivation generally increases as the cooking temperature decreases (Dubey et al., 1990). We factored in the possibility that temperatures were not consistently high enough to kill all *T. gondii* bradyzoites in an infected serving of breast muscle, since many Canadian consumers are not aware of the internal temperature of their meat after cooking (Murray et al., 2017). If inappropriately cooked, large tissues can contain viable bradyzoites at their core (Tenter et al., 2000). Though geese are often boiled whole, specific cooking duration and temperature data are currently unknown in Nunavik. Therefore, this exposure assessment factored in the possibility that meat and other tissues can sometimes be incompletely boiled, or

could be prepared in ways that do not fully inactivate bradyzoites (e.g. drying, roasting, grilling, smoking, microwaving). However, if goose tissues are consistently cooked at more than 67 ºC, regardless of method, this exposure assessment likely overestimates the monthly probability of exposure to *T. gondii*. Future research should aim to gather specific information on the duration and internal temperature of geese after cooking within the home.

While processed breast meat had the greatest contribution toward the monthly bradyzoite exposure dose, the second highest contributor was unprocessed (raw) gizzard. Although gizzard was not consumed as frequently as breast muscle, the fact that it is uncooked prevents the inactivation of bradyzoites. Therefore, a higher tissue bradyzoite load at the time of consumption, rather than a higher consumption frequency, could explain why raw gizzard is an important contributor to the monthly exposure bradyzoite dose. Processed heart was the third largest contributor, followed by processed gizzard, unprocessed liver, and processed liver. Processed liver contributed the least to the monthly bradyzoite exposure dose, which likely reflects that it is consumed only rarely, and in smaller quantities, relative to other tissues. For other months or in other communities, if consumption of goose liver (especially uncooked) is higher than that reported in this study, this exposure assessment would underestimate the probability of exposure. Results of this exposure assessment are specific to the unique consumer data reported for the month of May in Inukjuak, Nunavik, and are based on a small sample size. Future research is therefore needed to obtain consumer data from a larger sample size within and among communities for better estimating exposure probabilities to *T. gondii* from geese.

The probability of exposure to different bradyzoite doses varied according to each type of tissue-preparation combination (e.g. gizzard-cooked) in this model. There was a 3.5% monthly probability that people were exposed to a dose exceeding 1000 viable bradyzoites given the

consumption of an average of 12 servings of breast meat, which is almost comparable to 4% given the consumption of only 0.5 servings of raw liver. This could be because breast muscle is consumed more frequently and in larger quantities. Even for some tissues consumed raw, probabilities of absent exposure were low (91 and 98% probability of absent exposure for liver and gizzard, respectively). This suggests that, given most tissue consumption patterns reported by respondents for that month, exposure to *T. gondii* through goose was relatively low for some tissues. Waterfowl is not consumed with the same frequency throughout the year in Nunavik; in the spring, 86% of the regional population consumed geese compared to 51%-59% during all three other seasons (Nunavik Health Survey, 2004). Our exposure assessment for May therefore likely overestimates exposure for other seasons and other communities where not everyone consumes geese, or where geese are not as readily accessible.

Exposure assessment outcomes are based on several assumptions and uncertainties. Although species-appropriate data on tissue bradyzoite load greatly improved the reliability of this exposure assessment, the bradyzoite load in breast muscle was extrapolated to liver and gizzard since only one sample was available for each of these tissues (Bachand et al. 2018). The parasite load in breast muscle, rather than heart, was selected since tissue load in the hearts of experimentally infected turkeys, a domestic species for which data was available, was higher than in breast muscle based on the magnetic capture and real-time technique (Koethe et al., 2015). On the one hand, we may have overestimated the viable bradyzoite load in this assessment, as quantification of DNA of *T. gondii* does not discriminate between viable and non-viable bradyzoites (Guo et al., 2016). On the other hand, we may have underestimated prevalence of infection for individual goose tissues since it is possible that some geese had tissue loads lower than the limit of detection (445 bradyzoite-equivalents per 100 grams of tissue in

Bachand et al., 2018). This exposure assessment also assumed that all infected geese had similar tissue parasite loads and that they therefore present similar risks for exposure to *T. gondii* bradyzoites. However, the number and size of bradyzoite-containing cysts are highly variable and can range from a few bradyzoites to several thousands within a cyst (Tenter et al., 2000). Given that exposure occurs over their lifetime and that there is continued division of bradyzoites within tissue cysts, older geese likely harbor higher parasite loads within their tissues, in terms of both the number and size of cysts, compared to younger harvested geese. Therefore, accounting for the age of hunter-harvested geese could improve the reliability of the exposure assessment. However, variability in tissue bradyzoite loads was accounted for in this assessment. Bradyzoites are not distributed evenly within tissues (Dubey, 2010); therefore, it is possible that a portion of infected tissue does not contain cysts. This was not formally factored into our exposure assessment; however, heart and gizzard are usually consumed whole, and portions of breast muscle are usually consumed in high quantities (Table 4.1). It is still possible that this exposure assessment overestimated or underestimated the monthly exposure dose for breast meat given the uneven distribution of cysts within tissues.

As a method of inactivating bradyzoites, only cooking was considered in this exposure assessment. Freezing was not considered since all respondents reported freezing geese for less than 24 hours during the 30-day recall period, which would not inactivate bradyzoites (Dubey, 2010). For people who freeze geese at -12ºC for more than 72 hours, this exposure assessment would overestimate their probability of exposure to *T. gondii*. Cooking temperature was the most influential parameter on human exposure in this assessment. Internal temperatures were based on distributions reported by American consumers for chicken, which are not necessarily applicable to Inuit consumers with respect to the consumption of geese. Beside cooking and freezing, other

processing methods may be used (e.g. drying, smoking and salting), which was not factored into this exposure assessment. Since cooking is not always culturally acceptable to Inuit, public health messaging should perhaps focus on longer freezing periods, and future work should explore the efficacy of traditional methods of preparation for inactivating *T. gondii*.

Goose consumer data were averaged at the population level, based on a small sample size, and extrapolated to individuals under the assumption that everyone in the community consumes each of the four tissues throughout the month. Moreover, some Inuit may consume other tissues not included in this assessment. Further research is therefore needed to determine with more certainty whether goose consumption frequencies for different tissues and the number of different goose tissues consumed differ among Inuit. This being said, data from the goose consumption frequency survey did show that 100% of respondents consumed breast meat, which had the highest contribution to the monthly exposure dose of viable bradyzoites. A regional survey in Nunavik also reported that 61% of the population consumed geese more than 11 times yearly, compared to 32% who consumed goose 1-10 times yearly and 7% who reported never consuming goose (Nunavik Health Survey, 2004). Although the exposure assessment may overestimate the monthly dose for some individuals under the assumption that all four tissues are consumed, almost everyone consumes breast muscle. This exposure assessment was specific to the consumption of geese tissues at meal time and did not factor consumption of goose tissues at the time of carcass dressing (out in the land or in the home) or during processing at home before mealtime. This means that the probability of exposure linked to the consumption of geese tissues is likely underestimated, at least for individuals who are actively involved in harvesting and / or the preparation of geese prior to mealtime. Future research should aim to gather data specific to

preparatory steps such as harvesting and carcass dressing for a more accurate estimate of exposure.

Unlike previous quantitative microbial risk assessments for *T. gondii* where prevalence is extrapolated from serological data or meta-analyses, this exposure assessment factored prevalence and parasite tissue load data generated from hunter-harvested geese destined for consumption by Inuit in Nunavik. These data were generated to improve the certainty and relevance of the exposure assessment. Moreover, despite a small sample size, tissue consumer data at the community level were collected to fill gaps specific to people directly concerned by the outcome of this exposure assessment. More specifically, this study highlighted that different tissues posed different risks in terms of exposure (e.g. heart had the highest bradyzoite concentration, breast muscle is eaten more frequently and in larger quantities, and gizzard is more often consumed raw compared to most other tissues). Because goose consumer data are likely variable between communities, extrapolating outcomes of this exposure assessment to other communities should be done cautiously (Lemire et al., 2015). For example, the consumption of goose was reported to be higher in communities of the Hudson coast (where Inukjuak is located) compared to communities of Ungava Bay (Nunavik Health Survey, 2004).

Overall, exposure to *T. gondii* bradyzoites through the consumption of goose tissues, especially undercooked breast meat, is plausible for Inuit of Inukjuak in Nunavik. As well, our findings support the importance attached to harvesting and consumption of geese since all respondents consumed goose regularly. Recommendations to cook tissues are supported by this exposure assessment since the sensitivity analysis showed that cooking had the largest influence on the exposure assessment outcome. However, data are needed in Nunavik on actual finished cooking temperatures for different goose tissues. Until then, mitigation measures could promote freezing

of goose tissues for at least 72 hrs, which may be more culturally acceptable than cooking. In order to improve the reliability of the exposure assessment outcome, more precise information is needed on tissue load data (liver, gizzard), consumer data (the different types of tissues consumed, community-specific data, etc.), tissue processing data (whether processing methods other than cooking are used, whether processing methods vary by seasons, wildlife species, tissues and even communities, etc.). This risk-based exposure assessment could be a useful approach toward determining exposure probabilities for other country foods of animal origin using species-specific parameters. Next steps would also be to consider the consequences of such exposure in terms of clinical toxoplasmosis, to balance these risks against the benefits (nutritional, cultural, and economic) of goose consumption, and to manage and communicate these risks in a culturally-appropriate manner.

4.7 ACKOWLEDGEMENTS

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CHAPTER 5: FOOD SAFETY BEHAVIOR, AWARENESS AND PERCEPTION OF PARASITES IN INUIT EXPOSED TO WILDLIFE

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NB coordinated field data collection. NB, EJ, AR, BR and CA contributed to study design. AR contributed to the MCA analysis and figure. NB drafted the manuscript. All authors reviewed and approved the final manuscript.

5.1. CHAPTER TRANSITION

In Chapters 2 and 3, I determined the PCR prevalence and intensity of *T. gondii* in wildlife of Nunavik. In Chapter 4, I predicted the probability of Inuit exposure to *T. gondii* through consumption of geese in Nunavik. However, a quantitative appraisal of risk is not the sole factor considered by the general public when evaluating the importance of risk. Laypeople differ from experts in deciding which hazards are "risky" and in making decisions about mitigating risks. Based on the expectancy-value theory, the Protection Motivation Theory (PMT) is used to evaluate how individual risk perception and efficacy beliefs influence people's decisions to mitigate risks. Therefore, the first objective of this chapter was to describe Inuit perceptions of wildlife parasites in Nunavik based on data collected through survey work. Then, with a food safety perspective in mind, the second objective was to determine whether current levels of adoption of food handling practices are associated with risk perceptions and efficacy beliefs. Guided by the PMT model, I also determined whether Inuit believe that health-protective measures against exposure to wildlife parasites are effective (response efficacy) and whether they feel like they are capable of applying these measures effectively (self-efficacy). Information from this chapter can be used by public health in Nunavik to devise culturally-appropriate and effective risk communication messages linked to parasites from wildlife.

5.2. ABSTRACT

A high seroprevalence of *Toxoplasma gondii* and high levels of contact with wildlife occur among Inuit of Nunavik, Canada, where information on knowledge, perceptions of wildlife parasites, as well as adoption of wildlife handling behaviors remains scarce. This study described Inuit knowledge, awareness and risk perceptions of wildlife parasites, as well as patterns and influences on wildlife handling behaviors. In November 2015, 140 Inuit were surveyed in Inukjuak, Nunavik, Canada. Approximately 61% (95% CI: 53-69%) had heard of parasites and 47% were highly knowledgeable about parasite transmission (95% CI: 39-56%). About 30% (95% CI: 23-38%) had heard of *Trichinella* spp. compared to 16% (95% CI: 10-23%) for *Toxoplasma gondii*. A high level of perceived vulnerability occurred among 51% of respondents (95% CI: 43-60%) compared to 40% for perceived severity (95% CI: 30-48%). Half of respondents felt that wildlife handling practices were protective. Perceived severity and perceived response efficacy positively influenced the adoption of safe wildlife handling behaviors. Public health messages should strive to raise awareness of specific zoonotic parasites and clearly demonstrate the effectiveness of safe wildlife handling behaviors.

5.3. INTRODUCTION

Inuit have harvested wildlife for centuries (Bonesteel and Anderson, 2008). More than 12,000 Inuit live in Nunavik, Quebec, Canada where food insecurity affects 1 in 4 households (Blanchet and Rochette, 2008). Referred to as country food, wildlife contributes on average 40% to people's daily protein intake and is consumed by at least 50% of people throughout the year (Blanchet and Rochette, 2008). Although country food is beneficial, it can harbor human health hazards (e.g. chemical, physical and biological) (Havelaar et al., 2015). Wildlife can act as reservoirs for several zoonotic pathogens and contact with wildlife is a major risk factor for the transmission of these pathogens to humans (Jones et al., 2008). In Nunavik, human contact with wildlife occurs through hunting, the handling of carcasses, meat preparation and consumption.

Toxoplasma gondii and *Trichinella* spp. are zoonotic foodborne pathogens that can infect people who consume undercooked meat or organs from an infected animal (Dubey, 2010; Gajadhar and Forbes, 2010). Infection with *T. gondii* can cause abortion in susceptible pregnant women, whereas infection with *Trichinella* spp. can produce diarrhea, vomiting, fatigue and fever within 24 to 48 hours of consuming infected meat, and in more serious instances, fatal cardiac impairment (Dubey, 2010; Houzé et al., 2007). Investigation of an outbreak of *T. gondii* in pregnant women of Nunavik between 1987 and 1988 demonstrated strong associations between *T. gondii* seroconversion and consuming caribou and seal meat (McDonald et el., 1990). A separate study in Salluit and Kuujjuaq demonstrated *T. gondii* seroprevalences of 50% and 60%, respectively, associated with consumption of wildlife (Curtis et al., 1988). More recently, a regional health survey in 2004 demonstrated associations between handling/consuming wildlife and *T. gondii* exposure based on antibody detection in Inuit of Nunavik, where the average regional *T. gondii* seroprevalence was 60% (Blanchet and Rochette, 2008). Consequently, public

health authorities of Nunavik have recommended that pregnant women avoid handling or consuming raw country foods of animal origin throughout their pregnancy (Proulx, 1999). Several outbreaks and cases of trichinellosis have also occurred in Nunavik since the early 1980s linked to the consumption of walrus (*Odobenus rosemarus*) (Maclean et al., 1989; Larrat et al., 2012). Consequently, a walrus screening program was instituted for detection of *Trichinella nativa* based on walrus tongues submitted by hunters throughout Nunavik (Proulx et al., 2002). Serological and direct detection of *Trichinella nativa* and *Toxoplasma gondii* in wildlife species typically consumed by Inuit has also been reported in Nunavik (Leclair and Doidge, 2001; Forbes and Gajadhar, 2010; Bachand et al., 2018; submitted).

In order for food safety recommendations to be effective, public health authorities depend on the public's willingness to adopt health-protective behaviors (de Zwart et al., 2009), which in turn depends on whether or not a risk (threat) is perceived to be important (Leppin, 2009). Perceived threat, as defined by the Protection Motivation theory (PMT), is the product of two risk perception variables: perceived vulnerability (e.g. a person's judgment of the likelihood being exposed to a health hazard) and perceived severity (e.g. a person's judgment of how severe the impact of that exposure is on their health) (Rogers, 1975). This model contends that the level of perceived threat and perceived effectiveness of health-protective behaviors (e.g. response efficacy) influence people's willingness to adopt such behaviors (Prentice-Dunn and Rogers, 1986). The model also factors in people's beliefs of their own ability to effectively implement health-protective behaviors (e.g. self-efficacy). Generally, a higher level of perceived threat motivates people to adopt health-protective behaviors (Mullan et al., 2016). However, people may not always implement these behaviors if they don't believe or trust that they are effective or can be effectively implemented (Maddux and Rogers, 1983). More information is needed in

Nunavik on current wildlife food handling behaviors and on risk perceptions (e.g. perceived vulnerability, perceived severity) regarding wildlife parasites. Therefore, the objectives of this study were to determine levels of knowledge, awareness, risk perceptions and efficacy beliefs (e.g. self-efficacy, response efficacy) related to foodborne parasites from wildlife. The study also aimed to determine what factors influence the adoption of health-protective behaviors with respect to wildlife in Nunavik.

5.4. MATERIALS AND METHODS

5.4.1. Study design

A cross-sectional survey in the community of Inukjuak, Nunavik, during November 2015 included permanent Inuit residents older than 18 years old. To estimate the sample size, the number of required respondents was assessed based on the community's population size as per Statistics Canada (2013), a 5% acceptable error limit and a 90% confidence level (Dohoo, 2010). With approximately 1585 residents (the population size of Inukjuak in 2011), the targeted convenience sample size was 230 respondents (Statistics Canada, 2013). In order to maximise response rate, daily announcements were made in Inuktitut through the local radio station during a three-day period. Respondents came to the municipal conference centre where a local coordinator administered a 20-minute anonymized questionnaire. To test the survey instrument for comprehensibility, the questionnaire was piloted with 5 local Inuit volunteers. The first page of the questionnaire also contained written information on the nature of the study, the researcher's contact information, the University affiliation, details of the research permit, as well as a statement detailing the participant's right to refuse participation in the study. This study

protocol was approved by the University of Saskatchewan Behavioural Research Ethics board (BEH 15-235).

5.4.2. Data Collection

The questionnaire was adapted from a previous infectious disease risk perception questionnaire (Brug et al., 2004) and made available in both English and Inuktitut. Translation into Inuktitut was done by a resident of Nunavik. The first part of the questionnaire gathered information on: (a) sex, (b) age, (c) household size and (d) household composition (specifically the presence of children, pregnant women, or elders).

The questionnaire continued with a series of closed-ended questions on a) awareness of wildlife parasites, b) knowledge of wildlife parasite transmission, c) worry regarding the impact of wildlife parasites on the health of wildlife and people, d) adoption of food handling behaviors with respect to wildlife, e) perceived severity of infection with wildlife parasites from food, f) perceived vulnerability to wildlife parasites from food, g) perceived response efficacy, and h) self-efficacy. The full questionnaire is available as supplementary material.

5.4.3. Data Analysis

All data were numerically coded, entered into an Excel spreadsheet and analysed in R software version 3.4.4. Basic descriptive statistics for sociodemographic variables included the percentage frequency (± 95% confidence interval) of respondents. Perceived threat was calculated by multiplying the perceived vulnerability score (scale 1-5) with the perceived severity score (scale: 1-5) for a total possible score ranging between 0 and 25. Then, to normalise the skewed distribution of these scores, a square root transformation was done as per de Zwart et al. (2009) to obtain a final score outcome ranging between low (1) to high (5). A wildlife parasite

transmission knowledge score was devised based on the accumulation of correct answers to nine "True/False" items (knowledge score with a scale of 1 to 9). Finally, adoption of health-protective wildlife food handling behaviors was scored by the interviewer based on the sum of six correctly applied (yes, no) behaviors (Health Canada, 2012). Binary categories were then defined as low if scores were between 0 and 3 out of 6 (50% or less), and as high if scores were between 4 and 6 (more than 50%).

Multiple correspondence analysis (MCA) was done using the command MCA in the package FactoMineRfor R to explore relationships between the following variables: Knowledge (Know), self-efficacy (Seff), education (Educ), perceived thread (Rper), personal vulnerability (Pvul), personal severity (Psev), response efficacy (Reff), awareness (Awa) and worry (Wor). The two-dimensional plan resulting from this analysis was generated by the command fviz_mca of the factoextra package. Each variable category was colored according to its contribution to the plan. In addition, the variable Prot (adoption of protective behaviors) and Raw (consumption of raw tissues during carcass dressing) were used as supplementary variables to assess their association with each of the two dimensions visually and quantitatively based on the v-test.

A binary logistic regression model was developed to determine whether associations exist between explanatory variables (e.g. age, gender, education, knowledge, worry, awareness, perceived vulnerability, perceived severity, self-efficacy, response efficacy) and adoption of health-protective behaviors (the dependent variable). The following independent variables were categorized as binary outcomes: gender (male=1, female=0), knowledge of parasites (high=1, low=0), awareness of parasites (yes=1, no=0), worried about health effects from parasites (yes=1, no=0). Knowledge scores less than 50% of the overall possible score of 9 (5 or less correct answers) were classified as low, whereas scores of 6 or more correct answers were

classified as high (Siddiqui et al., 2016). Similarly, scores below 50% of the total possible score for each independent variable were classified as low, whereas scores exceeding 50% were classified as high as follows: personal vulnerability (scores 0 to 3 as low, scores 4 to 5 out of 5 as high), personal severity (scores 0 to 3 as low, scores 4 to 5 out of 5 as high), self-efficacy (scores 0 to 2 as low, scores 3 to 4 out of 4 as high) and response-efficacy (scores 0 to 2 as low, scores 3 to 4 out of 4 as high). Because information was needed for different generations of Inuit, age was categorized into three categories: ages 18 to 29 as young=0; ages 30 to 45 as older=1; and ages 45 or more as elder=2. To determine whether the level of education influenced the adoption of health-protective measures, categories reflected three different levels as follows: primary or less as a low level=0; incomplete high school as an intermediate level =1; and high school or above as a high level=2. A dummy variable was created for both age and education. Finally, based on whether global scores were below or above 50% of a total possible score out of 6, scores with respect to the adoption of health-protective behaviors were converted from an ordinal to a binary variable as follows: knowledge scores of 0 to 3 as low (=0) and scores of between 4 and 6 as high (=1) (Siddiqui et al., 2016)

A backward method with a $p > .05$ (likelihood ratio test) was used as a rejection criterion for keeping explanatory variables. To assess for confounding variables, odds ratios were calculated from regression coefficients during each step of the model building process to ensure that these did not vary significantly (<30%) on the log scale up to the final model (Dohoo, 2010). For the final model, first-order interactions were individually tested between all combinations of the retained explanatory variables and the lack of fit was evaluated using the Hosmer-Lemeshow test. Odds ratios were derived from regression coefficients.

5.5. RESULTS

In total, 175 people participated in the study. Although a convenience sample was used, the study population represented 11% of the community and proportions of respondents were similar to gender and age group proportions reported for the community of Inukjuak by Statistics Canada (2013). A total of 35 questionnaires were excluded from analyses because they had missing data: 2 had missing data for the presence of a child in the household, 7 had missing data on education level, 16 had missing data on personal susceptibility or personal severity, 2 had missing data on efficacy beliefs and 8 had missing data on adoption of safe wildlife food handling behaviors. In total, 140 respondents had complete data for all variables.

5.5.1. Sociodemographic variables

Of the 140 participants, 66 (47%) were women compared to 74 (53%) who were men (Table 1). The proportion of participants was similar for each age category with 44 people aged between 18 and 29 years-old (31%), 48 people aged between 30 and 44 years-old (34%) and 48 people aged more than 45 years-old (34%). Proportions for education and household characteristics are indicated in Table 5.1.

5.5.2. Awareness and knowledge of parasites

Among all 140 participants, 61% (95% CI: 53-69%) had heard about parasites. However, only 47% of respondents stated they had a high level of knowledge of parasite transmission (95% CI: 39-56%) compared to 29% (95% CI: 22-38%) and 24% (95% CI: 17-32%) for respondents with medium and low levels of knowledge, respectively. A proportion of 30% (95% CI: 23-38%) of

TABLE 5.1: CHARACTERISTICS OF 140 SURVEY RESPONDENTS FROM INUKJUAK, CANADA, AS WELL AS THEIR LEVEL OF AWARENESS, WORRY AND KNOWLEDGE OF WILDLIFE PARASITES

Variable	n	%	%95 LCI	% 95 UCI
Total	140			
Gender				
Female	66	47%	39%	56%
Male	74	53%	44%	61%
Age				
18-29	44	31%	24%	40%
30-44	48	34%	27%	42%
More than 45	48	34%	27%	42%
Education (Edu)				
Primary	41	29%	22%	37%
Incomplete high school	53	38%	30%	46%
High school or more	46	33%	23%	43%
Living with :				
Child	87	62%	54%	70%
Pregnant woman	13	9%	5%	15%
Elder	30	21%	15%	29%
Awareness of parasites (Awa)	86	61%	53%	69%
Worry of parasite impact on: (Wor)				
Human health	69	49%	41%	58%
Wildlife health	57	41%	33%	49%
Heard of *Toxoplasma gondii*	22	16%	11%	23%

Heard of *Trichinella* spp.	42	30%	23%	38%
Knowledge of parasites (Know)				
Low	33	24%	17%	32%
Intermediate	41	29%	22%	38%
High	66	47%	39%	56%

respondents had heard of *Trichinella* compared to 16% (95% CI: 10-23%) for *Toxoplasma gondii*. Overall, 49% (95% CI: 41-58%) of respondents were worried about the negative impact of wildlife parasites on human health and 41% (95% CI: 33-49%) were concerned about the negative impact of parasites on wildlife health (Table 1).

5.5.3. Risk perception variables

With respect to perceived vulnerability, 51% (95% CI: 43-60%) of respondents felt they were very likely to be exposed to parasites from wildlife compared to 34% (95% CI: 26-42%) who felt that exposure was very unlikely and 15% who fell somewhere in between (Table 2). In terms of perceived severity, 40% (95% CI: 32-48%) felt that infection with parasites from wildlife could lead to a severe impact in their health. Perceived threat, the product of perceived vulnerability and perceived severity, was high (a score of 4 or 5 out of 5) for 26% of respondents (95% CI: 19-35%).

5.5.4. Efficacy beliefs and adoption of health-protective wildlife food handling behaviors

Half (50%) of respondents agreed (95%: 41-59%) that actions can be taken to prevent infection with wildlife parasites. Moreover, 49% (95% CI: 42-59%) of respondents felt they were good at preventing infection with wildlife parasites. Finally, only 29% (95% CI: 21-36%) of respondents scored highly on adoption of safe wildlife food handling behaviors while processing and preparing hunted wildlife.

5.5.5. Multiple Correspondence Analyses (MCA)

The first two dimensions reflected 48% and 17% of the total inertia (equivalent to variance for qualitative variables), respectively. Nine categories from five variables contributed the most to the first dimension (Figure 5.1): Psev_y, Know_L, Reff_n, Reff_y, Rper_n, Awa_n, Rper_y, Know_H and Psev_n in decreasing contribution. The variables Pvul_n, Pvul_y, Rper_y, Rper_n, Seff_y and Seff_n had the greatest contribution in decreasing order to the second dimension. The variable education (Edu) did not contribute to either dimensions. The variable Raw (consumption of raw animal tissue during carcass dressing) was visually and significantly associated with the first dimension ($p<0.05$), while the variable Prot (adoption of health-protective behaviors) was near reaching significance ($p=0.059$). None of them were associated with the second dimension.

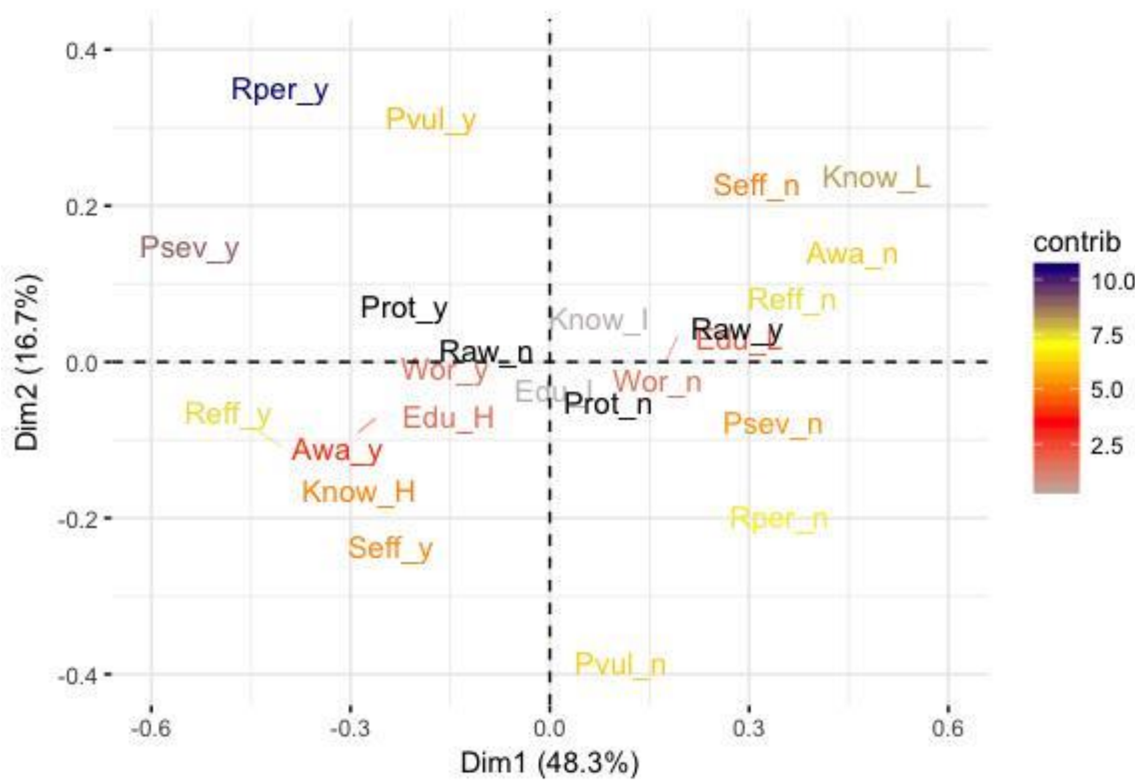

FIGURE 5.1: MULTIPLE CORRESPONDENCE ANALYSIS OF THE EXPLANATORY VARIABLES USED TO ASSESS ASSOCIATIONS WITH THE ADOPTION OF FOOD SAFETY HEALTH-PROTECTIVE MEASURES REPORTED BY 140 RESPONDENTS FROM INUKJUAK, NUNAVIK, IN NOVEMBER 2015

MCA plot on the first two dimensions. All variable categories are plotted in the two dimensional plane representing the maximum inertia (48% along the first dimension, and 17% along the second dimension). They are colored according to their contribution to the dimensions. Along the first dimension, there is a clear clustering of the positive answers to all variables related to risk perception on the left and a clustering of negative answers to the same variables on the right. Remarkably, eating raw meat and adopting protective food safety measures were distributed along the first dimension and consistently with the risk perception (i.e. adopting such practices and not eating raw meat are located on the left closest to the first dimension). The position of Pvul_y and Pvul_n was clearly closest to the second dimension and indicates that perceived vulnerability was not associated with the first dimension while it was the main contributor to the second dimension.

TABLE 5.2: PROPORTIONS AND THEIR 95% CONFIDENCE INTERVALS OF RESPONDENTS ACCORDING TO THEIR LEVEL OF PERCEIVED VULNERABILITY, PERCEIVED SEVERITY, PERCEIVED THREAT, SELF-EFFICACY AND RESPONSE-EFFICACY

Variable	n	%	%95 LCI	%95 UCI
Perceived Vulnerability (Pvul)				
Low	47	**34%**	26%	42%
Intermediate	21	**15%**	10%	22%
High	72	**51%**	43%	60%
Perceived Severity (Psev)				
Low	55	**39%**	32%	48%
Intermediate	29	**21%**	15%	28%
High	56	**40%**	32%	48%
Perceived Threat (Rper)				
Low	67	**48%**	39%	56%
Intermediate	36	**26%**	19%	35%
High	37	**26%**	19%	35%
Self-Efficacy (Seff)				
Low	71	**51%**	42%	59%
High	69	**49%**	41%	58%
Response Efficacy (Reff)				
Low	70	**50%**	41%	59%
High	70	**50%**	41%	59%

TABLE 5.3: PROPORTIONS AND THEIR 95% CONFIDENCE INTERVAL OF RESPONDENTS WHO APPLIED DIFFERENT PROTECTIVE WILDLIFE FOOD HANDLING BEHAVIORS IN INUKJUAK, NUNAVIK, CANADA

Description	n	**%**	**95% LCI**	**95%UCI**
Wash your hands with soap and water after handling an animal	130	93%	87%	96%
Wear gloves while handling or cutting an animal	43	31%	24%	39%
Wash and disinfect all equipment in contact with an animal	115	82%	75%	88%
Bleed and remove the intestines and stomach	73	52%	44%	60%
Keep the animal cool within 24 hours of consumption	88	63%	55%	70%
Eat some animal parts raw while handling the carcass	50	36%	28%	44%
Overall adoption of health-protective wildlife food handling behaviors*:				
Low score	100	71%	63%	79%
High score	40	29%	21%	36%

*This was calculated based on the collective application of all 6 behaviors. For example, if people applied 4 or more of the behaviors correctly, they had a "High" score.

TABLE 5.4: RESULTS OF MULTIVARIATE LOGISTIC REGRESSION MODELING FOR THE ADOPTION OF WILDLIFE SAFETY HANDLING PRACTICES ACCORDING TO SOCIOECONOMIC FACTORS, RISK PERCEPTION VARIABLES AND EFFICACY BELIEFS AMONG 140 RESPONDENTS SURVEYED IN INUKJUAK, NUNAVIK

	Null		**Full [1]**		**Reduced**			
Explanatory Variable	**β**	**95% CI**	**β**	**95% CI**	**β**	**OR**	**95% CI**	
Intercept	-0.52		-0.85		-0.83	0.43	0.15	1.2
Education Level								
High school vs primary			-1.31		**-1.25**	0.29	0.1	0.8
Higher education vs primary			-1.66		**-1.44**	0.24	0.07	0.7
Perceived vulnerability			0.53		0.47	1.6	0.7	3.6
Perceived severity			0.82		**0.89**	2.4	1.05	5.7
Response efficacy			1.37		**1.36**	3.9	1.7	9.9
AIC	186.72		178.07		170.74			
H-L (p)			*χ^2=9.2,Df=8,p = 0.32*		*χ^2=4.8. Df=8,p = 0.78*			
Likelihood Ratio Test (p)			*χ^2=34.2,Df=13,p < 0.001*		*χ^2=29.9. Df=7,p < 0.001*			
Pseudo R^2			*0.29 (p < 0.001)*		*0.26 (p < 0.001)*			

[1] Other variables included in the Full model included gender, knowledge of parasites, awareness of parasites, worry and self-efficacy

5.5.6. Factors associated with adoption of health-protective wildlife food handling behaviors

Results from the multivariable analyses are presented in Table 5.4. A higher education level, perceived severity and response efficacy were all significantly associated with the dependent variable (adoption of food safety protective practices) in the final binary logistic regression model. Although there was no significant difference for perceived vulnerability, the variable was retained since removal resulted in more than 30% change in the regression coefficient for perceived severity. Other than for perceived vulnerability, no confounders were detected in the final model. Moreover, no interaction was detected and model fit was adequate (Table 5.4). Respondents with the highest and intermediate education categories had lower odds of adopting health-protective behaviors compared to respondents in the lowest education level (OR=0.29 versus OR=0.24, respectively). Respondents who had higher perceived severity (OR: 2.4) and response efficacy beliefs (OR: 3.9) had higher odds of adopting safe wildlife food handling behaviors.

5.6. DISCUSSION

This paper highlights the influence of several factors on the adoption of health-protective behaviors with respect to handling of food from wildlife in Nunavik. A large proportion of Inuit in the community of Inukjuak were aware of wildlife parasites. However, less than 20% and 30% of respondents had heard about *Toxoplasma gondii* and *Trichinella nativa*, respectively, despite the existence of long-standing screening programs for these parasites in Nunavik (Mcdonald et al, 1990; Proulx et al., 2002). A higher awareness level for *Trichinella nativa* could be attributed to the regional screening program that directly involves community members (e.g. hunters). Moreover, trichinellosis outbreaks linked to the consumption of walrus meat that have led to the medical evacuation of clinically advanced cases for appropriate intensive care may have increased community members' awareness of this parasite (Larrat et al., 2012). However, awareness does not necessarily imply that people are knowledgeable of their significance as foodborne health hazards, which is partly reflected in this study by the fact that less than 50% of Inuit had high levels of knowledge concerning parasite transmission. A low level of awareness with respect to specific parasites may be explained partly by a lack of access to information on the types and risks of foodborne pathogens in some wildlife. It is also possible that Inuit do not believe that parasites from wildlife can negatively impact their health (Puffall et al., 2011).

Only one quarter of people perceived wildlife parasites as a threat to their health. It has been shown that even when risk is communicated to the general public, people may still perceive that benefits outweigh the risk (Frewer, 2000; Furgal and Rochette, 2007). The benefits of country food of wildlife origin are well recognized in Northern Canada (Kuhnlein et al., 2000). Optimistic bias, the belief that one's own risk is lower than for other people, could partly explain a low level of perceived threat and this was reflected by the fact that almost 45% of respondents

consumed raw tissues while processing wildlife carcasses (de Sousa Carvalho Rossi et al., 2017). This said, Inuit consider raw meat as health-promoting which makes the possibility of "risk" counterintuitive culturally-speaking (Food Safety Network, 2009). Cultural value, rather than optimistic bias, may therefore better explain the low levels of perceived threat in this study. Many foodborne parasites (including *Toxoplasma gondii* and *Trichinella nativa)* are not grossly visible, and some pathogens (i.e. *T. gondii*) can remain latent in healthy people (Dubey, 2010). A direct association between exposure to a parasite from wildlife and the subsequent development of disease (e.g. threat) is therefore not possible, which could also explain the low level of perceived threat for certain pathogens.

Perceived threat derives from the product of perceived vulnerability and perceived severity (Rogers, 1975). Perceived severity, but not perceived vulnerability, was significantly associated with the adoption of health-protective behaviors. This highlights the importance of separately analyzing both vulnerability and severity. Inuit with a high level of perceived severity had higher odds of adopting health-protective behaviors. This suggests that food safety messages which heighten levels of perceived severity could promote the adoption of health-protective behaviors in Nunavik. Doing so would require careful consideration, since labelling wildlife consumption as a "risky behavior" could also lead to decreased country food consumption, which in turn could promote food insecurity in the already highly food-insecure North (Power, 2008). One possible solution is to avoid the word "parasite" as a generic term and to differentiate "non-zoonotic" from "zoonotic" parasites in food safety messages by specifying the names of specific foodborne parasites that can be harmful to human health. This way, perceived benefits of wildlife can be maintained while informing consumers about the possibility of exposure to serious zoonotic

parasites. Ideally, the risk of human exposure to known host-pathogen combinations (e.g. *Trichinella nativa* in walruses) should be specified when possible.

Personal vulnerability was not shown to influence the adoption of health-protective behaviors, in contrast with what is usually predicted by the PMT model (Schafer et al., 1993). This was also supported by results of the multiple correspondence analyses where personal vulnerability was not associated with the adoption of protective behaviors. It is possible that the question format was not a good indicator for "vulnerability". Moreover, people with lower levels of perceived vulnerability may not have been knowledgeable on the existence of wildlife parasites or aware that parasites can be transmitted through food of wildlife-origin, though this was not shown to be the case statistically (data not shown). As well, consumption of wildlife is a habitual, centuries-old behavior with inherent heuristics that could play an important role in the cultural characterization of perceived vulnerability. For example, eating raw seal meat is an important component of the Inuit concept of health (Borré, 1994). It is therefore possible that perceived vulnerability is low because the benefits of a perceived health-promoting act (e.g. consuming raw meat) outweigh the threat.

Awareness of a threat does not necessarily translate into the adoption of health-protective behaviors since people may not feel confident in their ability to apply these behaviors (self-efficacy) and/or they may not believe that these behaviors are health-protective (effective) (Schafer et al., 1993; Parra et al., 2014). In this study, people with high levels of response efficacy had higher odds of adopting safe food handling behaviors. This suggests that people who are aware of safe behaviors and their effectiveness may be more likely to adopt such behaviors. Self-efficacy was not a significant determinant of health-protective behaviors. This makes sense if the avoidance of wildlife consumption is included as a health-protective behavior

since, as previously mentioned, the consumption of wildlife (sometimes raw) is considered as health-promoting by Inuit (Kenny et al., 2018). A lack of association between self-efficacy and the adoption of health-protective behaviors could also simply be attributed to a lack of awareness of safe behaviors and/or distrust of authorities (Frewer, 2000). Further research is needed to determine whether efficacy beliefs are specific to individual health-protective behaviors; for example, consuming certain wildlife species or certain tissues raw. This would help risk managers prioritise which behaviors to emphasize when devising risk communication messages. Further work is also needed to identify traditional practices around food inspection and handling behaviors that may already be protecting Inuit health against exposure to wildlife parasites. This could perhaps partially explain why a high proportion of Inuit in this study adopted low levels of non-traditional health-protective behaviors, yet had high levels of personal perceived severity.

Uneven levels of formal education among Inuit respondents and limits on the number of questions made it necessary to simplify the survey instrument by asking closed-ended questions with binary (yes, no) and simplified ordinal outcomes. This may have limited measurement accuracy for some risk perception and/or efficacy belief variables, which could also have prevented the detection of associations with adoption of health-protective behaviors. Therefore, it is possible that associations for perceived vulnerability and self-efficacy were underestimated in this study since higher odds of adopting health-protective behaviors are usually associated with these variables based on the Protection Motivation theory (Prentice-Dunn and Rogers, 1986; Schafer et al. 1993). Response-efficacy and perceived severity have been associated with increased odds of adopting health-protective behaviors, similarly to what is reported in this study (Levy et al., 2008; Bearth et al., 2014). Inuit with higher education levels had lower odds of adopting health-protective behaviors in this study, consistent with links between high-risk food

handling and higher education levels in other studies (Schafer et al., 1993; Zhang et al., 1999; Shifera et al., 2000; Hanson and Benedict, 2002). It is possible that, due to the social desirability bias, respondents who adopted low levels of health-protective behaviors falsely reported higher levels of education, and/or that respondents with lower education levels falsely reported adopting high levels of health-protective behaviors (Hanson and Benedict, 2002). It is also possible that hunting wildlife (and therefore exposure to it) is primarily restricted to people with higher education levels with the concordant higher income needed for expenses associated with hunting (Akande et al., 2015). Because proportions of respondents in the study population were similar to gender and age group proportions reported for the community of Inukjuak by Statistics Canada (2013), we are confident that results in this study are representative of the Inukjuak community. However, although Inuit values and beliefs around the importance of wildlife as country food are similar among all 14 communities of Nunavik, wildlife consumption and food handling trends vary between communities (Lemire et al., 2015). Therefore, extrapolating results from this study to the broader Nunavik Inuit population should be done cautiously.

Although awareness of parasites in wildlife was high in the community of Inukjuak, Nunavik, Quebec, knowledge of parasite transmission and awareness of specific zoonotic parasites (*Toxoplasma gondii* and *Trichinella nativa*) were low. This likely explains why perceived vulnerability, perceived threat, and adoption of health-protective behaviors were also relatively low. The small proportion of Inuit that routinely adopted health-protective behaviors when handling wildlife also felt that parasites could have a negative impact on their health, and that health-protective behaviors are effective at preventing infection. Because the word "parasites" should not be considered as a generic term when communicating risks to the general Inuit population, it may be important to raise awareness on specific zoonotic parasites, their

transmission routes (e.g. foodborne) and their specific negative health effects on people. It is also important to avoid promoting food insecurity by unknowingly creating a negative perception of wildlife as a source of "harmful" parasites. Therefore, conveying frequent, culturally-adapted risk communication messaging on the existence and effectiveness of health-protective behaviors could more effectively protect human health without compromising country food consumption as an important source of nutrition for Inuit.

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CHAPTER 6: DISCUSSION AND CONCLUSION

6.1 *Toxoplasma gondii* in wildlife of Nunavik

In the first part of this thesis, the aim was to determine whether *T. gondii* occurs in wildlife of Nunavik based on direct detection of DNA. For the first time, DNA of *T. gondii* was detected among 46% of foxes and 9% of geese from southern and western areas in Nunavik, but was not detected in other wildlife species. DNA of *T. gondii* has been detected among 51% of foxes in Spain, compared to 31% in Norway, 19% in Belgium, 16% in Germany and 69% in the United States based on parasite isolation using either conventional DNA extraction kits or bioassays followed by DNA characterization (Calero-Bernal et al., 2015; Prestrud et al., 2008; De Craeye et al., 2011; Hermann et al., 2012; Dubey et al., 2014). The variety of direct detection methods used in these studies, each with unknown test performance characteristics, makes comparison of these results challenging at best (Calero-Bernal et al., 2015). However, besides diagnostic test performance characteristics, ecological factors could also explain the variability in the apparent *T. gondii* prevalence in foxes. Indeed, a prevalence of 46% in this study was high considering that the aforementioned studies, except for the one in Norway, are from areas where cats are endemic. Cats are the only known definitive hosts for *T. gondii* and shed oocysts into the environment within their feces (Dubey, 2010). The *T. gondii* prevalence is therefore expected to be lower in fox populations where the post-natal route of exposure is limited to the transmission of tissue cysts through foodborne routes, but this was not the case in this study. Food consumption behaviors could be different between fox populations; for example, where certain prey species infected with *T. gondii* are consumed more frequently in one region versus another. This level of information is challenging to obtain. Epidemiological factors could also explain

regional differences in *T. gondii* prevalence; for example, if the *T. gondii* prevalence varies in prey populations from different regions. The *T. gondii* seroprevalence in arctic foxes of eastern Nunavut was 70.4% based on an IFAT, with a seroprevalence of 25% in Ross's geese and 26% in Lesser Snow geese, which is much higher than what we detected in foxes and geese of Nunavik. In this region of central Nunavut, geese constitute an important component of the arctic fox diet which could help explain why seroprevalence was much higher in this fox population (Elmore et al., 2014). These prevalences are based on serology rather than direct detection, so this should be interpreted with caution since serological exposure does not always correlate with active infection. Finally, it has also been reported that foxes can be infected through cannibalism which is known to occur in Svalbard, though this remains unconfirmed for Nunavik (Prestrud et al., 2008).

The finding of *T. gondii* DNA in geese tissues supports previous epidemiological findings of associations between the consumption of waterfowl and Inuit exposure to *T. gondii* in Nunavik (Messier et al., 2009). It also supports the hypothesis that migratory geese harbor *T. gondii* and the notion that migratory birds can act as healthy carriers of pathogens between southern and northern ecosystems (Prestrud et al., 2007; Elmore et al., 2014). Migratory birds are also known to act as transport vehicles for ectoparasites (ticks, fleas) infected with zoonotic pathogens (e.g. *Borrelia burgdorferi,* the agent of Lyme disease) and other pathogens such as avian influenza A viruses (Georgopoulou and Tsiouris, 2008; Bodewes and Kuiten, 2018). This is also the first time that DNA of *T. gondii* was detected in breast muscle, liver, and gizzard from naturally-infected seropositive geese. The ability to quantify tissue parasite load represents an important strength in this study. Tissue parasite loads were theoretically high enough to produce infection

in people given general daily goose consumer trends in Nunavik (Lemire et al., 2015) and infectious doses of bradyzoites (based on experimentally-infected mice and cats) (Dubey, 2006).

The *T. gondii* strain isolated from a single goose in this study was characterized as Type II based on the GRA6 gene, similarly to what was detected in three foxes from Chapter 2. This strain is the one most commonly involved in human cases (Dubey, 2010). In the absence of data on *T. gondii* strains in people of Nunavik, it is not known if geese carry the parasite strain responsible for the high *T. gondii* seroprevalence observed in Inuit of Nunavik. It is possible that geese can be co-infected with a mixture of clonal lineages and atypical *T. gondii* strains, which have increasingly been reported in North American wildlife (Dubey et al., 2011). Therefore, the use of more advanced methods for characterizing *T. gondii* strains present in migratory geese and other wildlife of Nunavik represents an important next step. Because recovering sufficient target DNA (needed for both detection and genotyping) from naturally-infected geese is no small challenge, future efforts should also be made to: 1. analyse more individual geese (increase sample size) to increase the chances of finding positive geese, as well as 2. analyse more tissues from confirmed positive geese (ideally both brain and heart should be analysed as the first step). This would improve chances of recovering sufficient target DNA for subsequent characterization and allow for a better description of *T. gondii* genetic diversity in geese that migrate to Nunavik.

No detection of *T. gondii* DNA occurred in ptarmigan, the only species to display consistent negative serological and molecular results. These results therefore do not support the hypothesis that oocysts occur in the terrestrial environment of Nunavik. This contrasts with the fact that *T. gondii* antibodies were detected in the sera of 23% of caribou, another endemic herbivorous

species of Nunavik; however, caribou are semi-migratory and have larger home range sizes than ptarmigan. This seroprevalence value is much higher than the *T. gondii* seroprevalence of 1.5% (n=268; in 1998) and <1% (n=120; in 2009) reported from the Leaf River herd based on a MAT test at a cut-off value of 1:25, but much lower than the 62.5% (n=40; in 1989) based on the Sabin-Feldman dye test (considered as the gold standard for *T. gondii* serology) detected in Kuujjuaq, Nunavik (Leclair and Doidge, 2001; Curry, 2012; McDonald et al., 1990). Assuming that exposure to *T. gondii* is possible, individual caribou could be exposed to oocysts in the southern part of their migratory route, where lynx occur (MDIFW, 2016). Although *T. gondii* has been detected in the sera of 44% of lynx (n=106) in Northern Quebec, oocysts have never been detected in the feces of lynx, nor has DNA been detected in tissues (Labelle et al., 2001). DNA of *T. gondii* was not detected in any of the caribou tissue samples in the current study. It is possible that the MC-PCR technique failed to detect *T. gondii* DNA in caribou since only small samples of tissues were available for the extraction of target DNA, which may have decreased the probability of detection. It is also possible that, although the MC-PCR technique is sensitive (large amounts of tissue in the order of up to 100 g can be analyzed), parasite loads in caribou tissues were below the detection limit. This is important since *T. gondii* cysts are not uniformly distributed among and within tissues of infected animals and this can make a significant difference if only small portions of tissues from larger species are used, in comparison to entire organs used in smaller species (Dubey, 2010). Our seropositive samples, in combination with findings of DNA in all tissues examined in reindeer experimentally exposed to high doses of Type III *T. gondii* oocysts (Bouchard et al., 2017), suggest that further work is needed to assess the tissue infection status of naturally-exposed caribou. For this, larger amounts (at least 100 g)

of different tissues should be included to provide more insight on the food safety risk of *T. gondii* in caribou.

DNA of *T. gondii* was not detected in any ringed seals or walruses, despite evidence of exposure based on serology in ringed seals. Seroprevalence was estimated at 20% on MAT and 30% on ELISA, which was comparable to previous estimates of 7-14% in seals of Nunavik (Leclair and Doidge, 2011; Simon et al., 2011). Following lipid removal as per Blanchet et al. (2011), seroprevalence dropped to 0% on MAT and only a third of the samples remained positive on ELISA. Unlike caribou, large amounts (up to 100 g) of three tissues were analysed for each individual ringed seal in this study, so we are more confident about the lack of DNA detection in ringed seals. The inclusion of brain tissue, considered as an important predilection site for *T. gondii* in several species, could have strengthened our results and should be considered in future research. This, in combination with positive findings on serology, suggests a need to validate serological assays for detecting *T. gondii* antibodies in marine mammals and to carefully interpret previous findings in which lipids were not removed or other confirmatory testing (such as PCR or bioassay) was not performed. Under the assumption that seropositive ringed seals were truly positive, it is possible that seals are exposed to oocysts shed in the feces of lynx from boreal regions (Labelle et al., 2001) and transported into the ocean via runoff or through the consumption of filter feeding fish or invertebrates that filter water contaminated with *T. gondii* oocysts (Dubey, 2003; Simon et al., 2013). Only tongue was analysed in walruses and no DNA was detected. Future research should assess serology and analyse several tissues for detecting *T. gondii* of DNA in order to shed more light on the occurrence of *T. gondii* in walruses.

There was no agreement between *T. gondii* detection based on serology versus direct detection in all species except for foxes (in which there was moderate agreement) and ptarmigan (all samples were negative for each test). Indeed, some seropositive individuals were negative for *T. gondii* based on direct detection, while some seronegative individuals were positive based on direct detection. Several limitations and explanations for discrepancies between antibody and DNA-based results have been discussed in previous chapters of this thesis, leading to the main following conclusions: 1) using serology and the MC-PCR technique in tandem can increase the sensitivity of detection of positive individuals, and 2) serology should not be used to infer the occurrence of an active infection. Because there was disagreement between serological and DNA detection results, tissues from seronegative animals should also be analysed to confirm whether active infection occurs in the absence of detectable antibodies. For now, our results do not support using serology to make decisions on the possible infection status of tissues from individual animals, especially where decisions impact food security such as in Nunavik. False-positives on serology, resulting in discarding a healthy animal as a source of food, could promote food insecurity if people who rely on or prefer to eat wildlife choose to discontinue their consumption.

This component of the thesis helped fill gaps relative to our knowledge of *T. gondii* in Nunavik. First, it confirmed that active infection does occur in at least two wildlife species and, therefore, that *T. gondii* is indeed present within wildlife reservoirs in Nunavik. Secondly, it highlighted that the MC-PCR technique can be used as an alternative, or complementary, detection method to serology in wildlife with respect to *T.gondii*. Several questions remain: Does *T. gondii* occur in other coastal communities of Nunavik? Do foxes and Inuit from different locations in Nunavik

share common sources of exposure? From a public health point of view, could *T. gondii* also occur in other wildlife species consumed by Inuit in Nunavik? Because both *T. gondii* prevalence in wildlife and consumer patterns (preparation method, consumption frequency) affect the risk of Inuit exposure to *T. gondii*, a better understanding of goose consumer trends is warranted. Finally, because direct detection of *T. gondii* in goose tissues was confirmed in this component of the thesis, another important question was whether Inuit can be exposed to viable *T. gondii* bradyzoites through the consumption of geese.

6.2 Inuit exposure to *T. gondii* in geese

This work demonstrated that, based on a study group of 30 respondents, people from Inukjuak were exposed to an average of 642 bradyzoites over a one-month period. There was a 32% probability that any one person was exposed to at least 1 bradyzoite, compared to a 23% probability that someone was exposed to a dose of more than 100 bradyzoites. Cooked (or partially cooked) breast meat contributed the most to the average monthly *T. gondii* bradyzoite exposure dose, followed by unprocessed (raw) gizzard and cooked heart. The assessment factored in inconsistency in the finished cooking temperatures, which can result in the incomplete inactivation of *T. gondii* bradyzoites, since most Canadian consumers are unaware of the internal temperature of their meat after cooking (Murray et al., 2017). Inappropriately cooked tissues (such as large portions of breast muscle) can contain viable bradyzoites at their core (Tenter et al., 2000). This exposure assessment therefore highlights that cooking to an internal temperature above 67 ºC can play an important role in decreasing the probability of exposure to *T. gondii* while still promoting consumption of geese. As for raw gizzard, this tissue is not consumed as frequently as other tissues. However, the absence of a cooking step circumvents the

possibility for bradyzoite inactivation so that a higher tissue load, rather than a higher consumption frequency, could explain why it had the second highest contribution. Cooked heart was the third largest contributor despite the fact that it is consumed in smaller quantities and at lower frequencies compared to breast muscle and gizzard. However, this can be explained by the fact that goose heart appears to have a higher parasite load compared to other tissues from naturally-infected geese (Bachand et al., 2019). Finally, liver contributed the least to the monthly exposure dose. This does not infer that the tissue itself presents a lower risk of exposure, but rather that its infrequent consumption is protective.

Not everyone in Inukjuak necessarily consumes each of the four tissues which means that exposure may have been overestimated for some people in this study. However, some people may consume tissues other than those considered in this assessment, which means that exposure may have been underestimated for others. This being said, every respondent in this study consumed breast meat which implies that the probability of exposure was likely not underestimated with respect to this specific tissue. Since every respondent in this study reported cooking breast meat prior to its consumption, this also means that *T. gondii* exposure from geese could significantly be reduced by encouraging people to ensure that breast meat is cooked at an internal temperature above 67 ºC or that it is frozen at less than -12 ºC for at least 72 hours as a more culturally-appropriate way of inactivating tissues cysts. Because exposure was assessed specifically at mealtime, future work is needed to factor exposure for individuals actively involved in harvesting and/or preparing geese during which goose tissues could also be consumed. Probabilities of exposure would have intuitively been higher if the exposure assessment had factored annual or lifetime geese consumption patterns since several studies have

reported a higher *T. gondi* seroprevalence in older people assumed to have had more opportunities for exposure. Lifetime, rather than monthly, exposure to *T. gondii* would have therefore provided better insight for explaining the high *T. gondii* seroprevalence observed in Inuit of Nunavik.

Unlike several quantitative microbial risk assessments previously done for *T. gondii* in domestic animals, where prevalence is extrapolated from serological data or meta-analyses, the major strength of this exposure assessment was that it factored prevalence and parasite quantification data generated from hunter-harvested geese actually destined for consumption by Inuit in Nunavik (Bachand et al., 2019). Moreover, despite a small sample size, consumer data at the community level were collected to fill gaps specific to the target group (Inuit) directly concerned with the outcome of this exposure assessment since granular information on goose consumption was not available. So, though goose consumption patterns were based on a small sample size, data generated from this group provided the best available data at this time. Because consumption patterns are variable between communities in Nunavik, extrapolating outcomes of this exposure assessment to other communities should be done cautiously (Lemire et al., 2015). Moreover, because consumer data were collected during the first month of the goose spring migration, when geese become accessible after a 7-month period while geese reside in their overwintering grounds, goose consumer patterns for this month are not necessarily representative for all months of the year in Nunavik. Moreover, because data were collected for a single month, these results also cannot be extrapolated to annual patterns. Despite these shortcomings, exposure to *T. gondii* through consumption of goose tissues is plausible for Inuit of Inukjuak in Nunavik. Undercooked breast meat had the highest contribution toward the monthly exposure

dose of viable bradyzoites. The recommendation to thoroughly cook tissues is supported by this exposure assessment and should be emphasized in risk communication. To reduce uncertainty, data is needed on actual (consumer-reported) finished cooking temperatures for different goose tissues in Nunavik. Moreover, in order to improve the reliability of the exposure assessment, information is needed on parasite load (liver, gizzard), additional consumer data (e.g. all types of tissues consumed, community-specific data, tissues consumed during carcass dressing, etc.), and tissue processing data (tissue processing methods other than cooking, variation in processing methods among seasons or communities, etc.). This exposure assessment can be used to predict *T. gondii* exposure probabilities for other country foods using food-specific parameters. Moreover, results from this exposure assessment could be used to estimate the health significance of *T. gondii* by performing a full risk assessment, which would integrate a risk characterization step specific to different target groups (e.g. pregnant women) once more data on *T. gondii* bradyzoite infectious doses for people are known. Finally, a comparative exposure assessment could be helpful in evaluating the relative importance of different wildlife species as sources of exposure to Inuit of Nunavik, once gaps concerning species-specific data (e.g. prevalence, tissue loads, and consumer patterns) are known.

6.3 Risk perceptions, knowledge and adoption of health-protective measures in Nunavik

Although country foods play an important role in the Inuit way of life, they can also be a source of contaminants and zoonotic parasites that may affect people’s health (Pufall et al., 2011; Proulx et al., 2002). This especially holds true in a culture where people consume raw and partially cooked meat (Food Safety Network, 2009). Because risk communication messages can compromise the positive views that Inuit have of country foods, it was important to better

understand Inuit awareness and knowledge of parasites from wildlife (O'Neil et al., 1997). Moreover, it was important to understand perceptions regarding the safety of country foods in the context of wildlife parasites, and how these influence the adoption of health-protective measures against exposure to parasites.

This thesis demonstrated that a large proportion of Inuit were aware that wildlife can harbor parasites. However, few Inuit knew about specific parasites including *T. gondii* and *Trichinella nativa.* A higher level of awareness occurred for *Trichinella nativa*, which could maybe be explained by the active involvement of community members (e.g. hunters) in the regional *Trichinella* screening program from walrus tongues, and that outbreaks of trichinellosis have sometimes led to the medical evacuation of community members (Larrat et al., 2012).
Less than half of Inuit had high levels of knowledge concerning parasite transmission from wildlife, similar to what has previously been found in Nunavik (Pufall et al., 2011). This could be explained by insufficient access to information surrounding risks of exposure to zoonotic parasites in wildlife. One study has also highlighted that people may not wish to know about parasites since it may affect how they view country foods and whether they choose to continue to consume it (Pufall et al., 2011). Only one quarter of Inuit perceived wildlife parasites as a threat to their health, and 45% of all respondents reported the consumption of raw tissues while processing wildlife. This was also similar to previous findings where people did not consider parasites in country foods as a major concern (Pufall et al., 2011). Inuit consider country food (and, in some circumstances, consumption of raw tissues) as health-promoting which likely makes the concept of "threat" counterintuitive, culturally speaking (Food Safety Network, 2009). Added to this, *T. gondii* is not grossly visible in tissue and remains latent in most healthy animal

and human hosts (Dubey, 2010). One study found that Inuit had a hard time believing that contaminants in country foods can affect their health since, similar to *T. gondii*, they cannot be seen (O'Neil et al, 1997). The same study reports that Inuit are resistant to invisible knowledge that cannot be validated through sensory experience. They also believe that there are obvious signs if an animal is unhealthy; for example, if it is infected with parasites. However, this may not always be the case for all parasites including *T. gondii*. It is therefore virtually impossible for people to make the association between exposure to wildlife infected with *T. gondii* and (later) development of disease (e.g. the threat). Lastly, people may simply not believe that parasites in country foods are harmful to human health if previous generations have consumed it for thousands of years, hence a low level of personal perceived vulnerability (Pufall et al., 2011).

Perceived severity and response efficacy were both elevated in people who reported adopting higher levels of health-protective behaviors while handling wildlife, which suggests that food safety messages that heighten people's level of perceived severity could promote the adoption of health-protective behaviors in Nunavik. This also suggests that awareness of health-protective behaviors and their effectiveness may lead to the increased adoption of public health recommendations. Uneven levels of formal education among Inuit respondents and limits on the number of questions to include in the questionnaire made it necessary to simplify the survey instrument by asking closed-ended questions with binary (yes, no) and simplified ordinal outcomes. This may have had limitations on measurement accuracy for some risk perception and/or efficacy belief variables, which could have prevented the detection of their associations with the adoption of health-protective behaviors. Therefore, it is possible that associations for perceived vulnerability and self-efficacy were underestimated in this study since higher odds of

adopting health-protective behaviors are usually associated with these variables based on the Protection Motivation theory (Prentice-Dunn and Rogers, 1986; Schafer et al. 1993). Lastly, though the sample size was smaller than expected, we are confident that results in this study are representative of the Inukjuak community since proportions of respondents in the study population were similar to gender and age group proportions reported for the community of Inukjuak by Statistics Canada (2013).

Because wildlife is valued by people as an important source of food and for cultural identity in Nunavik, food safety messaging should not deter Inuit from consuming wildlife. One possible solution could be to avoid use of the word "parasite" and to differentiate "non-zoonotic" from "zoonotic" pathogens by specifying the names of the specific zoonotic parasites that may be harmful to human health. Perceived benefits of wildlife can then be maintained while ensuring that consumers are informed of the risk of exposure to zoonotic pathogens through consumption of some wildlife species. Moreover, people should be made aware of health-protective measures that can decrease their probability of exposure to *T. gondii;* for example, cooking meat above 67ºC or freezing at -12ºC (or below) for at least 3 days.

6.4 Conclusion

This thesis showed that *T. gondii* occurs in the terrestrial ecosystem of Nunavik based on detection of DNA in tissues from foxes and geese using the MC-PCR technique. Detection of *T. gondii* in geese provides stronger support for the hypothesis that migratory birds carry the parasite between the North and their overwintering grounds. The absence of DNA detection in two terrestrial and two marine wildlife species endemic to Nunavik weakens the hypothesis that wildlife and people are exposed to *T. gondii* oocysts in Nunavik. However, consistent discrepancies between results of indirect and direct detection techniques for *T. gondii* in wildlife warrant more in-depth validation of these tests for use in wildlife.

Exposure to *T. gondii* through consumption of goose tissues is plausible for Inuit of Inukjuak in Nunavik. Undercooked breast meat had the highest contribution toward the monthly exposure dose of viable bradyzoites and recommendations to ensure that tissues are sufficiently cooked should be emphasized. Finally, Inuit awareness levels of parasites were generally high, but knowledge of specific zoonotic parasites was low. People do not consider parasites as a threat to their health, but the smaller proportion of Inuit who did adopted high levels of health-protective behaviors and high levels of response efficacy.

With respect to *T. gondii* in geese, a suitable message could therefore be that *T. gondii* can cause problems in pregnant women who have never been exposed to the parasite, that parasite detection has been confirmed in at least four goose tissues, that their consumption raw could possibly lead to exposure to *T. gondii*, but that cooking above 67 ºC or freezing at -12ºC for 3 days ensures that geese can continue to be safely consumed.

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APPENDIX A: SEAL SAMPLING SHEET

ᓄᓇᕕᒻᒥ ᒫᓚᔪᓂᑦ ᖃᐅᔨᑕᕐᓂᖅ 2015 ᒫᓚᔪᒥᒃ ᓇᓗᓐᒃᑯᔩᒍᑎᒃ:

ᑐᖅᑯᑕᐅᐱᕕᓂᖓᑕ ᐅᓪᓗᖓ: ᐅᓪᓗᖓ ____________ ᑕᖅᑭᖓ _________ 2015

(ᐊᖑᓇᓯᑦᑎᐅᑉ ᑕᑕᕐᓴᒥᐊᓕᖓ)

Natsiq (ᓇᑦᓯᒃ) ☐

ᓇᓂ ᒫᓚᔪᖅᑕᐱᓂᖅ: ☐☐☐☐☐☐ ☐☐☐☐☐☐

Latitude Longitude

ᑕᕐᓯᓗᒍ ᐊᑐᖅᑕᐱᓃᑦ: ☐ Degree Minutes Second ☐ Decimal degrees ☐ Degree Decimal

ᓱᓇᐅᓂᖓ: ☐ ᐊᖑᑎ ☐ ᐊᕐᓇᖅ ☐ ᓇᓗᓇᕐᑐᖅ

ᐅᑭᐅᖓ: ☐ ᓵᕐᖑᓕᐊᖅ (< 1 year) ☐ ᐅᕕᒐᕐᑐᖅ (1 to 4 years) ☐ ᒪᓇᒃ (≥ 5 years)

ᑎᒥᖓᑕ ᖃᓂᖓ: ☐ ᓴᓪᓗᖅ ☐ ᓈᒻᒪᑐᖅ ☐ ᖁᐃᓂᔪᖅ

ᐱᐅᓯᕐᓯᓯᐊᖕᖏᒍᑎᖏᓐᓂᒃ ᖃᐅᔨᒍᕕᑦ → ᓯᑰᓴᔭᐅᑉ ᑐᓄᐊᓂ ᐊᓪᓚᓗᒥᑦ

ᖃᐅᔨᓴᐅᑏᑦ ᐃᓗᓕᓅᑦ:

☐ 1. ᐅᖅᑲᖅ ᔫᕐᒦᑐᖅ #1

☐ 2. 200 ᑯᕿᒻᓯ ᐆᒪᑎᖓ ᔫᕐᒦᑐᖅ #2

☐ 3. 200 ᑯᕿᒻᓯ ᑲᓂᕈᐅᑎ ᔫᕐᒦᑐᑦ #3

☐ 4. 200 ᑯᕿᒻᓯ ᑎᖑᖓ ᔫᕐᒦᑐᖅ #4

☐ 5. ᐊᐅᒃ: →(2) ᓯᑯᑦᓴᔮᐲᑦ ᐊᐅᒥᑎᕐᓯᒪᔪᑦ ᔫᕐᒦᑐᑦ #5

→ (1) ᑐᕐᓯᐊᑯᑖᒦᑦᑐᖅ ᔫᕐᒦᓯᓂ #6

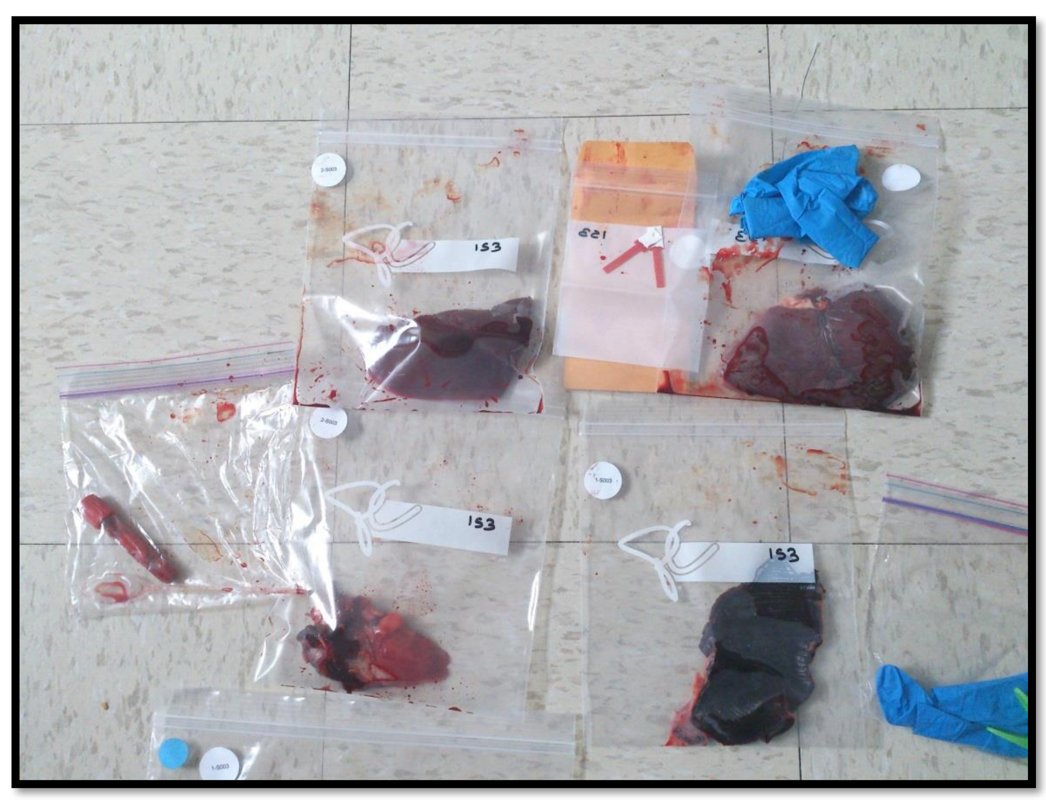
ᐊᑭᓕᕐᑕᐅᓂᕐᒧᑦ ᖃᐅᔨᓴᐅᑏᑦ ᑕᑕᕐᓴᒐᕐᓗ ᑐᓂᓗᒥᑦ ________________ ᓯᕓᓂᐅᑎᒃ#: ____________

____ ____

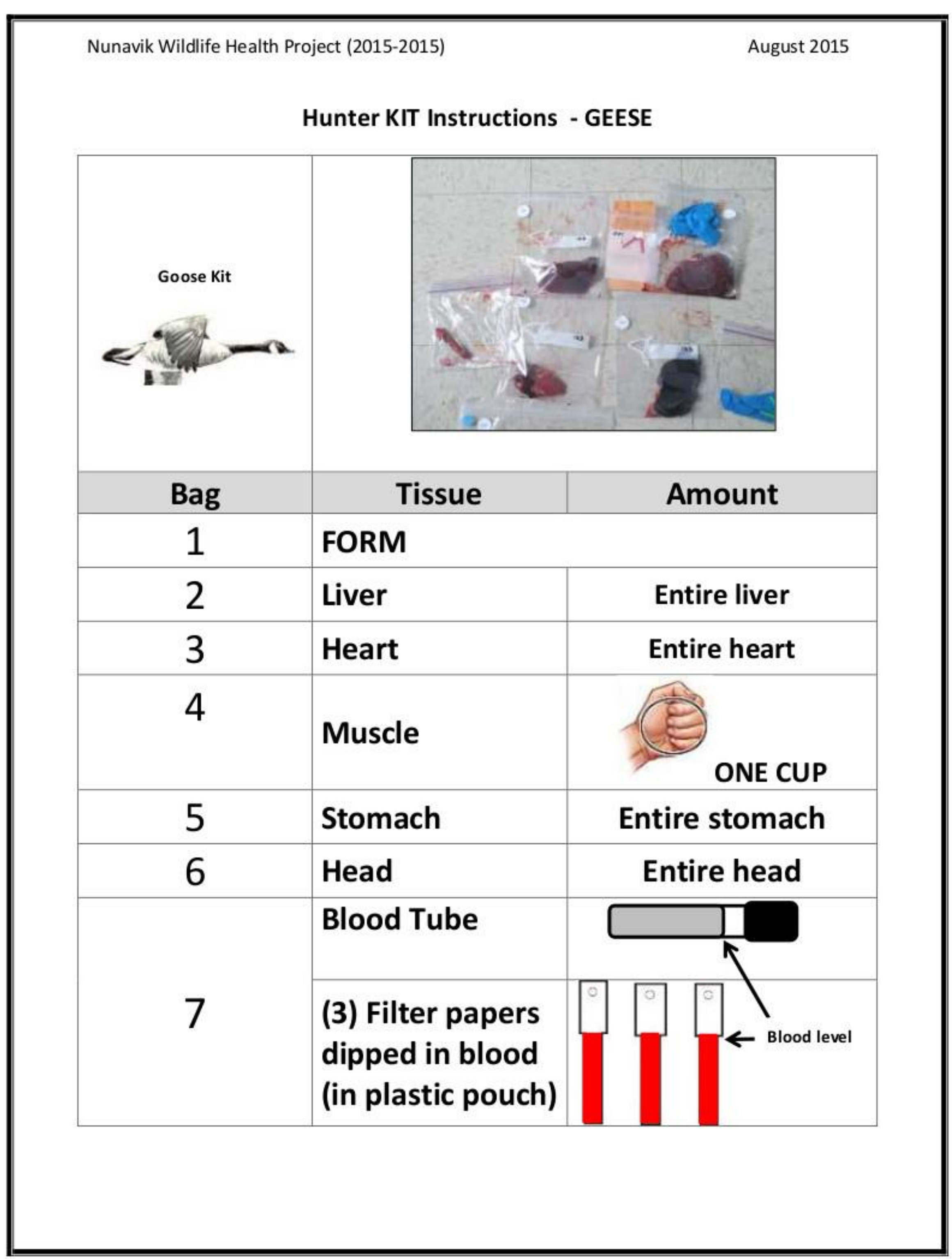
ᐊᑎᓕᐅᕐᕕᖓ (ᑐᑭᒧᐊᑦᑎᓯᔪᖅ) ᐊᑎᓕᐅᕐᕕᖓ (ᐊᖑᓇᓯᑦᑎᐅᑉ)

☐ ᐊᑭᓖᔪᖅ: ________________________________ $ 30 ᐊᑐᓂᑦ ᒫᓚᔪᒧᑦ

APPENDIX B: SEAL SAMPLING KIT



APPENDIX C: HUNTER SAMPLING INSTRUCTION SHEET (GOOSE)



Nunavik Wildlife Health Project (2015-2015) August 2015

Hunter KIT Instructions - GEESE

Goose Kit

Bag	Tissue	Amount
1	FORM	
2	Liver	Entire liver
3	Heart	Entire heart
4	Muscle	ONE CUP
5	Stomach	Entire stomach
6	Head	Entire head
7	Blood Tube	
	(3) Filter papers dipped in blood (in plastic pouch)	Blood level

APPENDIX D: GOOSE SAMPLING SHEET

Nunavik Wildlife Sampling 2015 **Unique Animal Code:**

Kill Date: Day ___________ Month _________ 2015

(To be filled by the hunter)

Specie (goose): ☐

Kill Location: ☐☐☐☐☐☐ ☐☐☐☐☐☐ Latitude Longitude

Check which system used: ☐ Degree Minutes Second ☐ Decimal degrees ☐ Degree Decimal Minutes

Animal gender: ☐ **Male** ☐ **Female** ☐ **Unknown**

Animal age: ☐ **Juvenile** (Less than 1 year) ☐ **Adult** (More than 1 year)

Animal condition: ☐ **Thin** ☐ **Average** ☐ **Fat**

Abnormal findings → write on back of sheet

Kit includes:

☐ 1. **HEAD** in bag # 1

☐ 2. **200 grams of BREAST MEAT** in bag # 2

☐ 2. **HEART** in bag # 3

☐ 3. **LIVER** in bag # 4

☐ 5. **GIZZARD in** bag # 5

☐ 6. **BLOOD:** → **(2)** filter papers filled with blood in bag # 6

→ **(1)** tube filled with chest fluid in bag # 6

For payment, submit kit & form coordinator

_________________________ **Signature (Coordinator)**

_________________________ **Name (Hunter)**

☐ Payment by: _________________________ **$ 15 per animal**

APPENDIX E: PTARMIGAN SAMPLING SHEET

ᓄᓇᕕᒻᒥ ᐆᒪᔪᓂᑦ ᖃᐅᔨᑕᕐᓂᖅ2015 ᐆᒪᔪᒥᒃ ᓇᓗᓂᒃᑯᔩᒍᑎᒃ:

ᑐᖁᑕᐅᕕᐱᓂᖓᑕ ᐅᓪᓗᖓ: ᐅᓪᓗᖓ ___________ ᑕᖅᑭᖓ _________ 2015

(ᐊᖑᓇᔪᑦᑎᐅᑉ ᑕᑕᕐᓴᒥᐊᓚᖓ)

ᐊᕐᑭᓪᒥᐅᑉ ᖃᓄᐃᑦᑑᓂᖓ: ᐅᕐᐱᒥᐅᑕᖅ ☐ ᖄᖅᑐᑦ ᖀᖅᑐᒥᐅᑕᖅ ☐

ᓱᓇᐅᓂᖓ: ☐ ᐊᖑᑎ ☐ ᐊᕐᓇᖅ ☐ ᓇᓗᓇᖅᑐᖅ

ᐅᑭᐅᖓ: ☐ ᐅᕝᕙᓚᖅᑐᖅ (Less than 1 year) ☐ ᒪᓐᒃ (More than 1 year)

ᑎᒥᖓᑕ ᖃᓂᖓ: ☐ ᓴᓪᓗᖅ ☐ ᓈᓪᒪᑐᖅ ☐ ᖁᐃᓂᔪᖅ

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Pointe Despins
Cox Island
Cape Dufferin
Inukjuak
Harrison Island
Frazier Island
Leonard Island
0 10 20km

Natural Resources Canada Ressources naturelles Canada

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Canada

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APPENDIX F: LOCAL INUIT DRESSING A GOOSE

APPENDIX G: COMMUNITY FREEZER WITH COUNTRY FOOD

APPENDIX H: WILDLIFE PARASITES QUESTIONNAIRE

Veterinarinary Microbioly,
University of Saskatchewan

Participant Consent Form

Project Title: **Wildlife parasites in Nunavik - Local knowledge and perception**

Researcher(s): Nicholas Bachand, PhD Student, Veterinary Microbiology, University of Saskatchewan, (514) 234-5032, nickbachand@gmail.com

Supervisor: Emily Jenkins, Veterinary Microbiology, 306-966-2569, emily.jenkins@usask,ca

Purpose(s) and Objective(s) of the Research: To understand local knowledge of parasites in wildlife

Procedures: Information will collected using a self-administered 25-minute questionnaire. Please feel free to ask any questions regarding the procedures and goals of the study or your role.

Funded by: *Arcticnet*

Potential Risks: There are no known or anticipated risks to you by participating.

Compensation: 25 $ dollars will be given once you submit the questionnaire

Confidentiality: You do not have to include your name or home address on this questionnaire. All information will be kept confidential and will be stored in a password-protected database.

Right to Withdraw: Your participation is voluntary and you can withdraw from answering the questionnaire at any time.

Follow up: We will present results from this study in November 2016 during a final to your community.

Questions or Concerns: If you have questions or concerns, you can contact the researcher (s) using the information at the top of this page. This research project has been approved on ethical grounds by the University of Saskatchewan Research Ethics Board. Any questions regarding your rights as a participant may be addressed to that committee through the Research Ethics Office ethics.office@usask.ca (306) 966-2975. Out of town participants may call toll free (888) 966-2975.

By completing and submitting the questionnaire, your free and informed consent is implied and indicates that you understand the above conditions of participation in this study.

Wildlife parasites in Nunavik

Good morning / afternoon / evening,

My name is Nicholas, I am a student at the University of Saskatchewan. We are conducting an important study on parasites of wildlife in Nunavik.

This questionnaire will take approximately 25 minutes. Your answers will help to improve knowledge of wildlife parasites in Nunavik. This survey is anonymous and confidential. Answers to all questions are voluntary, and answers will be treated with strict confidence.

You will be provided a voucher of 25$ dollars as compensation. Thank you for participating!

Please fill in the next five questions about yourself and your household

1- Gender

1/ Male
2/ Female

2- In what year were you born? _______

3- Which cultural group do you belong to?

1/ Inuit
2/ Cree
3/ Caucasian
4/ Other: ________________________

4- How many people currently live in your household (including you) _______

5- In your household, are there:

	Yes	No	Not applicable
Children under the age of 12 ? - - - - - - - - -	☐	☐	☐
Pregnant women? - - - - - - - - - - - - - - - - - -	☐	☐	☐
People older than 65 year's old? - - - - - - - -	☐	☐	☐

I would like to know more about your knowledge of wildlife parasites in Nunavik. Wildlife parasites are very small organisms that need to live in humans or animals in order to survive. In Inuktitut, they are sometimes referred to as "qumak".

6- **Have you ever heard of parasites?**

1/ Yes
2/ No

If yes, can you describe what a parasite is in your own words?
(answer briefly below)

7- **Have you seen parasites in wildlife?**

1/ Yes
2/ No

If yes, in which wildlife species and which body parts?
(answer briefly below)

8- **Are you concerned that parasites could be harming the health of wildlife?**

1/ Yes
2/ No

If yes, why are you concerned?
(answer briefly below)

9- **Are you concerned that parasites in wildlife could be harming your health? If yes, how concerned?**

1/ Extremely concerned
2/ Very concerned
3/ Somewhat concerned
4/ No very concerned
5/ Not concerned at all

If you're concerned, why are you concerned? (answer briefly below)

10- **Have you heard of *Toxoplasma (Toxoplasma* is a type of parasite that can cause problems in pregnant women)?**

1/ Yes
2/ No

If yes, where have you heard of *Toxoplasma*? (answer briefly below)

11- **Have you heard of *Trichinella* (*Trichinella* is also a type of parasite)?**

1/ Yes
2/ No

If yes, where have you heard of *Trichinella*? (answer briefly below)

Is each following statement *True*, *False* or *Don't Know*?
(check only one box per line)

	True	False	Don't Know
12- Wildlife parasites can infect people:			
a. By direct contact with healthy people, once they are infected	☐	☐	☐
b. Through air, if infected people cough or sneeze	☐	☐	☐
c. By drinking infected water from any source	☐	☐	☐
d. By eating infected animal meat or organs	☐	☐	☐
e. From mother to child during pregnancy	☐	☐	☐
f. Through contact with soil	☐	☐	☐
13- A person can have parasites without showing symptoms	☐	☐	☐
14- There are ways to prevent being infected with parasites	☐	☐	☐
15- Wildlife parasites can harm human health	☐	☐	☐

16 - Do you believe that the following wildlife species are likely or unlikely to carry parasites that can affect people?

	Very Unlikely 1	Unlikely 2	Not likely 3	Likely 4	Very Likely 5	Don't Know 6
1/ Caribou	☐	☐	☐	☐	☐	☐
2/ Ringed seals	☐	☐	☐	☐	☐	☐
3/ Canada or snow geese	☐	☐	☐	☐	☐	☐
4/ Willow or rock ptarmigan	☐	☐	☐	☐	☐	☐

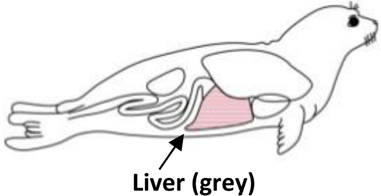
17- How concerned are you that wildlife parasites can harm human health?

1/ Extremely concerned
2/ Very concerned
3/ Somewhat concerned
4/ Not very concerned
5/ Not concerned

Questions **18 *to* 26** *have to do specifically with ringed seals (shown in diagram)*

Ringed seal

Questions **18 to 20** have to do with seal **LIVER (see diagram)**:

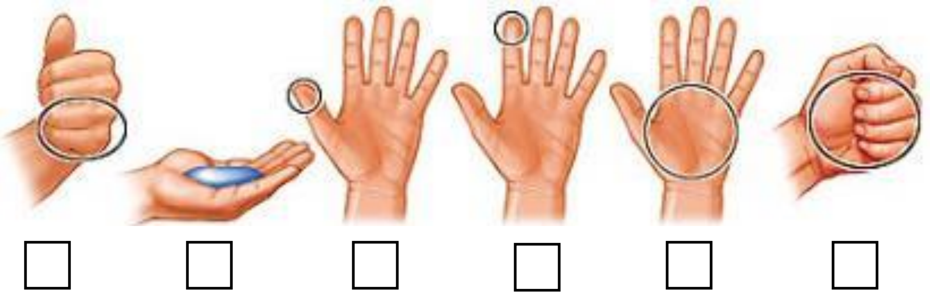


Liver (grey)

18- In the last year, how many times did you eat seal LIVER (from different seals)?

1/ Never in the last year
2/ Once
3/ Twice
4/ If more than twice, how many: ________

19- When you eat seal LIVER, what is a typical portion size?
(please check ONE (1) box according to the option that fits your usual portion size)

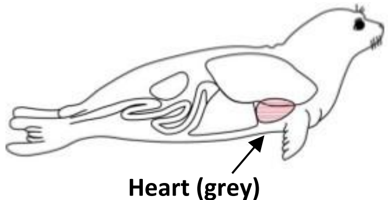


☐ ☐ ☐ ☐ ☐ ☐

20- In the last year, how often did you eat seal LIVER <u>raw or undercooked</u>?

1/ Never
2/ About half the time
3/ Always

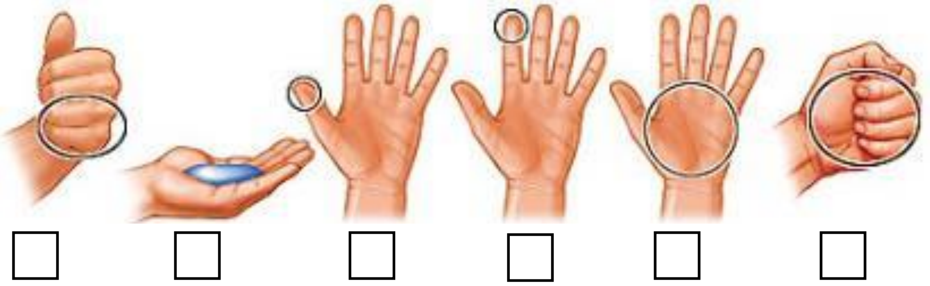
Questions **21** to **23** have to do specifically with seal **HEART**:



21- In the last year, how many times did you eat seal HEART (from different seals)?

1/ Never this last year
2/ Once
3/ Twice
4/ If more than twice, how many: _______

22- When you eat seal HEART, what is a typical portion size?
(please check ONE (1) box according to the option that best fits your usual portion size)

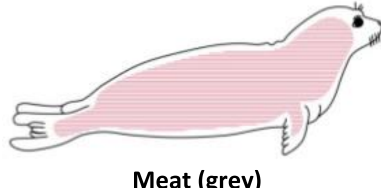


☐ ☐ ☐ ☐ ☐ ☐

23- In the last year, how often did you eat seal HEART <u>raw or undercooked</u>?

1/ Never
2/ About half the time
3/ Always

Questions **24** to **26** have to do with seal **MEAT (see diagram)**:

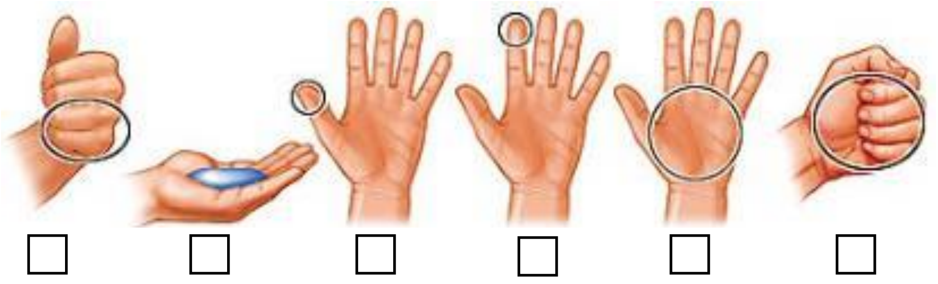


Meat (grey)

24- In the last year, how many times did you eat seal MEAT (from different seals)?

1/ Never this last year
2/ Once
3/ Twice
4/ If more than twice, how many: _______

25- When you eat seal MEAT, what is a typical portion size?
(please check One (1) box according to the option that best fits your portion size):



☐ ☐ ☐ ☐ ☐ ☐

26- In the last year, how often did you eat seal MEAT raw or undercooked?

1/ Never
2/ About half the time
3/ Always

27- In the last year, where did you get food from ringed seals?
(more than one answer is possible)**:**

1/ From within my own household (I live with a hunter)
2/ From family outside my household (in the community)
3/ From community members other than my own family
4/ From the hunter support program in my community
5/ From another community
6/ Other (please specify): ___________________________

28- When you handle food from hunted wildlife <u>in your home,</u> do you always:
(check only one box per line)

	Yes	No	Not *Applicable*
1/ Wash hands with soap and water after handling an animal	☐	☐	☐
2/ Wear gloves while handling or cutting the animal	☐	☐	☐
3/ Wash & disinfect all equipment that's been in contact with an animal	☐	☐	☐
4/ Bleed and remove the intestines and stomach quickly	☐	☐	☐
5/ Cool the carcass by keeping the chest open	☐	☐	☐
6/ Cut pieces into smaller pieces	☐	☐	☐
7/ Keep the hide (skin) on the animal	☐	☐	☐
8/ Keep the animal cool within 24 hours of consumption	☐	☐	☐
9/ Eat some parts raw while working on the carcass	☐	☐	☐
10/ Cut yourself accidentally while cutting the animal	☐	☐	☐
11/ Throw remainders of the animal back into the land or local garbage	☐	☐	☐
12/ Feed part of the animal to your dog (s)	☐	☐	☐

I would now like to ask you how serious you think each of the following diseases are and how likely you think it is that you will get these diseases in the next year.

29- In our own opinion, on a scale of 1 (not serious) to 10 (very serious), how serious do you think each following disease is? (check one box per line only)

	Not Serious at all 1	2	3	4	Somewhat serious 5	6	7	8	9	Very serious 10
1/ Diabetes	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2/ Food poisoning	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3/ Rabies	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4/ Heart disease	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5/ Cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6/ Wildlife parasites from food	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

30- Do you believe that you are likely or unlikely to get this disease in the next year?

	Very Unlikely 1	Unlikely 2	Not likely 3	Likely 4	Very Likely 5	Don't Know 6
1/ Diabetes	☐	☐	☐	☐	☐	☐
2/ Food poisoning	☐	☐	☐	☐	☐	☐
3/ Rabies	☐	☐	☐	☐	☐	☐
4/ Heart disease	☐	☐	☐	☐	☐	☐
5/ Cancer	☐	☐	☐	☐	☐	☐
6/ Wildlife parasites from food	☐	☐	☐	☐	☐	☐

I will now ask you some questions about actions you can take to prevent getting different diseases. First, I will ask if you think you can personally prevent yourself from getting these diseases.

	Not at all 1	A little bit 2	Quite a bit 3	Definitely 4	Don't Know 5
31- Can actions be taken to prevent people from getting wildlife parasites ?	☐	☐	☐	☐	☐
32- Are you good at preventing yourself from getting wildlife parasites?	☐	☐	☐	☐	☐

33- What can be done to prevent people from getting wildlife parasites?
(please provide your answer below)

34- What you like to have more information on wildlife parasites?

1/ Yes
2/ No

If yes, what type of information would you like to have and how should it be presented (written pamphlets, videos, etc…)? (answer below)

Now we just need a little more information about you if possible.

35- Which category is best to describe your current employment?

1/ Student
2/ Teacher
3/ Health care worker
4/ Factory worker
5/ Other type of employment (__________________________)
6/ Unemployed
7/ Stay at home parent
8/ Retired
9/ (partly) disabled

36- What is the highest education you completed or are still following?

1/ Primary school or no education
2/ Lower general secondary education or Lower vocational education
3/ Intermediate/higher general secondary education or Intermediate vocational education
4/ Higher vocational education or University education

Thank you very much for your participation!

APPENDIX I: GOOSE CONSUMPTION FREQUENCY QUESTIONNAIRE

Goose Consumption Survey

1. Gender: ☐ Male ☐ Female (Pregnant?: Yes___ No___)
2. Year of Birth: ______
3. Number of people in household: ______
4. Is there a hunter in your household? ☐ No ☐ Yes

1. How many Canada geese did you eat in the last 30 days? _______
2. How many snow geese did you eat in the last 30 days? _______
3. How many times was a goose frozen before you ate it? _______
4. Which of the following goose tissues do you USUALLY eat raw (uncooked):
   - ☐ Heart
   - ☐ Liver
   - ☐ Breast meat
   - ☐ Gizzard (stomach)
   - ☐ Other tissues: ____
   - ☐ I do not eat any goose tissues raw
5. How many meals does ONE GOOSE usually last for in your household?
   a. A single meal
   b. Two meals
   c. More than 2 meals (please specify the USUAL number of meals: ______)

Please estimate your average food use as best you can
and please answer every question by placing ONE tick (✓) per line

6. In the last month, how many times did you eat the following goose tissue while **CLEANING A GOOSE** after it was shot?
   *For example, if you cleaned two geese during the last month and ate the heart each time, please tick (✓) the box under the title "**2-3 times in the last 30 days**" for the line "1.heart"..*

Goose tissue	Never	Once	2-3 times	Once a week	2-3 times per week	5-6 times per week	Once per day	2-3 times per day
1.Heart								
2.Liver								
3.Stomach								
4.Meat								

Before moving on, please check if there is ONE tick (✓) per line

7. In the last 30 days, how many times did you eat EACH following <u>goose tissue</u> while **PREPARING A GOOSE** right before a meal? Please place ONE tick (✓) per line.

Goose tissue	Never	Once	2-3 times	Once a week	2-3 times per week	5-6 times per week	Once per day	2-3 times per day
1.Heart								
2.Liver								
3.Stomach								
4.Meat								

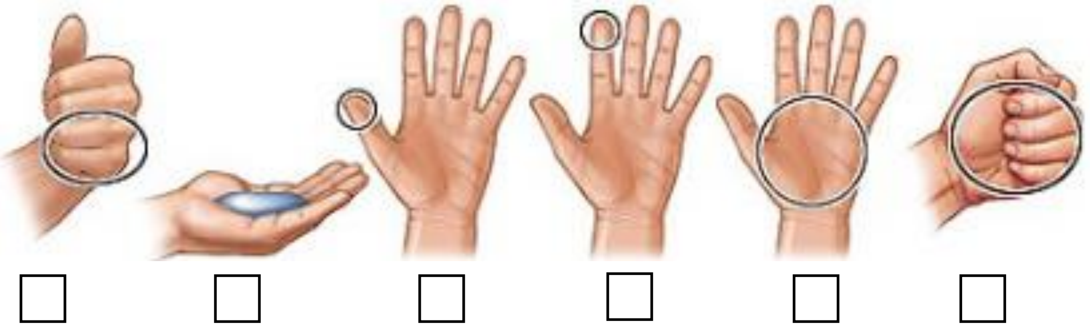
8. In the last 30 days, how many times did you eat each following <u>goose tissue</u> **AS A MEAL**? Cooking can include boiled, grilled, baked or smoked. Please place a tick (✓) per line.

Goose tissue	Never	Once	2-3 times	Once a week	2-3 times per week	5-6 times per week	Once per day	2-3 times per day
1.Heart cooked								
2. Heart raw								
3.Liver cooked								
4. Liver raw								
5.Stomach cooked								
6. Stomach raw								
7. Meat cooked								
8.Meat raw								

9. In the last 30 days, HOW MUCH of each tissue did you USUALLY eat at any given meal? Please place a tick (✓) per line.

	The entire organ	Half (1/2) of the organ	Less than one half the organ
1.Heart			
2.Liver			
3.Stomach			

10. In the last 30 days, how much do you <u>USUALLY</u> consume when you eat goose <u>**MEAT**</u>? Please tick only **ONE** box



☐ ☐ ☐ ☐ ☐ ☐

APPENDIX J: MAGNETIC CAPTURE LABORATORY CHECKLIST

Table 1. Samples

Sample ID	Tissue Type	Number of grams	mls of CLB

Table 2. Cell lysis buffer (2.5 mls per gram). Total mls needed:_____________________________

Reagent	Per gram	Number of grams	Total mls needed
100mM Tris.HCl pH8.0	0.25 mls		
5mM EDTA	0.025mls		
0.2% SDS (5ml 20%)	0.05mls		
Prot K (20 mg/ml)	5 ul		
Sterile Dist H20			

Table 3. Controls

Type	Tissue (animal, type)	Concentration	mls to spike with
Negative		n/a	n/a
Positive 1		$2.5x10^3$ /ml (250 tachys)	100 ul
Positive 2		$2.5x10^4$/ml (2500 tachys)	100 ul
Positive 3		$2.5x10^5$/ml (25000 tachys)	100 ul

DAY 1: Cell lysis buffer preparation

1. Get Sterile Distilled Water from GMP (order enough in advance) - - - - - - - - - - - - - -☐
2. Thaw proteinase K -☐
3. Thaw tachyzoites - ☐
4. Prepare Cell Lysis Buffer as per **Table 2** -☐
5. Place SDS last (since it is soapy) -☐

DAY 1: Tissue preparation

6. Thaw all tissues 5 hours before (or leave in fridge the night before) - - - - - - - - - - - - -☐
7. Pre-label all stomacher bags -☐
8. Weigh tissue without connective tissue, vessel wall or fat (min: 32 gr) - - - - - - - - - - ☐
9. Cut it into small pieces (approximately 1x1x1 cm) -☐
10. Clean knifes and cutting board using hot water and soap, followed by DNAzap) - - - ☐
11. Place cut tissue in a stomacher bag with filter add cell lysis buffer - - - - - - - - - - - - -☐
12. Add spike to controls – last 2 bags -☐
13. Stomacher for 2 min at 300 rpm (high speed) - ☐
14. Seal the bag -☐
15. Incubate overnight at 55°C -☐
16. Clean up (cutting board, knives and forceps in bleach overnight) - - - - - - - - - - - - - -☐

DAY 2: Supernatant transfer

17. Pre-label **two** 50 ml tubes and **two** 15 ml tubes -☐
18. Cut stomacher bag open with scissors and stomacher for 1min at 300 rpm - - - - - - - -☐
19. Disinfect scissors with alcohol between each step -☐
20. Transfer 50ml of crude lysate to 50ml tube -☐

21. Centrifuge tubes for 45min at 3500 x g - ☐
22. Transfer 12ml of supernatant to clean 15ml tube (to use in subsequent steps).- - - - - ☐
23. Transfer rest of supernatant to clean 50ml tube (to store at -20°C) - - - - - - - - - - - - - ☐

Day 2: Removal of biotin

24. Incubate 15ml tubes for 10min at 100°C (don't cap them too tightly!) - - - - - - - - - - ☐
25. Wash 50µl of streptavidin sepharose/sample in 1xPBS 500-1000µl, 3 times (short spin, 20s) - ☐
    a. Mark level of strep-seph taken the first time - ☐
    b. Once washed 3 times, resuspend in PBS to get the original volume - - - - - ☐
26. Cool the tubes down to approx. 40°C under running water - - - - - - - - - - - - - - - - - ☐
27. Centrifuge for 1 min at 3500 x g (to remove condensate from lids) - - - - - - - - - - - - ☐
28. Add 50µl of washed streptavidin sepharose to each sample - - - - - - - - - - - - - - - - - ☐
29. Incubate rotating for 45min at room temperature - ☐
30. Centrifuge the tubes for 15min at 3500 x g - ☐
31. Transfer 10ml of supernatant into a clean 15ml tube - ☐

(possible to continue on the next day, leave the samples in the fridge overnight)

DAY 2/3 : Hybridization

32. Set-up 95°C and 55°C beaker baths - ☐
33. Thaw capture oligos (Tox R & Tox F; 10pmol\uL per capture oligo) - - - - - - - - - - ☐
34. Add 10pmol of each capture oligo to each tube - ☐
35. Mix by decanting the tubes - ☐
36. Keep the tubes at 95°C for 15min to denature the DNA - ☐
37. Move tubes to a water bath at 55°C for 45min (mix a few times) - - - - - - - - - - - - - ☐
38. Wash dybabeads in the meantime - ☐
39. Afterwards cool tubes down to room temperature while rotating for 15 min - - - - - ☐

DAY 2\3: Washing the dynabeads

40. Resuspend the dynabeads by decanting the bottle (do not use vortex!) - - - - - - - - - ☐
41. Take 80µl of bead-suspension/sample in a 1.5ml tube (or 2) - - - - - - - - - - - - - - - ☐
42. Mark **original volume** of dynabead-suspension on tube - - - - - - - - - - - - - - - - - - - ☐
43. Place in magnet and remove supernatant after 2min - ☐
44. Resuspend the beads in 80µl 1x B&W buffer - ☐
45. Place in magnet and remove supernatant after 2min - ☐
46. Resuspend in 80µl of 1x B&W buffer at **original volume** - - - - - - - - - - - - - - - - - ☐

DAY 2\3 Capture

47. Add 2ml of 5M NaCl and 80µl of washed dynabeads PER tube - - - - - - - - - - - - - - ☐
48. Incubate at room temperature, while rotating (10rpm) for 60min - - - - - - - - - - - - - ☐
49. Switch on heating block at 100°C - ☐
50. Place tube in magnet and leave it horizontally on a shaking plate for 10min - - - - - - ☐
51. After, discard the supernatant - ☐
52. Resuspend the dynabeads in 500µl of 1x B&W - ☐
53. Transfer to 1.5ml eppendorf tube. - ☐
54. Place in small magnet for 2min - ☐
55. Pipet and discard the supernatant - ☐
56. Resuspend in 100µl 1x B&W, and place back in the magnet - - - - - - - - - - - - - - - - ☐
57. Pipet and discard the supernatant - ☐
58. Resuspend in 50µl of **sterile distilled water** (PCR-grade). - - - - - - - - - - - - - - - - - ☐

DAY 2\3: Release target from beads

59. Heat the eppendorf tubes at 100°C for 10min, vortex carefully now and then - - - - - ☐
60. Place the tube in magnet and directly pipet supernatant into clean tube - - - - - - - - - ☐
61. Store at - 20°C - ☐

APPENDIX K: *TOXOPLASMA* qPCR LABORATORY CHECKLIST

Step 1: Place PCR tubes in clean box and turn UV light on for 20 minutes - - - - - - - - - - - - - ☐

Step 2: Thaw reagents for Mastermix

- Itaq Probe Supermix - ☐
- Probe (Tox-TP1) (20uM) - ☐
- Primer 9F (10uM) - ☐
- Primer 11R (10uM) - ☐

Step 3: Calculate Amount of Mastermix Needed - ☐

# of samples: ____
# of NTC's: ____
CP's: ____
of Extras: ____
Total #:tubes ____ * 2 = ____

Mastermix (25 uL reaction): - ☐

iTaq Probe Supermix:	6.5 uL *	____ Tot # of tubes =	_____
Probe (TOX-TP1) (20uM)	0.25 uL *	____ Tot # of tubes =	_____
Primer 9 F (10 uM)	1.25 ul *	____ Tot # of tubes =	_____
Primer 11 R (10 uM)	1.25 uL *	____ Tot # of tubes =	_____
Distilled water	7.75 uL *	____ Tot # of tubes =	_____
Tot volume:			_____

Step 4: Return to clean room and reconstitute your Mastermix

- Gather pipettes, pipette tips, disposal container and rack with reagents - - - - - - - - ☐
- Label an Eppendorf tube with ''Mastermix'' - ☐
- Vortex all your reagents - ☐
- Transfer all the required amounts of each reagent into the MM Eppendorf tube - - ☐
- Place all unused reagents back into freezer - ☐
- Clean up - ☐

Step 5: Thaw your template DNA - ☐

Step 6: Return to clean box (once 20 minutes has expired)

- Place required amount of MM (17uL) per each NTC tube and close tubes - - - - - - - - ☐
- Place required amount of MM (17uL) per each sample tubes - - - - - - - - - - - - - - - - - ☐
- Place required amount of MM (8uL) per each sample tubes (including CP's) - - - - - - ☐
- Place plasmid DNA for standard curve (to quantify) - ☐
- Close all tubes, spin and go to BioRad machine - ☐